

The impact of the gut microbiota on the reproductive and metabolic endocrine system

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ABSTRACT

As the gut microbiota exerts various effects on the intestinal milieu which influences distant organs and pathways, it is considered to be a full-fledged endocrine organ. The microbiota plays a major role in the reproductive endocrine system throughout a woman's lifetime by interacting with estrogen, androgens, insulin, and other hormones. Imbalance of the gut microbiota composition can lead to several diseases and conditions, such as pregnancy complications, adverse pregnancy outcomes, polycystic ovary syndrome (PCOS), endometriosis, and cancer; however, research on the mechanisms is limited. More effort should be concentrated on exploring the potential causes and underlying the mechanisms of microbiota-hormone-mediated disease, and providing novel therapeutic and preventive strategies. As the gut microbiota exerts various effects on the intestinal milieu which influences distant organs and pathways, it is considered to be a full-fledged endocrine organ. The microbiota plays a major role in the reproductive endocrine system throughout a woman's lifetime by interacting with estrogen, androgens, insulin, and other hormones. Imbalance of the gut microbiota composition can lead to several diseases and conditions, such as pregnancy complications, adverse pregnancy outcomes, polycystic ovary syndrome (PCOS), endometriosis, and cancer; however, research on the mechanisms is limited. More effort should be concentrated on exploring the potential causes and underlying the mechanisms of microbiota-hormone-mediated disease, and providing novel therapeutic and preventive strategies.

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Introduction

The gut microbiota has various effects on the intestinal milieu that influence distant organs and pathways, and the gut microbiota is considered to be a full-fledged endocrine organ. The microbiota plays a major role in the reproductive endocrine system throughout a woman's lifetime by interacting with estrogens, androgens, insulin, and other hormones. Imbalances in the gut microbiota composition can lead to several diseases and conditions, such as pregnancy complications, adverse pregnancy outcomes, polycystic ovary syndrome (PCOS), endometriosis, and cancer; however, research on these mechanisms is limited. Research efforts should be focused on exploring the potential causes and underlying mechanisms of

microbiota hormone-mediated disease and identifying novel therapeutic and preventive strategies.

The gut microbiome contains a large amount of information. It is well known that the number of bacteria in the body is of the same order as the number of human cells¹ and that the amount of genetic information present in these microbes is at least 150-fold greater than that in the human genome.² Increasing evidence obtained in recent years has suggested that these microorganisms function almost as an extra organ by actively participate in shaping and maintaining our physiology. Numerous host and environmental factors, including diet, host genes, and hormones, are associated with variations in the gut microbiome. Sex hormones, such as progesterone, estradiol, and

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testosterone, also participate in communication between microorganisms and their hosts and play a number of important physiological roles in reproduction, differentiation, cell proliferation, apoptosis, inflammation, metabolism, homeostasis, and brain function.³ Commensal bacteria can produce and secrete hormones, and the crosstalk between microbes and hormones can affect host metabolism, immunity, and behavior. The human microbiome affects every stage and level of female reproduction, including follicle and oocyte maturation in the ovary, fertilization and embryo migration, implantation, and the whole pregnancy, even during parturition. Alterations in the microbiome, particularly the gut microbiome, have specific impacts on the reproductive endocrine system, and correcting abnormal microbiomes may lead to improved reproductive outcomes.⁴ Specific linear correlations between gut microbiota and serum hormone levels, which may have additional effects on the health of the body, have been reported in several studies. Sex hormone levels have a potential relationship with the gut microbiota, and this novel concept has been named the “*microgenderome*”⁵ Moreover, there may be a possible relationship between specific intestinal bacteria and female diseases, such as PCOS, endometriosis and bacterial vaginosis (BV). In this paper, we systematically review the interaction between the gut microbiota and the female endocrine system.

Interaction of estrogen and the gut microbiome

The gut microbiota is not only influenced by estrogens but also active in affecting estrogen levels. Estrogens are a principal regulator of the gut microbiome, and the gene repertoire of the gut microbiota that is capable of metabolizing estrogens is known as the “*estrobolome*”⁶

The expression of estrogen receptor β (ER β) and serum concentrations of steroidal hormones, especially estradiol, are known to change throughout the life cycle of the organism; therefore, regulation of estrogen is essential for women’s health. Intestinal bacteria play an important role in estrogen metabolism, evidenced by the observation that the use of antibiotics leads to lower estrogen levels.⁷ Microbially secreted β -glucuronidase can metabolize estrogens from their conjugate forms to their deconjugated forms.⁶ Dysbiosis and a reduction in gut

microbiota diversity reduce β -glucuronidase activity and result in decreased deconjugation of estrogen and phytoestrogen into their circulating and active forms. The decrease in circulating estrogens alters estrogen receptor activation and may lead to hypotrogenic pathologies: obesity, metabolic syndrome, cardiovascular disease and cognitive decline.^{4,6} Increased abundance of β -glucuronidase-producing bacteria can lead to elevated levels of circulating estrogens and drive diseases, such as endometriosis and cancer. In addition, estrogen levels can also affect the states of diseases and processes, including PCOS, endometrial hyperplasia, and ultimately fertility.⁸ It was reported that representative orders such as *Lactobacillales* and specific phyla such as *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* also differ as a function of murine ER β status, suggesting that steroid nuclear receptor status and dietary complexity may play important roles in microbiota maintenance.⁹ Alpha diversity has a negative correlation with estradiol concentrations, but the mechanism remains unclear. It is possible that gut microbes participate in the regulation of sex hormones and, conversely, that sex hormones modify microbial diversity.¹⁰

Recently, it was reported that the gut microbiome mediates the preventive effect of 17 β -estradiol against metabolic endotoxaemia and low-grade chronic inflammation. 17 β -estradiol-treated male and ovariectomized female mice have decreased *Proteobacteria* and lipopolysaccharide (LPS) biosynthesis, and these levels are similar to those of normal female mice. Estrogen or estrogen-like compounds can decrease the LPS produced by the gut microbiome and gut permeability, resulting in reduced metabolic endotoxaemia.¹¹ In addition, estrogen can modify gut epithelial barrier integrity in mice, evidenced by the observation that females are more resistant to gut injury than their male counterparts.¹²

Estrogen is also associated with a variety of sex hormone-driven cancers, such as endometrial, cervical, ovarian, prostate and breast cancer. The gut microbiota composition is altered in many of these cancers, and it may play an important role in promoting these cancers.¹³ For example, it was hypothesized that high-fat-diet (HFD)-associated steroids can influence the gastrointestinal microbiome by introducing a carcinogen that might act

on breast tissue or an estrogen that might contribute to tumor growth.¹⁴ Decreased ratios of estrogen metabolites to parental compounds and decreased fecal microbiota diversity are associated with an increased risk of breast cancer in postmenopausal women.¹⁵

In postmenopausal women, gut microbiota diversity is positively associated with the ratio of estrogen metabolites in urine.¹⁶ It has been reported that total fat mass and abdominal fat, critical factors in the future development of insulin resistance and type 2 diabetes, are increased in postmenopausal women compared with premenopausal women.^{17,18} The gut microbiota may play an important role in regulating estrogen levels and metabolism during menopause. Furthermore, the gut microbiota can metabolize estrogen-like compounds in foods such as soy isoflavones and promote the growth of some specific bacteria,¹⁹ and supplementation with soy isoflavones increases the concentration of *Bifidobacterium* and suppresses unclassified *Clostridiaceae* in postmenopausal women.^{20,21} In this regard, *Bifidobacterium* plays a beneficial role in promoting absorption and immunity and preventing infection in the intestine, while *Clostridiaceae* is known to be involved in inflammatory diseases and is associated with obesity.

