Gut and reproductive tract microbiota: Insights into the pathogenesis of endometriosis (Review)

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Abstract. Endometriosis is characterized by the presence of endometrial-like tissue outside the uterus and is associated with an inflammatory immune response. The gut and reproductive tract microbiota constitute a protective barrier against infection by pathogens and regulate inflammatory and immune functions. This review summarizes microbiota imbalance (i.e., dysbiosis) in endometriosis and discusses how dysbiosis influences disease development. The literature was searched for studies published from inception to March 2022 in the PubMed and Google Scholar databases using a combination of specific terms. An altered gut and reproductive tract microbiome has been reported in numerous conditions, such as inflammatory bowel disease, allergies, autoimmunity, cancer and reproductive disorders (e.g., endometriosis). Furthermore, microbial dysbiosis is a hallmark of endometriosis and is characterized by a decrease in beneficial probiotics and an increase in pathogenic microbes, which leads to a series of estrobolomic and metabolomic changes. Gut or reproductive tract microbiome dysbiosis was reported in mice, nonhuman primates, and females with endometriosis. Animal models of endometriosis demonstrated the effects of the gut microbiome on lesion growth and vice versa. The immune system mediated by the microbiota-gut-reproductive tract axis triggers an inflammatory response that damages reproductive tract tissue, which possibly leads to endometriosis. However, whether the alteration of eubiosis (a balanced microbiota) to dysbiosis is a cause or a result of endometriosis is unclear. In conclusion, this review provides an overview of the relationship between the gut and reproductive tract microbiome and endometriosis, focusing on the mechanisms by which dysbiosis may increase the risk of disease.

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1. Introduction

Endometriosis is a common estrogen-dependent inflammatory disorder, which affects ~10% of reproductive-age women (1). Endometriosis is defined by the presence of endometrial-like benign lesions located outside of the uterus (1). This disease is a heterogeneous disease that can present in different forms, including superficial peritoneal disease, ovarian endometrioma and deep infiltrating endometriosis (2). Endometriosis has been reported as a complex and multifactorial disease, which is characterized by estrogen dominance, increased cytokine levels, and innate and adaptive immune system activation (2,3).

Endometriosis is caused by endometrial cell engraftment outside of the uterus and is characterized by periodic hemorrhage from ectopic endometriotic lesions (3). Epidemiologic studies have reported that the risk of endometriosis is associated with increased exposure to menstruation (e.g., early age at menarche, shorter menstrual cycle length, longer menstrual flow duration, long menstrual period or reduced parity) (4). Typically, modern females marry later in life, have ≤2 children, and experience much shorter breastfeeding periods, which results in more frequent menstrual cycles (5). Females today menstruate approximately eight times more often than females in hunter-gatherer societies (i.e., ~400 vs. 50 menstrual cycles in their lifetime) (6). Menstruation is considered an inflammatory response with cyclic fluctuations in female steroid hormone levels and results in the production and release of inflammatory mediators, such as cytokines and prostaglandins (7,8). A surge in inflammatory mediators causes immune cell activation and recruitment, which results in further stressor production (e.g., cytokines, chemokines, angiogenesis factors and growth factors) (8). Multiple stressors are produced by different immune cells, mainly macrophages, and the regurgitated endometrial tissue (9,10) is eventually terminated by the host's immune system and immunoregulatory mechanisms.

Therefore, a higher frequency of menstruation may increase the risk of developing endometriosis.

Certain previous studies have suggested a role for microbes in the female reproductive tract in endometriosis (8,11). The female reproductive tract microbiota constitutes a defensive barrier to prevent infections, serves an important role in each of the structural, inflammatory, immune, metabolic, and endocrine systems, and may act as a key link between inflammation and immunity (12). Previous studies have suggested that the microbiota may be important for the prevention of non-communicable diseases (7,13). Furthermore, the abundance and composition of the gut and reproductive tract microbiota differ between females with and without endometriosis (8,14). Therefore, quantitative and qualitative imbalances between pathogenic bacteria and non-pathogenic environmental microbes (i.e., dysbiosis) may be involved in endometriosis pathogenesis (8). Dysfunction of the inflammatory and immune systems is a crucial aspect of endometriosis pathogenesis.

Thereby, both deleterious inflammations due to frequent menstrual cycles (an incessant attack from intrinsic stimuli) and pathogens or pathogenic microbes (an attack from extrinsic stimuli) are controlled by the host defense system. This review summarizes current knowledge about dysbiosis in endometriosis and discusses how this imbalance causes inappropriate immune responses, which can lead to the development of disease.

2. Search strategy and selection criteria

A literature search was performed to identify relevant studies. The PubMed and Google Scholar electronic databases were searched for literature published up to the 31st March 2022, combining the following keywords: 'Endometriosis', 'microbiome', 'dysbiosis', 'retrograde menstruation', 'inflammation' and 'immunity'. The search strategy combined these keywords with the Boolean operators 'and' and 'or', as described in Table I. Inclusion criteria were studies which specifically focused on the microbiome in endometriosis and included the publication of peer-reviewed original articles and reference lists in review articles. The exclusion criteria were duplicated studies, non-English language publications, letters to the editor, poster presentations, and unrelated literature. The identified articles were initially assessed for eligibility and then subsequently, the full-text of the articles was assessed. The first identification phase included an electric database search, hand search (manually searching for necessary documents), and the generation of a reference list of collected articles and review articles to identify additional relevant articles (Fig. 1). Duplicates were removed during the second, screening, phase. Titles and abstracts were read to remove inappropriate papers. The final eligible phase included full-text articles assessed as eligible.

