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REVIEW

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Reproductive tract microbiome and therapeutics of infertility

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Abstract

Background The microbiome is still a new area of research in human health and disease, especially in reproductive health. The present article aims to aid the perception on reproductive tract microbiome that may enable better management of its dysbiosis causing reproductive dysfunctions.

Main body In recent years, identification of microbiota in every part of human body has been eased by next-generation sequencing-based tools. It has been demonstrated that resident microbiota is vital for normal reproductive functions. The development of disease may result owing to changes in the microbiota brought about by internal or external factors. Female reproductive microbiota may be crucial in the success of assisted reproductive technologies such as embryo implantation and prenatal care. Though much has been learned about the vaginal microbiota, the uterine microbiome has gotten very little research attention. The impacts of well-known microorganisms including *Chlamydia trachomatis*, *Mycoplasma tuberculosis*, and *Neisseria gonorrhoeae* have been well documented, resulting in subclinical alterations that are considered risk factors for infertility and poor reproductive outcomes. Research on microbiota of male reproductive system is still in its early stages, and there are numerous questions concerning how inflammation and urogenital infections might impact male fertility. Certain microorganisms reportedly can directly affect spermatozoon function without even inducing oxidative stress or inflammatory cytokines, but via adhering to the spermatozoon or producing soluble factors capable of altering sperm motility and/or inducing apoptosis.

Conclusion The presence of specific microbiota in the reproductive tract, regardless of their pathogenicity, or the alteration of the reproductive tract resident microbiota may pose issues with fertilization, implantation, pregnancy as well as embryo development. This may result in the failure of fertility treatments and a reduction in the number of live births.

Keywords Infertility, Microbiota, Gonadotropins, Ovarian reserve, Polycystic ovary syndrome, Insulin resistance

Background

Genitourinary tract microbiota has thoroughly been analyzed through the past decades for the identification of various microorganisms as well as their innate influence on human reproductive pathophysiology [1, 2]. Since lately, a complete list of bacteria that typically reside in the reproductive tract has emerged with new biomolecular capabilities and/or methods for DNA sequencing like next-generation sequencing (NGS) [3]. In recent years, investigations concerning the human microbiome have contributed significantly to the understanding of microbial communities and their compositional diversity across various anatomical regions of the human body.

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These findings reveal that approximately 9% of the total bacterial burden within the human organism is localized within the female reproductive tract, emphasizing the importance of the microbiota in this particular anatomical niche [4]. In healthy individuals, Lactobacilli have been found to the chief bacteria in reproductive tract, along with some other microorganisms like *Gardnerella*, *Prevotella,* Bifidobacterium, *Sneathia*, Megasphaera, *Atopobium,* and *Anaerococcus* [5–7]. *Lactobacillus* dominates the microbiome of the human reproductive tract, which creates an acidic environment that was considered to protect women against sexually transmitted diseases and opportunistic diseases. Lactobacilli dominance appears to be peculiar to humans; whereas lactobacilli are generally found in > 70% of human vaginal microbiota, lactobacilli are seldom seen in more than 1% of vaginal microbiota in other mammals [8].

Infertile men and healthy men who donate their sperm for artificial insemination both exhibit robust microbiomes in their seminal fluid, indicating the presence of microorganisms in the male reproductive system [9, 10]. Surprisingly, the microbiome isolated from seminal samples are associated with semen parameters, where appearance of *Lactobacillus* reportedly facilitates the male reproductive functions whereas, other microorganisms like, *Anaerococcus*, *Pseudomonas*, or *Prevotella* are marked as a major cause of poor quality spermatozoa [9, 11].

Previous studies have demonstrated that the reproductive tract microbiota contributes to various aspects of reproduction, including gametogenesis, fertilization, gestation maintenance, and the initial colonization of neonatal microbiota [1]. It is believed that a 'normal/healthy' microbiome is distinct from one that is 'dysbiotic' or 'abnormal' [3] and deviates body from normal physiological state. This article aims to review the most recent reports on the human reproductive tract microbiome and the role of these microorganisms in reproductive functions.

Main text

Characterization of the reproductive microbiome

The uterine environment was postulated to be devoid of pathogenic microorganisms, an assumption stemming from its etymological derivation. Consequently, the preponderance of information pertaining to the female reproductive system has been primarily acquired through the examination of specimens derived from the vaginal *milieu*. In healthy female, the typical vaginal microbiome is often dominated by the bacteria *Lactobacillus spp*. [12], however age and hormonal changes may alter the microbiome environment [13]. For instance, in infants, the observed vaginal flora is a combination of aerobic and anaerobic microbial inhabitants together with the strains of *Prevotella*, *Enterobacteria*, *Streptococcus*, and *Staphylococcus* [14] later pubertal changes induce the vaginal environment to become estrogenic, causing glycogen levels to rise and pH levels to fall, with Lactobacilli as the prime microorganism. A second division was made into five community state types (CSTs) in order to further characterize the vaginal flora. It has been explained that, above 70% of female with vaginal microbiota directed by CST-I, CST-II, CST-III, and CST-V viz. *Lactobacilli crispatus, Lactobacilli gasseri, Lactobacilli iners*, or *Lactobacilli jensenii* [15]. A very few number of women show CST-IV distinguished by supremacy of anaerobic microorganisms like *Atopobium, Aerococcus, Gardnerella, Dialister, Prevotella, Megasphaera,* and *Sneathia* and less number of Lactobacilli [12]. Aside from that, reports on a healthy upper genital tract microbiota are scarce [16]. A quantitative polymerase chain reaction (qPCR) analysis of bacteria taken from most of the endometrial samples recovered from females (without any symptoms) undergoing hysterectomy did, however, classify the microbiome of the upper vaginal canal as a species [17]. It is generally known that the amount and quality of the microbiome change between the upper and lower genital tracts; however, the qPCR details were only focused on a specific unit of bacteria [17]. After doing a next-generation sequencing analysis of the 16SrRNA gene, it was possible to distinguish between vaginal and uterine microbiota in asymptomatic infertile and fertile female (nonpregnant) subjects [18]. In a rigorous study conducted by Mitchell et al., the researchers identified the presence of bacterial colonies in all endometrial samples obtained from the study participants. The predominant microorganism identified was Lactobacillus, followed by *Gardnerella, Prevotella, Atopobium*, and *Sneathia*. A noteworthy observation was that, in approximately 20% of the female subjects examined, there was a significant dissimilarity between the microbial communities found in the endometrium and those present in the vagina. This finding suggests that the composition of the microbiota in these two anatomical sites is not identical [18].

