Current findings in endometrial microbiome: impact on uterine diseases

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Abstract

Microbiome or microbiota is essential to regulate many mammalian physiological processes, including reproduction. Like other organs or tissues, the upper female reproductive tract used to be considered as devoid of microorganisms; however, a non-infection-related bacterial community was discovered in the uterus from humans and other mammals, and its composition is related to reproductive success. The dysbiosis of endometrial microbiota is associated with benign and malignant uterine diseases. Hence, this review addressed the current knowledge about uterine microbiota alterations and their association with common endometrial diseases, including endometrial polyposis, endometriosis, uterine myomatosis, endometrial hyperplasia, and endometrial cancer. There is a specific bacterial community in the endometrium in the most-analyzed uterine diseases. However, the constant finding consists in a reduced abundance of Firmicutes and Lactobacillus, while there is an increased abundance of Proteobacteria (such as Escherichia coli and Enterococcus), Bacteroidetes (Prevotella, for example), and Actinobacteria (as Gardnerella), in contrast to healthy endometrium. Besides, we discussed the future usefulness of the endometrial microbiota components as biomarkers to diagnose uterine diseases and their probable clinical outcomes. In addition, we analyzed their potential use as probiotics since they could provide an alternative or complement to existing therapies.

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Introduction

Knowledge of the human reproductive microbiome has long been based on studying the lower female reproductive tract (FRT). The vaginal microbiome has been extensively characterized, and its contribution to the maintenance of reproductive physiology has been determined. For example, it is known that vaginal microbiota from reproductive-age women is mainly composed of Lactobacillus crispatus, L. gasseri, L. iners, and L. jensenii and controls the growth of pathogenic microorganisms by maintaining a lower vaginal pH, competing for nutrients, inhibition of adherence, antimicrobial metabolites production, and regulation of local immune response (Verstraelen et al. 2022). However, information about microbiota from upper FRT tissues remains scarce.

Until recently, the uterus of healthy mammals, including humans, was considered as an environment lacking microorganisms. Nevertheless, in the last decade, studies focused on the characterization of the upper FRT microbiota, and the determination of its contribution to health and disease has been extensively achieved (Rampersaud et al. 2012). Omic sciences, particularly metagenomics and next-generation sequencing (NGS) techniques, have allowed the description of the complex and diverse human endometrial microbiota (Mitchell et al. 2015, Verstraelen et al. 2016, Li et al. 2018).

There is a growing body of evidence indicating that Lactobacillus is the most abundant genus in healthy endometrium, and in general, it appears that in pathological conditions and infertility, the presence of this genus is low (Verstraelen et al. 2016, Chen et al. 2017, Miles et al. 2017, Tao et al. 2017, Kyono et al. 2019). However, there is still no consensus on the healthy endometrial microbiota composition or the existence of a core microbiota (Peric et al. 2019).

Research lines about endometrial microbiota currently center on the elucidation of its role on fertility, pregnancy maintenance, and development of uterine diseases (Chen et al. 2017, Moreno & Simon 2018, Agostinis et al. 2019). This article reviews the current knowledge generated by this study area, analyzing the association and possible impact of the endometrial microbiome on common benign and malignant uterine diseases, considering the potential mechanisms involved in the pathogenesis of these diseases. In addition, the
existing methods used in their study are explored. We further expose the perspectives for research and the applications of the endometrial microbiome as potential biomarkers to diagnose uterine diseases and their use as probiotics to complement existing therapies.

The endometrial microbiome

The microbiota of each upper FRT organ or tissue, including the endometrium, contains a unique bacterial composition that differs from that of the lower FRT microbiota (Moreno et al. 2016, Chen et al. 2017). NGS of hypervariable regions (known as V1–V9) of the 16S rRNA gene has allowed the characterization of the endometrial microbiota at a more refined level than conventional bacterial culture techniques, which cannot detect most members of the upper reproductive microbial community (Moreno & Simon 2018). The combined use of next-generation and traditional molecular biology and culture techniques has led to a more detailed characterization of endometrial microbiota, detecting both culturable and non-culturable bacteria and showing a high concordance in results (Chen et al. 2017, Moreno et al. 2018).

Most reports have shown that the human endometrial microbiome is low in biomass in contrast to the vaginal microbiome. However, it is highly diverse and abundant in the phylum Firmicutes, with Lactobacillus as the predominant genus, followed by phyla Bacteroidetes, Proteobacteria, and Actinobacteria (Moore et al. 2000, Mitchell et al. 2015, Baker et al. 2018, Pelzer et al. 2018, Altmae & Rienzi 2021, Oberle et al. 2021, Sun et al. 2021). Other studies have reported that in the uterine microbiota predominates Bacteroidetes or Proteobacteria, with a low abundance or absence of Lactobacillus (Verstraeten et al. 2016, Chen et al. 2017, Winters et al. 2019, Wang et al. 2021). This latter composition appears to be related to gastrointestinal microbiota instead of the vaginal microbiota, albeit without significant correlation (Heil et al. 2019).

The tendency toward Lactobacillus abundance could result from the enrichment of vaginal or cervical microorganisms through transcervical sampling or reflect the uterine colonization with bacteria from the lower reproductive tract (Mitchell et al. 2015, Baker et al. 2018). Nevertheless, endometrial sampling is commonly performed after a hysterectomy, thus avoiding transcervical contamination; besides, vaginal, cervical, and uterine bacterial communities have shown to be different from each other, and technical controls of contamination have been used (Chen et al. 2017).

Interestingly, a continuum in the composition of the female reproductive system microbiota with gradual changes between adjacent tissues according to the finding of shared bacterial taxa and a positive correlation between upper and lower genital tract communities in the same patient has been reported (Walther- Antonio et al. 2016, Chen et al. 2017, Miles et al. 2017, Winters et al. 2019, Wang et al. 2021). These results suggest communication between the upper and the lower FRT. The anatomical proximity allows the migration of vaginal microbiota to the uterine cavity through the cervix, and this transfer could be regulated by the periodical physicochemical changes in cervical mucus or in the uterine immune status along the menstrual cycle or provoked by inflammatory processes (Agostinis et al. 2019, Peric et al. 2019).

Recently, an extensive study described the translocation of vaginal bacteria to the uterine cavity by using a combination of different strategies as metagenomic characterization of vaginal and uterine microbiota from women with benign gynecological pathologies, animal models, and analysis of public data. Vaginal lavage fluid was transplanted from endometritis patients or endometritis-associated Prevotella bivia and Clostridium perfringens into healthy rat vagina, inducing uterine inflammatory indicators related to the disease. Additionally, an increase of total bacteria and transferred bacterial biomasses was observed. Researchers also performed a source tracking between uterus and vagina by bioinformatic analysis of bacterial sequences at genus and strain levels; this analysis showed high proportions of shared single-nucleotide polymorphisms and the existence of common species between both localizations (as Lactobacillus iners). These findings support the theory of bacterial transfer from the vagina to the uterine cavity under specific conditions and its influence on endometrial physiology (Wang et al. 2021).