3. Results

Identification of studies. Searches of the PubMed and Google Scholar electronic databases yielded 422 literature citations (Fig. 1). After removing duplicates, 179 records were identified, of which 140 were excluded and 39 met the eligibility criteria.

Role of the microbiota in the gut and female reproductive tract. This subsection summarizes the mechanisms underlying the effect of microbiome dysbiosis with a focus on endometriosis. Mucosal barriers serve crucial roles in the defense against pathogens or pathogenic microbes (12). The gut, skin, lungs, mouth, vagina, etc., contain certain non-pathogenic commensal microbes (12). The human microbiota positively affects physical health by maintaining structural, immunological, metabolic and neurological homeostasis (12). Mammals, including humans, have co-evolved with host-resident microorganisms in a symbiotic relationship (15). Microbiota co-evolving with humans supported the development of a symbiotic relationship and accelerated immune system function (16). Furthermore, the microbiota has an essential role in the endocrine systems of the gut and reproductive systems by interacting with estrogen and other hormones via a healthy estrogen-gut axis (17,18).

Role of the gut microbiota in non-communicable disease development. The commensal microbiome sustains the host-microbiota symbiotic relationship and contributes to numerous interactions which affect human health, including the synthesis and absorption of essential nutrients to maintain energy homeostasis; maintenance of intestinal mucosal function and structural integrity; protection against pathogenic microbes or harmful agents; production of antimicrobial peptides that affect the epigenome (15); maturation, development and functions of the innate and adaptive immune system; modulation of host brain function and cognitive behavior; regulation of estrogen modulated by estrogen-metabolizing enzyme production; and dynamic modulation of the metabolome profile (19,20). The microbiota is recognized by innate immune receptors, in particular, toll-like receptors (TLRs) (21,22). Gut epithelial cell TLRs are the signaling pathways which can implement host defense mechanisms against invading microbes, and they not only eliminate pathogens via increased immunosurveillance (21), but also establish host-microbial symbiosis to maintain microorganism-induced homeostasis (22). Therefore, the microbial contamination theory or hygiene hypothesis, which claims the positive effect of exposure to non-pathogenic environmental microbes and pathogenic bacteria in early-life on human health, has attracted attention (7,8,13,23). The exposure of humans to non-pathogenic environmental microbes or pathogenic bacteria begins prenatally, and continues during parturition and postnatally (20). Generally, early-life exposures to microbial diversity may be associated with a reduced risk of developing allergies, including atopy and asthma (24). In contrast, abundant specific bacterial taxa which affect the gut microbiota composition are associated with disease susceptibility in adult life, including inflammatory bowel diseases (25,26), metabolic disorders (e.g., obesity, gestational diabetes mellitus, type II diabetes) (25), neurological diseases (e.g., autism) (26), and reproductive and endocrine disorders (e.g., pregnancy complications, adverse pregnancy outcomes, polycystic ovary syndrome) (27). For example,

Table I. The search strategy.

Search	Keyword and search term combinations
1	Endometriosis
2	Microbiome OR Microbiota OR Pathogenic OR Probiotics
3	Dysbiosis AND (Imbalance OR Impairment)
4	Retrograde menstruation OR Menses
5	Inflammation OR Inflammatory
6	Immunity OR Immune
7	Search 1 AND search 2
8	Search 1 AND search 3
9	Search 1 AND search 2 AND search 3
10	Search 1 AND search 4 AND search 5
11	Search 1 AND search 4 AND search 6

gut microbiome alteration increases inflammatory bowel disease susceptibility, as the relative abundance of beneficial organisms (e.g., *Lachnospiraceae*, Bifidobacterium species, *Roseburia*, *Sutterella* and *Faecalibacterium prausnitzii*) decreases whereas the relative abundance of pathogens (e.g., Proteobacteria, Fusobacteria species and *Ruminococcus gnavus*) increase (28). Furthermore, imbalances in the composition of the gut microbiota (i.e., dysbiosis) favor inflammation via specific inflammatory immune cell recruitment, proinflammatory cytokine production and compromised immunosurveillance (8,29). Diseases affecting the immune and inflammatory system, such as inflammatory bowel disease and metabolic disorders, are believed to be related to a decreased microbial community diversity. Similarly, a lifelong risk of non-communicable inflammatory diseases, including reproductive pathologies, may result from early-life microbial exposures (26).

Role of the reproductive tract microbiota in obstetric, gynecological, and reproductive diseases. The healthy human vagina is colonized by numerous types of microorganisms and is characterized by the beneficial microbiota, *Lactobacillus*. The presence of a vaginal microbiota dominated by *Lactobacillus* maintains an acidic vaginal environment with a pH of <4.5, which prevents the growth of pathogenic bacteria (30). This can protect the host from pathogenic microbial infections such as *Gardnerella* species (31) and *Neisseria gonorrhoeae* (32). Furthermore, the upper female reproductive tract, consisting of the uterus, fallopian tubes, ovaries and peritoneum, is not a sterile environment and demonstrates a highly diverse and unique microbiota. Therefore, the microbial community composition differs between the uterus and the vagina (33-35). Commensal microbes produce biological resources and other microbes can use valuable metabolic resources to persist in the ecosystem via resource-sharing/competition and cross-feeding, which limits the availability of resources to neighboring pathogenic bacteria and inhibits pathogenic microbial growth with symbiotic microbes (36). This indicates that pathogenic bacteria can survive and even grow with a decreased commensal microbial diversity and an abundance of microbial taxa.

