Review

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The effect of the female genital tract and gut microbiome on reproductive dysfunction

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SUMMARY Microorganisms are ubiquitous in the human body; they are present in various areas including the gut, mouth, skin, respiratory tract, and reproductive tract. The interaction between the microbiome and reproductive health has become an increasingly compelling area of study. Disruption of the female genital tract microbiome can significantly impact the metabolism of amino acids, carbohydrates, and lipids, increasing susceptibility to reproductive tract diseases such as vaginitis, chronic endometritis, endometrial polyps, endometriosis, and polycystic ovary syndrome. The gut microbiome, considered an endocrine organ, plays a crucial role in the reproductive endocrine system by interacting with hormones like estrogen and androgens. Imbalances in the gut microbiome composition can lead to various diseases and conditions, including polycystic ovary syndrome, endometriosis, and cancer, although research on their mechanisms remains limited. This review highlights the latest advancements in understanding the female genital tract and gut microbiomes in gynecological diseases. It also explores the potential of microbial communities in the treatment of reproductive diseases. Future research should focus on identifying the molecular mechanisms underlying the association between the microbiome and reproductive diseases to develop new and effective strategies for disease prevention, diagnosis, and treatment related to female reproductive organs.

Keywords microbiome, female genital tract, gut, reproductive disease, dysbiosis

1. Introduction

In recent decades, a focus of research in public health and translational medicine has been the human microbiome. Billions of microorganisms, including bacteria, archaea, fungi, and viruses, colonize the human body, affecting various aspects of human health such as growth, digestion, nutrient absorption, immune regulation, and metabolism (*1,2*). Imbalances in human microorganisms have been linked to diseases including dental caries, malnutrition, gastrointestinal ulcers, diabetes, cancer, depression, allergic asthma, and autoimmune diseases (*3*). Over the past two decades, the human microbiome has become a focus of research in public health and translational medicine. With advances in next-generation sequencing technology and related bioinformatic tools, the US and Europe conducted the Human Microbiome Project (HMP) and the Human Intestinal Tract (MetaHIT). These two large-scale human microbiome-related studies have brought about major advances in the entire field.

The intestinal and female genital tract (FGT) harbor stable microbial communities. The FGT, encompassing the vagina, cervix, endometrium, fallopian tubes, and ovaries, possesses a distinct microbiome, constituting around 9% of the total bacterial count in women (*4*). However, the FGT is not static, but rather a dynamically balanced ecosystem affected by factors including age, lifestyle, hormone levels, and environmental influences (*5*). The human symbiotic microbiota, exemplified by the gut microbiota, is often referred to as the "second genome" of the human body and is closely connected to female reproductive diseases. The gut microbiome is considered an extended endocrine organ, crucial in the reproductive endocrine system, interacting with hormones like estrogen and androgens throughout a woman's life (*6*). Disruptions to this microbiome, such as through epigenetic modifications, nervous system changes, and metabolic imbalances, can interfere with zygote formation, hinder embryo implantation and development, and increase susceptibility to diseases (*7*), significantly impairing reproductive capacity and pregnancy. Imbalances in the FGT and gut microbiome

composition can lead to the onset of reproductive-related diseases, including vaginitis, polycystic ovary syndrome (PCOS), endometriosis (EMs), chronic endometritis (CE), and endometrial polyps (EPs) (*8,9*).

This review delves into the interaction between the FGT and gut microbiome. It also examines the link between microbiome imbalance and reproductive diseases, emphasizing potential pathogenesis and therapeutic applications.

2. Characteristics of female microbiome

2.1. Female reproductive tract microbiome

The FGT consists of the upper genital tract (UGT), consisting of the fallopian tubes, ovaries, uterus, and endocervix, and the lower genital tract (LGT), consisting of the ectocervix and vagina. The use of high-throughput sequencing technology has provided deeper insights into the distribution of the FGT microbiome. In comparison to the LGT, the UGT exhibits lower bacterial density but higher diversity. Both domestic and international studies have indicated that the colonization of bacterial communities in the lower third of the vagina, posterior fornix, cervix, uterine cavity, fallopian tubes, and peritoneum in women of childbearing age undergoes continuous changes. Samples taken from various parts of the vagina and cervix have shown low species diversity, with *Lactobacillus* dominating (*10*). However, the FGT is highly dynamic and influenced by factors such as age, lifestyle, hormone levels, and the menstrual cycle (Figure 1).

2.1.1. Vaginal-cervical microbiome

The composition of the vaginal microbiome is closely related to reproductive health. In 2011, Ravel *et al.* (*11*) used 16srRNA sequencing technology to classify the community status types (CST) in the vagina of healthy women into five types based on the dominant species and pH level of *Lactobacillus*. CST-I, CST-II, CST-III, and CST-V involve the dominant species *Lactobacillus crispatus* (26.2%), *L. gasseri* (6.3%), *L. iners* (34.1%) and *L. jensenii* (5.3%), respectively. The abundance of *Lactobacillus* in CST-IV is low, while the abundance of specific anaerobic bacteria (*Dialister, Prevotella, Atopobium, Gardnerella,* and *Sneathia*) is high. A recent study based on a large sample dataset subsequently subdivided CST-IV into 7 subtypes, dominated by different non-lactobacilli species (*12*). This indicated that Streptococcus vaginalis may produce other end products of fermentation in the vagina and not just lactic acid as traditionally thought, and it may be related to elevated vaginal PH. Some studies have shown that CST-I tends to be the most stable, while CST-IV is prone to change. The vaginal microbiota dominated by *L. crispatus* is more likely to first transform into the vaginal microbiome dominated by *L. iners* or mixed Lactobacillus, rather than directly transitioning to complete dysregulation of the microbiome (*13,14*). Vaginal microorganisms interact with each other and are regulated by the host organism, which in turn regulates the host's local immune function. Some microorganisms interact with the host genome to maintain a relatively independent ecological environment (*15*). The female vaginal microbiome is dynamically changing due to multiple factors. At present, menstruation is generally believed to lead to changes in certain CST types, but the change in individual microbial community types does not necessarily accompany an increase in bacterial diversity (*16*). Srinivasan *et al.* (*17*) reported that during menstruation, the abundance of *Gardnerella* and *L. iners* in the vagina increased in 81%