Besides human microbiota studies, different research groups have characterized the endometrial microbiome of mammals with an economic or ecological conservation interest, such as mare, cow, dog, and giant panda (Heil et al. 2019). Interestingly, the phyla Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria are predominant in these animals, although their specific composition (at genus or species level) and abundances differ between hosts.

In contrast, related information in non-human primates is scarce. However, recently, Li et al. performed the characterization of the reproductive microbiota of captive hamadryas baboons, founding that the phylum Firmicutes and the order Clostridiales have a high abundance in endometrial tissue (Li et al. 2020). Data from animals can help standardize laboratory techniques and to establish study models to predict the origin and evolution of endometrial microbiota in humans and hosts with veterinarian importance and evaluate the true impact of an altered microbiota in the physiology of the female upper reproductive tract. The latter could be possible by evaluating host–bacterial interactions; however, it is necessary to consider the physiological and anatomical differences among species before extrapolating to human microbiota.

Despite the significant advances in the knowledge of the endometrial microbiota, there are still some...
challenges to overcome, as defining the core microbiota, mainly due to the lack of consensus between different findings. The discrepancies among studies are associated with various factors, including methodological issues, the low microbial biomass detected in the uterine cavity, and the clinical characteristics of the individuals, including the menstrual cycle phases at the time of sampling (Garcia-Grau et al. 2019, Odawara et al. 2021, Wang et al. 2021). Some of these issues are discussed in sections ‘Menstrual cycle, sex steroid hormones, and endometrial microbiota’ and ‘Future perspectives for endometrial microbiota research’.

Overall, the diverse findings suggest a significant role of endometrial microbiota in uterine physiology and reproduction (Moreno & Simon 2018). In this regard, recent studies have reported that the composition and abundance of the endometrial microbiota are different between fertile and infertile women, indicating its relationship with reproductive success. Infertile patients show a lower abundance of Lactobacillus in endometrial microbiota than fertile women (Wee et al. 2018). Additionally, the rates of embryo implantation, gestational development, and live births in patients undergoing in vitro fertilization (IVF) are higher when their endometrium is receptive and harbors mainly Lactobacillus species (abundance ≥90% or even ≥80%) at the time of embryo transfer (Moreno et al. 2016, Kyono et al. 2019). Hence, a dysbiotic endometrial microbiota is related to IVF failure (Hashimoto & Kyono 2019). In this sense, a novel study showed the increased abundance of Atopobium, Blifidobacterium, Chryseobacterium, Gardnerella, Haemophilus, Klebsiella, Neisseria, Staphylococcus, and Streptococcus in endometrial microbiota is associated with unfavorable conditions to reproductive success, causing clinical miscarriage or no pregnancy (Moreno et al. 2022).

Furthermore, the altered composition of the endometrial microbiota is associated with gynecological pathologies, showing different taxonomic diversity and abundance compared with healthy women. However, the microbial pathways involved in the pathogenesis of uterine diseases and obstetric complications, as well as the interactions with host pathways, are unknown; likewise, it is not clear whether particular microenvironmental conditions, as variable steroid hormonal levels, might modify the microbiota (Garcia-Grau et al. 2019). In the following sections, we will describe and discuss the microbiota composition reported along the menstrual cycle and in benign and malignant uterine diseases.

Menstrual cycle, sex steroid hormones, and endometrial microbiota

Since host conditions shape the microbiota composition, it is necessary to define the effect of different factors as physiological hormonal levels, mainly those of sex steroid hormones as estradiol and progesterone, age, menopausal status, and hormonal therapies, among others, on the composition, abundance, and physiology of the endometrial microbiota.

There are fluctuations in hormonal levels depending on the phase of the menstrual cycle, which are necessary for the proper regulation of the uterine and endometrial function. It is suggested that these fluctuations could act as regulators of FRT microbiota (Crittley et al. 2020).

In this regard, there are contradictory observations. For example, a study reported that the uterine microbiota did not change through the transition from the pre-receptive to the receptive phase of the menstrual cycle, suggesting that hormonal fluctuations do not influence the bacterial composition or abundance, and this community remains stable (Moreno et al. 2016).

Another study found that the endometrial microbiota shows light variations across the menstrual cycle phases, suggesting an influence of the hormonal status. Researchers found the enrichment of metabolic pathways is associated with bacterial growth in the proliferative phase in contrast to the secretory phase of the cycle, indicating an increase of bacterial biomass in this stage. However, particular bacteria such as Propionibacterium (now reclassified as Cutibacterium acnes), Pseudomonas sp., and Comamonadaceae showed an increased abundance in the secretory phase, while Sphingobium sp. or Carnobacterium sp. remained without changes along the cycle (Chen et al. 2017).

In contrast, significant differences have been reported between the menstrual cycle phases in the endometrial microbiota from menorrhagia and dysmenorrhea cases. The prevalence of Prevotella spp. was observed in the proliferative phase and enriched Sneathia spp. and L. crispatus in the secretory phase (Pelzer et al. 2018). Also, progestin administration to these patients was related to a reduced abundance of obligate anaerobes and an increment of facultative anaerobes in the proliferative endometrium. In contrast, the proliferative endometrium without progestin treatment showed high amounts of obligate anaerobes and reduced facultative anaerobes abundance, a condition usually related to a pathogenic status. Progestin administration was linked to the specific increase of L. crispatus but a general reduction of Lactobacillus spp., indicating that continuous hormonal dosage contributes to balance the uterine microbiota favoring the proliferation of species associated with a eubiotic state (Pelzer et al. 2018), which in turn could help to maintain a healthy endometrial status.

A more recent study based on metatranscriptomics analyzed the differences in the microbial function between the proliferative and mid-secretory phases in the endometrium from healthy women of reproductive age. In contrast to other studies, Proteobacteria was the most abundant phylum, and Klebsiella pneumoniae, Clostridium botulinum, Pasteurella multocida, and Hydrogenophaga sp. HN-16 were the most abundant
species in the human endometrium along all menstrual cycle, whereas Lactobacillus showed a very low abundance. The detected bacteria were alive, functional, and their metabolic functions vary along the menstrual cycle. Interestingly, the microbiota of the mid-secretory phase showed more transcriptionally active bacteria, and enrichment of metabolic pathways related to endometrial receptivity, as L-tryptophan degradation and C20 prostanoid biosynthesis. These findings indicate that host hormonal levels could influence the bacterial composition of the endometrial microbiota and its function (Sola-Leyva et al. 2021).

