REVIEW

Biologia Futura: endometrial microbiome affects endometrial receptivity from the perspective of the endometrial immune microenvironment

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Abstract
The existence of Lactobacillus-led colonized bacteria in the endometrium of a healthy human has been reported in recent studies. Unlike the composition of the microbiome in the lower genital tract, that in the endometrium is different and closely associated with the physiological and pathological processes of gynecological diseases. For example, changing the immune microenvironment affects the receptivity of the endometrium, thereby leading to abnormal reproductive outcomes, such as embryo implantation failure and recurrent spontaneous abortion. However, the concrete functions and mechanisms of the endometrial microbiome have not been studied thoroughly. This review elaborates the research progress on the mechanisms by which the endometrial microbiome affects endometrial receptivity from the perspective of endometrial immune microenvironment regulation. Considering the lack of a unified evaluation method for the endometrial microbiome, as well as the lack of an optimal treatment protocol against recurrent spontaneous abortion, we also discussed the application of combining antibiotics with probiotics/prebiotics as precautionary measures.

Abbreviations

AMP	Antimicrobial peptide
APC	Antigen presenting cell
ART	Assisted reproductive technology
DC	Dendritic cell
ERA	Endometrial receptivity array
EVT	Extra-villous trophoblasts
HLA	Human leukocyte antigen
IFN-γ	Interferon gamma
IL	Interleukin
IVF	In vitro fertilization
NK	Natural killer
PAMP	Pathogen-associated molecular patterns
PRR	Pattern recognition receptor
RIF	Recurrent implantation failure
RSA	Recurrent spontaneous abortion
TGF-β	Transforming growth factor-β
Th	T helper
TNF-α	Tumor necrosis factor alpha
Tregs	Regulatory T cells
uNK	Uterine natural killer
WOI	Window of implantation

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Introduction

The human endometrium was considered sterile until some studies using next-generation sequencing of the 16S rRNA gene revealed the existence of an endometrial microbiome represented by Lactobacillus and other bacteria (Baker et al. 2018; Moreno et al. 2020; Li et al. 2018). Despite the low biomass, the endometrial microbiome seems active (Bardos et al. 2019; Tomaiuolo et al. 2020). Recent studies have also reported the effect of the endometrial microbiome on implantation outcomes (Hashimoto and Kyono 2019; Franasiak et al. 2016; Garcia-Grau et al. 2019; Liu et al. 2018; Kitaya et al. 2019; Kyono et al. 2018, 2019; Verstraelen et al. 2016; Moreno et al. 2016;

Saxtorph et al. 2020). Furthermore, non-Lactobacillus-dominated endometrial microbiome has been reported to negatively influence pregnancy outcomes (Younes et al. 2018). Nonetheless, the mechanism by which the human endometrial microbiome affects embryo implantation remains unclear (Hashimoto and Kyono 2019; Franasiak et al. 2016). The synchronization of the development of high-quality embryos and an acceptable endometrium is crucial for the success of embryo implantation (Tohma and Esin 2017). About one-third of implantation failures are caused by embryo quality, whereas the rest are due to poor endometrial receptivity (Idelevich and Vilella 2020). Pregnancy failure is also associated with repeated in vitro fertilization (IVF) cycles in many infertile women undergoing assisted reproductive technology (ART), even if the selected and transferred embryos are high-quality (Benner et al. 2018). Recent studies on the endometrial microbiome have indicated the importance of evaluating the microbial environment to better comprehend endometrial proliferation, embryo apposition/attachment/invasion, and the development of early pregnancy (Tao et al. 2017).

To date, few studies have examined whether the endometrial microbiome can influence maternal intrauterine environment and thus control endometrial receptivity (Younes et al. 2018). Considering previous findings regarding chronic endometritis, vaginal or gut microbiome, and the positive influence of probiotics or prebiotics on IVF outcomes (Hashimoto and Kyono 2019; Al-Nasiry et al. 2020; Benner et al. 2018; Molina et al. 2020; Kayama et al. 2020; Zhou et al. 2018; Khalesi et al. 2019; Trush et al. 2020; Fu et al. 2020; Łaniewski et al. 2017; Pekmezovic et al. 2019; D'Ippolito et al. 2018; Xu et al. 2020), we speculate that the endometrial microbiome may play an important role in the regulation of endometrial receptivity.

The human endometrium is maintained by the equilibrium of several components, including the microbiome, cellular immune response, and cytokines that affect endometrial receptivity (Hashimoto and Kyono 2019; Al-Nasiry et al. 2020; Benner et al. 2018; Molina et al. 2020; Kayama et al. 2020; Zhou et al. 2018; Khalesi et al. 2019; Trush et al. 2020; Fu et al. 2020; Łaniewski et al. 2017; Pekmezovic et al. 2019). The balance of inflammatory factors is important for the regulation of blastocyst adhesion in the epithelial endometrial wall (Bardos et al. 2019; Tohma and Esin 2017). A non-Lactobacillus-dominated endometrium status may break the balance and trigger an inflammatory response in the endometrium, which would affect endometrial receptivity (Hashimoto and Kyono 2019; Moreno and Simon 2018; Haahr et al. 2020). Nevertheless, the role and contribution of the microbiome in endometrial receptivity by regulating the immune microenvironment remain unclear (Mor et al. 2017). In this review, we summarize current research achievements regarding the mechanism by which the endometrial microbiome affects endometrial receptivity from the perspective of immune microenvironment regulation.

The composition and source of the endometrial microbiome in healthy women

Many studies have investigated the composition and source of the "good" endometrial microbiome (Benner et al. 2018; Al-Nasiry et al. 2020; Franasiak and Scott 2017). However, so far, the composition and function of the endometrial microbiome remain controversial (Riganelli et al. 2020). It is generally recognized that a "healthy" female endometrium, like the vagina, is mainly colonized by *Lactobacillus* (Benner et al. 2018; Al-Nasiry et al. 2020; Franasiak and Scott 2017). However, some studies have expressed a different opinion. Verstraelen et al. (Winters et al. 2019) reported that three *Bacteroides* and one *Pelomonas* species were most abundant in 90% of all studied endometrial bacterial profiles. Winters et al. (2019) recently used 16S rRNA gene sequencing to investigate the presence of microbiota in the reproductive tract of 25 women who underwent total hysterectomy for fibroids or endometrial hyperplasia and found that endometrial bacterial profiles were dominated by *Acinetobacter*, *Pseudomonas*, *Comamonadaceae*, and *Cloacibacterium*. Chen et al. (2019) also reported that the endometrial microbiome was not dominated by Lactobacillus, but by Pseudomonas, Acinetobacter, Vagococcus, and Sphingobium.