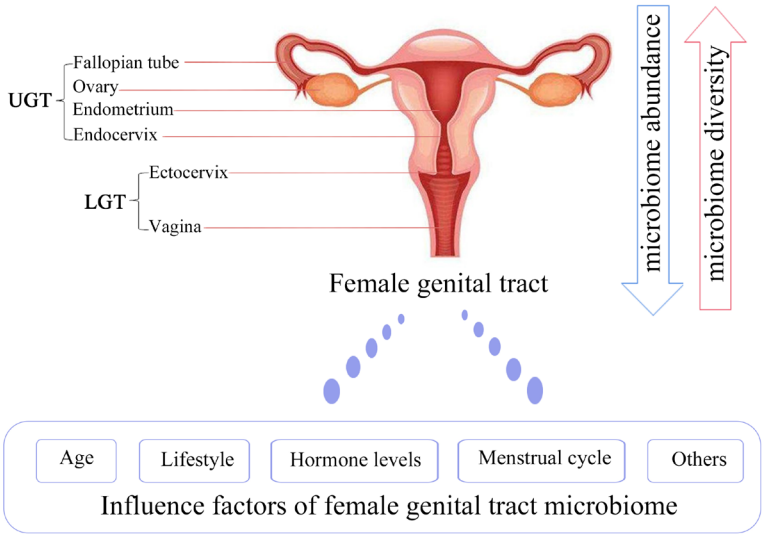

Figure 1. Female reproductive tract microbiome. The upper genital tract (UGT) consists of the fallopian tubes, ovaries, uterus, and endocervix, while the lower genital tract (LGT) consists of the ectocervix and vagina. Compared to the LGT, the UGT exhibits less bacterial density but higher diversity. The FGT can be influenced by various factors, including age, lifestyle, hormone levels, and the menstrual cycle.

of subjects, but the abundance of other *Lactobacillus* spp. decreased, and the microbiome gradually returned to a stable state before menstruation. Although the altered CST pattern has been described during menstruation, its relationship to reproductive health has not been fully determined. Gajer *et al*. (*18*) monitored the dynamic changes in the vaginal microbiome in 32 healthy women and they found that CST-I and CST-II are relatively stable and that the transformation of these two types is usually related to menstruation - the transition to CST-III dominated by *L. iners* during menstruation, which quickly returns to its original state after menstruation. The normal vaginal microbiome can prevent urogenital tract diseases (such as vaginitis, pelvic inflammatory disease, sexually transmitted diseases, and urinary tract infections) by sticking to the vaginal epithelium, preventing the invasion of pathogenic microorganisms, producing H_2O_2, bacteriocin and biosurfactants to maintain the acidic environment in the vagina and other mechanisms (Table 1).

In women of childbearing age, the composition of the cervical microbiome is usually similar to that of the vaginal microbiome (*25*). Punzón-Jiménez *et al.* (*9*) found that *Lactobacilli* accounted for 97.56% of cervical mucus according to qPCR detection, with *L. iners* and *L. crispatus* being the most abundant species. Pelzer *et al*. (*26*) reported that the highest content detected in cervical specimens is *Lactobacillus* spp., followed by *Gardnerella* spp., *Veillonella* spp., *Prevotella* spp., *Sneathia* spp., or *Fusobacterium* spp. In recent years, research on the cervical microbiome has mainly focused on its relationship to cervical cancer. The high diversity of species in the cervical microbiome and specific genera (such as *Gardnerella* spp.) are associated with the risk of human papilloma virus (HPV) infection in women (*27*). Persistent high-risk HPV infection increases the risk of cervical intraepithelial neoplasia (CIN) and even cervical cancer. Some taxa are associated with a vaginal microbiome imbalance, such as *Gardnerella*, *L. iners*, *Mycoplasma*, *Sneathia*, and *Fusobacterium*. These are reported to be risk factors for CIN and cervical cancer, inducing Toll like receptor 4 signaling, NF-κB activation and upregulation of pro-inflammatory cytokines (such as γ-interferon and IL-1), promoting the progression of cervical lesions by promoting inflammation and disrupting the cervical mucus barrier (*28-30*).

2.1.2. Endometrial microbiome

The endometrium plays a crucial role in female reproductive function. Despite the conventional belief that the uterus is a sterile environment, the endometrium has a unique microbial community (*31*). The bacterial load in the uterus is estimated to be 100 to 10,000 times less than that in the vaginal microbiome (*32*). Given the inertia but differences in the composition of endometrial and vaginal microbiota, the source of the endometrial microbiota is still controversial. Some researchers believe that microorganisms colonize the uterus through the vagina and cervix, but the vaginal microbiota is not a persistent source of endometrial microbiota and may be influenced by multiple factors (*33*). Another hypothesis suggests that uterine microbiota colonization involves multiple pathways such as gastrointestinal microbiota migration and blood transmission of respiratory and oral bacteria (*34*).

Thus far, most studies have reported that the uterine microbiota mainly consists of *Lactobacilli* (*35,36*). However, results of different studies in terms of the composition of the uterine microbiota are not consistent. Moreno *et al*. (*36*) found that *Lactobacillus* still accounts for the highest proportion (30.6%) in the endometrial microbiota and that there are also bacterial genera such as *Bifidobacterium*, *Gardnerella*, *Macrosporidium*, *Prevotella*, and *Streptococcus*. This is similar to the conclusions of Mitchell *et al*. (*37*), which found that the most common bacteria species in the uterine cavity were *L. iners*, and that *Gardnerella*, *Bacillus mirabilis*, and *L. plantarum* were detected in more than 40% of subjects. In contrast, Chen *et al*. (*38*) contends that the biomass of lactic acid bacteria is 1,000 times lower than that of the vagina and no longer dominates the endometrial environment, with *Pseudomonas* spp., *Acinetobacter* spp., *Vaginococcus* spp., and *Sphingobium* spp. being important components. Numerous studies have also shown that differences in the uterine microbiome may be related to different physiological or pathological states of the body. In 25 uterine samples from patients

Table 1. Types of vaginal microbiomes and the possible mechanisms by which they prevent reproductive and urinary diseases

CST	Vaginal PH	Dominant species	Role in reproductive diseases	References
I	4.0 ± 0.3	*L. crispatus*	Possible mechanisms for preventing reproductive and urinary diseases:	(*11,12,17*)
II	5.0	*L. gasseri*	Adhere to vaginal epithelium to prevent the invasion of pathogenic	(*12,17*)
III	4.4	*L. iners*	microorganisms	(*17-19*)
IV A	5.3 ± 0.6	*Gardnerella Atopotella*	Produce H_2O_2, bacteriocins, and biosurfactants to maintain an acidic environment in the vagina	(*20-22*)
		Campylobacter	Activates the NF- κB cascade	
IV B		*Atopobium Bifidobacteria*	Adhesins that promote epithelial colonization	
		Prevotella	Produce hemolysin to promote cytotoxicity	
V	4.4	*L. jensenii*		(*23,24*)

undergoing a hysterectomy for uterine fibroids, Winters *et al*. (*39*) found that the endometrial microbiome mainly consisted of *Acinetobacter*, *Clostridium*, *Pleuromonas*, and *Pseudomonas*.

2.1.3. Ovarian and fallopian tubal microbiome

The human ovaries and fallopian tubes are not always sterile and can be colonized by microorganisms (*30*). A study reported that 34.4% of women have microbial colonization in the follicular fluid (FF) (*40*). Previous studies have also reported that in infertile women, FF colonization rates range from 24% to 37%, with colonization rates of 40% and 32% in the left and right ovaries, respectively (*41*). Pelzer *et al*. (*42*) conducted a microbial culture of the FF obtained from 71 women undergoing sssisted reproductive technology (ART) during embryo retrieval and found microbial colonization in the FF, including *L. iners*, *Actinomyces* spp., *Corynebacterium aurimucosum*, *Fusobacterium* spp., *Peptoniphilus accharolyticus*, *Peptostreptococcus* spp., *Propionibacterium* spp., *Puccinia*, *Staphylococcus*, and *Candida parapsilosis*.