These results indicate that this host microbe-estrogen interaction contributes to a wide range of pathways that affect women's health and disease. Estrogens and gut microbiota might synergize to influence various aspects of women's health, including fertility, obesity, diabetes, and cancer. Therefore, a better understanding of the interactions between estrogens and gut microbiota will lead to new insights and new approaches toward reducing the risk of endocrine diseases in women.

Interaction between hyperandrogenaemia and intestinal flora

Androgens are also very important hormones in women, and hyperandrogenaemia (HA) is a salient feature of PCOS and a major contributor to hirsutism, acne, male pattern alopecia, and anovulation in affected women. In women with PCOS, elevated luteinizing hormone (LH) leads to the production of excess androgens by ovarian theca cells, and low FSH contributes to impaired folliculogenesis and anovulation, which is the most common reason for infertility

secondary to ovulatory dysfunction.²² HA conditions have serious health consequences, including increased risks for insulin resistance, type 2 diabetes, hypertension, obesity, and cardiovascular disease. Testosterone may influence the composition of the gut microbiome in females. A regression analysis showed that decreased abundances of several genera were correlated with higher circulating testosterone levels and impaired glucose metabolism in PCOS mice.¹⁰ Removal of the microbiota increases the circulating testosterone concentration in female mice but decreases the circulating testosterone concentration in male mice, suggesting a bidirectional interaction between the amount of male sex hormone and the microbiota.²³ Prenatal androgen (PNA) exposure has a powerful effect on a developing female fetus, as daughters of mothers with PCOS are more likely to be diagnosed with PCOS, and transgenerational effects are manifested in both reproductive and metabolic dysfunctions mediated by in utero and/or oocyte-derived factors.²⁴ In a dihydrotestosterone (DHT)-induced mouse model, the relative abundance of *Anaerococcus* is enriched in the high androgen group and positively correlated with the levels of testosterone and free testosterone.²⁵ The gut microbiota and its metabolites can activate the inflammatory pathway, brain-gut peptide secretion, and islet β -cell proliferation, leading to abnormal or excessive fat accumulation, insulin resistance, and compensatory hyperinsulinaemia.^{26,27}

In a testosterone cypionate-induced mouse model, the fecal microbiota profile of PNA animals contained an increased relative abundance of bacteria associated with steroid hormone synthesis and metabolite production of short-chain fatty acids (SCFAs). The cardiovascular function of the mice was also impacted, suggesting that early-life exposure to androgens in female offspring of women with PCOS may lead to long-term alterations in gut microbiota and cardio-metabolic function.²⁸ *Ruminococcus* has been reported to be significantly increased in neonatally androgenized rats and is positively correlated with serum testosterone levels.^{29,30,31} Shearmen *et al.* reported that there is a negative correlation between alpha diversity and total testosterone, hyperandrogenism, and hirsutism. In animal models of PNA exposure and maternal HA, the relative abundance of bacteria associated with steroid synthesis (*Nocardiaceae* and *Clostridiaceae*) and elongation of

unsaturated SCFAs is increased, while that of *Akkermansia*, *Bacteroides*, *Lactobacillus*, and *Clostridium* is decreased. These models also had increased body weight and mRNA expression of adipokines, and cardiovascular function was negatively impacted due to increased systolic and diastolic blood pressure and a decreased heart rate.²⁸ On the other hand, Insenser *et al.* found a positive correlation between alpha diversity and testosterone levels and the ratio of free testosterone to free estradiol, indicating that gut microbiota may play a role in the regulation of sex hormones and that sex hormones may modify microbial diversity.¹⁰ However, the limitation of these studies is that there was no in-depth assessment of the mechanistic basis for the interaction between gut microbiota and androgens. A variety of gut microbiota can express the enzymes involved in androgen metabolism and help to synthesize and transform androgens. The degradation of testosterone via microbial processes has been observed in several environmental matrices; for example, *Actinobacteria* and *Proteobacteria* are capable of degrading androgen,³² and *Clostridium scindens*, whose genome encodes 20 α -hydroxysteroid dehydrogenase (HSDH), is a human gut microbe with high potential to convert glucocorticoids into androgens.³³

In summary, excess androgen may result in dysbiosis of the host gut microbiota, and changes in the gut microbiome may influence the development and pathology of the endocrine systems of women, even in those with PCOS. Future studies should focus on mechanistic details to provide a comprehensive theoretical basis and therapeutic targets for the diagnosis and treatment of HA.

Gut microbiota and its role in thyroid disease

The thyroid is a vital endocrine gland. The main function of the thyroid is to secrete the iodine-containing thyroid hormones triiodothyronine (T₃) and thyroxine (T₄) and the peptide hormone calcitonin, which affects cardiovascular and reproductive diseases by influencing metabolism and tissue development.³⁴

The intestinal microbiota plays a prominent role in thyroid disorders, including Hashimoto's thyroiditis (HT) and Graves' disease (GD).³⁵ HT and GD are the major causes of hypothyroidism and hyperthyroidism, respectively, and the gut

microbiota plays a vital role in thyroid disorders.³⁶ It was reported that gut microbial diversity is different between patients with thyroid cancer and healthy control subjects. The abundances of *Neisseria* and *Streptococcus* are markedly elevated in the gut microbiota of patients with thyroid cancer and thyroid nodules, and the abundances of *Butyricimonas* and *Lactobacillus* are reduced.³⁷ In patients with HT, some genera of bacteria, such as *Blautia*, *Roseburia*, *Ruminococcus_toques_group*, *Romboutsia*, *Dorea*, *Fusicatenibacter*, and *Eubacterium_hallii_group*, are increased and correlated with clinical parameters.³⁸ In the Graves' orbitopathy or ophthalmopathy (GO) murine model, the abundance of *Firmicutes* in the intestine is higher and positively correlated with orbital adipogenesis.³⁹ The microbiota can also affect the uptake of iodine, selenium, iron, and zinc.³⁶ These findings demonstrate that the composition of the gut microbiota can cause and affect the progression of thyroid disorders.

