REVIEW

Female reproductive dysfunctions and the gut microbiota

Sangappa B Chadchan1,2, Vertika Singh1 and Ramakrishna Kommagani3,4,5

1Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas, USA
2Center for Reproductive Health Sciences, Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri, USA
3Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, USA

Correspondence should be addressed to R Kommagani: Rama.Kommagani@bcm.edu

This paper is part of a collection of articles exploring Gut Microbiome and Endocrinology, across the Journal of Endocrinology and the Journal of Molecular Endocrinology. The editor for this section was Dr Jonathon Schertzer.

Abstract

The gut microbiome is considered an endocrine organ that can influence distant organs and associated biological pathways. Recent advances suggest that gut microbial homeostasis is essential for reproductive health and that perturbations in the gut microbiota can lead to reproductive pathologies. This review provides an updated overview of the relationship between the gut microbiome and female reproductive diseases. Specifically, we highlight the most recent findings on the gut microbiome in gynecological pathologies including polycystic ovarian syndrome, endometriosis, and endometrial cancer. Most studies revealed associations between altered gut microbial compositions and these reproductive diseases, though few have suggested cause–effect relationships. Future studies should focus on determining the molecular mechanisms underlying associations between gut microbiota and reproductive diseases. Understanding this bidirectional relationship could lead to the development of novel and effective strategies to prevent, diagnose, and treat female reproductive organ-related diseases.

Introduction

The gut microbiome – comprised of the bacteria, viruses, fungi, and protozoa living commensally, symbiotically, or pathogenically in the digestive tract – has co-evolved with its hosts for millennia. These microbes, combined with their genomic pool, constitute the microbiome and participate in essential host activities such as digestion, immune cell maturation, and detoxification (Liang et al. 2018). Conversely, the perturbations in the microbiome disturb host energy homeostasis leading to the development of several diseases (Chassaing et al. 2012, Nicholson et al. 2012, Liang et al. 2018). Advances in the next-generation sequencing technologies and ‘omics’ tools over the past two decades have allowed comprehensive identification of the gut microbiota composition and the identification of microbial taxa associated with human diseases, including female reproductive tract pathologies (Tremellen & Pearce 2012, Fransasiak & Scott Jr 2015, Green et al. 2015). The functions of the female reproductive tract are regulated by the endocrine system in a well-coordinated manner, which, if disrupted, may lead to several disorders. Considered an extended endocrine organ, the gut microbiome acts as an important regulator of female reproductive health and associated diseases. In this review, we focus on associations and some causal connections between gut microbiota...
and polycystic ovarian syndrome (PCOS), endometriosis, and gynecologic cancers. Additionally, we review current knowledge regarding the use of pre/probiotics to manage some of these conditions.

**The human gut microbiome**

The human body harbors numerous microorganisms that reside in various tissues including the mouth, skin, vagina, and gut. Of these sites, the human gut is particularly enriched in microorganisms than the rest of the body (Quigley 2013). A total of all the microorganisms present inside the gastrointestinal (GI) tract is referred to as ‘gut microbiota’ which constitutes bacteria, fungi, viruses, protozoans, and archaea that co-evolved in an intricate and mutually beneficial relationship with the host (Backhed et al. 2005). The most dominant bacterial phyla in the gut include Firmicutes and Bacteroidetes, which constitute around 90% of the gut microbiome (Arunugam et al. 2011). About 200 genera belong to Firmicutes in the human gut and is dominated by the genus Clostridium (95%). The Bacteroidetes phylum is dominated by Bacteroides and Prevotella genera, while the phylum Actinobacteria is proportionally less abundant and is mainly represented by the Bifidobacterium genus (Rinninella et al. 2019).

The microbial diversities are usually measured in terms of alpha and beta diversities, which represent the ‘within sample’ and ‘between samples’ diversities, respectively. Alpha diversity is the measure of the species richness (number) or evenness (distribution) or both in a sample. Alpha diversity is measured as the ‘Shannon index,’ which is a quantitative measure of both the species abundance and evenness within a sample. On the other hand, beta diversity measures the variability in the microbial composition among different samples. These alpha and beta diversity indices identify broad differences in the microbiome compositions. Alterations in the ratio of this bacterial flora and their load, particularly involving the loss of beneficial microbes, can lead to ‘dysbiosis of the gut microbiota’ resulting in the development of various pathogenic diseases (Elias-Oliveira et al. 2020, Fan et al. 2021, Qi et al. 2021, Singh et al. 2021).

**Gut microbiome–estrogen axis**

The gut microbiome is known to influence the hormone levels in the host including estrogen levels in females (Flores et al. 2012). This functional link between gut microbiota and estrogen was first noted three decades ago when Adlercreutz et al. found that antibiotic supplementation decreased estrogen levels in women (Adlercreutz et al. 1984). The gut microbiota principally regulates the estrogen level by secretion of β-glucuronidase (gmGUS), an enzyme that converts conjugated estrogen into deconjugated estrogen in the GI tract facilitating it to bind to estrogen receptors, resulting in subsequent signaling and physiological downstream effects (Fig. 1). A decreased β-glucuronidase activity as a result of gut microbial dysbiosis can result in reduced deconjugation of estrogen and a decrease in the level of circulating estrogens (Flores et al. 2012). This further alters the activation of estrogen receptors leading to pathologies such as obesity and cardiovascular diseases. On the other hand, increased β-glucuronidase activity can result in elevated estrogens levels leading to pathologies, such as endometriosis and cancer (Plottel & Blaser 2011). Thus, optimal gmGUS activity is essential for maintaining estrogen levels in females.