Pelzer *et al*. used a microbial culture and NGS technology to analyze 16 female fallopian tube samples and confirmed that there was microbial colonization in the female fallopian tubes mainly consisting of *Staphylococcus* spp., *Enterococcus* spp., and *Lactobacillus* (*43*). Other common bacteria include *Pseudomonas*, *Burkholderia* spp., and *Propionibacterium* spp.. The right fallopian tube mainly has *Staphylococcus*, and the left fallopian tube mainly has *Lactobacillus*, *Enterococcus*, and *Pasteurella* (*43*). In analyzing the microbial community of fallopian tubes in patients with chronic salpingitis, Wang *et al*. (*44*) found that samples with salpingitis fluid contained a more abundant microbial composition, while samples with salpingitis pus were more likely to exhibit a single dominant bacterium. A study on laparoscopic examinations of 26 patients with acute salpingitis showed that gonococci were isolated from the fallopian tubes of 19% of patients, and 38% of patients had aerobic and/or anaerobic bacteria present in the fallopian tubes (*45*). Understanding the composition of these microbial communities may help propose alternative treatment options for patients undergoing salpingectomy due to certain pathological conditions.

2.1.4. FGT microbiome throughout the entire lifecycle

The reproductive tract microbiome of an individual undergoes changes throughout its lifetime, and especially during infancy, and then changes again in old age. Due to the presence of microbial colonization in the uterine cavity, the maternal microbiome has been assumed to be the main contributor to provide microbial strains to newborns through vertical transmission (*46*). The vaginal microbiome of newborns born naturally is similar to that of the mother, but colonies of microorganisms are only temporary, and the infant continues to obtain microorganisms from different maternal sources after birth (*47*). Many studies on the transmission of maternal microbiota have focused on the gut, skin, or oral microbiota of newborns (*47,48*). Because the pH level of the infant's vagina is neutral or alkaline and there is a lack of *Lactobacilli*, any microorganisms that are transferred at birth cannot survive (*49*). Only in early adolescence do common species in the vaginal microbiome of women of childbearing age (such as *L. crispatus*, *L. iners*, and *Gardnerella*) dominate the vaginal microbiome (*50*). Throughout a woman's life, the vaginal microbiota is not always dominated by *Lactobacilli*. During childhood, anaerobic bacteria and *Escherichia coli* dominate. After puberty, the increase in estrogen leads to the production and accumulation of glycogen, allowing *Lactobacillus* to maintain a dominant position in women of reproductive age. During the perimenopausal period, the proportion of *Lactobacilli* decreases again due to the decrease in endogenous estrogen. The vaginal microbiome in the premenopausal and perimenopausal stages consists of *Firmicutes*, while the postmenopausal stage is dominated by *Aspergillus*, *Anaplasma*, and *Actinobacteria* (*51*). A cross-sectional study (*52*) involving 70 patients showed that in the vaginal microbiome of premenopausal women, *Lactobacilli* accounted for 71.98%, and non-optimal microbiome accounted for 16.87%, while in postmenopausal women those proportions were 10.08% and 26.78%, respectively. The proportion of lactic acid bacteria in postmenopausal women is significantly low, while microbial diversity and vaginal pH are significantly high. A study has shown that *Lactobacillus* levels and lower vaginal pH levels can be maintained in women who receive hormone replacement therapy during perimenopause (*4*). In addition to age and hormone levels, the composition of the vaginal microbiome is also influenced by various factors, such as race, gestational age, sexual activity, stress, and dietary factors. An epidemiological study has shown that the abundance of *Lactobacillus* is related to race; compared to African/Hispanic women, Caucasian/Asian women have higher levels of *Lactobacillus* (*19*). A cohort study showed that as the gestational age increased, the relative abundance of the thick-walled portal increased. In addition, a new microbiome (*Atopobium*, *Aerococcus*, *Gemella*, *Sneathia*, *Parvimonas*, *Gardnerella*, and *Megasphaera*) was observed in the vaginal microenvironment during early pregnancy. By mid-pregnancy, the number of this new vaginal microbiota has sharply decreased, replaced by abundant *Lactobacilli* (*53*).

Due to the limited availability of upper reproductive tract specimens from children and adolescent women, the microbial sources and differences between them and adult women are still unclear. There are differences in the microbiome colonizing the fallopian tubes of premenopausal women and postmenopausal women (*54*),

indicating that sex hormones may play a regulatory role in the microbiome colonization of the female UGT.

2.2. Gut microbiome

2.2.1. Overview of the gut microbiome

There are approximately 10^{14} bacterial colonies in the human gut, including over 1,000 species of bacteria, that can provide various benefits to the host, such as enhancing the immune system and supporting intestinal function (*55*). In addition, the gut microbiome regulates host metabolism through various pathways and it participates in the regulation of the female reproductive endocrine system. The gut microbiome is dominated by five bacterial phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*, accounting for approximately 90% of the total gut microbiome (*56*). Approximately 200 genera of Firmicutes in the human gut are dominated by Clostridium (95%). The Bacteroidetes phylum mainly consists of the genera *Bacteroides* and *Prevotella*, while there is relatively small number of the Actinobacteria phylum, which mainly manifests as the genus *Bifidobacterium* (*57*). The relationship between the human microbiome and host is a mutual one. The composition and functional changes or destruction of gut microbiome caused by various factors are related to the development and progression of various diseases (*58*).

2.2.2. Gut microbiome-The foundation of an endocrine organ

The gut microbiome is a complex microbial community that not only plays a crucial role in gut microecology but also has significant impacts on external organs. These impacts extend to areas such as the host's mental state and neurological function, *via* the brain-gut axis, as well as insulin secretion and resistance, and participation in processes such as depression, autoimmune diseases, and cardiovascular diseases (*59*). The decrease in the abundance of the gut microbiome leads to a significant increase in lipopolysaccharides, thereby increasing intestinal permeability and activating the NF-κB signaling pathway, stimulating the release of pro-inflammatory factor tumor necrosis factor (TNF)-α, interleukin (IL)-8, and IL-6. Activated c-Jun amino terminal kinase and IκB kinase regulate serine phosphorylation of insulin receptor substrates, thereby inhibiting insulin signaling and leading to insulin resistance (IR) (*60*). The gut microbiome and its metabolites can act on TLRs and NLRs in the intestinal wall, inducing the production of TNF-α, IL-1, and IL-6, as well as locally resident and migrating antigen-presenting cells and circulating adaptive immune cells, causing a systemic immune response and trigger systemic autoimmune diseases (*61*). The gut microbiome can be viewed as a substantial endocrine organ based on a variety of key findings. Evidence of their direct effects comes from the ability of their produced and metabolized metabolites to reach the circulatory system and impact numerous organ and system functions throughout the host's body (*62*). Unlike other endocrine systems or organs that typically produce only a small number of hormone molecules, the gut microbiome has the potential to synthesize hundreds of functional metabolites (*63*). In fact, the complexity of the gut microbiome colonizing mammals actually exceeds that of the brain. Moreover, many hormones that microorganisms synthesize also function as neurotransmitters within the host's central nervous system. For example, several *Lactobacilli* synthesize γ-Aminobutyric acid, the most important inhibitory transmitter in the brain, while specific bacterial strains produce monoamines such as norepinephrine, dopamine, and serotonin (*64,65*). In the reproductive endocrine system, the gut microbiome plays an important physiological role by complex interaction with insulin, estrogen, androgen and other hormones.

