Beyond the Reproductive Tract: Gut Microbiome and Its Influence on Gynecological Health

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KEY POINTS

- Growing evidence defines the gut microbiome as an endocrine organ and suggests its potential role in gynecological physiology and pathophysiology due to its bidirectional relationship with female hormone levels.
- The estrobolome is defined as the collection of the gut microbial genes that encode enzymes implicated in estrogen activation, therefore potentially affecting different hormone-dependent gynecological functions.
- Gut microbial dysbiosis may lead to the activation of the immune responses and inflammation, and hormone dysregulation, thereby contributing to the onset and progression of multiple estrogen-driven inflammatory pathologies.
- The gut microbiome has been associated with endometriosis, polycystic ovary syndrome (PCOS), gynecological cancer, abortion and infertility.
- Current knowledge about the association of the gut microbiome with gynecological health is under-examined and requires well-designed studies based on standardized protocols for consistent findings.

ABSTRACT

Purpose of review: The analysis of microbiome in association with female health is today a "hot topic" with the main focus on microbes in the female reproductive tract. Nevertheless, recent studies are providing novel information of the possible influence of the gut microbiome on gynecological health outcomes, especially as we start to understand that the gut microbiome is an extended endocrine organ influencing female hormonal levels. This review summarizes the current knowledge of the gut microbes in association with gynecological health.

Recent findings: The gut microbiome has been associated with endometriosis, polycystic ovary syndrome, gynecological cancers, and infertility, although there is a lack of consistency and consensus among studies due to different study designs and protocols used, and the studies in general are underpowered.

Summary: The interconnection between the gut microbiome and reproductive health is complex and further research is warranted. The current knowledge in the field emphasizes the link between the microbiome and gynecological health outcomes, with high potential for novel diagnostic and treatment tools via modulation of the microenvironment.

Keywords: microbiota, endometriosis, polycystic ovary syndrome, cancer, infertility

1. INTRODUCTION

The human gastrointestinal tract harbors trillions of microorganisms including bacteria, archaea, viruses, protozoa, and fungi, being the largest and most diverse microbial ecosystem within the human body. The collection of the intestinal microbial genomes, the gut microbiome, is a research field of increasing interest since it represents a genetic pool more than one order of magnitude higher in genes than the human genome [1]. The vast majority of these microbial communities co-evolved symbiotically with the host and contribute to important metabolic, immune and epithelial functions, being crucial for the host physiology and pathophysiology [2–4].

Recent evidence refers to the gut microbiome as an extended endocrine organ due to its profound interaction with hormone levels [5]. Furthermore, a sex bias has been identified in microbiome-related diseases which are associated with sex hormones [6]. In this context, the term “microgenderome” has emerged to define the interactions between the microbiome, sex hormones and the immune system [7]. This interaction could unravel the molecular mechanisms underlying the gut microbiome influence on female reproductive health and how its dysbiosis could lead to different pathologies [8].

The gut microbiome-estrogen axis has been proposed as a cornerstone implicated in the pathogenesis of different gynecological conditions, such as polycystic ovary syndrome (PCOS), endometriosis, gynecological cancer, infertility and adverse pregnancy conditions [9] (Figure 1). This crosstalk between the gut microbiome and estrogens is regulated by the estrobolome, the aggregate of gut bacterial genes whose products are capable of metabolizing estrogens [10]. Certain enteric bacteria secrete β-glucuronidase, the main estrogen-regulator of the estrobolome, that converts the conjugated estrogen (glucuronic acid) into its deconjugated form that exerts its biological activity [11]. Thus,

an optimal β-glucuronidase activity reduces the inactivation of estrogen, leading to a balanced hormone circulating levels. However, a reduction in the gut microbial diversity as a result of dysbiosis and inflammation could reduce the β-glucuronidase activity and this reduction has been linked to hypoestrogenic pathologies such as obesity, metabolic syndrome, cardiovascular disease and cognitive decline [9]. Otherwise, an increased β-glucuronidase activity is associated with hyperestrogenic conditions and can lead to the progression of gynecological estrogen-driven diseases [9] (Figure 1). Since the estrobolome may have a profound influence on these pathologies, future interventions targeting the estrobolome-microbiome axis emerge as promising diagnostic and treatment tools for women's health [5]. However, the molecular mechanisms underlying the relationships between the gut microbiome, estrogen metabolism and gynecological outcomes are still in its infancy. In this narrative review, we summarize the whole body of knowledge of the gut microbiome and its interactions with different gynecological health conditions.