A study performed on the Japanese population found that the most common bacteria in the follicular phase were, from most to least abundant, Lactobacillus, Gardnerella, Prevotella, Bifidobacterium, Burkholderia, and Escherichia, whereas in the luteal phase were Lactobacillus, Burkholderia, Streptococcus, Gardnerella, Bifidobacterium, and Atopobium (Odawara et al. 2021). It is noteworthy that although Lactobacillus showed a slight reduction in the luteal phase compared to the follicular phase, it remains as the most abundant genus detected throughout the menstrual cycle, which coincides with the stability found in other reports, suggesting that in addition to participating in the regulation of the acquisition of endometrial receptivity, it could participate in the mechanisms of recovery of the endometrial lining after menstruation.

The available information about the effect of hormonal therapies on the endometrial microbiome is scarce, but studies in this regard continue to be carried out. Analysis of endometrial microbiota from women undergoing IVF subjected to ovarian stimulation (by administration of recombinant FSH) or progesterone supplementation showed differences compared to the endometrium before hormonal treatments, mainly the increase of bacterial diversity and potentially harmful bacteria as Atopobium and Prevotella. In contrast, Lactobacillus abundance was slightly decreased (Carosso et al. 2020). However, no follow-up of the results was performed after the procedures of IVF, which could indicate whether the individual microbial changes induced by hormonal management are correlated to success or unsuccessful reproductive outcomes.

Female steroid hormone levels also change throughout women’s lifetime (Graham et al. 2021). Hence, age is another aspect related to specific microbiota composition. Wang et al. recently reported that the endometrial microbiota from women above 40 years is characterized by a reduced diversity, in contrast to women under 40 years, whose endometrial microbiota tends to be more diverse. In addition, an elevated abundance of Bacteroides and Proteobacteria was detected in this age group, whereas Firmicutes and Actinobacteria showed low amounts; on the contrary, in endometrium from women above 50 years, the abundance of Proteobacteria decreased, whereas the abundance of Firmicutes increased. At the genus level, a reduction of Lactobacillus and the increase of Prevotella was observed, indicating a correlation of microbiota composition with age and the hormonal or menopausal status (Wang et al. 2021). Similarly, Odawara et al. reported a reduced Lactobacillus abundance in women older than 36 years, especially in multipara patients (Odawara et al. 2021).

Based on the described findings, it is essential to clarify whether hormonal fluctuations and changing uterine conditions throughout the menstrual cycle under healthy and pathological conditions influence the composition, structure, and physiology of the endometrial microbiota and how these bacterial characteristics, in turn, might influence reproductive function. The in vitro effect of different hormone levels on the growth and function of single or mixed cultures of the above bacteria and the possible impact on endometrial physiology must be evaluated. Figure 1 schematizes the main findings reported on the changes in the microbiota between the different phases of the menstrual cycle.

Microbiota in uterine-related diseases

Endometrial polyposis

Polyposis is a common benign endometrial disease defined as a localized overgrowth of glands, stromal cells, and blood vessels in the endometrial mucosa that projects into the uterine cavity; it is often associated with abnormal menstrual bleeding and infertility. Polyposis is present in women with these alterations at 50% and 32% rates, respectively. Some molecular factors involved in the etiology of endometrial polyposis are the elevated estrogen (ER) levels, modified expression of progesterone and estrogen receptors, and imbalance between mitotic activity and apoptosis. The risk factors include exogenous ER administration, hormone replacement therapies, obesity, and chronic inflammation of the endometrium (Nijkang et al. 2019), the latter possibly due to chronic endometritis provoked by a high load of microorganisms (Cicinelli et al. 2005, 2019).

Due to the correlation between chronic endometritis (CE) and endometrial polyps (EP), Fang et al. (2016) conducted a study aimed to characterize endometrial bacteria from Asian patients of reproductive age with EP, CE, and with both pathologies (EP/CE). Women without these conditions undergoing infertility diagnosis were enrolled as a control group. The 16S rRNA gene V4 region was sequenced using an Illumina platform. Their findings showed that Proteobacteria was the most abundant phylum, followed by Firmicutes and Actinobacteria, while that to genera level Lactobacillus, Enterobacter, and Pseudomonas dominate their composition. The results also showed that Firmicutes is the dominant phylum in patients with EP and EP/CE (Fang et al. 2016).
Moreover, *Lactobacillus*, *Gardnerella*, *Bifidobacterium*, *Streptococcus*, and *Alteromonas* were the predominant genera, while Proteobacteria and *Pseudomonas* abundances were low (Fang et al. 2016). These results differ from the finding of the requirement of a high abundance of *Lactobacillus* for adequate and healthy reproductive function (embryo implantation, pregnancy development, and live birth) (Moreno et al. 2016). These findings suggest that there should be a delicate balance in the *Lactobacillus* abundance to define a eubiotic or dysbiotic microbiota and its association with a pathological status. In general, endometrial communities associated with EP and EP/CE groups show higher bacterial diversity than control women (Fang et al. 2016). This study is one of the few that includes healthy women as a control group, who despite being candidates for IVF due to infertility of their male partners have regular menstrual cycles and did not show uterine abnormalities, thus eliminating confounding variables that could influence the detected composition and allowing to identify a group of bacteria specific to the disease. However, as in other studies, it is required to enlarge the sample size to confirm the findings and analyze the effect of the menstrual cycle phases on the endometrial microbiota due to altered hormonal response observed in polypsis pathogenesis.

Since EP and micropolyps (MP) are histological manifestations of CE and are related to endometrial inflammation (Cicinelli et al. 2005), another study developed in Caucasian women analyzed the association of MPs with the presence of bacteria through the vagina, cervix, and endometrium by culture techniques and PCR. Bacteria were recovered more frequently from patients with polyps than from control women (83% vs 77%), suggesting a higher bacterial abundance in the endometrium from the former. A remarkable finding of this research is the presence of *Enterococcus faecalis* in the endometrium from studied subjects, showing a light frequency reduction in patients with MP and CE in contrast with controls. *E. faecalis* is usually found in the colon, and the reported result suggests the possible translocation of this microorganism from the gastrointestinal tract to reproductive organs as a form of endometrial colonization (Tatarchuk & Herman 2016). This result must be taken carefully since *E. faecalis* presence could be related to vaginal contamination at the time of sampling since this microorganism has been associated with aerobic vaginitis (Kaambo et al. 2018). However, this microorganism may play a role in the endometrium in pathological conditions as it has also been detected in infertile patients subjected to IVF (Smolnikova et al. 2019) and patients with endometrial cancer (Mikamo et al. 1993). Therefore, it is required to perform studies with a larger sample size and avoid contamination during sampling through the transcervical route. The differences with the Fang study (Fang et al. 2016) could be attributed to the ethnic origins of the enrolled volunteers. There likely exists a microbial composition specific for each ethnic group in the same way that microbiome changes in the vaginal microbiota (Ravel et al. 2011).