2.2.3. Gut microbiome and estrogen regulation

Reproductive hormones play a direct role in regulating the reproductive activities of female animals. In recent years, many researchers have contended that further research on the regulation of reproductive microorganisms and sex hormones is needed, while the gut microbiome has been proven to have direct or indirect regulatory effects on sex hormones. Estrogen, a steroid reproductive hormone, undergoes metabolism *via* enterohepatic circulation. As a result, numerous studies have focused on its interaction with the gut microbiome, which was first identified over three decades ago by Adlercreutz *et al.* (*66*). The study in question found that supplementation with antibiotics led to a reduction in estrogen levels in women. Estrogen undergoes metabolism in the liver, which includes hydroxylation and coupling. Following this, some conjugated estrogen is excreted *via* urine, while some also enters the intestines and is eliminated through feces (*67*). The gut microbiome plays a critical role in regulating estrogen levels *via* the secretion of β-glucuronidase (GUS), which transforms conjugated estrogen into deconjugated estrogen (*68*) (Figure 2). The enterohepatic circulation of estrogens and their reabsorption are also regulated by the gut microbiome. Estrogen binds to the ligand binding region of receptors (ERα and ERβ) in the nucleus, causing conformational changes and promoting cell growth, apoptosis, proliferation, adhesion, and signal transduction through pathways such as MAPK, PI3K, and Src kinase (*69*). However, the imbalance of the gut microbiome may lead to decreased GUS activity, resulting in lower circulating estrogen levels, and ultimately lead to the development of obesity and cardiovascular disease. In addition, estrogen increases

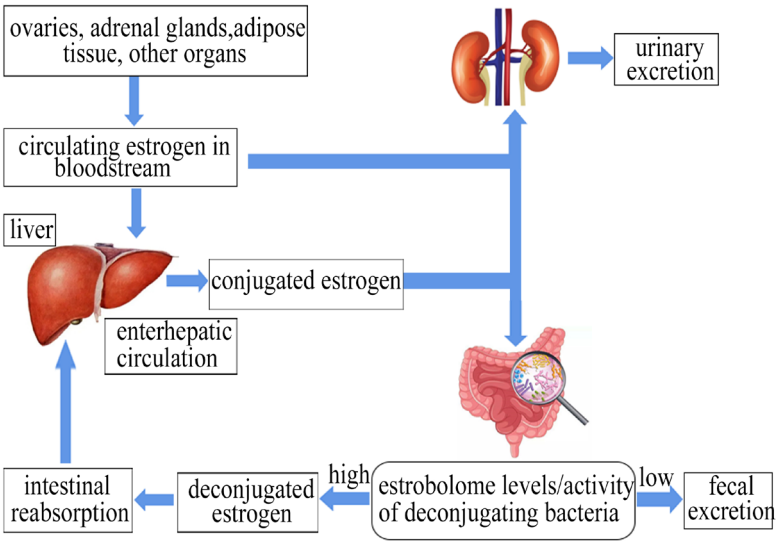

Figure 2. Gut microbiome and estrogen. Estrogen migrates in the blood, reaches the liver, and is inactivated by metabolism into conjugated estrogen. Some is excreted through urine and some is excreted through feces. The gut microbiome mainly secretes β-glucuronidase, which converts conjugated estrogen into deconjugated estrogen that enters the hepatic circulation through intestinal reabsorption.

circulating estrogen levels by reducing the abundance of bacteria producing glucuronidase, *i.e.* increasing the proportion of *Firmicutes*/*Bacteroidetes*, thereby increasing the interaction with ERα and ERβ, leading to endometrial cancer (EC), cancer breast cancer, EMs, and other diseases (*70*). Therefore, optimal GUS activity is essential for maintaining estrogen levels in women.

The gut microbiome may affect female sex steroid hormone levels through the production of short-chain fatty acids (SCFAs), such as abundant acetate, propionate, and butyrate. According to Lu *et al.* (*71*), the synthesis of progesterone and estradiol in pig granulosa cells can be regulated by butyrate through the cAMP signaling pathway. Recent research has shown that enteric-derived butyrate can cause non-alcoholic fatty liver disease due to an estrogen deficiency in premenopausal women (*72*). While a correlation between the gut microbiome and the endocrine system has been reported, the mechanisms of interaction, and particularly those relating to progesterone, remain poorly understood.

3. Role of female dysbiosis in reproductive and gynecological diseases

3.1. FGT dysbiosis in reproductive and gynecological diseases

Lactobacillus is dominant in the normal FGT microbiome. It produces active compounds, facilitates coaggregation based on cell wall molecules, promotes the integrity of the epithelium, and regulates immune responses. The disruption of the FGT microbiome significantly affects the metabolism of amino acids, carbohydrates, and lipids, thereby increasing disease susceptibility. Therefore, the interaction between the microbiome, metabolites, and host in the reproductive tract microenvironment is crucial for maintaining the balance of the reproductive tract. Dysbiosis in the FGT microbiome has been linked to a variety of gynecological disorders, including vaginitis, CE, EPs, EMs, and PCOS (Figure 3).

3.1.1 FGT dysbiosis and vaginitis

Vaginitis is characterized by inflammation of the vaginal mucosa and submucosal connective tissue, and the condition can manifest in several different forms. The most prevalent types of vaginitis include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis vaginitis. BV is the most commonly observed vaginal infectious disease affecting women of childbearing age. This condition is marked by the overgrowth of anaerobic bacteria, primarily *Xenobacteria*, *Gardnerella*, and human *Mycoplasma*. A longitudinal study conducted by Vodstrcil *et al.* (*73*) on 52 young Australian women found that *Gardnerella* was more likely to be detected in sexually active women, implicating sexual activity as an important risk factor for BV. Moreover, sexual activity increased the diversity of vaginal microbiome evolution in women both with and without BV, which could increase the pathogenic potential of the microbiome. Unfortunately, current treatments have a high rate of recurrence (> 50% rate of recurrence within 6 - 12 months) due to potential factors such as reinfection from sexual partners or endogenous sources, biofilm formation, or the failure for appropriate *Lactobacillus* bacteria to take hold (*74*). BV is closely linked to the metabolites present in the reproductive tract. In fact, researchers have found that alterations in specific metabolites (such as maltose, nicotinate, malonate,