Microbiome alterations in physiological and pathological conditions

Association of microbiome with the success of fertilization and continuation of early pregnancy has just recently been reported [19]. Understanding of the variations in microbiome in reproductive disorders may guide modern therapeutic interventions that might ameliorate the consequences in earlier non-curable clinical conditions. The connection linking clinically distinct infection, inflammation with modified reproductive functions have been reported [20–22]. Inflammation includes

release of numerous cytokines (which are proinflammatory in nature) and growth factors released by immune cells triggered by infested pathogens. Minute alterations in the microbiome via inflammatory mediators are not of clinical significance in the majority of instances [19]; nevertheless, the exact molecular pathways involved have not been completely studied and documented. Most likely, the impact of some microbiome components will not be felt immediately through interrelationships with the regional organ system. *Lactobacillus* is the most common bacteria that regulates the vaginal microbiota [5]. Despite this, the existence of particular subtypes of *Lactobacilli* that are effective as probiotics and are also responsible for preventing the overgrowth of other types of bacteria can be explained by the presence of a typical environment. They are capable of producing increased amounts of hydrogen peroxide (H_2O_2) and are generally considered to be beneficial. Because these bacteria can be toxic to microorganisms that generate little or no H_2O_2-scavenging enzymes (e.g., catalase), it is hypothesized that a lack of H_2O_2-producing *Lactobacillus* species could allow catalase-negative organisms to overgrow [23].

The growing body of evidence suggests that the human microbiome is not merely a collection of free-floating bacteria residing on human tissue surfaces. Multiple investigations have sought to construct comprehensive models of three-dimensional, network-like architectures, which in some instances consist of a single layer or incorporate both inner and outer layers. These protective outer layers, composed of polysaccharides, nucleic acids, and proteins, function as a defensive barrier against foreign entities. Consequently, such structures may attenuate immune responses and diminish the effectiveness of treatments employing antimicrobial agents [24]. Biofilms are the three-dimensional structures that have formed on the surfaces of tissues where they have settled and have been referred to as biofilms. Because biofilms have the capacity to execute essential physiological and pathological actions, they have sparked the interest of significant research undertakings in recent years.

Normally, biofilms exist in the vagina yet frequently extend into the space of endometrium [25] and sometimes into the fallopian tubes [21]. It is vital to recognize that the interaction between the microbiome and the müllerian system may be more complex than simply the presence or absence of specific microbe, or even their tissue load [21]. Future research should address the microbial interactions that causes various biofilms as well as their successive influence on reproductive physiology. In the Paramesonephric ducts, the microbiome has the potential to affect the reproductive system and maybe even alter gametogenesis [26]. Certainly, active microbiomes are also observed in ovarian follicles [21]. In addition to having an impact on the müllerian system, the microbiome may also have an impact on the reproductive axis, and it may even have an impact on gamete formation [1]. Indeed, it is possible that ovarian follicles contain active microbiome. Researchers have found that some bacteria can have negative impact on follicular growth and can even limit gonadotropin response in some women [27]. A similar effect may be seen in the male reproductive axis, with small changes in the microbiome being linked with changes in the semen parameters [21].

Majority of research assessing the effect of the microbiota on ART and clinical outcomes are association studies [21, 28, 29]. Detailed mechanistic investigations that might lead to the development of novel treatment techniques are required.

The microbiome of the male reproductive tract

The microbiota of seminal fluid has been the focus of most studies on male reproductive tract microbiome [9, 11]. Prostatitis is linked to a wide range of infections, including *gonorrhoea*, *chlamydia*, and more. Metagenomics methods have been utilised to characterise the seminal microbiota and the conventional semen analyses were used to identify individual specimens. Hou and his colleagues studied 77 samples taken from 58 infertile women and 19 semen donors [9]. The V1-V2 region of the 16S ribosome was successfully pyrosequenced. Naive Bayesian classification was used to categorize all sequence reads greater than 100 bp in length, which were then linked to the Silva database. Data from the various samples were found to be organized into six distinct groups. Semen parameters were similar across these groups. However, *Anaerococcus w*as shown to be the only taxonomic group to be related with aberrant semen parameters. These findings were again addressed by Weng et al. using 96 different specimens [11]. Out of the total of 96 specimens, 60 underwent conventional semen analysis, which revealed one or more anomalies in the parameters. The remaining 36 specimens with normal semen parameters were included as a control group. Despite the fact that this investigation used a targeted amplification strategy, primers specific to the 16S ribosomal DNA V4 region were employed. Next generation sequencing was carried out using the Miseq platform after barcoding. The ribosome database of the National Center for Biotechnology Information analyzed subsequent relevant trimming reads > 100 bp. Interpretation of the results revealed three distinct sets of findings. These categories were identified using principal component analysis. *Pseudomonas*, *Lactobacillus*, or *Prevotella* were the major species in each of the three groups. One of the