Imbalances in the composition of the reproductive tract microbiota can cause certain obstetric, gynecological and reproductive diseases. For example, a decrease in the abundance of the typical *Lactobacillus* and an abnormal increase in opportunistic pathogenic bacterial diversity cause pathologies, such as bacterial vaginosis, which is the most common cause of vaginal inflammation and infection (i.e., vaginitis) (37,38). Bacteria associated with bacterial vaginosis, such as *Gardnerella*, *Prevotella* and *Bacteroides*, induce increased levels of proinflammatory cytokine and mucosal epithelial barrier disruption (8,37). Moreover, elevated levels of inflammatory cytokine confer an increased risk for multiple gynecologic diseases, including endometritis, pelvic inflammatory disease and infertility (8,37). Additionally, patients with pelvic inflammatory disease are generally at increased risk of developing endometriosis (39). Furthermore, the lack of *Lactobacillus*-dominated microbiota species has been reported to be associated with poor fertility treatment outcomes (i.e., poor *in vitro* fertilization outcomes, with low fertilization and pregnancy rates) (35). Changes in microbiota distribution from eubiosis to dysbiosis can affect human health; however, the timing (i.e., pre-natal, intra-natal, post-natal or over time to the adult life) of these changes remains unknown. Research in this field is only beginning to apply microbiota analysis in the clinical setting, and its physiological and pathological roles in human reproduction and disease remain to be fully understood (34).

The clinicaltrials.gov electronic database was searched using the keywords 'endometriosis' and 'microbiome'. The electronic search yielded a total of six clinical trials, in which recruitment was completed, currently being performed, and not yet started in two, one and three studies, respectively. Currently, clinical studies in 'Microbiome and immunologic analysis in females with endometriosis (NCT04159740)' and 'Establishment of the human intestinal and salivary microbiota biobank in gynecological diseases (NCT04698109)' are currently being performed.

The role of the immune system is mediated by the microbiota in endometriosis. Immune cells, which are essential reproductive tract microenvironment components, secrete numerous cytokines and chemokines (40). Both innate [e.g., macrophages, neutrophils, dendritic cells and natural killer (NK) cells] and adaptive immune cells (e.g., T cells and B cells) contribute to regulation of tissue inflammation, immune cell recruitment and resolution, and the host defense response. Pelvic endometriosis is thought to arise from endometrial cell implantation through retrograde menstruation (2). *Escherichia coli* bacteria in menstrual blood and endotoxins in the peritoneal fluid are bioaccumulative contaminants which have been previously reported in females with endometriosis (41). Endometrial cell fragments that are shed during menstruation produce a damage-associated molecular pattern (DAMP) through the specialized pattern recognition receptors (e.g., TLRs) and activate inflammatory cytokine production, such as interleukin-1β (IL-1β), to trigger inflammation (9). Furthermore, DAMPs activate innate and adaptive immune cells, such as neutrophils, mast cells and Th17 cells, initiate immune cell response, and promote endometrial cell adhesion and angiogenesis (9). Particular TLR ligands (e.g., lipopolysaccharide, nucleic