Another mechanism by which the gut microbiome might influence sex-steroid hormone levels in females is by producing short-chain fatty acids (SCFAs). SCFAs are the primary by-products of bacterial anaerobic fermentation of dietary fibers in the intestine. Acetate (C2), propionate (C3), and butyrate (C4) are the most abundant SCFAs that are produced by gut microbes. Importantly, butyrate has been shown to regulate the synthesis of P4 (progesterone) and E2 (estradiol) in porcine granulosa cells (PGCs) via the cAMP signaling pathway (Lu et al. 2017). In this interesting in vitro study, the PGCs were treated with lower concentrations of butyric acid stimulate the progesterone secretion; however, higher butyrate concentrations significantly inhibited the progesterone secretion (Lu et al. 2017). Another study by Liu et al. showed that gut-derived butyrate contributes to nonalcoholic fatty liver disease in pre-menopause women due to estrogen deficiency (Liu et al. 2022). These studies highlight the plausible mechanism by which the dietary constituents and microbiota-derived metabolites may contribute to the regulation of estrogen and progesterone level in females; however, the underlying molecular mechanism is not clear yet. Nonetheless, the significance of gut microbiota in female reproductive pathologies is now well established, including PCOS, endometriosis, and other reproductive tract conditions.

**Gut microbiota and PCOS**

PCOS is a heterogeneous endocrine, neuroendocrine, and metabolic disorder that leads to difficult pregnancies or infertility (Tu et al. 2020, Wei et al. 2021) in 6.5–8.0% of reproductive-age women. The main characteristics of PCOS are hyperandrogenism, oligo/amenorrhea, and
polycystic ovarian morphology (Lindheim et al. 2016). Although genetic, lifestyle, and intrauterine factors have been suggested, the general etiology of PCOS is unclear. However, the gut microbiome contributes to several additional characteristics of PCOS such as obesity, insulin resistance, and low-grade inflammation (Lindheim et al. 2016, He & Li 2020, Lüll et al. 2021, Rizk & Thackray 2021).

Gut microbiome in PCOS patients

Several investigators have compared the gut microbial compositions in stool samples from people with and without PCOS. Lull et al. identified four genera that differed between 102 patients with PCOS (n=102) and 201 age- and BMI-matched healthy controls (n=201). The abundance of two genera from Clostridiales, Ruminococcaceae UCG-002, and Clostridiales Family XIII AD301 were correlated with several PCOS-related markers such as cystic ovarian morphology and higher testosterone levels. Moreover, patients with PCOS and pre-diabetes had significantly lower alpha diversity (Shannon index) and higher abundance of the Dorea and Bacteroides (Ruminococcus torques group and Lachnospiraceae UCG-004) genera than PCOS patients with normal glucose tolerance (Lüll et al. 2021). Liang et al. reported that gamma-aminobutyric acid-producing bacteria, including Parabacteroides distasonis and Bacteroides fragilis, were increased in PCOS patients, whereas Escherichia coli showed a positive relationship with serum luteinizing hormone (LH) levels and LH: follicle-stimulating hormone (FSH) ratios (Liang et al. 2021). As the higher level of LH is associated with PCOS condition (Zarei et al. 2021). Lindheim et al. found that the stool microbiome of PCOS patients (n=25) showed lower diversity and different phylogenetic composition than that of healthy controls (n=25). The authors did not observe any significant differences in any taxa with a relative abundance >1%. However, when assessing rare taxa, the relative abundances of bacteria from the phylum Tenericutes, specifically the order ML615J-28, and the family S24-7 (phylum Bacteroidetes) were significantly lower and associated with reproductive parameters in PCOS patients. Additionally, PCOS patients showed alterations in some, but not all, markers of gut barrier function and endotoxemia (Lindheim et al. 2017). Finally, Qi et al. examined 43 healthy controls (n=43) and 50 PCOS patients (n=50) and found that Bacteroides vulgatus was elevated in the gut microbiota of individuals with PCOS (Qi et al. 2019). B. vulgatus are gram-negative anaerobic bacteria inhabiting

Figure 1
Schematic representation of the modulation of uterine functions through the estrogen-gut microbiome axis. The secretion of β-glucuronidase by gut bacteria converts conjugated estrogen into deconjugated estrogen in the GI tract. The deconjugated estrogen is reabsorbed by the gut and translocated into the bloodstream, facilitating estrogen entry into the uterus, wherein estrogen exerts its downstream action.
the distal human gut and are typically non-pathogenic in healthy individuals (Takaishi et al. 2008). These bacteria deconjugate the bile acids synthesized in the liver, such as glycodeoxycholic acid and tauroursodeoxycholic acid (Qi et al. 2019). Qi et al. reported a negative correlation between the abundances of B. vulgatus and glycodeoxycholic acid and tauroursodeoxycholic acid in PCOS patients. Collectively, these studies revealed that PCOS is associated with alterations in the gut microbiome, but no cause–effect relationships have been determined.