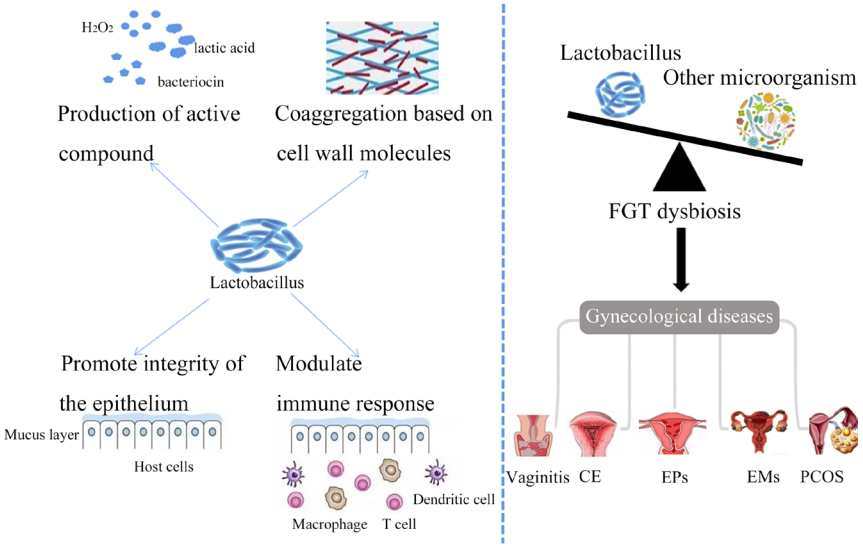

Figure 3. The relationship between FGT dysbiosis and diseases. *Lactobacillus* is dominant in the normal FGT microbiome. It produces active compounds, facilitates coaggregation based on cell wall molecules, promotes the integrity of the epithelium, and regulates immune responses. FGT dysbiosis increase the risk of diseases such as vaginitis, CE, EPs, EMs, and PCOS.

acetate, and nicotinamide adenine dinucleotide) can serve as metabolic markers, distinguishing individuals with BV from healthy individuals (*75*). Following successful treatment, these BV-associated metabolites decrease significantly. Given this, metabolic analysis of the reproductive tract is considered crucial to the identification and diagnosis of BV. According to a study conducted by McMillan *et al.* (*76*), an increase in 2-hydroxyisovalerate and γ-hydroxybutyrate in the vagina, as well as a decrease in lactate and tyrosine, are the most reliable indicators of BV. Nevertheless, further research is necessary to fully understand the effects of metabolites on both the microbiome and host immunity. The vaginal mucosa is an important line of defense for local immunity that can resist pathogen invasion by rapidly shedding epithelial cells and secreting cytotoxic cytokines. The imbalance of the gut microbiome activates the NF-κB pathway through Toll-like receptors, leading to increased release of pro-inflammatory cytokines and epithelial cell damage (*77*). *Lactobacillus* is able to inhibit HeLa cell apoptosis caused by pathogens, thus protecting epithelial cells (*78*).

VVC is the second most frequently occurring vaginitis following BV. Studies have estimated that around 10% to 15% of asymptomatic women carry *C. albicans* in their vaginas, and approximately 70% to 75% of women experience VVC at some point in their lives, impacting millions of women every year (*79*). A recent *in vivo* study indicated that colonization by *Lactobacillus* may not necessarily lower the risk of VVC, while colonization by *L. crispatus* can even lead to an increased risk of *C. albicans* colonization (*80,81*). Despite the association between VVC and the vaginal microbiome, whether specific *Lactobacillus* species are associated with this condition is still unknown. Further research is necessary to identify any potential links. *Chlamydia trachomatis* (CT) is a widespread sexually transmitted infection among women age 15-49, with global rates of infection ranging from 1.5% to 7% (*82*). Lactic acid present in the genital tract acts as a critical inhibitor of CT infection. Nevertheless, the *L. iners* species produces far less lactic acid than other *Lactobacillus* spp., making women with *L. iners* as the dominant flora particularly susceptible to CT infection (*83*). In addition, certain individuals with CT infections may have an abundance of anaerobic bacteria in their genital tract, such as *Gardnenella*, *Prevotella*, *Megalococcus*, and *Rosella*. In comparison to healthy controls, reproductive tract metabolites from individuals infected with CT demonstrated only a slight decline in several amino acids and biogenic amines (*18*). Upon experiencing CT reinfection or chronic infection, activated helper T cell (Th) - 1, Th2, and Th17 cells can stimulate tissue destruction, fibrosis, and scarring, ultimately contributing to pelvic inflammation (*84*).

3.1.2. FGT dysbiosis and CE and EPs

CE is a distinct alteration in the population of endometrial microorganisms present in the uterine cavity, predominantly caused by bacterial pathogens such as *Streptococcus*, *Escherichia coli*, *Enterococcus faecalis*, *Mycoplasma*, and other bacteria like tuberculosis bacilli and viruses (*85*). A study conducted by Liu *et al.* (*85*) found that CE is linked to a significant increase in the prevalence of 18 groups of bacteria within the endometrial cavity. Moreover, the relative abundance of *Lactobacillus* was notably higher in individuals with CE in comparison to those without CE (80.7% *vs.* 1.89%), while there was a lower prevalence of *L. crispatus* in

the CE microbiome. Antibiotic therapy administered to patients with CE has demonstrated an ability to markedly enhance reproductive outcomes, indicating a crucial correlation between the host and colonizing bacteria (*86*). In cases of CE, microbial infections, including those caused by Gram-negative bacteria, can trigger potent immune responses that may trigger B-cell entry into the endometrial stroma, subsequently leading to the aberrant expression of various pro-inflammatory molecules. Other studies have also found that *Streptococcus pyogenes* infects the human endometrium by limiting innate immune responses (*87*). Concurrently, endometrial microvascular endothelial cells express adhesion molecules and chemokines associated with B-cells in CE, resulting in alterations to the uterus's receptivity and unfavorable pregnancy outcomes (*88*). In addition, Chen *et al*. (*89*) found that endometrial microorganisms can interfere with glucose and lipid metabolism processes through the PWY-7347 and SUCSYN-PWY metabolic pathways to regulate immune cells, thereby affecting endometrial receptivity.