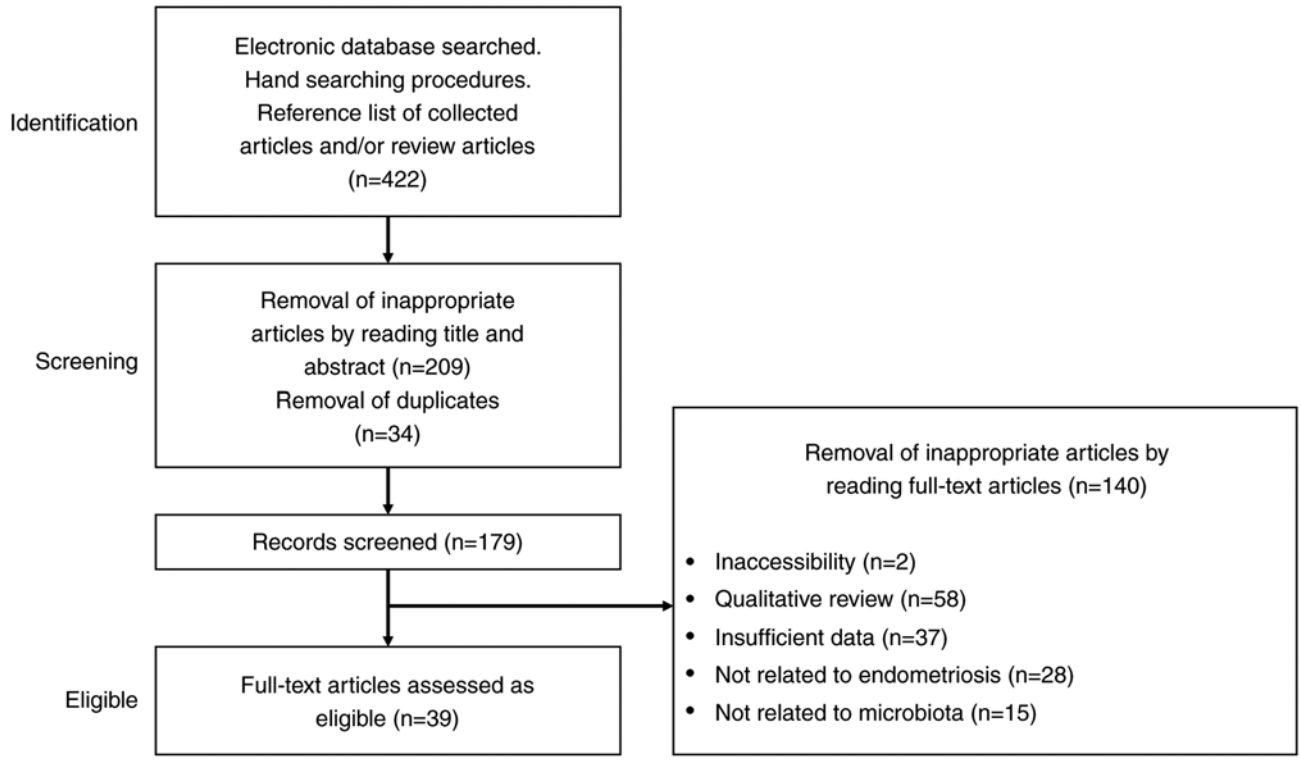

Figure 1. The number of articles identified by searching for keyword combinations. The number of articles identified by keyword combinations and the number of records identified through database searching. The number of records after duplicate removal, after records screened, after removal of inappropriate articles by reading full-text articles, and after assessing full-text articles for eligibility. The hand search procedure involved manually searching for necessary documents using magazines, textbooks, etc. that provided clues for research themes.

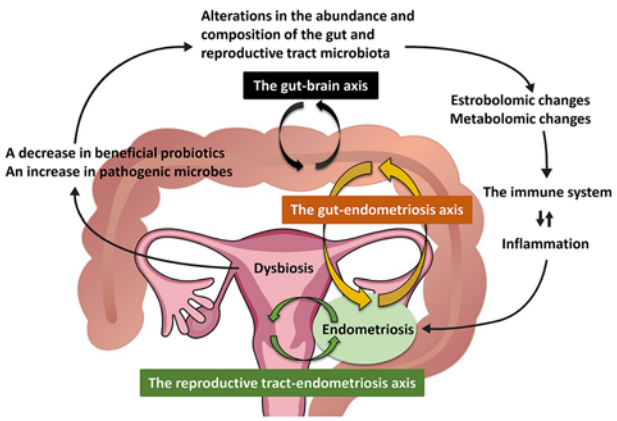

Figure 2. Role of the gut and reproductive tract microbiome in endometriosis development. An overview of the relationship between the gut and reproductive tract microbiome and endometriosis, which focuses on the mechanisms by which dysbiosis may increase the risk of disease. Endometriosis is caused by endometrial cell engraftment outside the uterus and is characterized by inflammation through periodic hemorrhage from ectopic endometriotic lesions. Dysbiosis favors inflammation via specific inflammatory immune cell recruitment, proinflammatory cytokine production and compromised immunosurveillance. The gut microbiota can accelerate inflammation and affects the pathogenesis and progression of endometriosis via dysregulation of the estrobolome, metabolome and gut-brain axis.

acids, flagellin, or zymosan of both commensal microbiota and pathogenic microbiota) are sensed by the immune system via the pattern recognition receptors (42). TLR-4-mediated nuclear factor κβ activation may be the main factor which affects endometriosis development (41). Over time, persistent immune stimuli may drive a vicious cycle of inflammation, adhesion and angiogenesis, which in turn facilitates ectopic endometrium implantation and growth (8). The immune system fights against bacterial infection but triggers an inflammatory response that damages reproductive tract tissue. The excessive inflammatory response may lead to endometriosis through host immune dysfunction. Therefore, the immune system mediated by the microbiota-gut-reproductive tract axis is thought to serve an important role in developing endometriosis.

Role of the gastrointestinal tract microbiome. This subsection summarizes the role of the gastrointestinal tract microbiome in endometriosis (Fig. 2). Data from mice and nonhuman primates are included, as microbiome research in females with endometriosis is still in its early stages (Table II). Previous *in vivo* studies have reported the influence of gut microbiota on animals with endometriosis. Mouse models of endometriosis demonstrated an increased ratio of Firmicutes to Bacteroidetes and an increased number and proportion of certain gut microbes (e.g., *Ruminococcaceae*, *Bifidobacterium* and *Parasutterella generae*) after endometrial fragment transimplantation (43). Moreover, a study which used rhesus macaques reported lower numbers of Lactobacillus bacteria and higher Gram-negative microbial loads in the endometriosis group compared with that in the non-endometriosis group (44). Furthermore, rhesus macaque with endometriosis were reported to have an increased risk of gastrointestinal inflammation (44). This finding was consistent with clinical observations that endometriosis is associated with an increased risk of gastrointestinal disorders, such as irritable

Table II. Summary of alterations in the microbiota in endometriosis.