most intriguing aspects of this study was the association among these groups and the clinical characteristics of the sperms. The *Lactobacillus* genus has a high incidence among standard specimens. Many *Lactobacillus* species, like those found in the female reproductive tract, may serve as both probiotics and protect against potentially other harmful bacteria. It is not yet determined whether or not H_2O_2-producing *Lactobacilli* are primarily capable of serving as probiotics in high-quality specimens. Moreover, it is not clear if the altered microbiome affects spermatozoa or if the variations in seminal contents produce an environment conducive to bacterial diversity. Finally, there is no evidence that a specific treatment can be provided, monitored, and improved in seminal quality to the point where clinical outcomes are impacted. Thus, these discussed facts leave immense scope for future research.

Female reproductive tract microbiome

Vaginal microbiome and associated pathologies

Preliminary consideration of the normal vaginal microbiome is necessary before that of the entire reproductive tract. During the human microbiome research, this was done in physically fit females [5]. The study examined 113 women and distinguished between the introitus, midway, and posterior fornix as three distinct anatomical regions of the vagina. A subset of research patients was evaluated after an additional 219(±69) days from the first specimen collection in order to establish inter-sample diversity. A total of 5,408 (±4,605) sequences per specimen with an average length of 448 (±99) bp base pairs were obtained using 454 pyrosequencing to evaluate 16 s rRNA in the hyper variable V3-V5 regions. RDP system of taxonomy used to assign species-level functional taxonomic characteristics. The research work permitted for the specification of alpha diversity (inter-sample) besides beta diversity (intra samples) also and contributed an amazing report in case of vaginal microbiome. The genital tract represented the fewest amount of alpha and very few of beta diversity when categorized using phylotypes contrasted with different part of body areas like oral cavity and skin [5]. In the above study, all specimens were taken from the three vaginal locations, hence sample variation was low and Lactobacilli predominated in all areas. Inter-sample difference decreased rapidly than intra-sample difference, demonstrating that microbiome distinctiveness is rational for all inhabitants. It is indeed true that vaginal communities in healthy persons are very simple compared to other regions of the body, which suggests that microbiome transfers may help categorize physical situations (healthy or ill).. Another study potentially assessed 152 cases receiving in vitro fertilization (IVF) [30]. The apex of the vagina, the cervix, and the nibs of the external and internal transfer catheters, as well as the culture medium used to flush the catheter after the transfer, were all tested prior to embryo transfer. When 50 colony-forming parts of each specimen were resurrected, the whole specimens in this study were found to be favorable. Patients with a positive culture from the vagina or cervix were labelled as such on the catheter tips or in the post-transfer flush medium. Only 19 of the 152 individuals were found to be completely free of microbial contamination, whereas 133 were found to be positive for one or more bacterial strains out of the total. The most typically isolated bacteria were Staphylococcus species, Lactobacillus species, and Enterobacteriaceae, which included *E. coli*, *Klebsiella*, and *Proteus*. According to the results, the implantation rates were 12.4% among those containing one or more than one microorganism verses 14% among those entirely negative ($P<0.001$). Moreover, patients containing *Enterobacteriaceae* and *Staphylococcus* found with low pregnancy rates as compare with patients lacking those strains. The work underscores the limitations of culture-based microbiome characterization and provides very limited insights into the microbiome during IVF treatments. Furthermore, 12.5% of patients tested purely negative for microbial contamination, suggesting that culture-based technologies significantly under depicts both the presence and the variety of microbial populations during embryo transfer. A successive work by using 16S sequencing technique captured an additional strong focus at the vaginal microbiome among the patients receiving IVF treatment [31]. The researchers hypothesised that as the vaginal microbiota varies during the regular menstrual cycle with fluctuating estrogen levels, the regulated ovarian hyperstimulation necessary for IVF success would likewise influence the vaginal microbiome [32, 33]. All specimens were obtained at stimulation baseline, during oocyte harvesting, before and after embryo transfer, and for women who became pregnant (6–8 weeks of pregnancy). A total of 99 vaginal samples were taken from 30 females, all of whom were analyzed. For the sequencing of the obtained samples, the Sanger sequencing method was utilized, and the RDP classifier was selected to identify the isolated bacteria. A 10% change in total readings across swab samples from the same patients was deemed significant. Microbiome density in vagina was classified using Shannon Diversity Index (SDI) and Chao1 techniques.

In 86% of the collected specimens, *Lactobacillus* was sustained by greater than half of the sequence reads. Only five patients among 30, did not display any variation in their microbiome on that time; in all the cases, patients showed predominance of monotypic *Lactobacillus* strain. In terms of outcomes, the SDI and Chao1 curves were able to differentiate between live births and stillbirths, with a lower diversity index being linked with a higher

likelihood of live birth. A small sample size and the need for further substantial, well-controlled investigations may have contributed to these rudimentary conclusions.