Bile acid, an important metabolite of gut microbiota, plays an essential role in thyroid diseases. The serum bile acid profiles of hyperthyroid and hypothyroid patients are different, and the composition of bile acid can reflect thyroid function.⁴⁰ The most prominent bile acid in hypothyroid patients is the secondary bile acid deoxycholic acid, whereas chenodeoxycholic acid is the most prominent bile acid in hyperthyroid patients. After medical treatment, the levels of bile acid return to normal in patients with thyroid dysfunction. The composition and level of bile acid are involved in the secretion of thyroid hormone, and the thyroid-stimulating hormone level is inversely associated with serum total bile acid in subclinical hypothyroidism.⁴¹ Thyroid hormone can also suppress bile acid synthesis in primary human hepatocytes.⁴² However, another study demonstrated that thyroid hormone could reduce circulating proprotein convertase subtilisin/kexin type 9 (PSCK9) levels and stimulate bile acid synthesis in humans.⁴³ Bile acids can induce energy expenditure and have beneficial metabolic effects by promoting cyclic-AMP-dependent thyroid hormone activating enzyme type 2 iodothyronine deiodinase (D2).⁴⁴

SCFAs interact with the thyroid hormone triiodothyronine and affect the secretion of hormones by regulating enterocyte gene transcription.⁴⁵ For instance, fecal SCFAs can affect women's emotional

health by influencing hyperlipidemia and thyroid disease.⁴⁶

Normal thyroid function is important to maintain healthy reproductive function in both sexes. During pregnancy, both hypothyroidism and hyperthyroidism cause increased rates of spontaneous miscarriage and preterm delivery.⁴⁷ Among women undergoing in vitro fertilization and embryo transfer (IVF-ET), treatment with levothyroxine does not reduce the miscarriage rate among those who test positive for anti-thyroperoxidase antibodies compared to those with normal thyroid function. Thyroid disorders have been demonstrated to be linked to reduced fertility, hypomenorrhea, and polymenorrhea and potentially linked to gestation-induced hypertension and placental abruption.⁴⁸ Given the above data, the gut microbiota may influence the reproductive endocrine system by inducing thyroid dysfunction, such as hypothyroidism or hyperthyroidism.

Obesity affects intestinal flora and reduces fertility

Obesity and overweight have become pandemic due to HFDs, low energy expenditure, dysbiosis in gut microbiota, or other environmental exposures,⁴⁹ which have adverse effects in terms of disorders of female reproductive endocrine processes. Of note, the role of gut microbiota in the development of obesity has become increasingly important. In an obese mouse model in which T cells disabled the Myd88 pathway, *Clostridia* colonization was reduced.⁵⁰ In mice, treatment against *Akkermansia muciniphila* by means of a purified membrane protein isolated from *A. muciniphila* or the pasteurized bacterium has been shown to prevent the development of obesity and associated complications.^{51,52} In a proof-of-concept exploratory study, supplementation with *A. muciniphila* in overweight and obese humans improved metabolism.⁵³ The genus *Bifidobacterium* has also been shown to have a consistent weight loss effect in humans.⁵⁴ These data suggest that the gut microbiota can modify host metabolism and that dysbiotic gut microbiota plays a driving role in the pathogenesis of obesity.

Although the mechanisms by which the gut microbiota regulates host metabolism are incompletely understood, small molecular metabolites derived from gut microbiota have been well investigated in

obesity (**Figure 1**).^{55–57} Bile acids are generated from cholesterol in the liver and are metabolized by the gut microbiota in the intestine;^{58,59} additionally, they play a pivotal role in nutrient absorption and biliary secretion of cholesterol.⁶⁰ The decreased levels of the gut microbiome component *Lactobacillus* expressing bile salt hydrolase (BSH) or the direct inhibition of bacterial BSH by caffeic acid phenethyl ester (CAPE) increase the production of tauro- β -muricholic acid (T- β -MCA).^{61,62} Bile acids are involved in obesity, and intestinal increased T- β -MCA, which is a farnesoid X receptor (FXR) nuclear receptor antagonist, improves obesity and hyperglycemia.⁶¹ TGR5 is another bile acid receptor that can be activated by secondary bile acids and induce cAMP/PKA signaling to increase energy expenditure in brown adipose tissue and muscle and increase the release of glucagon-like peptide 1 (GLP-1) in intestinal L cells.⁶³ SCFAs are the main metabolites produced by the bacterial fermentation of indigestible carbohydrates (for example, dietary fiber) and have an important role in the prevention and treatment of obesity.^{55,64} SCFAs stimulate enteroendocrine cells to secrete peptide YY (PYY) and GLP-1 through G protein-coupled receptors (GPR41 and GPR43).^{65,66} SCFAs have beneficial metabolic effects on protection against HFD-induced obesity via a PPAR γ -dependent switch from lipogenesis to fat oxidation.⁶⁷ It was also reported that butyrate could improve diet-induced obesity, dyslipidaemia and the development of hepatic steatosis, mainly by reducing appetite and activating brown adipose tissue.⁶⁸ Several human intervention studies also demonstrated that dietary fiber intake decreased body weight and increased the production of satiety-stimulating hormones.^{69,70} Although gut microbial metabolites have been demonstrated to play an important role in obesity-associated complications, further clinical studies are needed.

Gut microbiota dysbiosis in obesity is associated with reproductive endocrine diseases. A recent study showed that DHT and HFD shift the overall gut microbial composition and that PCOS rats have a lower Shannon and Simpson index. Moreover, these rats have abnormal estrous cycles with increasing androgen levels and exhibit multiple large ovarian cysts with diminished granulosa layers.²⁵ In a clinical study, compared with healthy controls, patients with PCOS showed an imbalance

in the intestinal microbiome. With the lower abundance of intestinal SCFAs in PCOS, the abundances of the *Faecalibacterium*, *Bifidobacterium*, and *Blautia* genera were decreased, while the abundances of *Parabacteroides* and *Clostridium* were increased. Furthermore, the administration of the probiotic *Bifidobacterium lactis* V9 could improve sex hormone levels in PCOS.⁷¹ Accumulative studies have demonstrated that obesity can disrupt processes related to female fertility, including sex hormone secretion, oocyte differentiation and maturation, and endometrial implantation, and other reproductive functions.^{72,73} Interestingly, bariatric surgery can improve fertility, reduce the risk of pregnancy complications, and improve fetal health.^{74,75} Imbalances in the gut microbiome in obese individuals induce increases in LPS, endotoxaemia, IL-6, and IL-1 β , which have proinflammatory functions.⁷⁶ Inflammation reduces oocyte quality, disrupts meiotic and cytoplasmic maturation and is involved in the development of reproductive diseases. Furthermore, the gut microbiota of maternal obesity during pregnancy influences microbiota colonization and metabolism in offspring.⁷⁷ In a Swedish nationwide register-based cohort and a clinical case-control study from Chile, they found that female offspring of mothers who were obese and had elevated androgen levels were more likely to be diagnosed with PCOS, but the role of the gut microbiome remains unclear.²⁴

The composition of the gut microbiome and its metabolites in obese individuals is different from that in healthy individuals. The specific microbiota or metabolites derived from the microbiota can influence the pathogenesis of obesity and further cause reproductive endocrine disorders by affecting host metabolism or the inflammatory stage.