While most investigators have examined the microbiota in stool, only a few have examined the bacterial composition in saliva. For example, one study of the salivary microbiome revealed that PCOS patients (n=24) had fewer bacteria from the phylum Actinobacteria than healthy controls (n=20). PCOS patients also exhibited a borderline significant shift in bacterial community composition in unweighted UniFrac analysis (Lindheim et al. 2016). UniFrac, one of the distance metrics used to measure beta diversity, collects phylogenetic information to compare microbial communities in different samples (Lozupone et al. 2006). It measures the evolutionary distance among sets of taxa in a phylogenetic tree as a fraction of the branch length of the tree (Lozupone & Knight 2005). An unweighted UniFrac analysis can be used to ascertain the incidence of variation among the samples; however, weighted UniFrac can additionally quantify the variation occurring in different lineages (Ito et al. 2019).

The alpha diversity and weighted UniFrac analysis were unchanged between PCOS patients and controls. No differences were observed at any taxonomic level (Lindheim et al. 2016). The authors also noted altered gut microbiota in stool samples, though the findings were not identical. One group used 16S rRNA sequencing to examine fecal microbial diversity profiles of healthy women (n=48), women with polycystic ovarian morphology (PCOM) (n=42), and women diagnosed with PCOS by the Rotterdam criteria (n=73). Patients with PCOS had lower microbial diversity than healthy controls and those with PCOM had an alpha diversity that was intermediate between that of healthy control and PCOS groups (Torres et al. 2018). Regression analysis showed that hyperandrogenism, total testosterone, and hirsutism were negatively correlated with alpha diversity (Torres et al. 2018).

Several investigators have examined associations between fecal bacteria (gut microbiota) and PCOS in patients with and without obesity. For example, Liu et al. examined the gut microbiome in 33 patients with PCOS (12 non-obese and 21 obese) and 15 healthy controls (9 non-obese and 6 obese) and found that the co-abundance groups (CAGs) increased in the PCOS patients. CAGs are the clustering of bacterial species based on the SparCC (Sparse Correlations for Compositional data) correlation coefficients of their relative abundance (Liu et al. 2017). SparCC is a mathematical approach to estimating correlation values from compositional data (Friedman & Alm 2012). Additionally, abundances of Bacteroides, Escherichia/Shigella, and Streptococcus were negatively correlated with Ghrelin expression and positively correlated with testosterone and BMI (Liu et al. 2017). Therefore, the downregulation of Ghrelin is associated with PCOS (Ibrahim & Alobaidi 2021). Liang et al. analyzed data from 20 patients with PCOS (10 lean and 10 overweight) and 20 healthy controls (10 lean and 10 overweight) and reported that the intake of dietary fiber and vitamin D was significantly decreased in the PCOS group (Kim et al. 2021). Dietary fiber plays a crucial role in the composition of the gut microbiota where it acts as a prebiotic to support beneficial gut bacteria (probiotics) and suppresses harmful bacteria (Kim et al. 2021). Future studies should further investigate the link between diet, PCOS, and the gut microbiome. Table 1A summarizes the list of studies that highlighted the gut microbiome changes in patients with PCOS.