An acquired physiological condition may have an impact on IVF, according to studies on the vaginal microbiome. Any change or modification at the point of embryo-endometrial interaction can be a cause of creating a difference in context of immunity, which may imprint either positive or negative impact on implantation.

Bacterial vaginosis

Lactobacillus predominates in the vaginal environment, and the presence of bacterial vaginosis (BV) indicates an upsurge in *Gardnerella vaginalis* in that environment. The 16S rDNA method is used to identify BV as the most common vaginal infection. Infertility, PTB, endometritis, pelvic inflammatory disease, and a high risk of infection with the human immunodeficiency virus are all associated with this disorder, which prompts millions of women of reproductive age to seek medical attention every year in the United States. As a result, the term "BV" is problematic because it refers to both the lower and higher reproductive tracts, both of which are infected [34]. *Lactobacillus* deficiency and increased proliferation of black-pigmented anaerobes, curved anaerobic motile rods, anaerobic cocci, and gram-variable diphtherial rods were seen in women with 'white discharge syndrome', according to Curtis (1932). Drs. Gardner and Duke, on the other hand, made a solid connection between uncomplicated vaginitis disease and *Haemophilus vaginalis* [35]. This microorganism was thought to be the source of the disease as straight inoculation of cultured *Gardnerella vaginalis* did not induce it.. Probably BV is a pathologic condition linked with poly-microbial agents mainly the predominance of anaerobic microbes has been observed which form a biofilm. Although, anaerobic microorganisms are extremely unfavorable to cultivate, sequencing techniques used by microbiome characterizing supply a novel technique to recognize more principal agents excluding *G. Vaginalis*. The Amsel criteria was applied for identification in the clinical background [36, 37]. For BV diagnosis in laboratories, the Nugent score is recommended as the gold standard and is deliberated by analyzing for the appearance of large gram-positive rods, small gram-variable rods (morphotypes), and curled gram-variable rods, and a score of 7–10 is predictable for BV diagnosis [38]. A number of studies have shown that infertile women are more likely to suffer from BV, which is linked to late foetal death and preeclampsia. Despite this, no studies have proven that BV has an adverse effect on pregnancy outcomes [39, 40]. Up to 40% of women undergoing IVF therapy have been found to have abnormal vaginal tract bacteria, according to previous studies [41, 42]. *Streptococcus viridans* was found to be associated with the recovery of the embryo transfer-catheter tip from 91 women undergoing IVF with embryo transfer (IVF-ET). However, the other virulent pathogens, such as H_2O_2-nonproducing Lactobacillus, Enterobacteriacea, *Staphylococcus epidermidis*, *E. coli* and anaerobic Gram-positive cocci were not found to be associated with LBR [43]. A 50% decrease in pregnancy rates was reported in another investigation when bacteria were extracted from the embryo transfer-catheter tips [44, 45]. A new ART study used the 16Ssequencing technique to identify the organization of the vaginal microbiome during IVF cycles. Only 31 women who underwent IVF were included in the study, and none of them showed signs of bacterial illness. The posterior fornix was swabbed on the day of USG initiation, the day of oocyte retrieval, the day of embryo transfer, and between the sixth and eighth weeks of gestation. IVF-ET technology's success can be attributed to a diverse vaginal microbiota during embryo transfer, with a lactobacillus-only vaginal microbiome delivering the best results [31]. A new meta-analysis study attempted to determine whether or not there are any negative effects of BV on fertilization rates. Nugent criteria were applied to 12 studies. Infertile women have a higher incidence of BV, according to the meta-analysis (OR 3.32; 95 percent CI, 1.53–7.20). Infertile women with a tubal component (OR 2.77; 95 percent CI 1.62 to 4.75) had a significantly higher rate of BV than those with other causes of infertility. While BV was negatively associated with lower fertility rates (OR, 1.03; 95% CI, 0.79–1.33), it was found to be positively associated with a significantly higher risk of preclinical preterm birth (OR, 2.36; 95% CI, 1.24–4.51) and a lower chance of pregnancy failure in the first three months of pregnancy (OR, 1.20; 95% CI, 0.53–2.75) [46].

Other pathological conditions

Pregnancy related vaginal microbiome studies may make use of relaying aids to evaluate obstetrical and perinatal problems proceeding to symptomatic appearance. In a variety of studies, researchers have examined the link between PTB and an array of microbial associations found along the vaginal pathway, including a wide range of species [47, 48]. They found that both Caucasian and African American women with PTB had altered microbial species in their vaginal microbiomes, although not to the extent that their term gestational counterparts had, thanks to the use of a culture-free procedure and chain termination method of sequencing [49]. Experiment on the varieties of Lactobacillus species at the time of gestational period reflected that the utterance of Lactobacillus origin was greatly associated with PTB [50]. Moreover,

it has been observed that the female with BV, an unusual microbial colonization of the vaginal space, are very much prone for PTB, pregnancy loss within first trimester [46] and unsuccessfulness of IVF [31]. These all results propose that depicting the pregnancy related vaginal microbiome via 'next-generation' advanced sequencing technique probably point out the women having greater possibility of PTB.