First author/s, year	Study design	Aims	Results and summary	(Refs.)
Bailey *et al*, 2002	*In vitro* experimental analysis	To identify an altered profile of intestinal microflora in female rhesus monkeys with endometriosis.	A significant decrease in Lactobacilli and an increase in gram-negative bacteria in rhesus monkeys with endometriosis. A higher prevalence of intestinal inflammation in monkeys with endometriosis in comparison to healthy controls. The microflora was affected by endometriosis-associated intestinal inflammation.	(44)
Seaman *et al*, 2008	A case-control study	To determine whether women with endometriosis were more likely to receive a diagnosis of pelvic inflammatory disease or irritable bowel syndrome than women without endometriosis.	Women with endometriosis were more likely to develop pelvic inflammatory disease or irritable bowel syndrome than women without endometriosis (Odds ratio 3.5).	(45)
Khan *et al*, 2010	A retrospective empirical research	To test the hypothesis that bacterial contamination of menstrual blood could be a local biologic event in the development of endometriosis	Elevated Escherichia coli contamination and endotoxin levels were identified in menstrual fluid or peritoneal fluid in women with endometriosis compared with control women. Endotoxin levels in menstrual fluid and peritoneal fluid in women with endometriosis may promote Toll-like receptor 4-mediated growth of endometriosis.	(41)
Attaman *et al*, 2014	*In vitro* experimental analysis	To elucidate the anti-inflammatory impact of polyunsaturated fatty acids in mouse endometriosis models.	Elevated systemic polyunsaturated fatty acid levels suppressed immune, angiogenic, and growth factors in endometriosis animal models. Omega-3 polyunsaturated fatty acids may suppress the establishment of endometriosis-like lesions through anti-inflammatory effects.	(69)
Hopeman *et al*, 2015	A cross-sectional study	To elucidate the relationship between serum polyunsaturated fatty acids and the risk of developing endometriosis.	A cross-sectional study of serum polyunsaturated fatty acids and clinical data from 205 women undergoing *in vitro* fertilization suggested that low eicosapentaenoic acid levels may be a risk factor for endometriosis.	(68)
Khan *et al*, 2016	A case-controlled biological study	To investigate microbial colonization in intrauterine environment and cystic fluid of women with and without endometriosis by molecular approach.	Molecular detection of intrauterine microbial colonization by 16S metagenome assay detected an increase in Streptococcaceae and Staphylococaceae in the cystic fluid of ovarian endometrioma comparing to that in cystic fluid of non-endometrioma cysts. Sub-clinical infection was identified in intrauterine environment and in the cystic fluid of ovarian endometrioma.	(54)

Table II. Continued.

First author/s, year	Study design	Aims	Results and summary	(Refs.)
Dols *et al*, 2016	A case-controlled biological study	To identify microbiota composition in the vagina of women with and without bacterial vaginosis.	Analysis of the bacterial communities by 16S rRNA amplicon sequencing revealed an increase in Gardnerella vaginalis, Lachnospiraceae, and Leptotrichiaceae in women with bacterial vaginosis. A simple molecular test may serve as a practical diagnostic method for the assessment of female reproductive tract dysbiosis.	(57)
Shi *et al*, 2017	A review article	To outline the interaction between the gut microbiota and the immune system and provide a reference for future studies.	Inflammation caused by abnormal immune responses may modulate the gut microbiome composition, resulting in intestinal diseases. This review summarizes the interaction between the gut microbiota and the immune system.	(60)
Khan *et al*, 2018	A retrospective empirical research	To measure the endotoxin levels in the menstrual fluid and peritoneal fluid in women with or without endometriosis.	The menstrual blood of women with endometriosis was contaminated with pathogenic bacteria including Escherichia coli. Lipopolysaccharide stimulates the growth of endometriosis via the LPS/TLR4 cascade-mediated pro-inflammatory response. A new concept called the 'bacterial contamination hypothesis' in endometriosis was proposed.	(42)
Yuan *et al*, 2018	*In vitro* experimental analysis	To investigate changes that occur in the gut microbiota during the development of murine endometriosis.	After transplantation of endometrial tissue, gain of pathogenic microbes and loss of probiotics were identified in mouse models of endometriosis. This study showed that endometriosis induces gut microbiota alterations in mice.	(43)
Ervin *et al*, 2019	*In vitro* experimental analysis	To provide the *in vitro* analysis of the ability of some human gut microbial gut microbial β-glucuronidase enzymes to reactivate estrogen glucuronides to estrone and estradiol.	This research showed that gut microbial β-glucuronidase enzymes participate in estrogen metabolism within the mammalian gastrointestinal tract. β-glucuronidase can reactivate estrogens from their inactive glucuronides, suggesting that this enzyme is involved in the estrobolome.	(18)
Ata *et al*, 2019	A prospective study	To compare vaginal, cervical and gut microbiota between women with stage 3/4 endometriosis and healthy controls.	The study compared vaginal, cervical and gut microbiota between women with stage 3/4 endometriosis and healthy controls. An increase in Gardnerella, Streptococcus, Escherichia, Shigella, and Ureoplasma was identified in the cervical microbiota of women with stage 3/4 endometriosis. Dysbiosis in the genital tract microbiome was identified in the affected women.	(48)

Table II. Continued.