The estradiol and progesterone serum levels possibly cause changes in the endometrial microbiome, so the composition of the endometrial microbiome and its function may differ in different phases of the menstrual cycle (Carosso et al. 2020). Chen et al. analyzed the microbiome in the reproductive tract of 110 women of reproductive age, showing that the endometrial microbiome varies according to the phase of the menstrual cycle. Specifically, the endometrial microbiome varied between the proliferative and secretory phase (Chen et al. 2017).

Most of the current research considers the endometrial microbiome as the transfer and colonization of the vaginal microbiome due to the continuity and similarity of the two tissues (Baker et al. 2018; Verstraelen et al. 2016) However, the characteristics of the microbiome in the endometrium and vagina are not exactly the same. In approximately 20% of cases, microbial populations between vaginal and endometrial samples were noted to have differences in terms of the identified bacterial taxa and the relative abundance of common bacteria (Moreno and Simon 2018). In addition, microbiome may be transferred from other parts of the body (gut or oral cavity) to the uterine cavity through blood circulation, because of the detection of a close resemblance between microbial communities, and some animal studies

have confirmed that microbiome can be passed through the blood circulating into the uterine cavity (Chen et al. 2019). Other colonization routes may also be related to invasive intrauterine operations (such as the placement or removal of a contraceptive ring, artificial abortion, and ART) (Baker et al. 2018; Verstraelen et al. 2016).

The relationship between endometrial microbiome and endometrial receptivity in healthy women

Endometrial receptivity is a process with intricate steps, during which the embryo can adhere, intrude, and grow into a fetus (Lessey and Young 2019). Implantation of the embryo into the endometrium occurs within a short period (3–4 days), known as the window of implantation (WOI). Under physiological conditions, this occurs on days 6–8 after ovulation (Lessey and Young 2019). Under some specific inflammatory or anatomic situations, this period may be shortened or shifted, thus inhibiting normal implantation and leading to pregnancy loss or infertility (Lessey and Young 2019). The prevention of normal implantation is due to the poor quality of both the embryo and endometrium, with one-third of cases caused by poor embryo quality and two-thirds caused by poor endometrial receptivity, indicating the influence of the latter in infertility and pregnancy loss (Idelevich and Vilella 2020).

Only a few factors that interfere with endometrial receptivity, including endocrine causes, inflammatory events, thin endometrium, granuloma, polyps, septum, and immune-mediated disorders, have been identified (Lessey and Young 2019). To monitor the WOI, several methods have been developed and applied, including endometrial histology, ultrasonography, endometrial receptivity array (ERA), measurement of the levels of blood hormones, integrin family members, leukemia inhibitory factor, and microRNAs (Lessey and Young 2019; Tan et al. 2018). The ERA, a practical molecular dating method, has been used to precisely and repeatedly identify the endometrial receptivity status by analyzing the gene expression profiles (Hashimoto and Kyono 2019; Tan et al. 2018). On the basis of ERA, studies have reported that endometrial factors are responsible for implantation failure in 25%–30% of cases, although the data are limited (Tan et al. 2018). Women implanted with frozen embryos after determining transfer timing by ERA were found to have a higher pregnancy rate per transfer (85.7% vs. 60.8%), suggesting that ERA can be used to guide decisions for improving pregnancy outcomes (Lessey and Young 2019). Unfortunately, each of the aforementioned techniques has limitations, such as high invasiveness, dependency on expertise, sample collection difficulties, and culturing limitations (D'Ippolito et al. 2018). Recent studies have also revealed that the endometrial microbiome affects the outcomes of implantation (Hashimoto and Kyono 2019; Franasiak et al. 2016; Garcia-Grau et al. 2019; Liu et al. 2018; Kitaya et al. 2019; Kyono et al. 2018, 2019; Verstraelen et al. 2016; Moreno et al. 2016; Saxtorph et al. 2020). In most studies, Lactobacillus, as the dominant microbiome species, has been suggested to benefit and support endometrial receptivity (Hashimoto and Kyono 2019; Franasiak et al. 2016; Garcia-Grau et al. 2019; Liu et al. 2018; Kitaya et al. 2019; Kyono et al. 2018, 2019; Verstraelen et al. 2016; Moreno et al. 2016; Saxtorph et al. 2020). However, most studies do not indicate which Lactobacillus species may be more supportive of pregnancy. Riganelli et al. reported that *L. iners* provided significant advantage in the emergence of good pregnancy outcome (Riganelli et al, 2020). In contrast, the pathological endometrial microbiome is associated with implantation failure (Punzón-Jiménez and Labarta 2021). This may be due to the dysregulated microbiome that can induce powerful immune stimulation, resulting in local destructive results, leading to transplantation failure (Makrigiannakis et al. 2021; Sola-Leyva et al. 2021). The precise regulatory mechanisms have not yet been elucidated.

Local immune response regulation of the endometrium

Increasing evidence has shown that the microbiome can affect the phenotypes and functions of immune cells (Benner et al. 2018; Agostinis et al. 2019). However, the interaction between the microbiome and the local immune system remains a complex issue. We hypothesized that healthy women have a normal endometrial microbiome ("eubiosis") under normal circumstances. The microbiome may be quite common and not pathological in many cases and are always checked by well-balanced immune regulation. A balanced inflammatory milieu may depend on the symbiotic relationship between the endometrial microbiome and the congenital and adaptive immune system (Al-Nasiry et al. 2020; Agostinis et al. 2019). Most key immune fighters are involved in uterine immunomodulatory events, including epithelial defenses and a conspicuous number of immune cells, such as natural killer (NK) cells and antigen presenting cells (APCs), such as macrophages and dendritic cells (DCs), T cells, neutrophils, and mast cells (Benner et al. 2018; Agostinis et al. 2019).