EPs are likely triggered by several factors, including genetics, endocrine imbalances, immune inflammation, irregular cell proliferation/apoptosis, angiogenesis, oxidative stress, and an imbalanced FGT microbiome. The composition of the LGT microbiome can markedly heighten the likelihood of developing EPs. A study conducted by Marchenco *et al*. (*90*) indicated that women with vaginal microbiome disorders are 3.5 times more susceptible to BV than healthy people. In addition, Kovalenko *et al*. (*91*) suggested that vaginal *Gardnerella*, commonly associated with BV, may fuel the emergence of EPs. Similarly, Horban *et al*. (*92*) found that 93.6% of individuals living with recurring BV had endometrial lesions, comprising 42% of all pathology results diagnosed as EPs. Fang *et al*. (*93*) examined the differences in the composition of the endometrial microbiome between patients experiencing EPs and healthy women, and they found that the bacterial diversity of the endometrial microbiome was higher in EPs patients than in their healthy counterparts. EPs patients exhibited higher ratios of endometrial *Lactobacillus*, *Bifidobacteria*, *Gardnerella*, *Streptococcus*, *Alternaria*, and *Prevotella* but lower proportions of *Pseudomonas*, *Enterobacteriaceae*, and *Sphingosine*. In specific terms, the proportion of *Lactobacillus* was significantly elevated in EP patients (38.64% *vs.* 6.17%), indicating that vaginal bacteria in individuals with EPs may also proliferate in the uterine cavity (*93*). When an intrauterine infection or changes in the microbiome of intrauterine colonization occur, immune cells can interact with microorganisms through pattern recognition receptors, recruiting circulating B cells to the endometrial stroma and stroma regions, reducing the antiviral and fungal infection function of natural killer cells, and severely reducing the abnormal immune microenvironment inside the uterine cavity, affecting sperm fertilization and embryo implantation (*94*). Nonetheless, there is still a need for further research concerning specific alterations in the microbial composition and pathogenic microbial species associated with EPs.

3.1.3. FGT dysbiosis and EMs

Despite the incidence of EMs increasing yearly to 5% - 15% (*95*), the pathogenesis of this condition remains unclear. In a cohort study conducted by Lin *et al*. (*96*) involving 79,512 patients with reproductive tract infections, the incidence of EMs was significantly higher compared to that in the control group. In addition, reproductive tract infections were identified as an independent risk factor for EMs, markedly increasing the likelihood of patients with a reproductive tract infection developing endometriosis (*96*). Moreover, Akiyama *et al*. (*97*) performed NGS analysis on the cervical mucus in women with and without EMs, and they found that the quantity of *Enterobacteriaceae* and *Streptococcus* in the cervical mucus of the EMs group was substantially higher than that of the control group, irrespective of the various phases of the menstrual cycle, except for the primary *Lactobacillus* species. Yang *et al*. (*98*) observed accumulation of several less abundant genera in the vaginal and cervical microbiome of EM patients, such as *Fannyhessea*, *Prevotella*, *Streptococcus*, *Bifidobacterium*, *Veillonella*, *Megasphaera*, and *Sneathia*. The microbial imbalance of EMs can promote disease progression through immune activation, impaired intestinal function of cytokines, changes in estrogen metabolism and signal transduction, and abnormalities in the homeostasis of progenitor cells and stem cells (*99*). Despite these findings, further research is required to clarify the mechanistic aspects that link these bacteria to the pathophysiology of endometriosis. Endometrial microbial infections may harm the contractility of the uterus and facilitate the implantation of retrograde endometrial cells (*100*), thus promoting the progression of this disease.

3.1.4. FGT dysbiosis and PCOS

The pathological and physiological mechanisms underlying PCOS are relatively intricate, encompassing multifaceted interactions within multiple mechanisms and pathways. Several studies have shown that the level of the *Lactobacillus* genus, and specifically *L. curlicus*, in the reproductive tract of PCOS patients is lower compared to that in the control group (*101*). In addition, PCOS patients also exhibit an increased abundance of species such as *Gardnerella*, *Chlamydia*, and *Prevotella* in the cervical canal, whereas the vaginal microbiome is enriched in species such as *Prevotella*, *Ageophilia*, and *Mycoplasma*, unlike in the control group (*101*). A Kyoto Encyclopedia of Genes and Genomes analysis showed

that oxidative phosphorylation, amino acid metabolism, and N-glucan biosynthesis were upregulated in the LGT of PCOS patients (*102*). These changes are beneficial to the colonization of several pathogenic bacteria (such as Gardnerella) but not to the growth of *Lactobacillus* (*103*).

Currently, the treatment for PCOS primarily involves Diane-35 (ethinylestradiol cyproterone). Reviews of the relevant literature have indicated that using hormonal contraception can decrease the risk of BV (*103*). The use of hormonal contraceptives can regulate the local inflammatory response of BV, which is significantly associated with high levels of IL, TNF, INF-r, IL-2, and IL-4 in contrast to the normal vaginal microbiome (*103*). These findings suggest that the use of hormone contraceptives may impact the immune system of the reproductive tract.

3.2.Gut dysbiosis in reproductive and gynecological diseases

The gut mucosal layer provides a vital protective barrier against microbial invasion. However, gut dysbiosis can disrupt the normal mucosal layer, leading to the increased incidence of various gynecological diseases, including PCOS, EMs, cancer, and obesity (Figure 4).

3.2.1. Gut dysbiosis and PCOS

PCOS is a multifactorial endocrine and metabolic disorder, and the gut microbiome is implicated in various metabolic activities in the human body. Indeed, exploring alterations in the gut microbiome of PCOS patients has become a focus of research in recent years. Notably, Torres *et al*. (*104*) found that both the α diversity (overall species richness) and β diversity (microbial community composition) of the gut microbiome in PCOS patients decreased compared to those in healthy women. In addition, they identified an increased abundance of specific gut microbiome in PCOS patients such as *Streptomyces* and *Candella* (*104*). Interestingly, these changes in the gut microbiome appear to be correlated with increased serum androgen levels in PCOS patients (*104*). In a mouse model of PCOS induced with dihydrotestosterone, the relative abundance of anaerobic bacteria in animals with a high dose of androgens increased (*105*). Multiple components of the gut microbiome can assist in the synthesis and transformation of androgens by synthesizing enzymes for androgen metabolism. *Actinobacteria* and *Proteobacteria* can degrade androgens (*106*). Due to its genome encoding 20α- hydroxysteroid dehydrogenase, Clostridium subtilis is involved in the conversion of glucocorticoids into androgens (*107*). However, the limitation of these studies lies in the lack of in-depth evaluation of the mechanism underlying the interaction between the gut microbiome and androgens. The observed decrease in gut microbiome diversity may contribute to decreased activity of the GUS, resulting in reduced circulating estrogen levels, and therefore a decrease in active estrogen receptors. As a result, this decrease in estrogen receptor activity may increase the risk of a variety of metabolic diseases including obesity, metabolic syndrome, and cardiovascular diseases, as well as a decrease in cognitive ability (*108*). Regarding the changes in microbiome composition observed in PCOS patients, several studies have reported an increase in the genus *Bacteroides*, which is consistent with findings from rodent models of PCOS. In contrast, the abundance of *Bifidobacterium* and *Faecalibacterium* apparently decreased in PCOS patients (*109,110*).