Endometrial microbiome and associated pathologies

Pathologic ascent of organisms from the vagina through the cervical canal was once supposed to explain upper genital tract microbial infestation. Due to its high concentration of cytokines, immunoglobulins, and antimicrobial peptides, cervical mucus is sterile in healthy women [51–53]. Due to the upward translocation of the reproductive tract, this is extremely unlikely. As it turns out, when radiolabeled macroaggregates the size of human spermatozoa was injected into the posterior vaginal fornix, they were detected in the uterus within two minutes [54]. During the follicular and luteal phases of every one of the 1,000 individuals studied, this uptake was taken into account. With culture-based technologies being used for uterine microbiome research prior to hysterectomies, early studies of this area suffered from a number of constraints. In a recent study of 58 women having hysterectomy, researchers used quantitative polymerase chain reaction (qPCR) technique focused at 12 specific bacterial species [17]. Vaginal sample was performed before hysterectomy, and uterine cavity sampling was performed afterward. In 95% of the cases, at least one species had colonized the upper vaginal tract. The median bacterial concentrations in the upper genital tract were lower than those in the vaginal tract by 2–4 log10 rRNA gene copies per sample. A partial filtering of germs as they ascend by the cervix or the immune system, or a combination of the two, was proposed in this study. Some preliminary studies of the uterine microbiota of infertile women taking ART have been conducted using hysterectomy materials. In one such trial, 33 patients had a single euploid blastocyst transferred [55]. The specimen was extracted from the inner sheath of the embryo transfer catheter. In order to prevent the lower genital system from becoming contaminated, the outer catheter was first inserted into the cervix. The 16S Metagenomics Kit (Ion Torrent; Life Technologies) was used to purify and examine the bacterial 16S ribosomal DNA, which consists of two primer sets amplifying hyper variable sections V2-4, 8 and V3-6, 7–9. As a result, the amplified were analyzed and classified into OTUs using the RDP classifier by the use of succeeding generation sequencing. Following this, the SDI and Chao1 diversity metrics were analyzed. Lactobacillus and Flavobacterium were the most common species found in both pregnant and non-pregnant subjects. Diversity indexes were also high even though they looked to be identical between the two groups of people. No correlation between individual bacteria and outcomes could be found after a series of corrections in subsequent tests. However, in this pilot study, the sample size was small, which means that future studies could yield different results based on a bigger sample size [55].

Chronic endometritis

When it comes to endometrial bacteria, chronic endometritis (CE) is the most prominent pathogenic occurrence. Endometrial inflammation caused by bacteria like *Enterococcus faecalis, E. coli, Gardnerella vaginalis, Klebsiella pneumonia, Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., and reproductive tract pathogens like *Mycoplasma* and *Ureaplasma* spp., as well as yeasts like *Saccharomyces cerevisiae* and *Candida* spp., are what distinguish [56, 57]. CE is present in the general population at a rate of about 19% [58], but in the infertile population, the incidence is over 45% [59]. In spite of this greater occurrence rate, it's assumed that RIF and RPL are more likely to be linked to infertility than the other causes of infertility [60, 61]. Vaginal ultrasonography cannot always detect CE because it can be asymptomatic and asymptote. The most recent method of determining whether a woman has cervical epithelioma (CE) is based on the presence of plasma cells in the stroma after office endometrial biopsy whether using conventional staining or immune-histochemistry for CD138. Hysteroscopy has been proposed as an alternative to the gold standard diagnosis of CE by certain studies [62, 63]. Cultivation ramifications have been explained in numerous studies of asymptote RIF and RPL patients in order to improve their reproductive outcomes [61, 64]. However, bacterial cultivation is not done on a daily basis since it is an expensive and time-consuming technique, and because not all of the microorganisms responsible for CE can be grown.

Endometriosis

When endometrial epithelial and stromal tissue can be seen outside of the uterus, it is called endometriosis, and it affects about 10% of women who are reproductively active [65]. Endometriosis symptoms include dysmenorrhea, pelvic discomfort, dyspareunia, and infertility, all of which have a negative impact on the quality of life for women who suffer from it [66]. Endometriosis onset is unknown despite basic investigations. Multiple studies suggest that uterine bacterial infection may induce endometriosis. A study found higher *E. coli* in endometriosis patients' menstrual blood [67]. The authors also observed Gardnerella, Enterococcus, Streptococcus,

and Staphylococcus as mostly recognized pathogenic genera found in endometrial samples of patients with endometriosis; consecutively other group of microbiota like Corynebacterium, Actinomyces, Prevotella, Fusobacterium, and Propionibacterium, in contrast with *Lactobacillus spp.* principally noticed among the control subjects [68]**.** Invariably, microbes from the families of Streptococcaceae and Staphylococcaceae were notably spread highly in the patients with ovarian endometriosis (in cyst fluid) in contrast to control group [69]. Overall, these outcomes suggest a significant link between CE and endometriosis [69, 70]; Endometrial cells seeded into the ectopic endometrium may have contributed to the dysperistalsis and weakening of uterine contractions in women with CE [71].

Other pathological conditions

As evidenced by the sequencing of Fusobacteria and Porphyromonas, microbial infections are also linked to colorectal cancer [72, 73]. Pelvic inflammation illness [74] and cervical intraepithelial necrosis [75] have both been linked to bacterial vaginosis in the reproductive system. Thirty-one patients who had undergone hysteroscopy for endometrial cancer or endometrial hyperplasia were referred to 16S rRNA gene sequencing to investigate the role of the microbiome in the development of these cancers, which included endometrial cancer, endometrial hyperplasia, and other benign clinical conditions [76]. In patients with endometrial hyperplasia and cancer, multiple microorganisms, including Dialister, Anaerostipes, Ruminococcus, Peptoniphilus, Bacteroides, Atopobium, and Porphyromonas, were found in the genital tract, indicating an infectious/inflammatory role of bacteria in the onset of endometrial cancer [76].