Similar to other mucosal tissues, the first line of defense of the female reproductive tract against pathogens is a physical barrier that consists of a mucous layer, IgA antibodies, and the commensal microbiome that limit the colonization of pathogens (Al-Nasiry et al. 2020). Epithelial cells located in the female genital tract prevent pathogens from invading and provide protection by secreting molecules, such as antimicrobial peptides (AMP) (Yarbrough et al. 2015).

Moreover, they are important for directly killing the microbiome, defending proteolytic enzymes from various pathogens, and regulating innate and adaptive immune responses (Al-Nasiry et al. 2020; Yarbrough et al. 2015). In humans, AMPs are associated with key regulatory processes during embryo implantation and the pathogenesis of various pregnancy complications (Al-Nasiry et al. 2020; Yarbrough et al. 2015).

Uterine NK (uNK) cells, as a critical counterpart of the innate immune system, are responsible for 70% of the endometrial leukocytes produced during early pregnancy and the menstrual cycle (Al-Nasiry et al. 2020; D'Ippolito et al. 2018). It remains unknown whether uNK cells are related to embryo implantation, although there is a possibility of successful implantation in NK cell-deficient mice (Le Bouteiller and Piccinni 2008). Notably, an indirect function of human CD56^{bright} and CD16 uNK cells during implantation was reported, considering the abundance of cells and the secreted cytokines, including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), granulocyte–macrophage colony-stimulating factor, and interleukin 10 (IL-10) (Benner et al. 2018).

Macrophages and DCs represent the second most abundant immune cell subset in the endometrium and account for 10%–20% of the decidual leukocyte population (Al-Nasiry et al. 2020). They are also responsible for the surveillance of microbiomes on mucosal surfaces and act as APCs (Al-Nasiry et al. 2020). APCs are equipped with pattern recognition receptors (PRRs), such as Toll-like receptors 1–10 and NOD-like receptors, allowing them to recognize microbial signals during infection, which is known as pathogen-associated molecular patterns (PAMPs), and elicit a protective immune response. PAMPs differ among species and possess molecules from cell membranes (e.g., lipopolysaccharides) and microbial cell walls (peptidoglycans). Immune cells can sense PAMPs through PRRs and establish host–microbe interactions. Receptor expression in endometrial cells decreases during the proliferative phase and increases during the secretory phase (Bardos et al. 2019; Al-Nasiry et al. 2020; Benner et al. 2018). Ligands for PRRs are not exclusive to pathogens and are abundantly produced by the commensal microbiome during healthy colonization (Zheng et al. 2020). Microbial stimulation of PRRs initiates cytokine/chemokine signaling cascades that lead to the secretion of IL-1β, IL-6, IL-8, and TNF-α, and then recruits or activates specialized immune cells (Benner et al. 2018; Villa et al. 2020).

Adaptive immune cells (B and T cells) are responsible for extra-specific and long-period cellular and humoral immunity against pathogens. While B cells are rare in the uterus, the number and phenotype of T cells highly varies between different stages of the menstrual cycle and pregnancy (Al-Nasiry et al. 2020). The number of T cells vary throughout the menstrual cycle, increasing to 50% of the leukocytes in the proliferative phase and decreasing to less than 10% in the secretory phase. Endometrial T cells consist of CD8$^+$ cells (66%) and CD4$^+$ cells (33%), with the latter playing a major role in early pregnancy. CD4$^+$ T cells, including T helper (Th) 1 cells, Th17 cells, regulatory T cells (Tregs), and Th2 cells, secrete specific cytokines that have many functions. However, experimental evidence regarding the role of T cells in the WOI is insufficient. Studies have reported that a shift in the Th1-to-Th2 ratio in the endometrium is necessary for the preparation of implantation (Benner et al. 2018; D'Ippolito et al. 2018). The implantation period is marked by an increase in pro-inflammatory Th1 cytokines, such as TNF-α and IL-12. Therefore, Th1 cells are recognized as a possible benefactor in pathological pregnancy. An increase in Th2 cells during the secretory phase toward early pregnancy is also observed. Th2 cells are stimulated by IL-4, IL-6, IL-10, and IL-11, and are responsible for producing anti-inflammatory cytokines, such as IL-4. Th1 and Th2 cells exert mutual inhibitory effects. In addition, the presence of Th17 cells depends on other factors, such as transforming growth factor-β (TGF-β), IL-6, and IL-21. Th17 cells are the source of pro-inflammatory cytokines, whereas Tregs are involved in inducing tolerance and in negative regulation of immune-mediated inflammation. The local microbiome on the uterine immune system can play a role regardless of whether the Th17 or Treg cell populations are enlarged by TGF-β (Bardos et al. 2019; D'Ippolito et al. 2018).

Immune regulation of sex hormones

The regulation of sex hormones on endometrial immune status may be one reason for the differences in the endometrial microbiome in different periods of menstruation. Sex hormones during the reproductive age dominate the secretion of pro-inflammatory cytokines, chemokines, and AMPs, and contribute to the selection of vaginal microbial species (Al-Nasiry et al. 2020). In particular, estradiol has been related to the shift of the vaginal microbiome from a Lactobacillus-deficient state to a proliferative Lactobacillus state by inducing glycogen synthesis in epithelial cells and the production of glycogen metabolites (maltose, maltotriose, and α-dextrines), which provides substrates for conversion to lactic acid by Lactobacilli (Al-Nasiry et al. 2020; Critchley et al. 2020).