IR is common in women with PCOS, and the incidence of IR varies from 25% to 70% (*111*). Zhang *et*

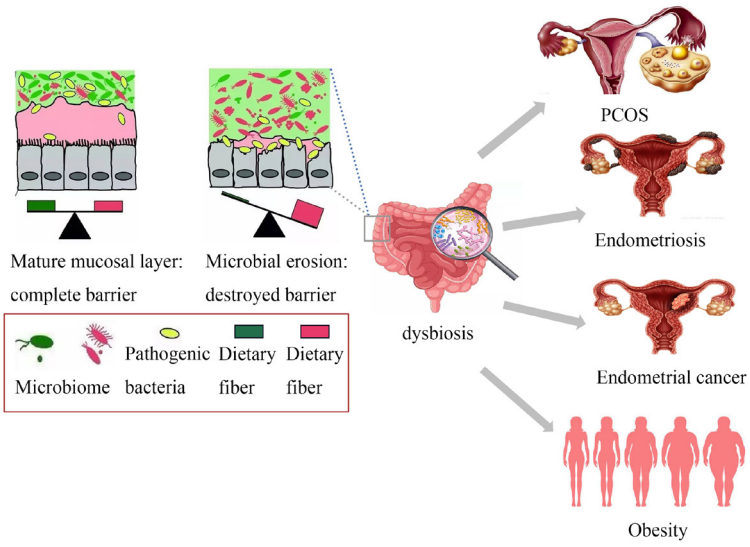

Figure 4. The relationship between gut dysbiosis and diseases. The gut mucosal layer is a protective barrier. When the gut is invaded by microorganisms, the normal mucosal layer is destroyed. Gut dysbiosis increases the risk of diseases such as PCOS, EMs, cancer, and obesity.

al. (*110*) also noted that the levels of acetate, propionate, and butyrate in the gut of PCOS patients decreased significantly compared to that in healthy women. Specifically, reductions of around 30% to 66% were observed. However, following probiotic treatment, the abundance of *Lactobacillus* in the gut of PCOS patients increased significantly, and SCFA levels in the intestines also increased. Overall, insulin secretion also increased (*110*). The gut microbiome has been found to impact insulin sensitivity *via* branched-chain amino acids such as leucine, isoleucine, and valine (*112*). This illustrates the complex and intimate relationship between PCOS and the gut microbiome. Further research is required to explore the potential mechanisms underlying the pathogenesis of PCOS in relation to the gut microbiome.

3.2.2. Gut dysbiosis and EMs

Endometriosis, an estrogen-dependent chronic inflammatory disease, has garnered significant attention due to its potential correlations with the gut microbiome. Mounting evidence suggests a link between the gut microbiome and the incidence of endometriosis. Svensson *et al.* (*113*) performed 16S rRNA sequencing on the gut microbiome of patients with EMs and healthy controls, and their results showed that compared to the healthy control group, the gut microbiome of patients with endometriosis showed lower levels of α and β diversity. Moreover, Svensson *et al*. (*113*) found significant variations in the abundance of twelve bacteria across the classes *Bacilli*, *Bacteroidia*, *Clostridia*, *Coriobacteriia*, and *Gammaproteobacteria* in the feces of patients with endometriosis. Similarly, Ata *et al*. (*114*) noted an increase in the number of Escherichia coli and Shigella, both belonging to the *Enterobacteriaceae* family, in the gut microbiome of patients with stage III-IV endometriosis.

Gut dysbiosis could potentially result in immune disorders, inflammatory reactions, and abnormal estrogen metabolism, and these factors may play significant roles in the emergence of endometriosis (*115*). Wei *et al.* (*116*) showed that Gram-negative bacteria can increase the number and volume of lesions and the number of macrophages in a mouse model of EMs by producing GUS. Other researchers have shown that there are significant differences in the gut microbiome in EMs patients compared to normal individuals, and the levels of expression of inflammatory factors, nuclear factors-κB p65, and cyclooxygenase-2 increased in EM patients (*117*). This suggests that an imbalance of the gut microbiome activates the inflammatory pathway of the gut-brain axis and participates in the progression of EMs. Moreover, disruption of the gut microbiome may lead to increases in the levels of specific neuroactive metabolites, including glutamate and aminobutyric acid. As a result, these metabolites can activate brain neurons (such as GnRH neurons) and increase estrogen production in the ovaries *via* the hypothalamic-pituitary-ovarian axis (*118*). Consequently, increased exposure to estrogen - caused by gut microbiome imbalances - may represent a significant risk factor for the development and progression of endometriosis.

3.2.3. Gut dysbiosis and EC

Over the past 20 years, the incidence of EC has continued to increase and affect younger women (*119*). Studies have shown that changes in the ratio of *Firmicutes*/*Bacteroidetes* can increase GUS active bacteria, increase estrogen levels, and enhance estrogen receptor binding, thereby promoting endometrial hyperplasia and the development of EC (*72*). In EC, estrogen upregulates the production of pro-inflammatory mediators such as IL-6 and TNF-α, which in turn synergistically upregulate the expression of enzymes involved in ovarian steroid production (17β-hydroxysteroid dehydrogenase, aromatase, and estrone sulfate esterase), thereby forming a feedback loop (*120*). An *in vitro* study showed that *Atopobium vaginae* and *Porphyromonas somerae* promote EC development by inducing the expression of inflammatory cytokines and chemokines (TNF-α, IL-1α, IL-1β, and IL-17α) in endometrial cells (*121*). The specific mechanisms underlying the disruption of normal homeostasis by gut microbiota leading to EC, as well as the competition between microorganisms for nutrition, signal transduction between microorganisms and hosts, and the impact of microbial metabolites, all require further research.

4. Use of microbial therapy

4.1. Dietary interventions

In recent years, interventions involving the FGT and gut microbiome as a treatment for female reproductive tract diseases has become a focus of research. More recently, nutrition has been recognized as another factor affecting women's reproductive health. Neggers *et al*. (*122*) and Tohill *et al*. (*123*) first demonstrated the role of malnutrition in BV and other gynecological infections in women of childbearing age. In 2007, Neggers *et al*. (*122*) described the subclinical deficiency of iron and vitamin D during pregnancy as associated with an increased risk of BV. A large cross-sectional study (*123*) subsequently confirmed that lower serum concentrations of vitamins A, C, E, and β-carotenoids were associated with BV, while lower iron levels were associated with increased *Candida* plaque measurement.

Although there is little understanding of the mechanisms by which nutrition affects female reproductive tract homeostasis, studies on gut microbiome in other parts of the body have revealed the impact of diet on bacterial community composition and function, with profound impacts on diseases such as obesity, metabolic

disorders, immune diseases, and cancer (*124*). In addition, the intestine is known to serve as the ultimate host of BV-related lactic acid bacteria and bacteria (*125*). Eating fiber-rich foods is important for the immune system by boosting the diversity of the microbiome, which boosts anti-inflammatory abilities. A high-fat diet is linked to a decrease in *Bacteroides* and *Coprobacillus* levels, while a low-fat diet promotes α diversity of the gut microbiome. Moreover, the concentration of SCFAs in the high-fat diet group is notably lower than that of the low-fat diet group. High-fat diets are also associated with an enrichment of arachidonic acid in feces and an increase in plasma proinflammatory factors (*126*).