2. ENDOMETRIOSIS

Endometriosis is defined as an estrogen-dependent chronic inflammatory gynecological disease characterized by endometrial-like tissue present outside of the uterus. It represents a major health concern since it affects 6-10% of women in reproductive age [12]. Regardless of the continuous research, the exact mechanisms of endometriosis are still undetermined. The most widely accepted hypothesis for the origin of endometriosis is Sampson's retrograde menstruation, which explains that women commonly have retrograde menstrual flow [13]. Nevertheless, only 10% of women are diagnosed with endometriosis [14]. Growing evidence proposes a multifactorial origin for endometriosis

development driven by genetic predisposition, environmental factors, inflammation, immune activation, hormone dysregulation and microbial dysbiosis [15, 16].

Much is known of the role of the gut microbiome in maintaining the integrity of the gastrointestinal epithelial lining and immune balance to prevent bacterial translocation, which can cause low-grade systemic inflammation [17]. While it is well-established that the gut microbes influence immunomodulation and the development of various inflammatory diseases [18], current studies have highlighted the potential implication of enteric microbes in the pathogenesis of endometriosis [11, 19]. The "bacterial contamination hypothesis" proposes that besides estrogen regulation, the gut microbiome could contribute to the onset of endometriosis through lipopolysaccharide (LPS) endotoxin as the initial trigger and bacterial contamination as its source in the intrauterine environment [20]. LPS is found in the cell wall of Gram-negative bacteria, and is a marker of inflammation which has been linked to endometriosis lesions activating the immune response by binding with Toll-like receptor 4 [20]. A systematic review concluded that increased abundance of *Proteobacteria*, *Enterobacteriaceae*, *Streptococcus* and *Escherichia* in the gut associated with the presence of endometriosis [21] (Figure 1). In line, a *Shigella*/*Escherichia* dominant gut microbiome in women with advanced stages 3/4 endometriosis have been described [22], and an increase in *Streptococcus* in the gut of patients with the 3/4 endometriosis stages has been reported [23]. Further, a recent study detected a higher abundance of *Shigella flexneri* (*Proteobacteria* phylum) in patients with external genital endometriosis compared to controls [24]. Additionally, a higher proportion of Gram-negative bacteria belonging to *Desulfobacterota* phylum have been detected in women with endometriosis when compared to healthy controls [25]. *Proteobacteria* and *Desulfobacterota* phyla are both characterized by Gram-negative staining, and, therefore, presenting LPS in the outer membrane [26]. Interestingly, a

recent translational study demonstrated a pathogenic mechanism via *Fusobacterium* (a Gram-negative bacterial genus) infection in endometrial cells through activation of transforming growth factor–β (TGF-β) signaling [27]. This activation leads to the transition from quiescent fibroblasts to transgelin (TAGLN)–positive myofibroblasts, which are able to proliferate, adhere, and migrate *in vitro* [27]. It was also observed that inoculation of *Fusobacterium nucleatum* in a murine model of endometriosis resulted in increased numbers and weights of endometriotic lesions [27].

Short-chain fatty acids (SCFAs) are microbial metabolites with pleiotropic beneficial effects for the host metabolism and immune regulation through their action on T-regulatory cells [28, 29]. *Lachnospiraceae*, *Eubacteriaceae* and *Ruminocacceae* family members are the main producers of SCFAs in the intestine, particularly producing acetate and butyrate [30, 31] (Figure 1). Several studies have found lower abundances of butyrate-producing microbes such as *Lachnospira*, *Ruminococcus*, *Eubacterium eligens* and *Coprococcus catus* in women with endometriosis [23, 24, 32, 33]. In particular, butyrate has been described as an anti-inflammatory mediator that could indirectly regulate endometriosis-related symptoms such as visceral inflammatory pain [34]. Considering the connection between the dysbiosis (through an increase of Gram-negative bacteria and/or depletion of SCFAs producers) and immune dysfunction, future studies are needed to study whether imbalances within these gut microbes are the cause, consequence or enhancer of endometriosis.