Overall, results show the alteration of uterine microbiota in patients with EP; however, it is still unknown if EP provokes changes in endometrial microbiota or if endometrial microbiota changes induce the growth of the polyps; hence, the role of microorganisms in EP development is still unknown. Since findings are not conclusive, it is necessary to perform more studies to...
clarify the role of specific bacterial groups in developing polyposis through the activation of inflammatory pathways and other molecular mechanisms.

**Endometriosis**

Endometriosis is a chronic inflammatory condition in which endometrial tissue develops outside the uterine cavity in abnormal locations such as the ovaries, fallopian tubes, and abdominal cavity (Zondervan et al. 2018). It affects up to 15% of women of reproductive age and is clinically characterized by chronic pelvic pain and infertility (Parasar et al. 2017). Patients also experience other symptoms such as dysmenorrhea, dyspareunia, dysuria, modified gut transit, intestinal pain, and other urinary disorders. This condition is usually classified into three subtypes based on its histopathology and anatomical locations: superficial endometriosis, deep infiltrating endometriosis, and ovarian endometriotic cysts (endometriomas) (Maruyama et al. 2020).

Endometriosis is a heterogeneous disease with a spectrum of biological features that vary depending on the type of endometriosis (Lagana et al. 2019, Wang et al. 2020). The etiology of this condition remains unknown, although there are three main theories: retrograde menstruation, coelomic metaplasia, and Müllerian remnants; however, a single etiological model is not enough to explain its pathogenesis (Maruyama et al. 2020). This disease seems to result from multifactorial processes involving several genetic, endocrine, immunological, environmental, and microbial factors (Lagana et al. 2019, Wang et al. 2020). Studies regarding the microbiome in endometriosis show a complex relationship between the taxonomical communities and the development of the disease (Leonardi et al. 2020). Some reports on mice models of induced endometriosis have shown changes in the gut microbiota composition induced by the disease through reducing its diversity and abundance (Yuan et al. 2018, Ni et al. 2020).

Since inflammation pathways activation is involved in the endometriosis pathogenesis, the involvement of bacteria and their metabolites has been suggested. The bacterial contamination hypothesis of endometriosis states that microbial components such as lipopolysaccharide (LPS) from *Escherichia coli* interact with TLR4 activating the immune response and promoting inflammation. It has been suggested that these microorganisms should either arrive at the pelvic cavity by translocation from the enterocytes or by ascending migration from the vagina (Khan et al. 2018). In this line, the presence of bacteria at the endometrial level was demonstrated in a case–control study in Asian women with endometriosis (age 21–52 years) using microbiological culture techniques from endometrial smears; the study showed a significantly higher colony formation of *Gardnerella, α-Streptococci, Enterococci*, and *E. coli* in the endometriosis group. They also reported that women treated with gonadotrophin-releasing hormone agonists (GnRHa) display a significantly higher bacterial colony formation of *Gardnerella, Enterococci*, and *E. coli* than the non-treated ones. The authors suggest that these findings could be related to the pathophysiological mechanisms involved in endometriosis, and the increased colony formation of some species could be explained by a direct effect of the higher prostaglandin E2 (PGE2) levels in menstrual and peritoneal fluid on bacterial replication or the indirect immunosuppressive effect of PGE2. Besides, it is proposed that the increased colony formation on GnRHa-treated women may be explained due to a hypo-estrogenic state (Khan et al. 2014). This study is one of the first reports on microbial association with endometriosis pathophysiology. The study is precise and straightforward regarding group criteria and endometriosis diagnosis.

The molecular detection of the endometrial microbiome in endometriosis has been explored and has allowed a better characterization of the taxonomical communities involved in endometriosis pathogenesis. Chen et al. (2017) reported a microbiota study along the FRT in Asian women of reproductive age (32–48 years). This study included samples from the vaginal lower third, posterior fornix, cervical canal, endometrium, and peritoneal fluid and was performed in a large cohort using gene amplicon sequencing (Ion Torrent) and microbiological cultures. This study allowed a comprehensive characterization of the dominant microbiota phenotypes along the upper and lower reproductive tract. It analyzed the correlation of several variables that could be influencing the reproductive tract microbiome, showing that age, body temperature, menstrual cycle, and factors such as infertility and anemia correlate with the microbial phenotypes from some regions (Chen et al. 2017). Although this study was not explicitly addressed to describe the microbiome in pathological conditions, data showed that endometriosis is associated with different microbiota compositions. Moreover, they developed a microbiota-based model that distinguishes subjects with endometriosis-associated infertility, showing that this group of women harbors many operational taxonomic units being bacteria from the phylum Firmicutes such as *Erysipelothrix* sp. the dominant.

Besides, a growing body of literature reports changes in the endometrial microbiota communities in endometriosis patients. Khaleque Khan et al. (2016) conducted a case–control study in Asian women with endometriosis (21–52 years). The molecular characterization of the microbiome was performed from endometrial swabs, endometrioma, and non-endometrioma cystic fluid using a MiSeq system (Illumina, San Diego, CA, USA). Results were reported at the family level, showing a decrease in *Lactobacillaceae* and an increase in *Streptococcaceae* and *Moraxellaceae* in samples derived from women.
with endometriosis. They also found an association with GhRHAs treatment and changes in the microbiota composition in the endometriosis group, with an increase in Streptococcaceae, Staphylococcaceae, and Enterobacteriaceae and a decrease in Lactobacillaceae. In endometrioma cysts, a higher percentage of Streptococcaceae, Staphylococcaceae, Enterobacteriaceae, and Moraxellaceae and a decreased abundance of Lactobacillaceae were found when compared to non-endometrioma cysts (Khan et al. 2016).

A recent study also addressed the microbiota composition in the upper and lower reproductive tract from endometriosis and non-endometriosis women aged 23–44 years of Asian ethnicity. This study analyzed bacterial V4-V5 regions (Ion Torrent Platform) from samples from the lower third vagina, posterior vaginal fornix, cervical mucus, endometrium, and peritoneal fluid from pelvic endometriosis and women without endometriosis who underwent laparoscopic surgery; thus, this research provides detailed microbiota data explicitly associated with endometriosis. Following the previous reports, a reduction of the genus Lactobacillus was detected in endometriosis. Moreover, a higher proportion of Pseudomonas, Acinetobacter, Vagococcus, and Comamonas was found compared to non-endometriosis samples. Like for the signature species reported in endometrium from endometriosis patients, the results showed enrichment of Sphingobium sp., Deltia sp., Pseudomonas viridiflava, and Acinetobacter sp. Interestingly, both Sphingobium sp. and P. viridiflava were also enriched in peritoneal fluid from endometriosis patients, suggesting a general increase in these species, together with a decrease in the genus Lactobacillus, that should play an essential role in endometriosis pathogenesis (Wei et al. 2020).