Endometrial microbiome and endometrial receptivity in healthy women

The connection between a receptive endometrium and competent blastocyst involves a series of phases that constitute a successful implantation, including apposition, adhesion, and invasion (Bardos et al. 2019; Idelevich and Vilella 2020). The immune system plays different roles in each step

(Bardos et al. 2019). However, the contribution of the endometrial microbiome to endometrial receptivity by regulating the immune microenvironment remains unclear (Mor et al. 2017). Based on previous findings and currently available knowledge regarding chronic endometritis, the vaginal/gut microbiome (Hashimoto and Kyono 2019; Al-Nasiry et al. 2020; Benner et al. 2018; Molina et al. 2020; Kayama et al. 2020; Zhou et al. 2018; Khalesi et al. 2019; Trush et al. 2020; Fu et al. 2020; Łaniewski et al. 2017; Pekmezovic et al. 2019; D'Ippolito et al. 2018; Xu et al. 2020), the following three possible mechanisms at the feto-maternal interface are proposed (Al-Nasiry et al. 2020) (Fig. 1a):

(1) The commensal microbiome maintains the integrity of the epithelial barrier by activating the production of various AMPs secreted by endometrial cells, preservation of epithelial tight junctions, and stable production of mucus.

(2) Once encountered by immune cells such as APCs in the endometrium, the commensal microbiome triggers signal transduction via PRRs through the respective PAMPs.

(3) The commensal microbiome produces metabolites, including short-chain fatty acids and polysaccharides, which potentially influence the immune responses in endometrial epithelial cells and T cells or change the endometrial fluid pH to form a competitive niche microenvironment to resist the pathogenic microbiome.

These mechanisms may be the reason for the activation of uNK cells and the formation of specific T cells, which are shaped by high Tregs, low Th17, and a shift in Th1-Th2 cytokine secretion. These adaptive changes guarantee an immunotolerant milieu for a semi-allogenic fetus and function as key procedures in normal placentation. The interaction of activated uNK cells via specific receptors (killer-cell immunoglobulin-like receptors) with human leukocyte antigen (HLA)-C and HLA-G from extra-villous trophoblasts (EVT) from the implanting embryo enhances EVT invasion, stromal matrix degradation, angiogenesis, and the reformation of maternal spiral arteries, so as to play an active role in early pregnancy.

The relationship between endometrial microbiome and endometrial receptivity in early pregnancy disorders

The implantation phase resembles the process of tissue injury and subsequent repair. This mild microbial stimulation caused by the commensal microbiome can induce a potentially favorable microenvironment for embryo implantation (Al-Nasiry et al.

2020; Agostinis et al. 2019; Mor et al. 2017; Leshem et al. 2020). However, abnormal composition and/or dysbiosis of the reproductive tract in the endometrial microbiome have been implicated in early pregnancy disorders, such as infertility, recurrent implantation failure (RIF), and recurrent spontaneous abortion (RSA).

Endometrial microbiome and infertility

Recently, a growing number of studies have highlighted the correlation between infertility and the endometrial microbiome (Hashimoto and Kyono 2019; Kyono et al. 2019; Riganelli et al. 2020). The percentage of Lactobacilli in the endometrium of women undergoing IVF was shown to be dramatically lower than that in healthy individuals (Kyono et al. 2018). In addition, studies have shown that various microbiome pollutants present at the tip of the catheter, such as Enterobacter, Streptococcus, and Staphylococcus, may have a negative impact on IVF outcomes during embryo transfer (Al-Nasiry et al. 2020; Mlodzik et al. 2020; Moreno and Simon 2018). Many studies have attempted to correlate the endometrial microbiome with the IVF outcomes of women with infertility, but only one study found a statistically significant difference (Molina et al. 2020). Moreno et al. used 16S rRNA sequencing technology to analyze the endometrial microbiome of 13 women of child-bearing age and of 35 infertile women. The results showed significant increases in implantation rate (61% vs 23%), pregnancy rate (71% vs. 33%), persistent pregnancy rate (59% vs. 13%), and live rate (59% vs. 7%) of women wherein Lactobacillus dominated the microbiome (> 90% Lactobacillus) (Moreno et al. 2016). Kyono et al. used similar research methods and concluded that the content of Lactobacillus in the endometrium of infertile patients was more than 80%, which was sufficient to show a good IVF outcome (Kyono et al. 2019). However, most studies have not found a valid correlation between the endometrial microbiome and IVF outcomes. Despite the above, most studies agree that Lactobacillus is the main component of the endometrium, and that the balanced state of the less diverse endometrial microbiome increases the success rate of IVF in infertile patients.