First author/s, year	Study design	Aims	Results and summary	(Refs.)
Hernandes *et al*, 2020	A case-controlled biological study	To identify microbiome profiles in vaginal fluid, endometrium, and lesion of women with deep endometriosis.	An increase in Lactobacillus, Gardnerella, Streptococcus, and Prevotella in vaginal fluid, eutopic endometrium and endometriotic lesion. An increase in Alishewanella, Enterococcus, and Pseudomonas in women with deep endometriotic lesions.	(55)
Wei *et al*, 2020	A case-controlled biological study	To identify microbiota composition and distribution along the female reproductive tract of women with endometriosis.	The specific microbiota composition in upper reproductive tract may be an indicator for the risk of endometriosis.	(56)
Jiang *et al*, 2021	A review article	To investigate and summarize intricate connections between the gut and reproductive tract microbiota and endometriosis.	16S rRNA amplicon analysis revealed altered microbiota composition and distribution along the female reproductive tract in women with endometriosis. Dysbiosis disrupts normal immune function, impairs immunosurveillance, alters immune cell profiles, and leads to the elevation of proinflammatory cytokines, which may contribute to the pathogenesis of endometriosis.	(8)
Kovács *et al*, 2021	A review article	To discuss the identification and diagnostic potential of novel disease biomarkers of microbial origin.	Altered profile of gut microbial composition and function may be a potent source of biomarkers of endometriosis. Endometriosis-specific microbial profiles may also represent targets for novel diagnostic options for endometriosis.	(47)

Key articles were categorized into *in vitro* studies, *in vivo*/animal studies and human studies, the study design, aim, results and summary are presented. Studies demonstrating altered microbiota composition and distribution along the gut and reproductive tract in women with endometriosis, as well as studies from mice and non-human primates. Articles with only basic research for the implications of dysbiosis, such as the role of immune dysfunction, cytokine-induced dysfunction via inflammatory pathways, altered estrogen metabolism, microbiota-derived metabolome or altered metabolites, were not included.

bowel syndrome (45). Gut microbiota diversity and abundance decreased in animals with endometriosis compared with healthy controls (46). Further, previous studies have reported an increased absolute number of *Gardnerella*, *Streptococcus*, *Enterococci* and *Escherichia coli* in the vagina of females with endometriosis compared with healthy females, which indicated an increased proportion of potentially pathogenic microbes and a reduced microbiome diversity (47,48). Therefore, the type and abundance of gut microbiota differ between females with and without endometriosis (49).

Experimental induction of endometriosis by fecal microbiota transplantation has been previously reported; the transplantation of fecal microbiota obtained from a surgically induced endometriosis mouse donor into the gastrointestinal tract of other endometriotic mice promoted lesion growth and associated inflammation (50). Furthermore, *in vivo* experiments demonstrated that broad-spectrum antibiotic treatment in mice with endometriosis reduced the endometriotic lesion size (43). The mouse model demonstrated that endometriosis-specific gut microbiota can alter disease progression by modulating inflammation (50). Non-pathogenic and pathogenic fecal bacterial contamination, followed by a process of proinflammatory cytokine- and immune-activation-mediated gut dysfunction, provides a plausible explanation of gut microbial dysbiosis in endometriosis. Furthermore, preliminary studies on the efficacy of probiotics for women with endometriosis have revealed positive effects in pain relief (8,51,52) (see Treatment of endometriosis by modulating the microbiome).

Conversely, studies with endometriosis models which used mice and rhesus macaques have reported that endometriosis induced gut microbiota alterations or dysbiosis (43,44). Previous animal studies have reported that gut microbiota composition alterations promote the development of endometriosis, and that experimental endometriosis affects the composition of the gut microbiota (43,44). However, changes in the gut microbiota in the same female patient before and after the onset of endometriosis are unknown. In contrast, other *in vivo* mouse studies did not report changes in the gut microbiome composition profiles (53). Therefore, a gut microbiota composition imbalance could cause endometriosis exacerbation; however, there are conflicting results that endometriosis itself may or may not change the gut microbiota.

Role of the reproductive tract microbiome. This subsection summarizes the role of the reproductive tract microbiome in endometriosis (Fig. 2). Numerous studies have analyzed the microbiome composition of the reproductive tract in females with endometriosis and reported an increased abundance of opportunistic pathogens associated with bacterial vaginosis and diminished *Lactobacillus* dominance along the reproductive tract (from the vagina up into the cervix, endometrium, fallopian tube and peritoneal fluid) of females with endometriosis (8,47,48,54-57). Opportunistic pathogens, including *Streptococcaceae* and *Staphylococaceae*, were reported to be enriched in the cystic fluid of females with ovarian endometrioma (54). Moreover, *Alishewanella*, *Enterococcus*, *Ureaplasma* and *Pseudomonas* are often detected in endometriotic lesions (9), whereas *Atopobium* is completely absent in the vagina of patients with endometriosis (48). Additionally, greater numbers of bacteria such as *Gardnerella*, *Streptococcus*, *Enterococci* and *Escherichia coli* are present in the endometrium of females with endometriosis compared with controls (14). Furthermore, an increased absolute number of bacterial pathogens, including *Gardnerella*, *Streptococcus*, *Escherichia*, *Shigella* and *Ureaplasma*, was reported in the cervical microbiota of females with stage III-IV endometriosis (48). Endometriosis may affect the composition of the reproductive tract microbiota, which in turn increases the risk of lower genital tract infection, endometritis, pelvic inflammatory disease and surgical site infections after hysterectomy (14). Additionally, increased numbers of *Firmicutes* and decreased *Actinobacteria* and *Bacteroidetes* in the cervical microbiota have been reported to increase the risk of developing endometriosis (58). Increasing disease severity (e.g., advanced stage, the coexistence of deep infiltrating endometriosis, disease burden and symptom severity) have been reported to be associated with decreasing abundance of *Dialister* and an increasing abundance of *Lactobacillus* and *Streptococcus* (58). Endometriosis is characterized by the loss of beneficial microorganisms (e.g., *Lactobacilli*) and the gain of pathogenic microorganisms. However, the specific bacteria identified as the causative microbiota remains unknown. Furthermore, evidence of the effects of the host site on the reproductive tract microbiota composition is currently scarce.