Gut microbiome and its role in insulin resistance

Insulin resistance and compensatory hyperinsulinism contribute to androgen excess in PCOS and other reproductive diseases because insulin can induce androgen secretion from the adrenal glands and regulate the level of luteinizing hormone.^{22,78} Accumulative evidence has demonstrated that the gut microbiome plays an important role in regulating the secretion of insulin.⁷⁹

Insulin is an important hormone that increases membrane permeability to glucose and lowers the level of glucose by activating the insulin receptor. The binding of insulin to insulin receptors causes the activation of insulin receptor tyrosine kinase and the tyrosine phosphorylation of insulin receptor and insulin receptor substrate (IRS) proteins.^{80,81} Phosphorylation of IRS stimulates the binding of the lipid kinase phosphatidylinositol-3-kinase (PI3-K) at the plasma membrane, which phosphorylates the Thr308 residue of AKT by synthesizing PtdIns(3,4,5)P3 (PIP3).⁸² AKT activation leads to glucose production, utilization and uptake, as well as the synthesis of glycogen, lipids, and proteins.⁸² Interestingly, mounting evidence suggests that the gut microbiome, as well as the metabolites of bacteria, are involved in the progression of insulin resistance.^{57,83,84}

A recent study showed that the serum metabolome of insulin-resistant individuals is characterized by increased levels of branched-chain amino acids (BCAAs), which are associated with an increased abundance of the species *Prevotella copri* and *Bacteroides vulgatus* (*B. vulgatus*). *P. copri* can also induce insulin resistance, aggravate glucose intolerance, and increase the levels of BCAAs.⁸⁵ The administration of *Phellinus linteus* polysaccharide extract (PLPE) to mice was found to change the gut microbiota composition and increase SCFA levels, which reduces LPS content and systemic inflammation and improves insulin resistance by inhibiting JNK and NF κ B activation.⁸⁶ SCFAs, which are the main fermentation products of the intestinal microbiota and include propionate, acetate, and butyrate, affect metabolic processes of the host, especially insulin resistance.⁸⁷ Human and animal studies have suggested that acetate beneficially affects host metabolism and improves insulin resistance through the gut hormone GLP-1 secreted from colonic L cells, which inhibits appetite and reduces lipolysis and systemic proinflammatory cytokine levels.⁸⁸ Additionally, it was reported that the microbiota-generated metabolites propionate and butyrate activate intestinal gluconeogenesis through complementary mechanisms.⁶⁴

Fecal microbiota transplantation (FMT) is a method of modulating microbial composition and has been widely explored for the treatment of a growing range of microbiome-associated diseases,

including insulin resistance and obesity.⁸⁹ Administration of *Collinsella* can reduce the expression of tight junction proteins in intestinal cells and increase the permeability of the intestinal wall. Moreover, *Collinsella* is highly correlated with serum insulin levels, suggesting that the gut microbiota can significantly affect glucose metabolic function.⁹⁰ The transplantation of fecal microbiota from lean donors to obese male recipients with metabolic syndromes improves insulin sensitivity and is accompanied by an altered microbiota composition.⁹¹

Insulin resistance and compensatory hyperinsulinaemia induce excess androgen and cause reproductive diseases such as PCOS. Insulin can induce the secretion of androgen from the adrenal glands and modulate LH pulsatility.^{78,92} Conversely, hyperandrogenism induces inflammation of abdominal and visceral fat and causes insulin resistance and metabolic dysfunction, thus forming negative feedback in PCOS.⁷⁸ Transcriptional and epigenetic changes in skeletal muscle may promote metabolic abnormalities (such as insulin resistance) in women with PCOS, and the investigators identified differentially expressed genes that were associated with muscle function and metabolism, such as *COL1A1* and *MAP2K6*.⁹³ *Bacteroides* is a proinflammatory bacterium that plays a critical role in insulin resistance via an inflammatory mechanism. It was reported that *Bacteroides* was increased in patients with PCOS²⁵ and displayed a negative correlation with fasting insulin levels. Administration of IL-22 or glycodeoxycholic acid (GDCA) could help relieve insulin resistance in PCOS mice.⁹⁴

Metformin is widely used for the treatment of individuals with type 2 diabetes (T2D) because of its distinct glucose-lowering effect and safety profile. Recently, it was reported that the gut microbiota plays an important role in the mechanism of action of metformin and that metformin can alter the gut microbiota composition and affect metabolic pathways.^{95–97} The gut microbiome profiles and gut-derived metabolites are related to host insulin sensitivity.⁸⁵ Metformin can increase several SCFA-producing microbiota with increased butyrate and propionate, which are involved in glucose homeostasis, and can improve insulin resistance.⁹⁸ Sun *et al.* further investigated the role

of microbiota and its metabolites in the hypoglycemic effect of metformin and its detailed molecular mechanism. They found that the species *Bacteroides fragilis* was decreased and that bile acid glycodeoxycholic acid (GDCA) was increased in the gut after metformin treatment, with concomitant inhibition of intestinal FXR signaling. Briefly, metformin improves metabolic dysfunction in part through the *B. fragilis*–GDCA–intestinal FXR axis.⁹⁹ However, Ara Koh *et al.* reported that the microbial metabolite imidazole propionate impairs the glucose-lowering effect of metformin through p38γ-dependent inhibitory AMPK phosphorylation.¹⁰⁰

Overall, these studies indicate that the microbiota and its metabolites are involved in the pathogenesis of insulin resistance (**Figure 1**). Insulin resistance is one of the symptoms of female reproductive diseases such as PCOS, which has been demonstrated to cause gut microbiota dysfunction; however, the underlying mechanisms of microbiota-insulin resistant reproductive diseases remain to be further studied.

Microbiota, the female reproductive tract, and embryo development

The gut microbiota and its products may influence all of embryonic development from the formation of gametes to the processes involved in fertilization, implantation of the conceptus, placentation, abortion, delivery of the new-borns, and metabolic programming and reprogramming during critical periods of the life cycle (**Figure 2**). According to reports, many bacteria present in the digestive tract are also present in the female reproductive tract, including the vagina, endometrium and placenta.^{101–103} Functionally, the microbiota is a continuum in the reproductive tract and can be perturbed at multiple sites in a disease.

The vaginal microbiome is dynamic and seems to be affected by the menstrual cycle, the dominant microbiota, and sexual activity.¹⁰⁴ Women who deliver preterm exhibit significantly lower vaginal species levels of *Lactobacillus crispatus* and higher levels of *Sneathia amnii*, which are correlated with proinflammatory cytokines in vaginal fluid.¹⁰⁵ A meta-analysis including five independent studies across 3,201 samples demonstrated that there are significant differences between the microbial within-sample