Given the hyperestrogenic conditions associated with endometriosis, there is growing interest focused on the estrobolome as a key factor contributing to the progression of the disease. Alterations in the gut microbiome that result in overexpression of estrobolome associated genes could both trigger formation and maintenance of the lesions. Interestingly, β-glucuronidase activity has been found in Gram-negative bacteria [25].

Moreover, an analysis of microbial genomes associated enteric *Bacteroides*, *Bifidobacterium*, *Escherichia* and *Lactobacillus* with β-glucuronidase production [35] (Figure 1). In endometriosis population, several studies have reported higher abundances in these bacteria [23, 32]. Nevertheless, an enzymatic activity study of fecal samples did not reveal significant differences in β-glucuronidase activity in women with and without endometriosis [36]. We have recently performed the first whole metagenome study on a cohort of 1,000 women with and without endometriosis, and did not find any microbial features (species or pathways) associated with the disease [37, 38]. Furthermore, we analyzed the estrobolome-associated genes and did not find any significant differences between the two study groups [38].

Altogether, the previous results pave the way for future strategies targeted to the gut microbiome-estrobolome axis, nevertheless the studies in the field lack consensus and the identification of an endometriosis-associated microbiome profile still constitutes a debate without a definitive answer. It is clear that endometriosis is a complex heterogenic disease and further studies applying well defined, adequately powered study groups are warranted to determine the core microbial composition in endometriosis.

3. POLYCYSTIC OVARY SYNDROME

The polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine disorders in women of reproductive age, affecting up to 20% of women [39]. Despite its high prevalence, its multifactorial complexity has made it challenging to understand the underlying etiology. Possible triggers include genetic factors, intrauterine environment, lifestyle, and, in an increasingly explored approach, alterations in the gut microbiome [40, 41].

Considering the role of the gut microbes in metabolic disorders, the search of the link between the gut microbes and PCOS is plausible. Numerous studies have detected alterations in the microbial richness, diversity, and microbial composition in PCOS [41]. Specifically, PCOS patients have been observed to exhibit a decrease in α-diversity indices (diversity within a sample) compared to controls [42–45]. Regarding β-diversity (dissimilarity of the microbial community between samples), previous studies have identified differences in the microbial composition among samples from PCOS patients compared to healthy controls [42, 44–47]. While other studies have not observed any significant differences in microbial composition in PCOS [48–52]. Our recent systematic review summarizes that the women PCOS have decreased microbial diversity in the gut and that the prevalent taxa are *Bacteroides spp., Parabacteroides spp., Prevotella, Megamonas spp., Megasphaera massiliensis, Escherichia/Shigella*, while *Bifidobacterium spp., Lactobacillus spp., Faecalibacterium*, and *Blautia* are reduced [41].

The presence of obesity and insulin resistance also emerges as crucial factors in the study of the gut microbes in PCOS. Obesity, a common characteristics in PCOS patients, has been analyzed in relation to the intestinal microbiome, revealing significant differences in the gut microbiome β-diversity in PCOS patients with obesity compared to non-obese patients [53, 54]. In PCOS groups with high BMI, an increased abundance of *Erysipelotrichaceae*_UCG-003 [45], *Prevotellaceae* [54], *Streptococcus*, *Fusobacterium, Rhizobacter*, and *Achromobacter* [49] was observed. Nevertheless, other previous studies have not observed any microbiome differences between the groups [48, 49, 51]. Regarding insulin resistance, studies have shown a decrease in the α-diversity when compared to controls [48, 54, 55], and the most abundant bacteria such as *Prevotella,*

211 *Megamonas*, *Dialister* [54], *Prevotella stercorea* [48] and *Faecalibacterium* [55] have
212 been identified in PCOS patients with insulin resistance.