Two studies have investigated the effects of treatments on the fecal microbiome of patients with PCOS. First, when non-diabetic PCOS patients with human immunodeficiency virus (HIV) taking antiretroviral therapy (n=23) were treated with metformin, the abundance of gut anti-inflammatory bacteria such as butyrate-producing species and the protective Akkermansia muciniphila increased in the gut (Ouyang et al. 2020). By producing SCFAs, they protect the gut epithelial barrier and reduce inflammation levels in patients with HIV receiving antiretroviral therapy. Secondly, Garcia-Beltran et al. examined the gut microbiota composition of non-obese females with PCOS (age 15.8 years; BMI 25 kg/m²) who were randomized to receive treatment with either an oral contraceptive (n=15) or with spironolactone–pioglitazone–metformin (n=15). The authors reported that adolescent girls with PCOS had decreased alpha diversity, an altered microbiota pattern, and a taxonomic profile with more abundant Family XI and less abundant family Prevotellaceae, genus Prevotella, and genus Senegalimassilia than in healthy controls. Additionally, Family XI abundance showed a positive relationship to hepato-visceral fat (Garcia-Beltran et al. 2021). Treatment with spironolactone–pioglitazone–metformin treatment, but not with oral contraceptives, normalized the abundance of Family XI. Prevotellaceae, Prevotella, and Senegalimassilia
Table 1  The main findings of the human and rodent studies in association with gut microbial alterations in (A) PCOS, (B) endometriosis, and (C) cervical and ovarian cancer.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Strain</th>
<th>Main findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS</td>
<td></td>
<td>↑ Actinobacteria and ↓ Bacteroidetes in PCOS women</td>
<td>Jobira et al. (2020)</td>
</tr>
<tr>
<td>PCOS</td>
<td></td>
<td>↑ Clostridiales, Ruminococaceae UCG-002 and Clostridiales Family XII AD3011 in PCOS group</td>
<td>Lüll et al. (2021)</td>
</tr>
<tr>
<td>PCOS</td>
<td></td>
<td>↓ Alpha diversity, altered microbiota patterns in girls with PCOS</td>
<td>Garcia-Beltran et al. (2021)</td>
</tr>
<tr>
<td>PCOS</td>
<td></td>
<td>↑ Parabacteroides distasonis and Bacteroides fragilis in PCOS patients</td>
<td>Liang et al. (2021)</td>
</tr>
<tr>
<td>PCOS</td>
<td></td>
<td>↓ Diversity and a dissimilar phylogenetic composition in PCOS group</td>
<td>Lindheim et al. (2017)</td>
</tr>
<tr>
<td>Rodent studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS</td>
<td>Sprague-Dawley rats</td>
<td>↓ Lactobacillus, Ruminococcus, and Clostridium and ↑ Prevotella in PCOS rats</td>
<td>Guo et al. (2016)</td>
</tr>
<tr>
<td>PCOS</td>
<td>C57BL/6N mice</td>
<td>↓ Alpha diversity after 5 weeks of letrozole treatment in letrozole-treated mice</td>
<td>Kelley et al. (2016)</td>
</tr>
<tr>
<td><strong>Human studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometriosis</td>
<td></td>
<td>↑ Escherichia and Shigella in the endometriosis group</td>
<td>Ata et al. (2019)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td></td>
<td>No differences in the gut microbiomes</td>
<td>Perrotta et al. (2020)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td></td>
<td>↑ Bacteroides and Clostridio in endometriosis patients</td>
<td>Svensson et al. (2021)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td></td>
<td>Low alpha diversity and high Firmicutes-to-Bacteroidetes ratio in endometriosis group</td>
<td>Shan et al. (2021)</td>
</tr>
<tr>
<td>Rodent studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometriosis</td>
<td>C57BL/6N mice</td>
<td>Low alpha and beta diversity in mice with endometriosis</td>
<td>Ni et al. (2020)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>C57BL/6N mice</td>
<td>No difference in the diversity</td>
<td>Hanschel et al. (2019)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>C57BL/6N mice</td>
<td>↑ Firmicutes-to-Bacteroidetes ratio and ↑ Bifidobacterium in endometriosis group</td>
<td>Yuan et al. (2018)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>C57BL/6N mice</td>
<td>↑ Bacteroides and few Firmicutes in the endometriosis group</td>
<td>Chhadan et al. (2019)</td>
</tr>
<tr>
<td><strong>Human studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td></td>
<td>↑ Abundance of Prevotella in early cervical cancer patients</td>
<td>Kang et al. (2020)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td></td>
<td>↑ Abundance of Prevotella, Porphyromonas, and Dialister and ↓ abundance of Acterooides, Alstipes, and Lachnospiraceae in cervical cancer patients</td>
<td>Sims et al. (2019)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td></td>
<td>↑ Bacteroides and ↓ Firmicites in cancer patients</td>
<td>Wang et al. (2019)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td>↓ Phylogenetic diversity in patients with the primary platinum-resistant disease</td>
<td>Jacobson et al. (2021)</td>
</tr>
</tbody>
</table>

abundance remained unchanged after either treatment (Garcia-Beltran et al. 2021). More work is needed to determine whether these microbiome changes reflect the direct effects of the treatments or the resolution of PCOS.

**Gut microbiota in rodent models of PCOS**

To address whether associations between altered gut bacteria and PCOS in humans reflect cause/effect relationships, several researchers have turned to rodent models of this disease. The most common methods of inducing PCOS in mice and rats are to treat them with Letrozole or DHEA. The abundances of *Lactobacillus*, *Ruminococcus*, and *Clostridium* were lower and *Prevotella* was higher in rats with letrozole-induced PCOS than in control rats (Guo et al. 2016). Similar results were observed in the letrozole-induced PCOS mouse model (Kelley et al. 2016). However, Guo et al. took their work one step further by transplanting fecal microbiota from healthy rats into rats with PCOS. This treatment improved the estrous cycles in all of the rats (n=8), and the ovarian functions were normalized. Interestingly, transfer of just *Lactobacillus* improved the estrous cycle in 75% of the rats, suggesting a single genus might have a profound impact on the estrous cycle in PCOS patients. In both cases, estrous cycle improvements were coupled...
with decreasing androgen biosynthesis and normalized ovarian morphology. Moreover, the composition of the gut microbiota was restored in both of the treated groups, with levels of Lactobacillus and Clostridium increasing and Prevotella decreasing. These results indicate that dysbiosis of the gut microbiota contributes to the pathogenesis of PCOS in rats (Guo et al. 2016), highlighting the need for investigations into probiotic-based treatment strategies for women with PCOS. The data also highlighted the significance of fecal microbiota transfer (FMT) and Lactobacillus transplantation for the treatments of PCOS.