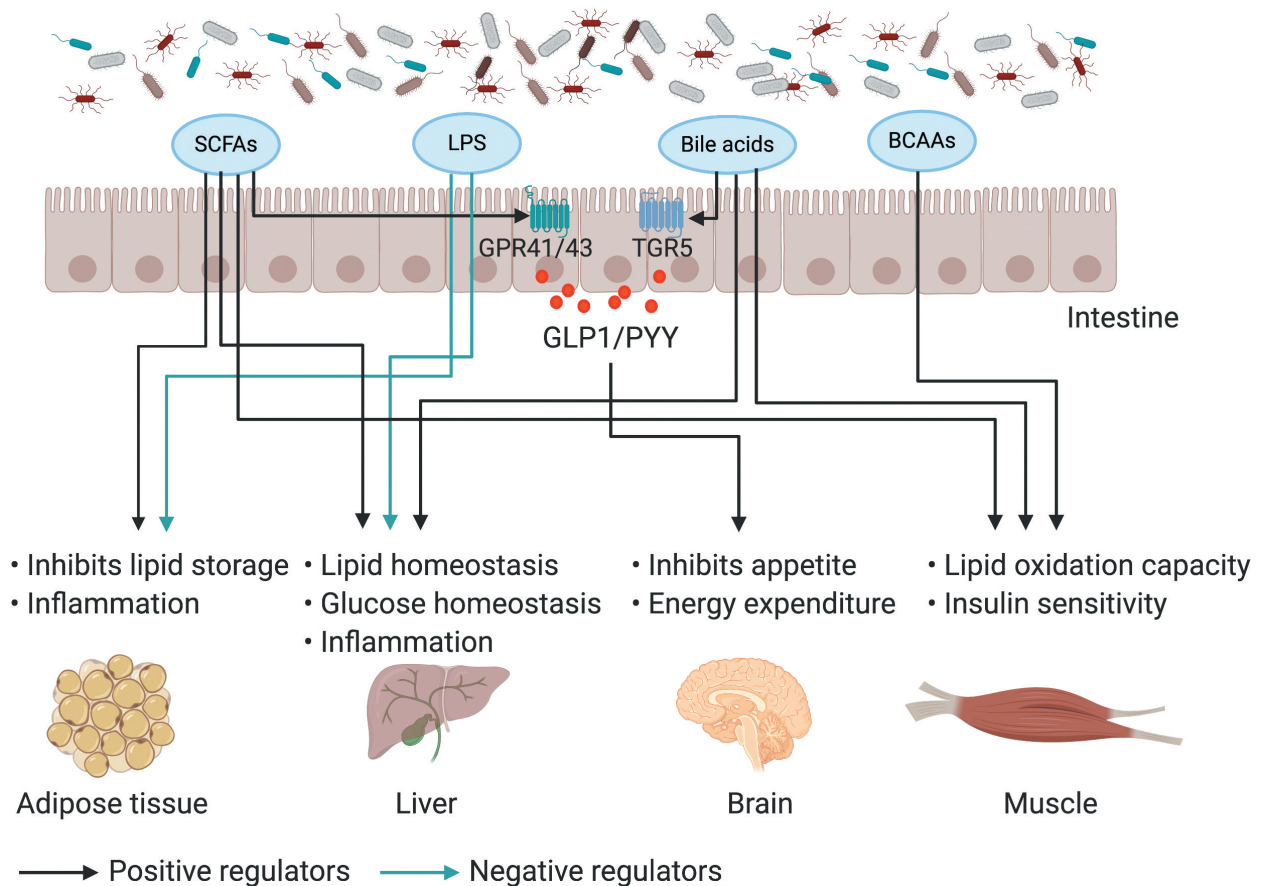


Figure 1. The impact of gut microbiota and its metabolites on inter-organ crosstalk contributes to obesity and insulin resistance. The gut microbiota and its metabolites communicate with distant organs (host adipose tissue, muscle, liver and brain). SCFAs derived from the fermentation of gut microbiota, LPS, bile acids and BCAAs play important roles in inter-organ crosstalk by regulating gut integrity and influencing peripheral tissue function and metabolism; furthermore, they could contribute to obesity and insulin resistance.

variance among women who delivered prematurely compared to those who delivered at term. Moreover, the differences were observed across pregnancy trimesters and are consistent across different racial or ethnic backgrounds.¹⁰⁶ The maternal vaginal microbiome can alter the composition of the microbiota colonizing the neonatal gut. Microbiota diversity, structure, and composition in non-inoculated cesarean-delivered offspring are significantly decreased compared with those in vaginally delivered offspring.¹⁰⁷ Prenatal stress is also associated with alterations in the fetal intestinal transcriptome and niche, as well as with changes in the adult gut that occur due to additional exposure to stress in adulthood.¹⁰⁷

Recently, the molecular identification of bacterial species in the endometrium confirmed that the uterine cavity is not sterile.¹⁰⁸ Furthermore, endometrial communities also arise from colonization of specific macroflora, with a number of *Bacteroidetes*

and *Proteobacteria* taxa primarily associated with the gastrointestinal tract.¹⁰⁹ There are several differing opinions suggesting that the presence of uterine microbiota is likely reflective of bacterial tourists or invaders rather than a resident population that contributes to health and homeostasis.¹¹⁰ The presence of bacterial species in the uterine cavity for patients undergoing in vitro fertilization (IVF) has negative effects on the implantation and pregnancy rates.¹¹¹ The presence of a non-*Lactobacillus*-dominated microbiota in a receptive endometrium is associated with significant decreases in implantation, pregnancy, ongoing pregnancy, and live birth rates,¹⁰² but the pathogens and the mechanisms by which they interfere with embryonic implantation remain unclear. The intestinal microbiome and its metabolites can affect endometrial and uterine immunity during implantation and placentation as a possible interaction partner of the local microbiota.¹¹² For example,

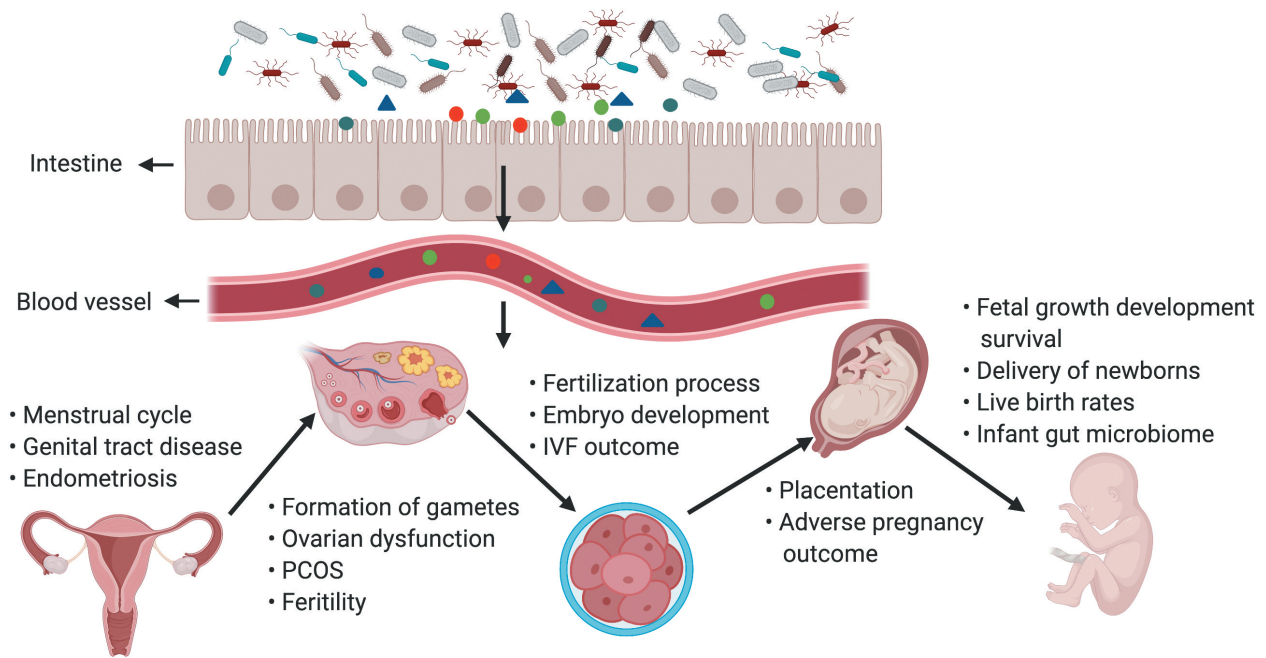


Figure 2. The gut microbiota and its impact on the female reproductive tract, embryo development and pregnancy. Products of gut microbiota may be transported through the circulation and influence the female reproductive tract (e.g., may cause disruptions in the menstrual cycle, genital tract disease and endometriosis), ovarian function, embryonic development and the health of the mother and fetus. The gut can affect the formation of gametes, embryo development and fertilization processes, and alteration of intestinal flora can lead to ovarian dysfunction, PCOS, infertility, and adverse IVF outcomes. In pregnant women, the gut microbiota has an effect on placentation, pregnancy outcomes, the delivery of new-born babies, the infant microbiome, the live birth rate and fetal growth, development, and survival.