Endometrial microbiome and RIF and RSA

Early repeated pregnancy failure is a special case of infertility, mainly including RSA and RIF. RSA represents the loss of two or more clinically or biochemically established pregnancies. RIF is defined as the loss of two or more pregnancies after the transfer of high-quality embryos; the two may also occur at the same time. At present, few studies have focused on the endometrial microbiome in women with early
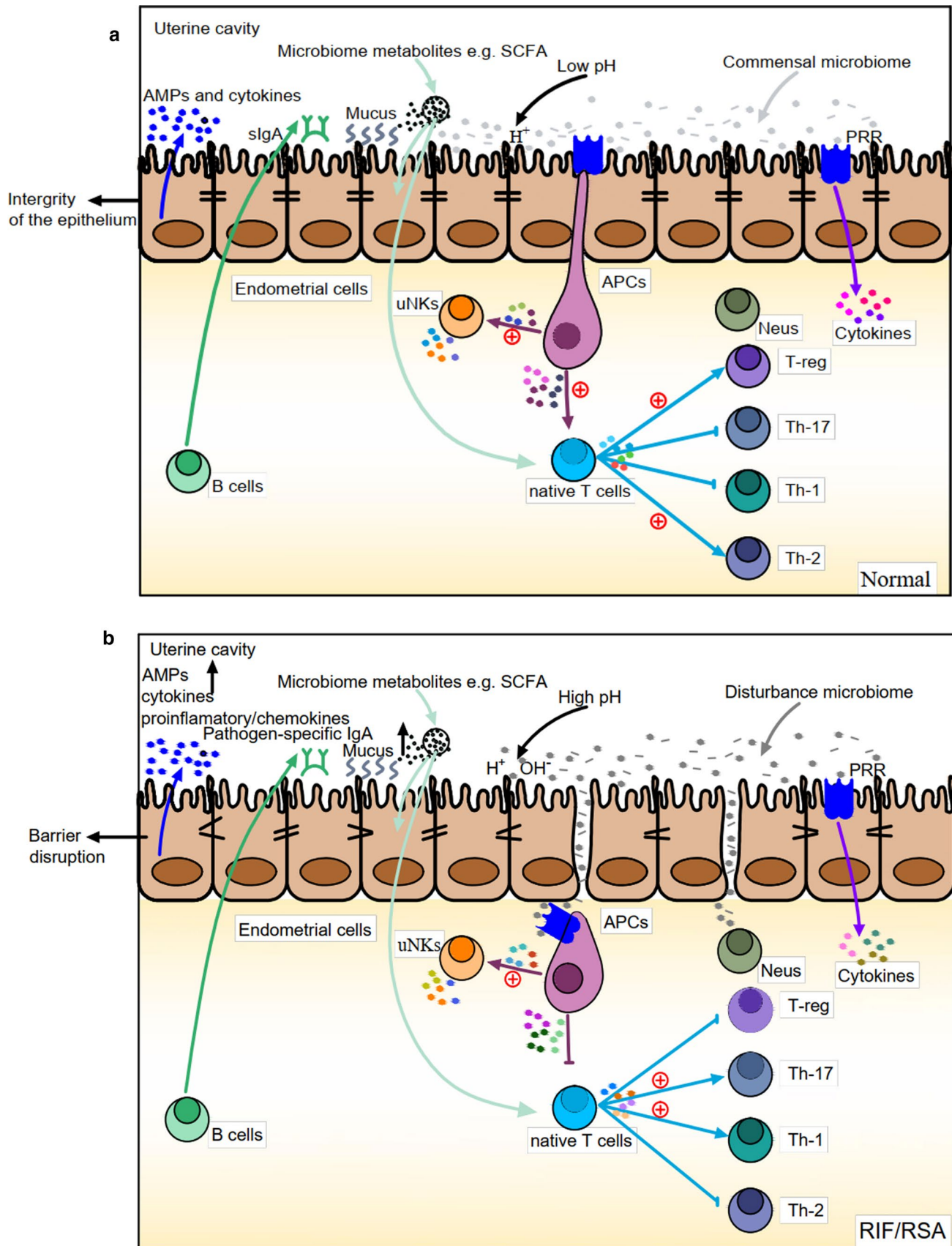
◄**Fig. 1** Interaction between the endometrial microbiome and local immune mediators in normal conditions (**a**) and in RIF/RSA (**b**). **a** In normal conditions, (1) the commensal microbiome maintains the integrity of the epithelial barrier via the activation of AMP production secreted by endometrial cells and the preservation of epithelial tight junctions and stable mucus production; (2) once encountered by immune cells in the endometrium, e.g., APCs, the commensal microbiome triggers signal transduction via PRR through the respective PAMPs; (3) the commensal microbiome produces metabolites, including SCFAs and polysaccharides, thus potentially influencing the immune responses in endometrial epithelial cells and T cells or changing the endometrial fluid pH to form a competitive niche microenvironment to confront the pathogenic microbiome. These mechanisms are probably the reason of the activation of uNK cells and the formation of specified T cells, which are shaped by high Tregs, low Th17, and a shift in Th1-Th2 cytokine secretion. These adaptive changes guarantee an immunotolerant milieu for the semi-allogenic fetus and constitute key procedures in normal placentation. **b** Disturbance in the normal endometrial microbiome can negatively impact the endometrial receptivity by the following mechanisms: (1) dominance of the non-commensal microbiome, thus weakening the epithelial barrier; (2) limited host defense mechanisms and facilitated entering of pathogens in the endometrial stroma. If APCs are encountered, signal transduction is triggered through PRRs and PAMPs. (3) These mechanisms may lead to the activation of uNK cells and changes in specific subpopulations of T cells, either due to the direct effect caused by the invasion of pathogens or the indirect effect caused by the absorption of bacterial products. Such changes in T-cell subpopulations include a decreased number of Tregs, increased number of Th17 cells, and transformation of Th2 cells into Th1 cells, predominated by TNF-α, IFN-γ, IL-2, and IL-10. AMPs, antimicrobial peptides; APCs, antigen presenting cells; IFN-γ, interferon gamma; IL, interleukin; Neus, neutrophils; PRR, pattern recognition receptor; RIF, recurrent implantation failure; RSA, recurrent spontaneous abortion; SCFAs, short-chain fatty acids; Th, T helper; TNF-α, tumor necrosis factor alpha; Tregs, regulatory T cells; uNKs, uterine natural killer cells

repeated pregnancy failure. Verstraelen et al. analyzed the endometrium of 19 women with reproductive failures (RIF, RSA, or both) and found that the majority of colonizing microbiomes belong to the Bacteroidetes and Proteobacteria phyla, which belong to the Bacteroides and Pelomonas genera, respectively. Lactobacillus spp., as the dominant genus, were only observed in four patients (Verstraelen et al. 2016). Kitaya et al. recruited 28 infertile women with a history of RIF and 18 infertile women who initially underwent IVF attempt and embryo transfer (the control group) to analyze endometrial fluid samples. The results showed significant differences in the composition of the endometrial microbiome between women who experienced RIF and the control group. Burkholderia was detected in one-fourth of samples in those who experienced RIF but not in samples from the control group. Reports regarding this bacterium in the human female reproductive tract have not been sufficiently published, and environmental pollution has not been excluded as a possible source (Kitaya et al. 2019). Due to the complex pathogenesis of RSA and RIF and the limited sample size of clinically relevant studies on the endometrial microbiome, it is difficult to obtain meaningful research conclusions at present.

Disturbance of the normal endometrial microbiome negatively impacts endometrial receptivity

In general, early pregnancy disorders have some similarities in terms of their pathogenesis and various risk factors, such as genetics, metabolism, hormones, and immunity. However, the etiology in approximately 50% of patients remains unknown (Al-Nasiry et al. 2020). The endometrial microbiome may constitute a breakthrough point. The mechanism by which the disturbance of the endometrial microbial ecosystem negatively impacts the implantation process is not yet fully understood. The inflammatory response triggered in the endometrium, which adversely affects embryo implantation, may be a possible explanation considering that inflammatory mediators are tightly regulated during the WOI (Bracewell-Milnes et al. 2018).