In a different approach, Qi et al. transplanted feces from women with PCOS to B. vulgatus-colonized recipient mice and noted increased disruption of ovarian functions, insulin resistance, altered bile acid metabolism, reduced interleukin-22 secretion, and infertility (Qi et al. 2019). These features of PCOS were reversed when the mice were treated with glycodeoxycholic acid, which induced intestinal group 3 innate lymphoid cell IL-22 secretion through GATA-binding protein 3 (Qi et al. 2019). Whether a similar treatment would be effective in humans has not been determined. The summary of the rodent studies is presented in Table 1A.

### Gut microbiota and endometriosis

#### Gut microbiota and endometriosis in humans

Endometriosis is a disease in which cells from the epithelial lining of the uterus (the endometrium) implant and proliferate on peritoneal surfaces in the abdomen. Endometriosis affects approximately 196 million people worldwide. Nearly half of these patients experience chronic pelvic pain, and 30–50% are infertile (Missmer et al. 2004, Smoliarz et al. 2021). Currently available treatments such as hormonal therapy and surgical excision have adverse side effects and do not prevent recurrences (Abbott et al. 2003). Endometriosis is thought to be caused by retrograde menstruation. However, whereas 90% of menstruating people experience retrograde menstruation, only 10% develop endometriosis (Mehedintu et al. 2014). The American Society for Reproductive Medicine and American Fertility Society have classified endometriosis as stage I–IV depending on the size, number of lesions, presence of adhesions, and ovarian vs peritoneal involvement. Presence of the ovarian cysts and adhesions are assigned with higher stages but they do not correlate with the severity of the pain (Zondervan et al. 2016). This has led many researchers to explore other factors that may contribute to the development of this disease.

Recent evidence suggests that endometriosis is associated with gut microbial dysbiosis, though the data are far from consistent across studies. For example, in one study, patients with stage three or stage four (moderate to severe) endometriosis (n=14) were more likely to have abundant Shigella/Escherichia in their colonic microbiota than healthy participants (n=14) (Ata et al. 2019). In contrast, another study identified no differences between the gut microbiomes of patients with endometriosis (n=35) and healthy controls (n=24) during the proliferative and secretory phases of the menstrual cycle (Perrotta et al. 2020). However, this study did not specify the stages of endometriosis. A larger study conducted on human stool samples suggested that both alpha (the microbial diversity of a single sample) and beta (a measure of similarity or dissimilarity between two communities) diversities, as well as the Firmicutes-to-Bacteroidetes ratio (Svensson et al. 2021), were higher in the stool samples of controls (n=198) than in patients with endometriosis (n=66) (Svensson et al. 2021). This study appears to contradict another study in which those with stage three or stage four endometriosis (n=12) had lower alpha diversity of gut microbiota and a higher Firmicutes-to-Bacteroidetes ratio than healthy controls (Shan et al. 2021). In the largest study to date, Svensson et al. reported the abundance of 12 bacteria belonging to the classes Bacilli, Bacteroidia, Clostridia, Coriobacteriia, and gammaproteobacteria that differed significantly between stool samples from endometriosis patients (n=66) and those from matched healthy controls (n=198) (Svensson et al. 2021). Two bacteria from class Bacteroidia (Bacteroides and Parabacteroides) and two belonging to class Clostridia (Oscillospira and Coprococcus) were present in higher abundances in endometriosis patients, whereas two bacterial species from the Bacteroidia (Paraprevotella and one unidentified) and Clostridia (Lachnospira and one unidentified) classes were at lower abundances in the stool samples of endometriosis patients. The variability of findings across these studies points to the need for larger studies in which potential confounders (e.g. stage of endometriosis, age, race/ethnicity, other health conditions, medication, and diet) are assessed. Moreover, to date, no causal relationships between gut microbiota and endometriosis have been established in humans. Table 1B summarizes the studies that analyzed the gut microbiome changes in patients with endometriosis.

#### Gut microbiota and endometriosis in rodents

To address some of the limitations of human experiments, some researchers have turned to rodent models of...
endometriosis. In these models, autologous or donor uterine tissue is injected into the peritoneal space or sutured to a peritoneal surface, resulting in endometriotic lesions that grow to up to 50 mm² within 21 days (Chadchan et al. 2019, 2021).

As with human studies, the rodent studies of the gut microbiome thus far have reached somewhat divergent conclusions. For example, Ni et al. examined mouse fecal samples and found that the diversity (alpha and beta) and abundance of the gut microbiota were lower in mice with endometriosis than in mice without endometriosis (Ni et al. 2020). In contrast, another study conducted on feces reported no difference in the alpha and beta diversity between mice with and without endometriosis (Hantschel et al. 2019). Additionally, in another study, dysbiosis of the gut microbiota was observed 42 days after endometriosis induction, with an elevated Firmicutes-to-Bacteroidetes ratio and elevated abundance of *Bifidobacterium*, a commonly used probiotic (Yuan et al. 2018). This elevated Firmicutes-to-Bacteroidetes ratio was similar to the finding of (Shan et al. 2021). In a study of rats, those with endometriosis had an elevated Firmicutes-to-Bacteroidetes ratio and decreased abundance of *Ruminococcaceae* in stool samples (Cao et al. 2020). In contrast, we showed that the feces of mice with endometriosis had more Bacteroidetes and fewer Firmicutes than mice without endometriosis. Additionally, we reported that mice treated with antibiotics had reduced Firmicutes and Bacteroidetes in their feces and developed significantly smaller endometriotic lesions than vehicle-treated mice (Chadchan et al. 2019). The summary of the rodent studies is presented in Table 1B.