T cells are a large fraction of immune cells in the human endometrium, and a proper ratio of Th1 and Th2 cells within the endometrium is needed to prepare for implantation. The intestinal microbiome and metabolites can provide immunostimulatory signals and activate innate and downstream adaptive immune responses, and the differentiation of various T cell subsets is impaired in mice with disrupted gut microbiota.^{113,114} Because T cells reside mostly in the deeper layers of the endometrium, they are more likely to have a role in early placenta formation after implantation.

Establishment and maintenance of placental integrity and function are critical for fetal growth, development, and survival. Whether the placenta has its own colonized flora remains controversial. A recent report demonstrated that there was no evidence of the presence of bacteria in the large majority of placental samples from both complicated and uncomplicated pregnancies. However, in approximately 5% of pregnant women, there is an important pathogen, *S. agalactiae*, in the placenta before the onset of labor.¹¹⁵ Another study

indicated that the placenta harbors a low-abundance but metabolically rich microbiome, and variations in the placental microbiome are associated with a remote history of antenatal infection, which may have a profound influence on intrauterine infection and preterm birth.¹⁰³ According to one recent study of new-borns, the genus *Escherichia* has a high abundance in meconium and is a strong contributor to early-onset sepsis among extremely low-birth-weight neonates, and the placenta is a likely source of *Escherichia* in the new-born meconium.¹¹⁶ In a recent study, SCFAs in the bloodstream were able to pass from a non-germ-free mother's gut microbiota across the placenta and into developing embryos. Embryonic insulin regulation was impaired in embryos from germ-free (GF) mothers, and insulin levels were significantly elevated in the adult stage, providing evidence for the crucial contribution of the maternal gut environment to the metabolic programming of offspring.¹¹⁷ Patients with preeclampsia showed reduced bacterial diversity with obvious dysbiosis. In the placenta of both patients and mice with

preeclampsia, the total bacteria, *Fusobacterium* genus, and inflammatory cytokine levels were significantly increased, suggesting that the gut microbiome is symbiotic and contributes to disease pathogenesis.¹¹⁸

Intestinal microflora disorder can lead to ovarian dysfunction, including oocyte developmental disorder, disruption of the estrous cycle, and abnormal ovulation. Changes in the gut bacteria of *Drosophila* can affect its germ line. Removal of the gut bacteria represses oogenesis, expedites the maternal-to-zygotic transition in the offspring and unmasks hidden phenotypic variation in mutants, and the main impact on oogenesis is linked to the lack of the gut *Acetobacter* species.¹¹⁹ Antibiotic amoxicillin may induce gut dysbiosis and alter the estrous cycles of mice, and the oocyte area from the dysbiotic group was lower than that from nondysbiotic mice with increased thickness of the pellucid zone.¹²⁰ Fecal transplantation from women with ovarian disorders to mice leads to ovarian dysfunction and impaired fertility,⁹⁴ and treating PCOS rats with *Lactobacillus* and fecal microbiota from healthy rats leads to recovery of the estrous cycle, with decreasing androgen biosynthesis and normal ovarian morphologies.¹²¹ Obesity-dependent gut dysbiosis is a contributing factor to the development of ovarian dysfunction associated with metabolic syndrome.¹²² Obesity negatively impacts ovarian function and oocyte quality, and bariatric surgery in obese women can help to normalize ovulatory patterns, improve conception rates and fetal health, reduce pregnancy complications, and improve metabolic function. All of these improvements may be due to changes in the composition of the gut microbiome.^{76,123} The levels of oocyte-specific marker transcripts and proinflammatory signals are significantly higher in the ovaries of obese mice than in the ovaries of lean mice. In addition, there is a positive correlation between the relative ovarian abundance of oocyte-specific transcripts and the cecum microbiota composition in obese mice, indicating that the gut microbiome in obese mice may contribute to reduced oocyte quality.¹²⁴ The microbiome in follicular fluid influences the IVF outcome, and the presence of some microorganisms, especially *Lactobacillus spp.* is associated with better embryo quality and higher rates of embryo transfer and pregnancy.¹²⁵ A recent report demonstrated

that the levels of trimethylamine N-oxide (TMAO) and its gut-derived precursor gamma-butyrobetaine are lower in follicular fluid from normally fertilized oocytes that develop into top-quality embryos than in those that do not. These results indicate that the microbiota may play an important role in embryo development and that microbiota-dependent metabolites can be significant predictive biomarkers in the future.¹²⁶

Overall, the gut microbiota is closely related to female reproductive tract health, and the impetus of these studies in the future will be to identify targets that can be used to therapeutically improve the fertility of women with reproductive endocrine disorders.

Changes in the microbiota and host hormone levels during pregnancy

The composition of the gut microbiota shifts among the different stages of pregnancy, particularly in the third trimester, and these changes lead to differences in metabolic, immunological and hormonal changes, which are necessary and highly beneficial to support a healthy pregnancy and fetal development.¹²⁷ In turn, dramatic shifts in hormone levels, such as those of estrogen and progesterone, also impact gut function and bacterial composition, accompanied by unique inflammatory and immune changes. For example, problems associated with infection by *Listeria monocytogenes* in pregnancy are partly due to elevated estrogen and progesterone levels, leading to adverse outcomes, including preterm delivery or stillbirth.¹²⁸ Progesterone, the principal gestational hormone, affects the contents of several bacterial species, such as the relative abundance of the *Bifidobacterium* genus, which is increased; progesterone facilitates the health of the pregnant mother and is perhaps also helpful to the transmission of beneficial species to the neonate.¹²⁹

The microbial diversity in the gut at the start of pregnancy appears to be similar to that in nonpregnant women, and the abundance of gut bacteria associated with inflammatory states increases as pregnancy advances.¹³⁰ A high abundance of the family *Ruminococcaceae* in early pregnancy may be related to adverse metabolic health, and a high abundance of the families *Lachnospiraceae*, *Prevotellaceae*, and *Bacteroidaceae* is associated

with maternal energy metabolism.¹³¹ A high abundance of species in the *Coriobacteriaceae* family and *Collinsella* genus may serve as markers of impaired glucose metabolism.¹³¹ During the third trimester, the abundances of *Proteobacteria* and *Actinobacteria*, especially the *Enterobacteriaceae* family and *Streptococcus* genus, increase substantially, and the gut microbiota may induce greater adiposity and insulin resistance in the mother as they promote energy storage and provide for the growth of fetal metabolism. In addition, compared to the first trimester, the third trimester has elevated low-grade inflammation and reduced oral glucose tolerance, and these disorders can be transferred to GF mice.¹²⁷ The relative abundances of the *Bacteroides* and *Staphylococcus* genera are higher in overweight pregnant women during gestation. The two genera are also associated with enhanced accretion of white adipose tissue in pregnant women and the fetus, indicating that the gut microbiota may regulate maternal and fetal whole-body energy metabolism.^{132,133}