Corresponding to the reported mechanism of connection between the microbiome and endometrial cells is a disturbed balance, which may occur according to the following mechanisms (Fig. 1b): (1) weakening of the epithelial barrier due to the dominance of the non-commensal microbiome (Al-Nasiry et al. 2020; Benner et al. 2018; Baker et al. 2018); (2) limited host defense mechanisms and facilitated entering of pathogens in the endometrial stroma. If APCs are encountered, signal transduction is triggered through PRRs and PAMPs (Al-Nasiry et al. 2020; Benner et al. 2018; Molina et al. 2020); (3) activation of uNK cells and changes in specific subpopulations of T cells either due to the direct effect caused by the invasion of pathogens or the indirect effect resulting from the absorption of bacterial products. Such changes in T cells may decrease the number of Tregs, increase Th17 cells, and allow transformation of Th2 cells into Th1 cells, predominated by TNF-α, IFN-γ, IL-2, and IL-10. Abnormal uNK cell maturation, either primary or secondary to shallow EVT invasion, may be a potential bridge between a disturbed endometrial microbiome and reformation of the maternal spiral, which negatively impacts pregnancy (Al-Nasiry et al. 2020; Benner et al. 2018).

Current possible interventions based on the microbiome

Due to its diverse etiology, complicated pathogenesis, limited treatment options, and clinical problems in treating infertility, RSA and RIF have yet to be resolved (Kuroda et al. 2021). Therapies based on the endometrial microbiome may provide new diagnosis and treatment modalities. At present, treatments that involve the microbiome as a target mainly comprise antibiotics, probiotics, prebiotics, and microbiome transplantation (Molina et al. 2020). Some companies have developed commercial tests, such as iGenomix®'s EMMA test and Varinos Inc.'s endometrial microbiome test, which can be used to evaluate the

endometrial microbiome (Molina et al. 2020). Endometrial samples are divided into Lactobacillus-dominant and as non-Lactobacillus-dominant. When the sample is classified as non-Lactobacillus-dominant, appropriate treatments including antibiotics, probiotics, and prebiotics are considered (Molina et al. 2020). Hence, providing patients with alternative treatment recommendations still requires more in-depth research on the "core" endometrial microbiome.

Probiotics are active microorganisms that are beneficial to the host. They are biologically active molecules that can promote health when used rationally. They are mainly composed of Lactobacilli and Bifidobacteria (Molina et al. 2020; Trush et al. 2020) and may be an effective alternative to antibiotics for regulating the endometrial microbiome and preventing infection (Molina et al. 2020). Notably, probiotics are not drugs, which means that product information is limited, and only a few probiotics have been fully clinically tested (Haahr et al. 2020). In particular, their application in improving reproduction outcomes remains limited (Garcia-Grau et al. 2019; Kyono et al. 2019). Garcia-Grau et al. (2019) have reported on a patient with RSA, involving ectopic pregnancy and two clinical abortions, who continued to be infected with Gardnerella and other microbes during the 18 months of follow-up, as evaluated by analysis of the microbiome in endometrial fluid samples six times. After using the corresponding antibiotics and probiotic tampon, the microbiome imbalance and pregnancy outcomes did not improve. Combined with sequencing analysis, it was determined that Gardnerella in the uterine cavity was resistant to metronidazole. This shows that probiotics in clinical practice may be effective only if the endometrial microbiome reaches a balanced state.

Prebiotics refer to organic substances that are not digested and absorbed by the host but can selectively promote the metabolism and reproduction of a beneficial microbiome in the body, thus improving the preservation of health. To improve the endometrial microbiome in infertile women. It has been found that the proportion of Lactobacilli in the uterine cavity can be increased by oral prebiotics after antibiotic treatment in 67% (6/9) of patients that are non-Lactobacillus-dominant, indicating that prebiotics are effective in restoring the uterus dominated by Lactobacilli. Nevertheless, the application of prebiotics in the field of human reproduction is still inconclusive, and future research is required to reveal the role of prebiotics in the microbiome of the female reproductive tract. In addition, no case of uterine microbiota transplantation has ever been reported, and such a technique may constitute a future research direction.

In general, although there are limited reports of antibiotics, probiotics, prebiotics, and so on, directly acting on the endometrium, considering that the endometrial microbiome may originate from other parts of the body (vaginal, oral, gut, etc.), from an overall point of view, the above treatment in other parts of the body may also be helpful to adjust the balance of the endometrial microbiome, thereby improving the outcome of pregnancy. However, further research is needed to confirm this indication.

Conclusions for future biology

It is increasingly recognized that the endometrial microbiome influences endometrial receptivity. The currently published data have indicated that Lactobacillus dominancy in the endometrium may benefit embryo implantation, whereas a non-Lactobacillus-dominant endometrial microbiome may impair endometrial receptivity and lead to implantation failure and pregnancy loss. The hypothesis that the endometrial microbiome regulates endometrial receptivity by modulating the endometrial immune microenvironment remains controversial. At present, there are many techniques for detecting endometrial receptivity, but each has limitations. Instead, the microbiome may serve as a new biomarker and may play a role in personalized medicine based on probiotics/prebiotics and uterine microbiome transplantation to improve receptivity, thus ultimately contributing to the health of the female reproductive system. Given the limited understanding of the endometrium microbiome, more randomized controlled clinical trials and mechanistic studies on commensal–pathogen–immune interactions are required in the future. For instance, except for Lactobacillus, some of the microbes detected in the endometrium may be simple residents rather than pathogens. At this time, excessive therapeutic intervention for the endometrial microbiome should be avoided.