Possible mechanisms of endometriosis development by alterations of microbiota abundance and composition. This section summarizes the mechanisms by which alterations in gut and reproductive tract microbiota composition and function cause endometriosis. The growth and maintenance of endometriosis are influenced by estrogen metabolism (59). The gut microbiome is an important regulator of circulating estrogens (17). The gut microbiota is involved in regulation of estrogen levels via the β-glucuronidase enzyme, which is responsible for the deconjugation of conjugated estrogens into their active forms (18). Thus, estrogen metabolism is modulated by the estrobolome (i.e., the aggregate of genes capable of metabolizing estrogens in the gut microbiome) (17). Gut microbiome dysbiosis caused by the imbalance between the commensal and pathogenic microbiomes or reduced microbial diversity dysregulates the bidirectional crosstalk between the gut and uterus, which results in not only immune dysfunction but also altered estrogen signaling (8,60). Microbial diversity and dysbiosis reduction lower β-glucuronidase activity (17). Conversely, the estrobolome, through the increased abundance of β-glucuronidase-producing bacteria, increases circulating estrogens, thereby developing endometriosis (8,17). Investigating the number of β-glucuronidase-producing bacteria in the gut microbiome of patients with endometriosis is of great interest.

The gut microbiome communicates bidirectionally with distant tissues via the gut-brain axis (61). The gut microbiota modulates neurophysiological processes by altering immune, endocrine, reproductive and neural signaling pathways via the gut-brain axis (62). Estrogen has been reported to stimulate neural growth and differentiation both *in vitro* (63) and in animal studies (64). The gut-brain axis promotes the central sensitization of chronic pain (65). Therefore, endometriosis-associated pain may be affected by regulation of microglia and astrocytes via the dysbiotic gut-brain axis (65). Severe

pain and infertility associated with endometriosis have been reported to be associated with decreased cervical microbial diversity and abundance (66).

Finally, changes in the microbiome abundance and composition can affect the metabolic profile, and vice versa (17). A metabolomic study in animals has reported that bile acid biosynthesis and α-linolenic acid metabolism were characteristic pathways in the feces of endometriosis mice (46). Gut dysbiosis in mice with endometriosis appears to cause fecal metabolomic alterations, but its significance in the progression of endometriosis is unclear.

Collectively, these results suggest that the gut microbiota initiates inflammation and affects the pathogenesis and progression of endometriosis via dysregulation of the estrobolome, metabolome and gut-brain axis (58,66). However, whether the composition, abundance and functional alteration of the gut microbiota causes disease progression is unknown.

Treatment of endometriosis by modulating the microbiome. This section first discusses the beneficial effect of probiotics on endometriosis. A recent review summarized dietary supplements (i.e., anti-inflammatory, anti-oxidant, anti-proliferative and immune modulators) used for treating endometriosis (67). Targeting the gut microbiota with probiotics, prebiotics and symbiotics may be beneficial dietary interventions for certain patients, including those with endometriosis, osteoporosis and obesity (67-70). Data on the oral administration of Lactobacillus and omega-3 polyunsaturated fatty acids (PUFAs) are well documented and were presented as examples. See reference (67) for the remaining drugs. The previous study reviewed the up-to-date evidence on dietary supplements used as a complementary treatment, including vitamin C, vitamin D, vitamin E, zinc, magnesium, selenium, omega 3, propolis, quercetin, curcumin, N-acetylcysteine, probiotics, resveratrol, alpha lipoic acid and epigallocatechin-3-gallate (67). Oral Lactobacillus administration reduced the growth of mouse endometriotic lesions by increasing IL-12 concentration and NK cell activity (51,71,72). Randomized, placebo-controlled trials reported that oral *Lactobacillus* administration ameliorated endometriosis-associated pain in females (52,73). Furthermore, *Lactobacillus* probiotic treatment has been reported to prevent endometriosis proliferation in rats (71). Additionally, a high intake of omega-3 PUFAs may reduce the risk of developing endometriosis by modulating inflammatory and oxidative stress (68). A mouse model of endometriosis demonstrated that a high-dose PUFA treatment inhibited lesion growth (69). Therefore, available data suggest the important role of dietary supplement-induced beneficial changes in the gut microbiota in human health and reduce the risk of inflammatory diseases, including endometriosis.

There are numerous available therapeutic approaches, such as modulating the microbiome, to treat endometriosis. Preclinical studies have evaluated the possibility of antibiotic administration, fecal microbial transplantation or administration of bacterial components for the treatment of endometriosis in addition to probiotic treatment. Chadchan *et al* (50) reported that antibiotic therapy with metronidazole reduced endometriotic lesion progression in mice through gut microbiota modulation. Moreover, fecal microbiota transplantation is a promising therapeutic option to target *Clostridium difficile* infections and holds promise in developing novel therapeutics for multiple sclerosis, autism, obesity and other systemic diseases (74). Additionally, the gut microbiota could induce inflammatory pain in endometriosis by increasing glutamate and decreasing gamma-aminobutyric acid levels through modulation of microglia, astrocytes and immune cells (65). Fecal microbiota transplantation has been reported to be a potential treatment option for endometriosis due to its beneficial effects in reducing pain despite the absence of no clinical data (74). Furthermore, a preclinical mouse model demonstrated that gut microbiota-derived short-chain fatty acids (e.g., n-butyrate) protected against endometriosis progression (75).