A more recent publication reported a high bacterial diversity in the endometrial microbiota of endometriosis patients, in contrast to patients with pelvic pain diagnosed with other benign gynecological diseases (by sequencing of V4 region of 16S rRNA gene on Miseq platform). The endometrial taxa enriched in endometriosis were Actinobacteria, Oxalobacteraceae, Streptococcaceae, and Tepidimonas (Wessels et al. 2021); some of them had not been previously reported in other studies of the endometrial microbiome; however, results confirm the alteration of the bacterial composition in women with endometriosis. The authors suggested that the increased bacterial diversity in these patients is an inducer of the immune response involved in the physiology of the disease.

As a whole, these findings highlight the role of the endometrial microbiome in endometriosis pathophysiology; however, the specific role of the enriched species in endometriosis endometrium and the impact of the Lactobacillus decrease should be further explored.

Uterine myomatosis

Uterine myomatosis (hysteromyoma, uterine fibroids, or leiomyoma) is a common benign gynecological condition responsible for most hysterectomy procedures worldwide in women at fertile age (Torres-de la Roche et al. 2017). Myomatosis consists of tumor-like growths of muscular and fibrous tissue embedded in or attached to the uterus wall (classified as submucosal, intramural, or subserosal depending on the profundity of the lump). Patients with this disease suffer from heavy, prolonged, and painful menstrual periods, abnormal bleeding between menses, and infertility (Liu et al. 2013). Despite the growing knowledge about the myriad of molecular factors involved in myomatosis development, such as genetic, epigenetic, hormonal, environmental, pro-inflammatory, angiogenic, and growth factors (Torres-de la Roche et al. 2017), the exact pathogenesis and the participation of the endometrial microbiota in the process is still unknown.

Some authors have proposed the possible involvement of bacteria through inflammation in myomatosis pathogenesis. The effect of E. coli LPS was assayed on primary cultures of isolated fibroblasts from human uterine fibroids. The authors reported the activation of the TLR4/MyD88/NFKB signaling pathway from studied cells upon LPS treatment; besides, a specific TLR4-inhibitor (a viral inhibitory peptide of TLR4, also known as VIPER) and siTLR4 suppressed this effect. These results suggest that inflammation provoked by bacteria also participates in the pathogenesis of uterine fibroids, possibly through inducing cell proliferation (Guo et al. 2015). It is likely that other molecules produced by different bacterial species from a dysbiotic microbiota, both Gram-negative and Gram-positive, participate in the dysregulation of diverse host signaling pathways involved in myomatosis. Therefore, it is relevant to identify the individual species of the endometrial microbiota associated with each gynecological condition and assess their role and the molecular and metabolic pathways involved, individually and as a whole, using in vitro and in vivo assays.

A preliminary study performed by species-specific quantitative PCR determined that endometrial microbiota from women who underwent a hysterectomy due to abnormal bleeding, fibroids, and pain was abundant in L. iners, Prevotella, and L. crispatus, in contrast with the low abundance of G. vaginalis, A. vaginae, and L. Jensenii. The importance of this study lies in the confirmation of a bacterial endometrial community and the fact that their abundance is lower than vaginal microbiota. Significantly, the detected bacteria were not associated with inflammatory processes, indicating that they are not related to infection and suggesting the possible participation of different bacteria-activated signaling pathways in patients diagnosed with benign gynecological diseases (Mitchell et al. 2015). According to these findings,
a 16S rDNA metagenomic analysis (Illumina MiSeq) revealed that Lactobacillaceae, Staphylococcaceae, and Enterobacteriaceae are the groups showing the higher abundance at the family level in endometrium from patients with myomatosis (Khan et al. 2016).

Another study performed by analyzing the 16S rDNA V3-V5 region (Illumina MiSeq) reported the dominance of genera Shigella, Barnesiella, Staphylococcus, Blautia, and Parabacteroides in the endometrial microbiome from a small group of patients with benign diseases, which included endometrial fibroids (Walther-Antonio et al. 2016). Differences with the Khan study (Khan et al. 2016) rely on the chosen sequencing method and the analyzed region of the 16S rRNA gene since sequencing specific 16S regions increases or diminishes the detection resolution of specific taxonomic groups (Abellan-Schneyder et al. 2021). However, in both cases, sequencing does not allow to distinguish between living, dead, or inactive bacteria (García-Grau et al. 2019). Therefore, as mentioned above, some studies simultaneously used molecular and culture techniques to validate their findings (Chen et al. 2017, Moreno et al. 2018).

In contrast, a study performed along the reproductive tract (vagina, cervix, uterus, Fallopian tubes, and ovaries) from a reduced group of patients who underwent hysterectomy and salpingo-oophorectomy due to diverse pathologies found that Proteobacteria and Firmicutes predominantly compose the endometrial microbiota from a patient diagnosed with uterine fibroids in almost equal abundance. Furthermore, Lactobacillus was the most abundant genera (about 50%) (Miles et al. 2017). This reduction in contrast to the Lactobacillus abundance in functional endometrium (Moreno et al. 2016) could be a distinctive disease characteristic. In this case, the authors used 454 pyrosequencing of the 16S rRNA V1-V3 region (Miles et al. 2017).

In general, the reviewed studies about uterine diseases share some limitations, such as the lack of an adequate number of samples, the exclusion of healthy women as controls, the clinical and demographic characteristics of the study population are not homogeneous, the prophylactic administration of antibiotics, and the use of different molecular and sequencing techniques. It is necessary to reach a consensus in the study design to perform more robust analyses that reflect the precise microbiota composition associated with a specific disease.

**Endometrial hyperplasia**

Endometrial hyperplasia is a common uterine alteration characterized by evident morphological changes manifested as an increased number of dilated glands with an augmented gland-to-stroma ratio in contrast to the healthy endometrium in the menstrual cycle proliferative phase and often evolves into cancer (Kubyshkin et al. 2016, Sanderson et al. 2017).

Overgrowth of endometrial tissue is associated with a hyperestrogenic microenvironment and a continuous and increasing inflammatory response (Kubyshkin et al. 2016). According to its inflammatory characteristics, endometrial hyperplasia could be related to microbiota alterations; it has been shown that endometrial hyperplasia samples are enriched with the genera Parabacteroides and Capnocytophaga. Since their structure and α-diversity differ from those of control samples and resembles those of cancer patients, the authors suggest that this bacterial community is a transition to microbiota related to endometrial cancer and hence is involved in the early stages of carcinogenesis (Walther-Antonio et al. 2016).