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Declarations

References

Agostinis C, Mangogna A, Bossi F et al (2019) Uterine immunity and microbiota: a shifting paradigm. Front Immunol 10:2387. https://doi.org/10.3389/fimmu.2019.02387

Al-Nasiry S, Ambrosino E, Schlaepfer M et al (2020) The interplay between reproductive tract microbiota and immunological system in human reproduction. Front Immunol. https://doi.org/10.3389/fimmu.2020.00378

Baker JM, Chase DM, Herbst-Kralovetz MM (2018) Uterine microbiota: residents, tourists, or invaders? Front Immunol. https://doi.org/10.3389/fimmu.2018.00208

Bardos J, Fiorentino D, Longman RE, Paidas M (2019) Immunological role of the maternal uterine microbiome in pregnancy: pregnancies pathologies and altered microbiota. Front Immunol 10:2823. https://doi.org/10.3389/fimmu.2019.02823

Benner M, Ferwerda G, Joosten I, van der Molen RG (2018) How uterine microbiota might be responsible for a receptive, fertile endometrium. Hum Reprod Update 24(4):393–415. https://doi.org/10.1093/humupd/dmy012

Bracewell-Milnes T, Saso S, Nikolaou D et al (2018) Investigating the effect of an abnormal cervico-vaginal and endometrial microbiome on assisted reproductive technologies: a systematic review. Am J Reprod Immunol 80(5):e13037. https://doi.org/10.1111/aji.13037

Carosso A, Revelli A, Gennarelli G et al (2020) Controlled ovarian stimulation and progesterone supplementation affect vaginal and endometrial microbiota in IVF cycles: a pilot study. J Assist Reprod Gen 37(9):2315–2326. https://doi.org/10.1007/s10815-020-01878-4

Chen C, Song X, Wei W et al (2017) The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nat Commun 8(1):875. https://doi.org/10.1038/s41467-017-00901-0

Chen HJ, Gur TL (2019) Intrauterine microbiota: missing, or the missing link? Trends Neurosci 42(6):402–413. https://doi.org/10.1016/j.tins.2019.03.008

Critchley H, Babayev E, Bulun SE et al (2020) Menstruation: science and society. Am J Obstet Gynecol 223(5):624–664. https://doi.org/10.1016/j.ajog.2020.06.004

D'Ippolito S, Di Nicuolo F, Pontecorvi A et al (2018) Endometrial microbes and microbiome: recent insights on the inflammatory and immune "players" of the human endometrium. Am J Reprod Immunol 80(6):e13065. https://doi.org/10.1111/aji.13065

Franasiak JM, Scott RT (2017) Endometrial microbiome. Curr Opin Obstet Gyn 29(3):146–152. https://doi.org/10.1097/GCO.0000000000000357

Franasiak JM, Werner MD, Juneau CR et al (2016) Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. J Assist Reprod Gen 33(1):129–136. https://doi.org/10.1007/s10815-015-0614-z

Fu M, Zhang X, Liang Y et al (2020) Alterations in vaginal microbiota and associated metabolome in women with recurrent implantation failure. Mbio 11(3):110. https://doi.org/10.1128/mBio.03242-19

Garcia-Grau I, Perez-Villaroya D, Bau D et al (2019) Taxonomical and functional assessment of the endometrial microbiota in a context of recurrent reproductive failure: a case report. Pathogens 8(4):205. https://doi.org/10.3390/pathogens8040205

Haahr T, Jensen JS, Humaidan P (2020) Research and business-the yin and yang in modern medicine. Reprod Biomed Online 40(5):613–616. https://doi.org/10.1016/j.rbmo.2020.01.023

Hashimoto T, Kyono K (2019) Does dysbiotic endometrium affect blastocyst implantation in IVF patients? J Assist Reprod Gen 36(12):2471–2479. https://doi.org/10.1007/s10815-019-01630-7

Idelevich A, Vilella F (2020) Mother and embryo cross-communication. Genes-Basel 11(4):376. https://doi.org/10.3390/genes11040376

Kayama H, Okumura R, Takeda K (2020) Interaction between the microbiota, epithelia, and immune cells in the intestine. Annu Rev Immunol 38(1):23–48. https://doi.org/10.1146/annurev-immunol-070119-115104

Khalesi S, Bellissimo N, Vandelanotte C et al (2019) A review of probiotic supplementation in healthy adults: helpful or hype? Eur J Clin Nutr 73(1):24–37. https://doi.org/10.1038/s41430-018-0135-9

Kitaya K, Nagai Y, Arai W et al (2019) Characterization of microbiota in endometrial fluid and vaginal secretions in infertile women with repeated implantation failure. Mediat Inflamm 2019:1–10. https://doi.org/10.1155/2019/4893437

Kuroda K, Matsumura Y, Ikemoto Y et al (2021) Analysis of the risk factors and treatment for repeated implantation failure: OPtimization of Thyroid function, IMmunity, and Uterine Milieu (OPTIMUM) treatment strategy. Am J Reprod Immunol. https://doi.org/10.1111/aji.13376

Kyono K, Hashimoto T, Kikuchi S et al (2019) A pilot study and case reports on endometrial microbiota and pregnancy outcome: an analysis using 16S rRNA gene sequencing among IVF patients, and trial therapeutic intervention for dysbiotic endometrium. Reprod Med Biol 18(1):72–82. https://doi.org/10.1002/rmb2.12250

Kyono K, Hashimoto T, Nagai Y, Sakuraba Y (2018) Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study. Reprod Med Biol 17(3):297–306. https://doi.org/10.1002/rmb2.12105

Łaniewski P, Gomez A, Hire G et al (2017) Human three-dimensional endometrial epithelial cell model to study host interactions with vaginal bacteria and neisseria gonorrhoeae. Infect Immun 85(3):7441. https://doi.org/10.1128/IAI.01049-16

Le Bouteiller P, Piccinni M (2008) Human NK cells in pregnant uterus: why there? Am J Reprod Immunol 59(5):401–406. https://doi.org/10.1111/j.1600-0897.2008.00597.x

Leshem A, Liwinski T, Elinav E (2020) Immune-microbiota interplay and colonization resistance in infection. Mol Cell 78(4):597–613. https://doi.org/10.1016/j.molcel.2020.03.001