One mechanism by which mammalian gut bacteria affect host physiology is by processing otherwise indigestible nutrients into biologically active metabolites (Forbes et al. 2016, Kho & Lal 2018, Li et al. 2018), including SCFAs. Ni et al. conducted an advanced metabolomics analysis revealing higher abundances of chenodeoxycholic and ursodeoxycholic acids and lower abundances of alpha-linolenic acid and 12,13s-epoxy-9z,11,15z-octadecatrienoic acid in feces of mice with endometriosis than in feces of mice without endometriosis (Ni et al. 2021). Moreover, they noted that exogenous supplementation of alpha-linolenic acid restored the abundance of Firmicutes and Bacteroidetes, improved the intestinal wall barrier, reduced abdominal inflammation, and reduced the abundance of lipopolysaccharide in mice with endometriosis (Ni et al. 2021).

Although the aforementioned studies identified the altered gut microbiota in endometriosis, it is not clear whether altered gut microbiota itself affects disease progression. Toward this, our group demonstrated that altered gut microbiota, in fact, drives the endometriotic lesion growth in mice (Chadchan et al. 2021). Moreover, we found that feces derived from mice with endometriosis have fewer SCFAs, specifically n-butyrate, compared to feces from mice without endometriosis. Consistent with this, treatment with n-butyrate attenuated the endometriotic lesion growth in mice and a pre-clinical mouse model. Molecular mechanistic studies found that n-butyrate acts via G-protein-coupled receptors, histone deacetylases (HDACs), and a GTPase-activating protein, RAP1GAP, to inhibit human endometriotic cell survival and lesion growth (Chadchan et al. 2021). Interestingly, multiple reports found HDAC overexpression in endometriotic lesions (Colón-Díaz et al. 2012, Gujral et al. 2020). Importantly, SCFAs are also known to play crucial roles in immune cell regulation by regulation of Treg cell expansion (Blander et al. 2017). This may serve as another possible mechanism driving the development of endometriosis, as it is known that alteration in the Treg cell population promotes inflammation and angiogenesis, facilitating the attachment and growth of endometrial implants (Tanaka et al. 2017). In conclusion, across all the studied species, endometriosis is generally associated with an imbalance in the Firmicutes-to-Bacteroidetes ratio, suggesting that dysbiosis of the gut microbiota is linked with endometriosis pathophysiology. Importantly, supplementation of n-butyrate in the form of analogs or engineered bacteria with n-butyrate overproduction could be used as an effective treatment regime for patients with endometriosis (Chadchan et al. 2021).

Although mouse studies are beginning to suggest mechanisms by which the gut microbiota affects endometriosis, we thus far lack definitive cause/effect relationships between gut microbiota and endometriosis in humans. The relevance of specific gut-derived metabolites in female reproductive health still needs to be determined. This is a promising area of research, as it could lead to simple diet interventions to reduce the burden of endometriosis.

**Gut microbiome in gynecologic cancers**

The most common gynecological cancer is endometrial cancer, affecting 66,570 women in the United States with 12,940 deaths predicted in the year 2021, according to the American Cancer Society. The next most common gynecologic cancer is cervical cancer, which is also the fourth most common female malignancy in the world (Small Jr et al. 2017, Zhang et al. 2020). The third most common gynecologic cancer is ovarian cancer, which
accounts for 2.5% of all female malignancies. However, 80% of patients are diagnosed with advanced-stage ovarian cancer, so the disease accounts for 5% of all cancer deaths (Zhang et al. 2020). Thus far, only a few studies have examined relationships between the gut microbiome and gynecologic cancers. We highlight a few examples below.

In endometrial cancer, estrogen plays an important role (Poppi et al. 2020) in modulating the inflammatory response (Baker et al. 2017). Given that the gut microbiota can metabolize estrogen and thereby alter the circulating estrogen concentration (Baker et al. 2017), gut bacteria could influence the development of endometrial cancer. However, no studies have thus far provided a direct link between gut bacteria and endometrial cancer. In one study, investigators examined the effect of urolithin A, a gut-derived bacterial metabolite from ellagic acid, on the endometrial cancer cell line, Ishikawa. This treatment disrupted Rac1 and Pak1 activity, caused actin depolymerization, and decreased cell migration (Alauddin et al. 2020).

Wang et al. found that the gut microbial composition differed significantly between stool samples of patients with cervical cancer (n=8) and those of healthy controls (n=5). Specifically, cancer patients had more Bacteroidetes and fewer Firmicutes than healthy controls (Wang et al. 2019). Another study revealed that Prevotella, Porphyromonas, and Dialister were significantly more abundant, and Acteroides, Alistipes, and Lachnospiracea were less abundant in the gut microbiomes of cervical cancer patients (n=42) than in those of matched healthy controls (n=46) (Sims et al. 2019). Similarly, Kang et al. reported that Prevotella was more abundant in fecal samples of early cervical cancer patients than in those from healthy controls (Kang et al. 2020).