**Endometrial cancer**

Endometrial cancer is a frequent malign disease in the FRT (Constantine et al. 2019); the specific molecular mechanisms involved in this pathology, such as genetic susceptibility, epigenetic changes, or increased inflammatory response, are well known (Banno et al. 2014); however, its linkage with the microbiota remains undefined. The role of dysbiosis in inflammatory pathways activation during the pathogenesis of endometrial cancer has been suggested, and different research lines have been developed to demonstrate this hypothesis.

In a preliminary study performed with Japanese volunteers, the uterine bacterial composition was analyzed by microbiological culture-based techniques in enrolled patients with different endometrial cancer stages who underwent a hysterectomy. Streptococcus agalactiae, E. coli, Klebsiella pneumoniae, Bacteroides distasonis, and Prevotella bivia were the microorganisms predominantly isolated, in contrast to those found in control group participants (Staphylococcus epidermidis, L. acidophilus, and E. faecalis). Moreover, authors have proposed the potential participation of carcinogenic metabolites, such as n-butyril and n-valeric acids, produced by bacteria in stimulating cell growth during endometrial cancer (Mikamo et al. 1993). However, this hypothesis is yet to be proved.

Interestingly, a risk factor for endometrial cancer is post-menopause since, in this period, an increase in uterine bacterial diversity is observed, which is associated with disorders and pathologies in the FRT. The endometrial bacterial community in post-menopausal women could create conditions that allow establishing a bacterial community related to endometrial cancer (Walsh et al. 2019). In a study that involved nonreproductive-age Caucasian patients diagnosed with endometrial cancer and undergoing hysterectomy, microbial signatures associated with this disease by sequencing V3-V5 regions of the 16S rRNA gene in the endometrium, vagina, cervix, Fallopian tubes, and ovaries were found. Despite sharing some bacterial taxonomic groups, each analyzed
organ contained a specific composition, confirmed by correlation analysis. Endometrial microbiota in cancer patients showed a specific structure, with high diversity and dominance of Bacteroides (Bacteroidetes) and Faecalibacterium (Firmicutes).

In contrast, the endometrium from the control group showed the dominance of Staphylococcus, Blautia (Firmicutes), and Parabacteroides (Bacteroidetes). These findings suggest that endometrial cancer-related bacteria are probably associated with chronic inflammation and disruption of host cellular functions, leading to a carcinogenic process (Walther-Antonio et al. 2016). Nonetheless, like other studies, it lacked a control group composed of healthy women, usually restricted mainly due to ethical issues and failed to validate their findings in a larger cohort.

A more recent study based on V3-V4 16S rRNA gene sequencing (HiSeq, Illumina) reported that Micrococcus (phylum Actinobacteria) is the most abundant genus in patients with endometrial cancer when compared with patients affected by benign uterine pathologies. Genera such as Pseudoramibacter, Megamonas, Eubacterium (Firmicutes), Rhodobacter, Vogesella, Bilophila, and Rheinheimera (Proteobacteria) also showed a high abundance in cancer patients. Interestingly, a positive correlation between Micrococcus abundance and IL-6 (protein and mRNA) and IL-7 (mRNA) levels were observed, which are inflammatory markers associated with the physiopathology of endometrial cancer. This finding supports the hypothesis of an association between endometrial dysbiosis and inflammation during endometrial cancer. However, it was difficult to determine whether the detected bacteria are the cause or the consequence of endometrial cancer (Li et al. 2021). Notwithstanding, this is the first study that found a correlation between uterine cancer-associated inflammation markers and dysbiosis. It is necessary to isolate by culture techniques the species or strains identified through sequencing and determine by individual or co-culture treatments their inflammatory and tumorigenic capacity using both in vitro and in vivo models to confirm the bacterial role in the development of endometrial cancer.

In order to elucidate the uterine microbiome role in endometrial cancer pathogenesis, a transcriptomic analysis has also been performed. Li et al. recently reported the reduction of bacterial diversity and high prevalence of Pelomonas and Prevotella at stage I endometrial cancer, in contrast to patients with benign gynecological diseases. Remarkably, Prevotella positively correlated with burden tumor and hematologic biomarkers of endometrial cancer as D-dimer (DD) and fibrin degradation products. Transcriptomic data identified the upregulation of eight genes involved in pathways associated with fibrin degradation, including those with proteolysis and transcription factor activities. Hence, Prevotella may promote fibrin degradation by inducing the expression of specific genes. Moreover, the authors propose that their identity could be used to predict or diagnose endometrial cancer (Li et al. 2021).

Discovering the molecular mechanisms underlying endometrial cancer has led to searching for alternative treatment strategies. For example, the increased expression and activity of COX-2 in endometrial cancer is related to inflammation, apoptosis inhibition, angiogenesis, and metastasis (Ohno et al. 2005). For this reason, a conventional treatment indicated to reduce the risk of cancer development consists of the administration of non-steroidal anti-inflammatory drugs (NSAIDs) (Zhang et al. 2016). Interestingly, some NSAIDs, such as diclofenac, ibuprofen, and particularly aspirin, have antimicrobial properties by inhibiting processes such as bacterial growth, adhesion, and biofilm formation. Considering the potential role of the cancer-associated endometrial microbiota in the induction of inflammation by stimulating the production of COX-2 in carcinogenesis, NSAIDs will have additive effects in inhibiting the disease progress through inhibiting bacterial growth and inflammation (Kuzmycz & Staczek 2020). However, it is necessary to perform more studies to ascertain the carcinogenic processes induced by bacteria, the influence of NSAIDs in microbial viability, and their potential secondary effects.

As described, recent findings have associated endometrial microbiota alterations with endometrial carcinogenesis, probably involving a pro-inflammatory status. As a result, chronic inflammation could modulate the microbiota composition and structure. Also, the association of specific bacterial taxa with different pathways involved in cancer pathogenesis has been identified, indicating its involvement beyond the alteration of inflammatory processes. However, it is necessary to elucidate the exact composition of endometrial microbiota in healthy women and its variations between ethnic groups and age ranges, which will allow understanding clinical differences and outcomes among diverse populations. It is also crucial to continue identifying the specific bacterial mechanisms involved in the disruption of host cell physiology during carcinogenesis; this will allow managing the cancer progression by altering bacterial—host interactions or blocking the activated signaling pathways.

Perspectives for the endometrial microbiota research

Many studies have demonstrated a female reproductive microbiota and the presence or absence of specific bacterial groups associated with uterine diseases or infertility. However, the road to knowing it thoroughly and understanding its role in maintaining and regulating the human endometrial physiology to be used in clinical and medical practice is still long. Here, we presented the current findings on the endometrial microbiome...
and uterine diseases, from endometrial polyposis to cancer (Fig. 2).