An important parameter in the pathogenesis of PCOS is the presence of advanced glycosylation end products (AGEs). Research has confirmed that an unhealthy diet in PCOS patients can lead to an inflammatory state, inducing oxidative stress and stimulating androgen synthesis in ovarian tissue, leading to an increased inflammatory response and ultimately forming a vicious cycle (*127*). In contrast, a high-fiber diet based on polyunsaturated fatty acids can increase insulin sensitivity and thus alleviate hyperandrogenism (*128*). The impact of vitamin D on the diversity of the gut microbiome has been validated in multiple studies. Supplementing vitamin D increases the overall diversity of the gut microbiota and increases the proportion of *Firmicutes/Bacteroidetes*, thereby maintaining intestinal homeostasis (*129*). *In vitro* and animal studies have shown that dietary vitamin D supplementation contributes to the regression of endometriosis lesions with reduced invasion and proliferation (*130*).

4.2. Probiotics

The efficacy of probiotics in the treatment of microbial disorders has been reported in several studies (*131-133*). Currently, the available probiotics can be classified into three categories: *Lactobacillus*, *Bifidobacteria*, and Gram-positive cocci, such as *Streptococcus* and *Lactococcus.* Studies have revealed that probiotics help regulate gut microbiome, treat metabolic disorders, and restore gut microbiome damaged by a high-fat diet to a healthy state (*131*). Chenoll *et al.* (*132*) isolated *Lactobacillus rhamnosus* BPL005 from vaginal samples and co-cultured it with a primary endometrial epithelial cell model with the colonization of *Atopobium vaginae*, *Propionibacterium acnes*, *Gardnerella vaginalis*, and *Streptococcus agalactiae*. They noted a significant reduction in *Propionibacterium acnes* and *Streptococcus agalactiae*, suggesting that microbial agents have the potential to facilitate improved reproductive health. Studies have demonstrated that the combination of a vaginal lactobacillus preparation is more effective in curing vaginal infectious diseases and decreasing the rate of recurrence compared to antibiotic treatment alone (*133*).

Probiotics have been proven to treat other reproductive diseases, including those not transmitted by microbial pathogens. A study in human subjects revealed that, compared to the control group, PCOS patients who received probiotic supplements for 12 weeks experienced an increase in sex hormone binding protein, a decrease in their hirsutism score and extremely low density lipoprotein levels, and improvement in insulin sensitivity (*134*). Zhang *et al.* (*110*) found that oral administration of *Bifidobacterium lactis* V9 can alleviate PCOS by increasing the release of gastrin and peptide YY, thereby restoring sexual hormones at the brain level in a manner similar to normal hormone levels. In addition, probiotic therapy is also quite effective in the treatment of endometriosis. Animal experiments have shown that oral *Lactobacilli* can reduce the growth of mouse EM lesions by increasing IL-12 and NK cell activity (*135*). Itoh *et al*. (*136*) also found that oral probiotics can activate NK cells in the body and inhibit the development of EM lesions. A randomized controlled trial found that oral *Lactobacilli* can alleviate pain related to EMs in women (*137*). In addition, probiotics have been found to improve the fertilization rate, pregnancy success rate, and offspring survival rate of several animals by altering the activity of the microbiota, promoting nutrient absorption and digestion, and improving the immune system (*138,139*).

4.3. Fecal microbial transplantation(FMT)

Fecal microbiota transplantation (FMT) is a rapidly evolving therapy that aims to reconstruct a patient's dysbiotic microbiota with the beneficial fecal microbiota of a healthy individual. In recent years, FMT has displayed great potential for the treatment of female reproductive tract diseases. A study in a rat model of induced PCOS confirmed that after FMT treatment, the estrus cycle of PCOS rats improved, androgen biosynthesis decreased, ovarian morphology returned to normal, and the intestinal microbiota composition was characterized by an increase in *Lactobacilli* and *Clostridium* and a decrease in *Prevotella* (*140*). Preclinical mouse models suggest that short-chain fatty acids derived from the gut microbiota can prevent the progression of endometriosis, thereby reducing EM-related pain (*141*). Despite the lack of clinical data, FMT is a potential treatment option for endometriosis due to its beneficial effect in reducing EM-related pain. In breeders, FMT can regulate increased hormone secretion, intestinal health, and ovarian function through SIRT1-related cell apoptosis and cytokine signaling pathways (*142*). In addition to animal models, prospective data should be obtained from laboratory studies for further research in humans.

5. Methods of studying the microbiome

Early microbiology research relied on optical microscopy

and microbial culture techniques, but microbial culture took a long time and the results obtained were incomplete and unrepresentative, posing numerous obstacles to microbiology research (*143*). In recent years, molecular biology theory and techniques have greatly promoted the innovation of microbial classification and identification and expanded our understanding of microbial structure and function. From the perspective of genetic evolution, people classify and identify microorganisms at the molecular level, making the classification of microorganisms increasingly refined and precise. The main techniques used are: denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, terminal-restriction fragment length polymorphism, temporal temperature gradient gel electrophoresis, single-strand conformation polymorphism, western blot hybridization, quantitative PCR, and 16S rRNA library detection (*144*). However, the above methods all have the drawbacks of low throughput (fewer samples can be analyzed and fewer data can be obtained) and high cost, precluding their widespread use by ordinary laboratories.

Sequencing is the most commonly used technique in modern molecular biology research. With the completion of the Human Genome Project, research has entered the era of functional genomics. Conventional Sanger sequencing can no longer meet the requirements of large-scale gene sequencing. After more than 30 years of development, next generation sequencing (NGS) has arrived. At present, NGS has become the mainstream method for classifying and identifying bacteria by detecting the sequence of 16S rRNA genes in bacteria. 16S rRNA widely exists in prokaryotic cells. In the process of bacterial evolution, the evolution of rRNA genes is relatively conserved. A large amount of information is retained, facilitating amplification and sequencing, and is considered to be the most ideal yardstick to measure the evolutionary history of life. The unique advantages of 16S rRNA enable bioinformatic analysis of the generated sequences within a relatively short sequencing range (V1-V9), effectively distinguishing bacterial communities (*145*). Understanding the composition and function of microorganisms helps to understand the potential ecological adverse states represented by microbial species or community types. Future research on metabolome composition may reveal complex interactions between species and host microorganisms.

6. Conclusion

A balanced and healthy microbiome is crucial for maintaining reproductive health as it assists in protecting the host from pathogens, enhancing reproductive potential, and reducing the likelihood of adverse pregnancy outcomes. Implementing effective measures, such as addressing dysbiosis, monitoring dynamic changes in the microbiome, and bolstering levels of beneficial bacteria, can significantly improve reproductive health. To gain a comprehensive understanding of all microbial species undergoing modifications in reproductive pathology, an essential task is to conduct large-scale longitudinal studies encompassing bacteria, fungi, and viruses. As further research on the FGT and gut microbiome unfolds, microbial therapy has the potential to serve as a prospective approach to enhance female reproductive health.

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