Only one study has examined the gut microbiota composition in ovarian cancer patients, revealing that patients with primary platinum-resistant disease had lower phylogenetic diversity than platinum-sensitive patients (Jacobson et al. 2021). Table 1C provides a summary of the studies that investigated the association of gut microbiome alterations in women with cervical and ovarian cancer.

Unlike other types of cancer, a link between gut microbiota and gynecological cancers is not widely investigated, and there is much room for exploration to identify connections between the gut microbiome and ovarian as well as other gynecologic cancers.

Few studies explored the role of the uterine, cervical, and vaginal microbiota in patients with endometriosis and infertility, albeit found differential microbial patterns (Table 2). One possible reason for this variance could be due to study design differences and a lack of defined normal uterine microbiome. The healthy vaginal microbiota exhibits fewer different bacteria than the gut microbiota, that is, low taxonomic diversity which is typically dominated by Lactobacillus species (Ravel et al. 2011). It is worth noting that the continuous change of the microbiota distribution was identified along the reproductive tract (Chen et al. 2017, Wei et al. 2020). However, enrichment of Gardnerella was seen in both endometriosis and infertility (Wee et al. 2018, Ata et al. 2019). Further, the study of vaginal microbiota in healthy controls (n=18) and endometriosis patients (n=16) also found a significant enrichment of Atopobium as well as Gardnerella, while Lactobacillus was found to be lower in patients with endometriosis (Lu et al. 2022). Similarly, the decrease in the Lactobacillus level of the vaginal microbiome was highlighted in the patient with endometriosis (Wei et al. 2020). Furthermore, the level of Anaerococcus genus in vaginal samples can be utilized to predict the revised American Society for Reproductive Medicine stages of endometriosis (Perrotta et al. 2020), which showed a positive correlation with advanced stages of endometriosis. In addition, the analysis of the vaginal microbiome of patients with endometriosis/adenomyosis associated with chronic pelvic pain syndrome (CPPS) displayed significantly higher alpha diversity, as well as higher counts of Clostridium butyricum, Clostridium disporicum, Alloscardovia omnicolens, and Veillonella montpellierensis when compared to either CPPS patients without endometriosis/adenomyosis or women without CPPS (Chao et al. 2021). In contrast, Hernandez et al. showed that there was no difference in the vaginal mucus microbiome of control and deep infiltrating endometriosis patients (Hernandez et al. 2020). Analysis of the cervical microbiome by Chang et al. revealed that significantly reduced richness and diversity were detected in endometriosis patients with more severe clinical symptoms when compared to control patients (Chang et al. 2022). The presence of Lactobacillus crispatus was found to be associated with a higher rate of insemination success and fertility in two of the studies (Campisciano et al. 2017, Amato et al. 2020). It is interesting to note that the gut and the vaginal microbiome are in dynamic crosstalk with each other. In fact, the vaginal microbiome is known to have evolved from the translocation of species from the gut or by mother-to-child transfer at the time of delivery (Amabebe & Anumba 2020).

The gut microbiome alterations are linked to several other female reproductive disorders such as preterm birth and intra-uterine growth retardation (IUGR). Dahls et al. found that mothers delivering prematurely have lower alpha diversity in the gut than term deliveries (Dahl et al. 2017). Interestingly, the gut microbiota composition in
Table 2  Study design and results of recent literature describing the studies of uterine, cervical, and vaginal microbiota in association with endometriosis and infertility.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Microbiome</th>
<th>Major findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometriosis</td>
<td>Gastrointestinal (GI) and urogenital (UG) microbiomes</td>
<td>Identification of Clostridiales_Incertae_Sedis XI Anaerococcus as a characteristic biomarker in AMEM patients</td>
<td>Chen et al. (2021)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Gut and vaginal microbiome</td>
<td>Strong positive association between the GI/UG bacteria and the concentrations of urinary estrogen and its metabolites in the P-EOSIS group</td>
<td>Le et al. (2021)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Endometrial and vaginal microbiome</td>
<td>The occurrence of a non-Lactobacillus-dominated microbiota in a receptive endometrium was correlated with a significant decrease in implantation rate</td>
<td>Moreno et al. (2016)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Endometrial microbiome</td>
<td>Enrichment in Actinobacteria phylum, Oxalobacteraceae and Streptococcaceae families, and Tepidimonas genus in endometriosis group</td>
<td>Wessels et al. (2021)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Vaginal microbiome</td>
<td>Significant enrichment of Gardnerella and Atopobium and reduction in Lactobacillus spp. in patients with endometriosis</td>
<td>Lu et al. (2022)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Gut and vaginal microbiome</td>
<td>Higher OTU (operational taxonomic unit) of Anaerococcus genus in vaginal samples with advanced stages of endometriosis</td>
<td>Perrotta et al. (2020)</td>
</tr>
<tr>
<td>Endometriosis/adenomyosis with CPPS</td>
<td>Vaginal microbiome</td>
<td>Higher alpha diversity, as well as higher counts of Clostridium butyricum, Clostridium disporicum, Alloscardovia omnicolens, and Veillonella montpellierensis in endometriosis/adenomyosis patient with chronic pelvic pain syndrome (CPPS) when compared to either CPPS patients without EM/AM or women without CPPS.</td>
<td>Chao et al. (2021)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Cervical microbiome</td>
<td>Reduced richness and diversity of cervical microbiota were detected in endometriosis patients with more severe clinical symptoms</td>
<td>Chang et al. (2022)</td>
</tr>
<tr>
<td>Infertility</td>
<td>Cervical-vaginal microbiome</td>
<td>Differential presence of Lactobacillus iners, Lactobacillus crispatus, and Lactobacillus gasseri in idiopathic infertile women</td>
<td>Campisciano et al. (2017)</td>
</tr>
<tr>
<td>Infertility</td>
<td>Vaginal, cervical, and endometrial microbiome.</td>
<td>Increased Ureaplasma and Gardnerella vagina in the cervix of infertile women</td>
<td>Wee et al. (2018)</td>
</tr>
<tr>
<td>Infertility</td>
<td>Vaginal and seminal microbiome</td>
<td>L. crispatus correlated with a higher rate of intrauterine insemination success</td>
<td>Amato et al. (2020)</td>
</tr>
</tbody>
</table>