Nonetheless, the reported composition of control groups in the different studies may not represent the eubiotic or ‘healthy’ endometrial microbiota due to fertile women heterogeneity, the demographic and clinical characteristics of the study population, and other factors such as study design, endometrial sampling, sample storage, contamination controls, sequencing techniques, and parameters of bioinformatic analysis among the performed studies must be considered (Molina et al. 2021). Hence, it is necessary to perform more carefully designed studies that allow the discovery of the microbiota signature of each region of the FRT, specifically from the endometrium, and to identify the more susceptible taxa that present variations between a healthy status and benign or malign diseases.

Controversial findings show the requirement of standardizing sampling, culture, molecular, sequencing, and bioinformatics techniques used to perform the microbial characterization of the endometrial microbiota. These adequacies require the appropriate use of contamination controls in each step of the process. From our point of view, one of the most critical factors that influence the results is that in most studies, the enrolled participants cannot be considered representatives of a healthy microbiota. For example, they experienced recurrent implantation failure and pregnancy loss or underwent pre-diagnostic procedures and artificial reproductive techniques (ART) treatments due to infertility or sub-infertility, despite not showing apparent uterine or pelvic cavity abnormalities and had good reproductive outcomes after IVF procedures (Franasiak et al. 2016, Verstraelen et al. 2016). Likewise, some studies recruited patients diagnosed with benign uterine pathologies as control participants. Hence, their uterine microbiota cannot be considered healthy (Khan et al. 2014, Mitchell et al. 2015, Walther-Antonio et al. 2016, Chen et al. 2017). Therefore, results must be considered with caution; even then, their invaluable contributions to the growing knowledge in the area are unquestionable. Table 1 summarizes the studies discussed above, describing sampling strategies, detection methods, and study participants.

The effect of preoperative antibiotics, which gynecologists routinely use for standard procedures like a hysterectomy (Van Eyk et al. 2012), is another crucial factor that can influence the obtained results, which

Figure 2 Schematic representation of the endometrial microbiome composition in uterine-related diseases. The most representative genera from each bacterial phylum are shown across the physiological and pathological conditions. *Lactobacillus* and some *Proteobacteria* are abundant in the eubiotic endometrium, while changes in the microbiome from uterine diseases are observed. Myomatosis displays a high diversity of bacteria, including *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. *Lactobacillus*, *Bifidobacterium*, and *Gardnerella* predominantly compose the endometrial microbiome in polyposis. In endometrial cancer, the bacterial community is mainly composed of *Streptococcus*, *E. coli*, and *Prevotella*. The presence of *Prevotella* and *C. cytoxypophaga* characterizes endometrial dysplasia. Endometriosis is characterized by a predominance of *E. coli*, *Pseudomonas*, *Acinetobacter*, and *Gardnerella*, whereas a decrease in *Lactobacillus* is observed.
Table 1  Endometrial microbiome and microbiological studies in uterine diseases.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Endometrial disease/study group</th>
<th>Subjects, n</th>
<th>Endometrial sampling</th>
<th>Detection method</th>
<th>Abundance of microbiota</th>
</tr>
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<td>Increased</td>
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<td></td>
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<td>Reduced</td>
</tr>
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<td>Mikamo et al. (1993)</td>
<td>Cancer</td>
<td>Swab</td>
<td>Conventional culturing</td>
<td>Streptococcus agalactiae, Escherichia coli, Klebsiella pneumoniae, Bacteroides distasonis, Prevotella bivia</td>
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<tr>
<td></td>
<td>Endometrial cancer</td>
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<td></td>
<td>No endometrial cancer</td>
<td>20</td>
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<td>Walther-Antonio et al. (2016)</td>
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<td>Biopsy</td>
<td>16S rDNA gene V3-V5 region sequencing (Illumina MiSeq)</td>
<td>• Cancer: Bacteroides, Faecalibacterium • Endometrial hyperplasia: Parabacteroides, Capnocytophaga</td>
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<td></td>
<td>Benign uterine conditions</td>
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<td>Cancer</td>
<td>Biopsy</td>
<td>16S rDNA gene V3-V4 region sequencing (Illumina HiSeq)</td>
<td>Micrococcus</td>
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<td>Benign uterine lesions</td>
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<tr>
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<td>Cancer</td>
<td>Biopsy</td>
<td>16S rRNA gene V3-V4 region sequencing (Illumina Miseq)</td>
<td>Pelomonas, Prevotella, Nocardioides, Muribaculum</td>
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<tr>
<td></td>
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<td></td>
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<td>10</td>
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<td>Swab</td>
<td>16S rRNA gene V4 region sequencing (Illumina Miseq)</td>
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<td>Healthy women</td>
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<td>Biopsy</td>
<td>Conventional culturing and targeted PCR</td>
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<td>Khan et al. (2014)</td>
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<td>Swab</td>
<td>Conventional culturing</td>
<td>Gardnerella, α-Streptococci, Enterococci, E.coli</td>
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<td>Swab and cystic fluid</td>
<td>16S rDNA sequence analysis (Illumina MiSeq)</td>
<td>Streptococcaceae, Moraxellaceae lactobacillae</td>
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<td>Lavage fluid</td>
<td>16S rDNA gene V4-V5 region sequencing (Ion Torrent, PGM)</td>
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<td>Biopsy</td>
<td>16S rDNA gene V3 region sequencing (Illumina MiSeq)</td>
<td>Actinobacteria, Oxalobacteraceae, Streptococcaceae, Tepidimonas</td>
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<tr>
<td></td>
<td>Other benign gynecological conditions</td>
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</table>
other authors have also discussed (Mitchell et al. 2015, Miles et al. 2017, Baker et al. 2018). The antibiotic administration potentially alters the microbiota content and abundance in endometrial samples and, in the future, leads to the emergence of antibiotic-resistant strains that eventually act as pathogens. Interestingly, in a recent study performed by Winters et al., the prophylactic antibiotics were administered to the patients after removing the uterus, and bacterial profiles differed from those Lactobacillus-dominated (LD) profiles found by other studies (Winters et al. 2019). Hence, it is necessary to evaluate the effect of commonly used antibiotics during gynecologic surgeries on the viability and physiology of endometrial microbiota and define which antibiotics can prevent post-surgical infections and dysbiosis.

Besides, it is necessary to consider social, cultural, and geographical features of enrolled participants, such as ethnic origin, since it could be related to their endometrial bacterial community structure and composition, as it happens in the vaginal microbiome. For example, Hispanic and Afro-American women predominantly possess the vaginal community state type-IV (non-Lactobacillus-dominated (NLD) microbiota), and this specific composition represents a significant risk for these women to develop bacterial vaginosis (Ravel et al. 2011, Molina et al. 2021). Similarly, the uterine microbiota composition can differ between women from different racial origins (Mitchell et al. 2015). Since studies include a single ethnic group, it is necessary to develop more extensive studies that include healthy women from diverse origins (Verstraalen et al. 2016, Walther-Antonio et al. 2016), and hence establish if a specific group has more risk than others to develop a disease based on its microbiota composition.