The microbiota of ovarian follicles

Human follicular fluids have been studied extensively, and it has been revealed that many patients have a diverse active microbiome. Follicular aspirates from the time of transvaginal oocyte retrieval were used to collect certain specimens; others were retrieved laparoscopically [77–80]. For transvaginal oocyte aspiration, it's not clear whether the follicular fluid is contaminated or if it is truly colonized when it is punctured for aspiration [78, 80, 81]. Contaminated follicular fluid specimens include the same species as vaginal swabs. However, the significant concentration of uncommon species suggests that the follicle has been occupied for longer. Labeling a pathogen as a "possible pathogen" may overlook situations when it has colonized the upper genital canal from the vagina. Simultaneously study the vagina, endocervix, uterus, fallopian tube, follicular fluid, and abdominal cavity. Recent studies employ cell culture techniques [79, 82, 83]. An active follicular microbiome has been shown in early trials to have a significant impact on the success of ART. The clinical diagnostic of the female spouse has an effect on the microbiome's influence. Endometriosis has been linked to decreased fertility and development rates, as well as decreased transfer and implantation rates, although ovulatory dysfunction and male-factor infertility have not correlated with it [79, 82, 83]. Endometriosis may cause a more complicated immune response and a different reaction to an active microbiome, which might harm the developing egg. Thus, an active microbiome is not always harmful. Pelzer et al. found improved outcome with presence of *Lactobacilli*. [80]. The relevance of the follicular microbiota is just beginning to emerge, and it has to be examined thoroughly. Scientifically, further investigations employing metagenomic techniques could be advantageous.

Placental and amniotic fluid microbiome

The presence of pathogens in even supposedly 'sterile' places like the uterus and fetal tissues [84] has been known for decades. Pregnant women's placenta and amniotic fluid (AF) are not sterile environments, and the presence of bacterial species affects gestation and parturition, recent studies have confirmed this conclusion [85–87]. A study of previous experiments that evaluated bacteria detected in the AF has revealed that even in women with 'good' term pregnancies, bacteria are still present in the bloodstream [88]. Bacteria found in the placenta and the mouth cavity have been linked in an unusual way [89]. This study's limitations include utilizing placental tissue from known chorioamnionitis patients and culture-based bacterial species identification. DNA sequencing has shown that even "healthy" placental tissue, which is not infectious, has a range of microbial species, similar to that of the oral cavity [85, 86]. Because of this, bacterial transmission from the mouth to the placenta and the AF may be beneficial for the pregnant mother and her unborn child [90, 91]. Streptococcus, *E. coli*, Shigels, Proteus, Enterobactera, and Candida have been shown to negatively impact pregnancy [92], but they have also been shown to play a pivotal role in amino acid metabolism and positively impact sperm transport and fertility [93]. Bifidobacteria and lactobacilli, for example, have been shown to play a significant role in protecting the fetus from activated maternal immune cells by establishing an early framework for programming the naive fetal immune system [94]. Research suggests that bacterial species transported from other mucosal tissues to the placenta and amniotic fluid during pregnancy may have both symbiotic and harmful effects.

Pregnancy

Higher levels of estrogen and progesterone are thought to support pregnancy's status as a 'healthy' parasite-host physiological condition [95]. Placental components and the presence of a growing embryo (parasite) alter the mother's (host) microbial network in an unpredictable way. Pregnancy-related changes in the vaginal microbiome have been reported as early as 18–24 weeks gestation, according to a comprehensive study of microbial migrations [86]. When pregnant women were compared to non-pregnant women, this study found that while factors such as gestational planning and vaginal area had an impact on the structure of the microbial network, there were overall decreases in both microbial abundance and variety throughout the entirety of the pregnancy [86]. Examining the effects of pregnancy on the explicit bacterial scientific categorization articulation revealed that the declaration of Lactobacillus, Bifidobacteriaceae, and Streptococcaceae operational ordered units was unusually linked to pregnancy. According to newer research, pregnancies activate vaginal microbial strength which is then overrun by Lactobacillus species [50]. To protect the hatchling from the maternal safe reaction and to protect the uterine condition from microorganism invasion, improved articulation of individuals within these specific bacterial families may have the power to improve immune system responsibilities [96, 97].

Placental bacteria can be distinguished by their metabolic profiles using a metagenomic approach, such as that used by Aagaard and colleagues (2014) [98]. Placenta bacterial quality profiles supported digestion of cofactors and nutrients more than other tissues examined, according to this study (stool, tongue, back vagina, and so on). For starch and amino corrosive digestion, they saw a decrease in bacterial quality profiles. Both gestational age and the history of previous maternal contaminations had an effect on the abundance of metabolic pathways overall. Overall, this study indicated that placental capacity may be a defining aspect of placental and maternal well-being when the microorganisms invading placental tissues alter [98].