preterm neonates also differs significantly from that of born at full-term (Hiltunen et al. 2022). Additionally, a study analyzing the gut microbiota profiles in IUGR and normal birth weight piglets demonstrated that the metabolome profile in the IUGR piglets was significantly altered in comparison with controls (Zhang et al. 2019).

**General conclusions, limitations, and future perspectives**

Humans acquire a unique, dynamic ecosystem of gut microbiota in early life and maintain this metabolically active ‘organ’ throughout their existence (Gagliardi et al. 2018). Recent advances well established that gut microbiome perturbations are linked to several female reproductive tract disorders, commonly endometriosis, PCOS, gynecological cancers, and infertility (Fig. 2) (Ata et al. 2019, Lüll et al. 2021, Svensson et al. 2021). However, most studies merely report snapshots of the perturbed microbial diversity in reproductive disorders. Nonetheless, these alterations are disease-specific in nature, thus offering a unique opportunity for non-invasive-based early detection of reproductive tract pathologies. Emerging findings well established that
the gut microbiota affects host physiology through the production of metabolites including SCFAs. However, limited data are available on how the gut microbiota or -derived metabolites regulate reproductive function and dysfunctions. Moreover, it is not clear how gut microbiota might influence the peritoneal microenvironment, which could influence pathogenic manifestations of these diseases. Thus, efforts toward identifying novel treatment strategies by modulating bacteria via dietary intervention, microbial supplementation, or FMT are of high clinical relevance. However, a significant knowledge gap exists on the mechanisms by which specific bacterial species or groups of species may drive or influence reproductive tract function and dysfunctions. Importantly, additional studies are needed to determine whether dysbiosis in the gut bacteria causes these diseases or is itself a consequence of disease progression. Decoding the microbe–microbe–host interactions is key to linking gut microbiota alterations with microbial regulation of host processes and to optimally realizing the potential of gut microbiota in combating these diseases. Importantly, large and longitudinal integrative studies are much needed to identify all the microbial species, including bacteria, fungi, and viruses that are altered in reproductive pathologies. Although much remains to be unearthed, the data thus far provided tantalizing hints that modifying the gut microbiome could be a valuable avenue for treating many female reproductive tract pathologies. Importantly, these studies indicate that the gut microbiome and derived metabolites represent a new frontier in the management of reproductive tract diseases.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
R K received the funding from National Institutes of Health/National Institute of Child Health and Human Development (grants R01HD102680 and R01HD065435) and a Research Scholar Grant from the American Cancer Society.
Author contribution statement
S B C, V S, and R K wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Acknowledgements
The authors thank Drs. Deborah J Frank and Reilly Woodson (Department of Obstetrics and Gynecology, Washington University), Himanshi Bhatia, and Jennifer Brazill (InPrint, Washington University) for assistance with manuscript editing.

References
Blander JM, Longman RS, Ilyev ID, Sonnenberg GF & Arts D 2017 Regulation of inflammation by microbiota interactions with the host. Nature Immunology 18 851–860. (https://doi.org/10.1038/nli.3780)
Friedman J & Alm EJ 2012 Inferring correlation networks from genomic data. PLoS Computational Biology 8 e1002687. (https://doi.org/10.1371/journal.pcbi.1002687)


Liu L, Fu Q, Li T, Shao K, Zhu X, Cong Y & Zhao X 2022 Gut microbiota and butyrate contribute to nonalcoholic fatty liver disease in premenopause due to estrogen deficiency. PLoS One 17 e0262855. (https://doi.org/10.1371/journal.pone.0262855)


Wessels JM, Domínguez MA, Leyland NA, Agarwal SK & Foster WG 2021 Endometrial microbiota is more diverse in people with endometriosis than symptomatic controls. *Science Reports* 11 18877. (https://doi.org/10.1038/s41598-021-98380-3)


Received in final form 30 June 2022
Accepted 18 July 2022
Accepted Manuscript published online 18 July 2022