Another factor to consider is the bacterial colonization of the endometrial epithelia through biofilms; these structures were reported in the endometrium from patients diagnosed with bacterial vaginosis provoked by G. vaginalis (Swidsinski et al. 2013, Fransaciak et al. 2016). However, so far, no other study has reported the presence of these structures in the endometrium. Due to the scarce information about biofilms, more studies are needed to determine their presence, components, and function in the human endometrium under healthy and pathological conditions. This information will allow us to analyze the possibility of these biological structures as a target in modulating uterine physiology.

On the other hand, uterine immune cells play relevant roles in decidualization, fertilization, embryo implantation, placentation, and pregnancy maintenance; hence, it is required to define their interactions with endometrial microbiota (Agostinis et al. 2019). This knowledge will define if the uterine bacterial community has immunomodulatory properties by participating in the differentiation and function of the endometrial immune cells. It will also be possible to understand how the microbiota alteration is related to infertility and uterine pathologies associated with inflammatory processes.

Finally, the study of the host–microbiota interactions in the upper reproductive tract environment is another promising field that will elucidate the role of this microbial community in regulating uterine physiology and in the pathogenesis of diverse diseases. In this sense, it is necessary to develop in vitro studies using co-cultures containing human endometrial cells and selected microorganisms, evaluating the host and microbial signaling pathways involved. According to Chen and collaborators, bacterial pathways associated with uterine diseases are proliferation, cell metabolism, biosynthesis, motility, secretion systems, genetic information maintenance, and cell communication (Chen et al. 2017). Besides metatranscriptomics, the latter must be analyzed and confirmed in culture-based studies and by metabolomics and metaproteomics methods (Zhang et al. 2019).

Present and future applications of the endometrial microbiome

Even with the relatively recent confirmation of a bacterial community that inhabits the endometrium under no-infectious conditions, the applied analysis of the endometrial microbiome in clinical practice is increasing. Molina et al. (2020) recently published a comprehensive review of the current diagnostic use of endometrial microbiota and treatments to improve uterine bacterial dysbiosis associated with benign diseases and the potential use of healthy endometrial bacteria for regulating the uterine microbial community. This section briefly discusses the role of endometrial microbiota as a biomarker and its potential use in transplantation.

Commercially, the knowledge about endometrial microbiota is applied to routine diagnostic studies for patients undergoing ART. Specifically, Igenomix, a Spanish biotechnology company specialized in reproductive genetics and medicine, created the Endometrial Microbiome Metagenomic Analysis (EMMA) (Molina et al. 2020). EMMA was developed from a study that reported a major reproductive success when the endometrial microbiota is abundant in Lactobacillus (>90%), a community named LD, in contrast with microbiota NLD (Moreno et al. 2016). Similarly, Varinos Inc., a Japanese start-up, performs an endometrial microbiome test based on LD and NLD microbiota (Elnashar 2021). Determining a dysbiotic microbiota allows predicting the reproductive results to propose adequate treatment to infertile patients (Moreno & Simon 2018). Similarly, Igenomix developed the Analysis of Infectious Chronic Endometritis (ALICE) test, which detects pathogenic bacteria associated.
with chronic endometritis in patients with infertility (Moreno et al. 2018).

As mentioned in previous sections, the bacterial components of endometrial microbiota can also be diagnostic and prognostic tools for benign and malign uterine conditions, even in asymptomatic women. It has been possible to identify different bacterial patterns between the endometrium patients undergoing the studied diseases compared to a control group (Fang et al. 2016, Walther-Antonio et al. 2016, Li et al. 2021). However, to use the endometrial microbiome signatures as disease biomarkers, it is required to reach a consensus about its ‘normal’ composition.

Transplantation is another possible application for the endometrial microbiota as a complement to the traditional treatment of patients diagnosed with infertility and uterine diseases. Recent reports suggest the potential for microbiota transplantation into sites other than the gastrointestinal tract, such as the vagina, urinary system, sinuses, and skin, in a strategy called selective microbiota transplantation (Zhang et al. 2018). Vaginal microbiota transplantation has been recently assayed in clinical trials, and it has diminished the recurrence of intractable bacterial vaginosis (Lev-Sagie et al. 2019), which opens the possibilities for the development of uterine microbiota transplantation (Molina et al. 2020). However, it is necessary to consider multiple factors to enable the clinical application of microbiome transplantation, from the good clinical trial design to the ethical, medical, and regulatory issues (DeLong et al. 2019).

It is convenient to evaluate the possible benefits of the most abundant components of the ‘healthy’ community to improve microbiota transplantation in the endometrium. Lactobacillus strains isolated from the lower reproductive tract were recently analyzed by their potential as probiotics in an in vitro model of human endometrial epithelial cells. Among these, L. rhamnosus strain BPL005 is capable of producing short-chain fatty acids, lowering the pH, inhibiting in co-culture the growth of pathogens such as P. acnes and Streptococcus agalactiae, and reducing the levels of pro-inflammatory molecules IL-6, IL-8, and MCP-1 induced by these pathogens in the treated cell cultures. According to these results, the authors propose using L. rhamnosus as part of an intervention strategy that allows restoring the bacterial balance and the healthy physiology of the female reproductive system (Chenoll et al. 2019).

In line with the above, it is required to perform studies directed to isolate by culture techniques uterine Lactobacillus strains or other representative species from healthy fertile women and evaluate their effects and potential benefits on endometrial cells cultures and in vivo models, to be further used in clinical trials. Thus, the endometrial microbiota transplantation or the administration of selected strains that are part of this microbial community as probiotics could be a successful therapy for uterine diseases involving pro-inflammatory processes.

Conclusion

The study of the human endometrial microbiota has been a growing and promising field of knowledge in recent years. It has made it possible to determine the existence of microbial signatures in different uterine pathologies, highlighting the importance of a eubiotic microbiota in maintaining reproductive health.

The characterization of the endometrial microbiota by integrating new generation and traditional techniques will allow us to understand a part of the complex regulation of female reproductive physiology and how its balance is altered in response to a dysbiotic endometrium through the activation of specific bacterial and host pathways. As discussed throughout this review, information obtained from the endometrial microbiota could be applied as a diagnostic tool in gynecological diseases and modify the dysbiotic endometrium to restore a healthy microbiota. However, it is necessary to establish reproducible methodological and analytical techniques to obtain an unbiased bacterial composition and understand how its changes relate to specific environmental and host factors.

Declaration of interest

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

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Author contribution statement

E G-G, and I C-A, conceived the idea and contributed to writing and editing the manuscript; D M-B, and E G-G contributed to the literature survey and writing manuscript; D M-B, I C-A, and E G-G wrote and edited the manuscript for submission; E G-G and D M-B prepared figures and table. All the authors have read and agreed to publish the present version of the manuscript.

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