Preterm birth (PTB)

In the world, PTB is the most common cause of newborn illness and death [99]. The choriodecidual space, amnion, chorion, placenta, amniotic fluid and/or umbilical cord are all places where microbial infections can arise within the uterus, and this accounts for 25–40% of all PTB cases [49, 100]. Changing the normal intrauterine microbiome, which includes *Sneathia sanguinegens*, *Streptococcus* spp., *Ureaplasma parvum, Mycoplasma hominis, E. coli, Bacteroides spp, G. vaginalis*, and *Fusobacterium nucleatum*, has been linked to PTB in some studies. This is because the normal intrauterine microbiome includes *L. crispatus*, *Ureaplasma* [101–103]. PTB is caused by the production of pro-inflammatory molecules like IL-1b, IL-6, and IL-8, as well as macrophage colony stimulating factor-1 (MCF-1), tumor necrosis factor-alpha (TNF-α-), and prostaglandins, which trigger uterine contractions and, ultimately, cervical epithelial barrier breakdown and preterm labor [104, 105]. Prophylactic antibiotics throughout the second and third trimesters of pregnancy have been studied in an effort to avoid the bacteria related with PTB. Antibiotics lowered the likelihood of maternal infection, but did not reduce PTB, according to the findings of these trials [106, 107]. It is probable that antibiotics have a harmful effect on not only pathogenic bacteria but also helpful microorganisms in the reproductive system [108].

Chorioamnionitis

Inflammation of the fetal membranes induced by bacterial infection is the etiology of the obstetrical problem known as chorioamnionitis Infections of Streptococcus agalactiae, *F. nucleatum*, and Ureaplasma parvum have been linked to this intra-amniotic infection, while the reduced bacterial multiplicity on the fetal side of the placenta is a characteristic of severe chorioamnionitis with colonisation of *Corynebacterium* spp., *E. coli*, *Peptostreptococcus magnus*, *Prevotella bivia*, *Streptococcus* spp., and the genital mycoplasmas [101, 109, 110]. Microorganisms may arise from vaginal bacteria that climb and enter the uterine cavity, deviating from previous findings that linked amniocentesis with bacteria in the vaginal tract [111]. Having these microbes flourish in the fetal membranes triggers an immune and inflammatory response, resulting in PTB, but antibiotic therapy is highly recommended for this condition due to the reduced risk of chorioamnionitis and delivery time, as well as the prevention of neonatal infection [112].

Preeclampsia

In the United States, preeclampsia accounts for up to 8% of pregnancy problems and 16% of maternal mortality [113]. Based on bacterial cultures and targeted PCR for the 16S rRNA gene from preeclampsia patients' samples, it has recently been claimed that bacterial infections and preeclampsia may be linked [110, 114]. In a study of preeclamptic women, researchers found that the placenta contained bacterial species that are generally found in the oral cavity: *Actinobacillus actinomycetem comitans, F. nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis*, and *Treponema denticola* [114]. Patients with preeclampsia had amniotic fluid samples that contained Lactobacillus, Leptotrichia, Sneethia, Streptococcus, and Ureaplasma genera, according to

another research study [110]. Preeclampsia patients' placentas have been found to have a rise in bacteria when compared to normotensive primiparous mothers. The 16S rRNA meta-genomics analysis of preeclamptic placentas revealed bacteria usually associated with gastrointestinal tract infections (Bacillus, Escherichia coli, Listeria, and Salmonella), respiratory tract infections (*A. noxybacillus* and *K. pneumoniae*), and periodontal infections (Dialister, Porphyromonas, Prevotella, and Variovorax) in addition to other infections [115]. There may not be a single pathogen causing preeclampsia, given the wide range of bacterial species that have been linked to the condition. Preeclampsia is linked to the polymicrobial community by the activation of inflammatory and anti-angiogenic pathways, resulting in an altered trophoblast and endothelial function and an elevated blood pressure, according to this hypothesis [115].

Microbiota to improve reproductive outcomes

The human microbiota is the collection of microorganisms, including bacteria, fungi, and viruses, that live on and within the human body. These microorganisms have crucial roles in maintaining health, and imbalances in the microbiota can be associated with various diseases. In recent years, there has been growing interest in understanding the role of microbiota in reproductive health and improving reproductive outcomes.

Towards the close of the twentieth century, Egbase et al. made a definitive case for the importance of the microbiota of the reproductive tract for successful reproduction. After oocyte retrieval and 48 h after embryo transfer, the investigators compared the bacteria from the transfer catheter tip of a mock transfer done with prophylactic antibiotics to their pregnancy results [44]. Endometrial pathogens (Enterococcus, Staphylococcus, *E. coli*, and other mixed cultures) were found to be associated with a lower clinical pregnancy rate per transfer compared to those with negative cultures or those that had reacted to prophylactic antibiotics after positive cultures at egg retrieval (18.7%, vs. 41.3%, and 38.1%, $P<0.01$, respectively) [44]. This study suggests that lowering harmful reproductive tract bacteria and promoting Lactobacillus bacteria may enhance reproductive outcomes in women with aberrant microbiotas. Antibiotics and probiotics are being investigated as remedies.

For the treatment of BV and the prevention of premature birth, antibiotics have been extensively studied [116, 117]. It is still debated whether or not antibiotics are advantageous prior to embryo transfer; while they are consistent in minimizing infection of the upper vaginal canal, no beneficial function has been detected in the pregnancy outcome [118]. When using broad-spectrum antibiotics, it is possible that not only dysbiotic bacteria, but also Lactobacilli, could be harmed. There have been a number of investigations on the effects of prescribed antibiotics on infertile patients with CE, and the results have been mixed. RIF patients who had CE antibiotic therapy were shown to have better pregnancy outcomes after an organized review and meta-analysis [119]. Antibiotic therapy is not only effective at eliminating the source of infection by targeting particular bacteria, but it can also improve infertility in RIF patients with CE, according to the results of this study. Following the successful treatment of CE, successive IVF cycles saw an increase in implantation rates, clinical pregnancy, continued pregnancy, and live birth rates. There were no statistically significant differences between patients with and without resolved CE in these outcomes. In light of these findings, it appears that detecting and treating CE in RIF patients prior to embryo transfer may be an effective way to eliminate the source of infection, enhance endometrial microbial health, and boost live birth rates in these women [119].