213 Beyond the bacteria, a number of studies have explored the mycobiome and virome in
214 PCOS patients, where the increased abundance of fungi: *Saccharomyces, Lentinula*, and
215 *Aspergillus* [46], *Candida*, *Malassezia, Kazachstania, Microascus, Coniochaeta,*
216 *Xepicula, Paraphoma, Pyrenochaetopsis, Cephaliophora, Epicoccum,* and *Sclerophora*
217 has been observed in PCOS patients [45]. The analysis of the gut virome detected lower
218 viral diversity and significant alterations in virome composition in women with PCOS,
219 where the most enriched taxon was *Quimbyviridae* when compared to healthy controls
220 [56].

221 Altogether, there seems to be a common trend of reduced microbial diversity in PCOS,
222 nevertheless, the studies are performed on limited sample size and lack consensus.

223

224 **4. GYNECOLOGICAL CANCER**

225 Within the recent years, there has been a growing interest in understanding the connection
226 between the human microbiome and various types of cancers, including gynecological
227 cancers. Among these, the most common are endometrial, cervical, and ovarian cancers,
228 where the endometrial cancer is the most prevalent [57]. However, the most lethal is
229 ovarian cancer, accounting for 5% of total cancer-related deaths [8, 57]. These cancers
230 are characterized by being estrogen-mediated tumors [58]. Estrogens have the capacity to
231 modulate the inflammatory response and increase the production of pro-inflammatory
232 mediators (IL-6 and TNF-α) [59]. This can establish a feedback loop that influences the
233 expression of enzymes associated with ovarian steroidogenesis. The gut microbiome is
234 able to metabolize these estrogens, increasing their concentration and thereby enhancing

the development of endometrial cancer [59]. Simultaneously, this increase in the estrogen levels also has the potential to induce changes in the gut microbiome, indirectly contributing to the cancer progression [8].

Although there is still limited literature of the relationship between the gut microbiome and endometrial cancer, existing studies provide contradicting results. One study found a significant reduction in the gut microbial α-diversity and differences in β-diversity among endometrial cancer patients [60], while other did not detect any significant differences [61]. These discrepancies are also reflected in the phylogenetic composition, with variations in the abundances of *Firmicutes, Proteobacteria, Actinobacteria,* and *Bacteroidetes* in the gut microbiome among endometrial cancer patients when compared to controls [60, 61].

In ovarian cancer studies, changes in β-diversity were consistently observed between patients and controls, however, in richness, no significant differences were detected [62,63]. Moreover, increase in specific bacterial abundances such as *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* phyla have been reported in ovarian cancer patients [62].

The research of the gut microbiome in gynecological cancers is very preliminary and future research is needed to clarify the potential cancer-associated microbial profile and unravel the complexity of the relationship between gut microbes and gynecological cancers.

5. INFERTILITY

The vaginal microbiome has been the subject of extensive research in relation to female fertility; however, the influence of the gut microbiome is still relatively unstudied. Recent

investigations suggest that the gut microbiome may play a crucial role in the modulation of the reproductive system through the gut-uterus axis. It has been observed that even a small alteration in the commensal and symbiotic gut microbes can trigger dysbiosis, disrupting intestinal homeostasis and increase the risk of inflammatory processes associated with adverse reproductive pathologies [64] (Figure 1).

A previous study highlighted that the diversity and composition of the intestinal microbiome, along with its metabolite profiles, show significant alterations in patients who had experienced spontaneous abortions [64]. When analyzing the fecal microbiome in association with spontaneous abortions, an overrepresentation of various opportunistic pathogens (*Prevotellaceae*_NK3B31_group, *Bacteroidales*_S24_7_group, and *Eubacterium ruminantium*) was identified in the affected group, while other microorganisms (*Prevotellaceae*, *Prevotella*_1, and *Gammaproteobacteria*) were more abundant in the control group [64]. Additionally, a significant correlation was found between the metabolites associated with these microorganisms and an increase in cytokines linked to Th1 and Th17 [64]. The reduction in the richness and diversity of the microbiome in patients who had suffered abortions supported these findings, corroborating previous results linking microbiome composition to infertility [65-67].