Lessey BA, Young SL (2019) What exactly is endometrial receptivity? Fertil Steril 111(4):611–617. https://doi.org/10.1016/j.fertnstert.2019.02.009

Li F, Chen C, Wei W et al (2018) The metagenome of the female upper reproductive tract. Gigascience 7(10):4110. https://doi.org/10.1093/gigascience/giy107

Liu Y, Wong KK, Ko EY et al (2018) Systematic comparison of bacterial colonization of endometrial tissue and fluid samples in recurrent miscarriage patients: implications for future endometrial microbiome studies. Clin Chem 64(12):1743–1752. https://doi.org/10.1373/clinchem.2018.289306

Makrigiannakis A, Makrygiannakis F, Vrekoussis T (2021) Approaches to improve endometrial receptivity in case of repeated implantation failures. Front Cell Dev Biol. https://doi.org/10.3389/fcell.2021.613277

Mlodzik N, Lukaszuk K, Sieg W et al (2020) Endometrial microbiota-do they mean more than we have expected? Ginekol Pol 91(1):45–48. https://doi.org/10.5603/GP.2020.0010

Molina NM, Sola-Leyva A, Saez-Lara MJ et al (2020) New opportunities for endometrial health by modifying uterine microbial

composition: present or future? Biomolecules (Basel, Switzerland) 10(4):593. https://doi.org/10.3390/biom10040593

Mor G, Aldo P, Alvero AB (2017) The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol 17(8):469–482. https://doi.org/10.1038/nri.2017.64

Moreno I, Codoñer FM, Vilella F et al (2016) Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol 215(6):684–703. https://doi.org/10.1016/j.ajog.2016.09.075

Moreno I, Garcia-Grau I, Bau D et al (2020) The first glimpse of the endometrial microbiota in early pregnancy. Am J Obstet Gynecol 222(4):296–305. https://doi.org/10.1016/j.ajog.2020.01.031

Moreno I, Simon C (2018) Relevance of assessing the uterine microbiota in infertility. Fertil Steril 110(3):337–343. https://doi.org/10.1016/j.fertnstert.2018.04.041

Pekmezovic M, Mogavero S, Naglik JR, Hube B (2019) Host-pathogen interactions during female genital tract infections. Trends Microbiol 27(12):982–996. https://doi.org/10.1016/j.tim.2019.07.006

Punzón-Jiménez P, Labarta E (2021) The impact of the female genital tract microbiome in women health and reproduction: a review. J Assist Reprod Gen. https://doi.org/10.1007/s10815-021-02247-5

Riganelli L, Iebba V, Piccioni M et al (2020) Structural variations of vaginal and endometrial microbiota: hints on female infertility. Front Cell Infect Mi. https://doi.org/10.3389/fcimb.2020.00350

Saxtorph MH, Hallager T, Persson G et al (2020) Assessing endometrial receptivity after recurrent implantation failure: a prospective controlled cohort study. Reprod Biomed Online 41(6):998–1006. https://doi.org/10.1016/j.rbmo.2020.08.015

Sola-Leyva A, Andrés-León E, Molina NM et al (2021) Mapping the entire functionally active endometrial microbiota. Hum Reprod 36(4):1021–1031. https://doi.org/10.1093/humrep/deaa372

Tan J, Kan A, Hitkari J et al (2018) The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers. J Assist Reprod Genet 35(4):683–692. https://doi.org/10.1007/s10815-017-1112-2

Tao X, Franasiak JM, Zhan Y et al (2017) Characterizing the endometrial microbiome by analyzing the ultra-low bacteria from embryo transfer catheter tips in IVF cycles: next generation sequencing (NGS) analysis of the 16S ribosomal gene. Hum Microbiome J 3:15–21. https://doi.org/10.1016/j.humic.2017.01.004

Tohma YA, Esin S (2017) A novel contributor to endometrial receptivity: endometrial microbiota. Gynecol Obstet Res Open J. https://doi.org/10.17140/GOROJ-4-e006

Tomaiuolo R, Veneruso I, Cariati F, Argenio DV (2020) Microbiota and human reproduction: the case of female infertility. High-Throughput 9(2):12. https://doi.org/10.3390/ht9020012

Trush EA, Poluektova EA, Beniashvilli AG et al (2020) The evolution of human probiotics: challenges and prospects. Probiot Antimicro 12(4):1291–1299. https://doi.org/10.1007/s12602-019-09628-4

Verstraelen H, Vilchez-Vargas R, Desimpel F et al (2016) Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1–2 region of the 16S rRNA gene. PeerJ 4:e1602. https://doi.org/10.7717/peerj.1602

Villa P, Cipolla C, D'Ippolito S et al (2020) The interplay between immune system and microbiota in gynecological diseases: a narrative review. Eur Rev Med Pharmaco 24(10):5676–5690. https://doi.org/10.26355/eurrev_202005_21359

Winters AD, Romero R, Gervasi MT et al (2019) Does the endometrial cavity have a molecular microbial signature? Sci Rep-Uk 9(1):1140. https://doi.org/10.1038/s41598-019-46173-0

Xu J, Bian G, Zheng M et al (2020) Fertility factors affect the vaginal microbiome in women of reproductive age. Am J Reprod Immunol 83(4):e13220. https://doi.org/10.1111/aji.13220

Yarbrough VL, Winkle S, Herbst-Kralovetz MM (2015) Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Hum Reprod Update 21(3):353–377. https://doi.org/10.1093/humupd/dmu065

Younes JA, Lievens E, Hummelen R et al (2018) Women and their microbes: the unexpected friendship. Trends Microbiol 26(1):16–32. https://doi.org/10.1016/j.tim.2017.07.008

Zheng D, Liwinski T, Elinav E (2020) Interaction between microbiota and immunity in health and disease. Cell Res 30(6):492–506. https://doi.org/10.1038/s41422-020-0332-7

Zhou JZ, Way SS, Chen K (2018) Immunology of the uterine and vaginal mucosae. Trends Immunol 39(4):302–314. https://doi.org/10.1016/j.it.2018.01.007