Thus, the microbiota plays a significant role in various aspects of reproductive health. Further research into the interactions between microbiota and reproductive processes may lead to new strategies for improving reproductive outcomes and personalized treatments for couples experiencing infertility or recurrent pregnancy loss.

Therapeutics

Microorganism pathogenicity can be prevented or diagnosed individually. The mucosal or slimy surfaces of the reproductive and digestive tracts are one of the key passageways for microorganisms. Mucosal barriers and immune systems prevent tissue degeneration from damaging reaction to pathogens [120]. These are very much affectionately controlled to create a balance within assimilation of useful nutrients and prevention from antigen and harmful pathogens [121, 122]. Disruptions in this system can lead to inflammatory bowel disease (reactions to commensal organisms), celiac disease (reaction to gluten and specific foods), Helicobacter related gastritis (Peptic ulcer diseases, gastric cancers related to Helicobacter), allergic disorders that are linked to food reaction, aero-antigens, and fungi. Commensal organisms, prebiotics and probiotics and fecal transplantation and immunization techniques for the mucosal immune system are of interest to few poor countries. As a result, it is important to ensure that either the microenvironment's formational changes result from or are driven by various pathogenecities, and that various forms of microbial therapies can affect the development of a variety of diseases.

Probiotics

Probiotic treatment involves eating "good bacteria" or live creatures. Probiotics may minimize preterm birth by improving dietary absorption and immunity. Probiotics are claimed to improve immunological, intestinal, placental, and CVS functioning, although there is no evidence relating them to reproductive issues [123]. Nutritional supplementation with some specific probiotic combination has been found to reduce inflammation in pregnant women, possibly by preventing colonization of harmful microbes [124]. Pregnancy outcomes may be positively influenced by probiotic use, despite the fact that changes in the vaginal and gut microbes may not specifically target the uterine microorganisms, according to these studies.

In addition, probiotic treatment takes part in remedy of genitor urinary tract disorders [123]. Probiotic supplement was observed in pilot research studies to be an equivalently (or more) successful, small period, medicament contrast to ideal antibiotic therapeutics among the patients suffering from vaginitis and BV [125, 126]. Moreover, another pilot study made use of probiotics among the patients of BV unveiled that probiotics usually do not remove unfavorable species, as done by antibiotics, instead extinguished the excessive growth of microbial varieties linked with BV enhancing its efficacy [126]. Additional research has shown that injecting pathogenic bacteria with Lactobacillus crispatus into uterine microorganisms can speed up implantation time and reduce the spread of pathogenic microbes [7].

Probiotics may alter genital tract microbes. These live biotherapeutic substances include one or more suitable bacteria, such as Lactobacillus, to enlarge the niche and replace dysbiotic microorganisms. Oral and vaginal probiotics such *L. crispatus, L. gasseri, L. plantarum, L. reuteri*, and *L. rhamnosus* are available. This might reestablish a healthy LD microbial population while overcoming antibiotic resistance, a higher risk of periodic infections following treatment, and certain medication side effects from the eradication of endogenous off-target microbes in other regions of the body [122, 127]. Probiotics alone may not reduce BV and other genital tract infections. Probitiotic therapy using a single cycle of vaginal L. Crispatus therapy showed spread and growth of this strain in up to 60% of women[128]. On the other hand, a twostep therapeutic protocol using vaginal probiotics succeeding antibiotic therapy might be beneficial to first combat the sedulous bacteria and followed by repopulation of the genital tract with Lactobacillus species [129, 130].

Conclusion and future perspectives

Microbial-related pathogenicity can be strategically anticipated for prophylactic measures or assessed as individualized criteria for diagnosis. Utilization of probiotics may facilitate the establishment or preservation of a salubrious microenvironment, in conjunction with managing dietary alterations through the incorporation of atypical gut or reproductive tract-associated commensal organisms. The principal conduits through which various environmental constituents gain entry predominantly encompass the mucosal or lubricated interfaces of the reproductive and gastrointestinal tracts. The attenuation of tissue deterioration, ensuing from detrimental responses to foreign substances, is mitigated by the mucosal barriers and the mucosal immune system, which orchestrate a significant preventative contribution encompassing both antigenic and bacterial components [120]. Mucosal annexations are not that much of enriched with immune cells. These are very much affectionately controlled to create a balance within assimilation of useful nutrients and prevention from antigen and harmful pathogens [121, 122]. Disruptions to this delicate balance may result in various health issues such as inflammatory bowel disease, allergic reactions to commensal organisms, celiac disease, food allergies, Helicobacter-induced gastritis, peptic ulcers, and stomach malignancies. Furthermore, food allergies, aeroantigens, and fungi can also arise. The mucosal entourage offers insight into strategies to minimize the risk of infection or re-infection, thus safeguarding not only the individual but also those in close proximity. In conclusion, maintaining a balanced and healthy mucosal environment is crucial in preventing infections, mitigating pathogenicity, and preserving overall well-being, while probiotics and dietary alterations can play a vital role in supporting this delicate equilibrium and protecting individuals and communities from adverse health consequences.

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