In a similar context, notable differences in the composition of the gut microbiome between patients with infertility (recurrent implantation failure –RIF- and unexplained infertility) and controls have been detected [65]. *Bacteroides* and *Hungatella* stood out as the most abundant genera in the gut in infertilite women, especially in cases of unexplained infertility. A decrease in the genera *Prevotella* 9, *Ruminococcaceae* UCG-004, *Ruminococcaceae* UCG-010, and an increase in *Bacteroides*, *Dorea* oral clone FR58, and *Peptoniphilus* were detected in the gut microbiome when compared to controls [65]. Further studies have detected higher abundance of *Verrucomicrobia*, and members

of *Barnesiellaceae* and *Phascolarctobacterium* in the gut [67], while the genera *Stenotrophomonas, Streptococcus,* and *Roseburia* showed a decrease in patients with infertility [67]. Altogether, regardless of these preliminary studies, there seems to be a consensus in the increase of the gut *Firmicutes/Bacteroidetes* ratio in infertile women when compared to controls [64, 66].

6. CONCLUSIONS

There is growing body of evidence demonstrating that the gut microbes play important role in female physiology and pathophysiology and that via its endocrine and hormonal regulation, specifically estrobolome regulation can influence female reproductive health. In this review we gather the knowledge of the gut microbiome involvement in endometriosis, PCOS, cancer and infertility, and with the time, the list of different gynecological disorders in association with the gut microbiome will definitely grow.

The current knowledge of the microbe-disease associations encompasses the microbial diversity analyses and identification of specific bacterial genera. The majority of the studies of the gut microbiome in female gynecological health have applied the 16S rRNA gene sequencing technique that does not have sufficient specificity to identify bacteria on species level, which makes the generalization of the findings of the current studies imprecise. The whole metagenome sequencing method, although more expensive and requiring advanced bioinformatics skills, would provide more detailed information of the exact bacterial species and detects also other microorganisms within the sample, such as viruses, fungi, archaea and other microeukaryotes.

Another important aspect that calls for caution when interpreting the previous findings is that most of the studies have been performed on limited sample size, lacking detection

power and negative/positive controls. It is known that inherent elements of study design, such as sample size, sample collection method, DNA extraction process, type of sequencing employed, and data analysis, represent limitations that can influence the accurate detection of microorganisms [68, 69].

Furthermore, the currently applied next-generation sequencing-based microbiome analysis techniques assess DNA sequences, which do not necessarily equate with the presence of live bacteria [70]. Thus, DNA-based techniques characterize a microbiome but do not mean that the detected sequences are functionally active microbes. RNA analysis-based technique (i.e. meta-transcriptomics [71] and culturomics [72], together with integration with other omics analysis platforms would provide further knowledge of the functionality of the microbes in the gut in reproductive health and disease.

In conclusion, the gut microbiome studies in the female gynecological health are in its infancy, and future research on bigger, well-designed studies together with novel methods are warranted to unravel the core microbial compositions in gynecological health and to understand the function of specific microbes in the disease development.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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FIGURE LEGEND

Figure 1. Potential gut microbiome-driven mechanisms underlying gynecological physiology and pathophysiology. In healthy (eubiotic) conditions, the estrobolome contributes to estrogen activation through the secretion of beta-glucuronidase. Homeostatic circulating estrogen levels regulate menstrual cycle and contribute to uterine health. Moreover, several gut microbes release short-chain fatty acids (SCFA; e.g., butyrate, acetate and propionate), which participate as anti-inflammatory mediators maintaining gut barrier function and physiological inflammation. When the gut microbiome is disrupted (dysbiosis), the overgrowth of β-glucuronidase-producing bacteria may lead to hyperestrogenic levels commonly reported in different gynecological pathologies. On the other hand, gut dysbiosis can be linked to a reduction of SCFA-producing bacteria, resulting in increased pro-inflammatory mediators and systemic inflammation. *Reported β-glucuronidase-producing genera associated with any gynecological disease in case-control studies. (Created with BioRender.com).