4. Discussion

Microbial profile abnormalities (e.g., the composition and abundance) of the gut and reproductive tract microbiome have been reported in numerous diseases, such as inflammatory bowel disease, allergies, autoimmunity, psoriasis, arthritis, reproductive disorders, cancer and endometriosis (6,7,23-28,30-37,76,77). Both animal and human studies have reported that pathogenic bacteria are enriched in the endometriosis group compared with controls (8,9,14,41-48,54-57,73). Additionally, abnormal gut and cervical microbiomes may be associated with endometriosis severity, including pain and infertility, possibly through the impact of the estrobolome and metabolome (58,76). Reproductive tract microbiota alterations were also identified in mice, nonhuman primates and females with endometriosis, which has been universally observed across species (8,43,44,50-52). Microbiota dysbiosis and endometriosis development and progression are thought to be supported by a bidirectional relationship (76); however, no evidence suggests a cause-effect relationship. Additionally, altered specific microbiota composition in endometriosis has no clear consensus. The microbiota has been reported to be a major regulator of such physiological processes, not only within the gut and reproductive tract but also at distant sites, e.g., microbiota-brain crosstalk (78). As mentioned above, microbial profiles in the gut and endometriosis and in the genital tract and endometriosis have been reported, but the crosstalk between the gut and genital tract environments remains unclear. Collectively, microbiome composition and abundance alterations may cause endometriosis and its associated symptoms, including infertility and pelvic pain, possibly through the gut-brain axis.

The microbiota is not merely composed of microorganisms, such as commensals and symbionts, and they are increasingly recognized as serving beneficial roles in human health and reproduction beyond infection (34). The immune system is unable to eliminate the microbial symbiont population once the commensal microbiomes (e.g., non-pathogenic environmental bacteria, certain commensals and probiotics) are established in the host (79). Females infected with commensals or non-pathogenic microbiota early in life maintain a symbiotic relationship, thereby avoiding excessive tissue damage, preserving immune homeostasis and suppressing the inflammatory response (7,23). However, modern females often face a rapidly changing microbial environment, with serious biodiversity loss due to industrialized Western lifestyles. Environmental changes (e.g., diet and lifestyle), which lead to

reduced microorganism exposure can affect the microbiota composition and diversity. A microbiota imbalance or impairment (e.g., a combination of increased pathogenic microbes and loss of probiotics) in the gut and female reproductive tract alters immune cell profiles, disrupts normal immune function and compromises immunosurveillance, which leads to chronic states of aberrant immune activation and persistent inflammatory responses (8). This concept may be supported by epidemiological data that the prevalence of autoimmunity, allergy, inflammatory bowel disease and reproductive disease, such as endometriosis, is increasing in high-income countries (7,25,76,80). The so-called hygiene hypothesis initially focused on allergic diseases, but a reduced non-pathogenic commensal microbial diversity may cause numerous types of disease, including autoimmune diseases, through impaired immunoregulatory mechanisms (23). Microbiotas, inherited at birth, serve a role in an individual's predisposition to developing certain diseases (2). Moreover, endometriosis is generally more common in females with irritable bowel syndrome because both diseases are characterized by chronic inflammation (45,47). Interestingly, decreased NK activity (81) and increased risk of pregnancy complications (82,83) remain unchanged after surgery for endometriosis.

Microbiota dysbiosis and frequent retrograde menstruation can contribute to the development of endometriosis. Alterations of eubiosis to dysbiosis (the first event) may induce immune, metabolomic and estrobolomic disturbances, which can trigger systemic inflammation and contribute to the development and progression of endometriosis (7,8). Furthermore, frequent retrograde menstruation (the second event) causes repeated inflammation and serves a critical role in the development of endometriosis. The current retrograde menstrual theory does not provide an adequate explanation for why endometriosis occurs only in certain females while almost all females have retrograde menstruation. It can be hypothesized that only females who experience the first and second events, not just one of the two events, are more likely to develop endometriosis. The increased risk of developing endometriosis in daughters of mothers with endometriosis may be related to the effects of microbiota exposure (84). This evidence is at least partly explained by the hygiene hypothesis, which suggests that infections by microorganisms, such as commensals and symbionts, early in life prevent non-communicable inflammatory diseases (7,13).

Finally, preclinical and clinical studies have demonstrated no commonality in gut microbiota composition profiles across species. Several studies have reported that the microbial composition of the gut and reproductive tract is altered in females with endometriosis; however, its clinical relevance needs to be evaluated. Future studies will focus on the immune and molecular mechanisms which underlie the host-microbiota relationship to identify effective strategies to diagnose, manage and prevent endometriosis. In conclusion, this review provides an updated overview of the relationship between the gut and reproductive tract microbiome and endometriosis, and discusses how dysbiosis increases the risk of disease.

5. Summary and future perspective

Endometriosis has become a significant public health problem in Japan because of its higher prevalence in Asian females compared with that in Caucasian females (85). In recent years, the impact of symbiotic and pathogenic microorganisms on human health has attracted considerable attention. This review summarized the latest findings on the role of microbiomes in the study of endometriosis. The gut and reproductive tract microbiota components of females with endometriosis differ from those in healthy females, which may help identify potential biomarker candidates. Therefore, an in-depth analysis of microbiota compositions should be performed to assess the most appropriate prevention, diagnosis and treatment in patients with endometriosis. Further research is required to identify specific microbiota compositions that are altered in females with endometriosis and to explore the causal relationship.

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Competing interests

The author declares that they have no competing interests.

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