The gut microbiome may regulate the stability of maternal metabolism by influencing SCFAs, inflammatory cytokines, bile acid metabolism, and metabolic hormones, leading to the occurrence of diabetes and obesity regardless of pregnancy.¹³⁴ The metabolites of the gut microbiome, including bile acids and SCFAs, have a distinctive position among maternal microbial metabolites that affect the fetus because they can penetrate the placental barrier and are critical to ensuring organ function for development, growth, and immunity.¹³⁵

The metabolic hormone levels (insulin, C-peptide, glucagon, incretin, and adipokine), and microbiome profiles of women who are overweight or obese differ, indicating that metabolic hormones participate in the process in which gut microbiota influences metabolic health during early pregnancy in women who were overweight or obese.¹³¹ Indeed, the gut microbiota plays an important role in the development of gestational diabetes mellitus (GDM), and the differences in the microbiota between women with GDM and healthy pregnant women are correlated with blood glucose levels;¹³⁶ pregnant women with GDM have gut microbiota dysbiosis, and the gut plays an important role in the development of GDM.¹³⁴

Progesterone and estrogen levels rise dramatically during pregnancy, with significant immunity-related changes, and these are likely to affect the microbiota. Elevated concentrations of progesterone during pregnancy can inhibit the development of Th1 responses and the production of proinflammatory cytokines, such as IFN- γ , and promote Th2 immune responses, including the synthesis of anti-inflammatory cytokines such as IL-4, IL-5, and IL-10.¹³⁷ On the other hand, the maternal microbiota have been shown to shape the offspring's immune system in terms of immunity-related gene expression and the numbers of cells involved in innate immunity, and it may be of great importance for preventing allergic disease in the offspring.¹³⁸

Due to the changed hormone levels, the vaginal microbiome is also changed significantly during pregnancy, with decreased overall diversity, increased stability, and increased abundance of the *Lactobacillus* species, but these changes occur mainly in early pregnancy; the communities at the later stages resemble those of the nonpregnant state.¹³⁹ Some vaginal communities in early pregnancy stages are associated with preterm birth.¹⁴⁰

Obesity and hyperglycemia during pregnancy can affect the risk of adverse pregnancy outcomes and the establishment of early gut microbiota in infants, thus affecting the long-term health of mothers and offspring. The most recent belief is that the maternal microbiota shapes the phenotypes of offspring. The infant gut microbiome may be influenced by the maternal microbiome through vertical transfer of maternal microbes to infants during vaginal delivery and breastfeeding.¹⁴¹ The maternal diet dramatically alters the intestinal microbiome of mothers and their offspring at one year of age, and a HFD results in significant variation in intestinal taxa among dams with metabolic disorders in a primate study.¹⁴² Compared to control offspring, offspring from GF mothers are highly susceptible to metabolic syndrome characterized by an exacerbation of obesity and glucose intolerance.¹¹⁷ The colonization of microbes in mothers is of great importance to babies during early life and plays a critical role in the establishment and maturation of developmental pathways.¹⁴³ In a mouse model, maternal microbial metabolites drive the development of the pups' innate immune system and maturation of the intestinal epithelium.¹⁴⁴

In short, pregnancy is an important and modifying physiological period for women of reproductive age, and the gut microbiome exhibits significant changes during pregnancy. Reconstruction of the gut may change the states of the mother's endocrine, metabolic, and immune systems, which are necessary for a successful pregnancy (**Figure 2**).

Reproductive endocrine disorders related to gut microbiota

PCOS

PCOS is one of the most common reproductive endocrine and metabolic disorders and is defined as a combination of signs and symptoms of excess androgens (hirsutism and/or hyperandrogenaemia) and ovarian dysfunction (anovulation, oligo-ovulation and/or polycystic ovarian morphology). The prevalence of PCOS among women of reproductive age ranges from 6% to 20%.⁷⁸ Patients with PCOS usually have insulin resistance, obesity, low-grade inflammation, and cardiovascular risk factors.⁷⁸ Due to the complex etiology and its heterogeneous nature, the cause of PCOS is unknown, but studies suggest that PCOS might be a complex multi-genic disorder with strong epigenetic and environmental influences, including diet and other lifestyle issues.^{22,145} Recent studies have demonstrated that gut microbes play an important role in the etiology of PCOS. In 2012, Pearce *et al.* proposed an assumption that disturbances in bowel bacterial flora ("Dysbiosis of Gut Microbiota") brought about by a poor diet create an increase in gut mucosal permeability, releasing LPS into the systemic circulation, activating the immune system and driving up serum insulin levels, leading to increased androgen production in the ovary and interference with normal follicular development.¹²² In recent years, many papers clarifying the relationship between the gut microbiota and PCOS have been published and have shed light on the pathogenesis and therapy of this disease. Our previous study showed that the beta diversity of microbiomes in women with PCOS was significantly decreased compared with that in healthy controls, and *B. vulgatus* was markedly increased in PCOS patients. Transplantation of fecal microbiota from women with PCOS or *B. vulgatus*-colonized recipient mice resulted in PCOS-like mice, accompanied

by decreased IL-22 and GDCA, indicating that the gut microbiota–bile acid–interleukin-22 axis regulates PCOS.⁹⁴ Another study showed that PCOS mice had decreased abundances of gut microbiome species and decreased phylogenetic diversity and that *Firmicutes* levels were significantly higher in PCOS mice than in control mice.¹⁴⁶ The abundance of the *Tenericutes* phylum in women with PCOS was significantly lower than that in healthy women.^{147,148} In addition, there was a decrease in *Akkermensia* and *Ruminococcaceae*, and gram-negative bacteria belonging to the *Bacteroides* and *Escherichia/Shigella* genera were significantly increased in PCOS women, especially women who were obese. Under this condition, LPS produced by gram-negative bacteria was demonstrated to induce chronic inflammation, obesity, and insulin resistance in LPS-infused mice. Compared with controls, PCOS patients have significant decreases in the serotonin, ghrelin, and PYY levels, indicating that the mediators of the brain-gut axis are associated with PCOS.¹⁴⁹ A recent study showed that compared with that of BMI-matched normal adolescents, the gut microbiome of obese adolescents with PCOS is altered and that these alterations include decreased alpha diversity, which is strongly associated with higher testosterone concentrations.¹⁵⁰

Treating PCOS rats with the *Lactobacillus* genus and FMT from healthy rats can improve estrous cycles and ovarian morphologies and decrease androgen biosynthesis, indicating that improving the structure of the intestinal flora can relieve the symptoms of PCOS.¹²¹ In two randomized, double-blind, placebo-controlled trials, probiotic supplementation for women with PCOS had favorable effects on weight loss, insulin resistance, triglycerides and VLDL cholesterol concentration,^{151,152} and probiotics also helped improve total testosterone, SHBG and proinflammatory cytokine production.¹⁵² Another clinical trial found that coadministration of probiotics and selenium had beneficial effects on mental health parameters, serum total testosterone levels, hirsutism, and biomarkers of inflammation and oxidative stress, such as hs-CRP, the total antioxidant capacity, and total glutathione, in women with PCOS, indicating that probiotics can modulate inflammation in women with PCOS.¹⁵³

Metformin is one of the most effective drugs used in the treatment of type 2 diabetes and insulin resistance and has been shown to improve fertility outcomes among females with insulin resistance associated with PCOS. It can help to reinstate menstrual cyclicity, improve hyperandrogenism and ovulation, decrease the incidence of cesarean sections, and limit the number of premature births.^{154,155} However, the exact mechanism by which metformin helps to improve PCOS remains unclear, though the gut microbiome may play an important role. In a recent study, inflammatory indicators from the plasma and ovary, including TNF- α , IL-6, and IL-17A, were decreased in a metformin-treated PCOS mouse model compared with the control group, and the *Helicobacter* genus was decreased in the metformin-treated group. Metformin can alleviate PCOS via its anti-inflammation properties and modulation of the gut microbiota, which may contribute to potential clinical therapy for the disease.¹⁵⁶

This offers a new therapeutic strategy for the clinical diagnosis and treatment of PCOS.

Endometriosis

Endometriosis is a condition defined by the presence of endometrial glands and stroma outside of the uterine cavity; in this condition, dysbiosis influences estrogen levels in circulation, and increased estrogen levels can stimulate the growth of ectopic endometriotic foci and inflammatory activity.¹⁵⁷ Endometriosis is considered a typical multifactorial condition that may be determined by genetic, immunological, and environmental factors. Because gut flora affects estrogen metabolism, inflammation and homeostasis, recent studies have shown that gut microbiota may be closely involved in the onset and progression of endometriosis. Because the microbiota is involved in the regulation of estrogen cycling, gut dysbiosis increases the levels of circulating estrogen, which may markedly stimulate the growth and cyclic bleeding of endometriotic lesions. On the other hand, a number of immune cell subtypes and inflammatory factors are altered in endometriosis, as are inflammatory cytokine levels in the peritoneal fluid and serum, which are increased, reflecting dysregulation and alterations in the gut microbiome and gut permeability.¹⁵⁸ The gut microbiota plays an important role in the systemic inflammatory response and activation of immune

cells, and imbalances in the intestinal flora are thought to play a role in the pathogenesis of endometriosis. Once the balance between estrogen levels in circulation and the gut microbiome is disrupted, increased estrogen exposure can stimulate the development and progression of endometriosis.¹⁵⁹ There is a 50% increase in the risk of inflammatory bowel disease in women with endometriosis, indicating a strong interaction between immunological processes in the gut and endometriotic lesions.¹⁶⁰ In a murine model of endometriosis, the *Firmicutes/Bacteroidetes* ratio was elevated, and *Bifidobacterium* was also increased.¹⁶¹ In another study, endometriotic lesions and inflammatory responses in mice treated with broad-spectrum antibiotics were significantly reduced compared to those in control mice. Oral gavage of feces from mice with endometriosis restored endometriotic lesion growth and inflammation, suggesting that gut bacteria promote endometriosis progression in mice.¹⁶² Endometriosis is associated with lower *Lactobacilli* concentrations and more gram-negative bacteria as well as a higher prevalence of intestinal inflammation in rhesus monkeys, while the mechanisms linking these effects remain unclear.¹⁶³ A recent study showed that the vaginal, cervical and gut microbiota compositions were similar between women with stage 3–4 endometriosis and controls, but patients with endometriosis had more *Escherichia/Shigella* in their stool.¹⁵⁹

Taken together, these observations indicate there may be a direct link between pathological changes in the gut microbiota and the onset and progression of endometriosis. Thus, there is an urgent need to characterize the complex pathophysiological condition of endometriosis, to establish the basis for the development of novel diagnostic and therapeutic strategies.

Female genital tract diseases

The vaginal microbiota typically changes throughout the menstrual cycle, and microbiota stability is associated with the estradiol peak at ovulation and the progesterone rise in the midluteal phase. However, alterations in the vaginal microbiota can lead to several pathologies. BV is a dysbiosis of the vaginal microbiome that has a close relationship with significant adverse healthcare outcomes, including an increased risk of abnormal pregnancy outcomes, pelvic inflammatory disease, and increased susceptibility to sexually

transmitted infections.¹⁶⁴ BV is characterized by low levels of “healthy” *Lactobacilli* and overgrowth of numerous bacteria, including *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella*, *Bacteroides*, *Sneathia*, *Leptotrichia*, and members of the Clostridia class, among others.^{165,166}

The most prevalent vaginal *Lactobacillus* species have been shown to colonize the rectum, and cocolonization of the vagina and rectum correlates with the lowest prevalence of BV.¹⁶⁷ The rectum is a key reservoir for vaginal lactobacilli, and rectal colonization with these microorganisms contributes to the maintenance of health-associated vaginal microbiota; furthermore, gut microbiota can indirectly influence genital microbiota through the estrobolome.¹⁶⁸ A meta-analysis that included 12 studies comprising a total of 2980 patients showed that tubal factor infertility was significantly more prevalent in IVF patients diagnosed with BV than in patients without BV and that BV was significantly associated with early spontaneous abortion in IVF patients.¹⁶⁹ The promotion of *Lactobacillus* colonization with probiotics, modulation of vaginal pH, hormonal administration, and eradication of pathogenic bacteria with antibiotics could alter the cervicovaginal microbiota and have beneficial effects on women with BV.¹⁷⁰

There is strong evidence that intestinal-vaginal interactions play a key role in BV. The challenge is to find tools and strategies capable of modulating microbial populations in the cervicovaginal microenvironment; understanding the connection between intestinal and vaginal microbiota may be a goal for new treatments of female genital tract disorders.

Conclusion and future perspectives

In conclusion, the unique dynamic ecosystem of the gastrointestinal microbiota humans acquire in early life is regarded as a metabolically active “organ”, which is responsible for the health of the individual and development of diseases throughout life. Steroids and metabolic hormones play vital roles in intestinal homeostasis, and dysbiotic microbiota may trigger intestinal barrier dysfunction and affect other organs, leading to chronic low-grade inflammation, metabolic disorders, and immune dysfunction. Together, estrogens, androgens, progesterone, insulin, and other hormones regulate women’s health from the womb to the tomb. Although studies have shown that the gut

microbiota has a vital function in the female reproductive endocrine system, further mechanistic studies are still needed. In the future, extensive and functional studies will make it possible to use the gut microbiota as a biomarker for certain diseases, and manipulating the gut microbiota in specific and directed ways will serve to move the field forward. By using probiotics or fecal transplantation, we will be able to treat dysbiosis and prevent disease development.

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Disclosure of potential conflicts of interest

The authors declare no conflicts of interest.

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