Received: 28 August 2018 | Revised: 18 September 2018 | Accepted: 25 September 2018

DOI: 10.1002/rmb2.12249

REVIEW ARTICLE

WILEY Reproductive Medicine and Biology

Deciphering the effect of reproductive tract microbiota on human reproduction

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Abstract

Background: The female reproductive tract contains an active microbiome comprising mainly bacteria from the *Lactobacillus* genus, which is associated with a healthy microbiome state. However, spatio-temporal fluctuations of this microbiome that occur in response to internal and external factors may impact the physiology of the reproductive tract organs and even lead to pathological states.

Methods: Current literature covering the reproductive tract microbiome is summarized and contextualized in this review.

Main findings: This review presents the current knowledge about the bacterial composition of the lower and upper reproductive tract as well as the impact of the microbiota on women's health and reproduction. We place special focus on the impact of the endometrial microbiome in infertility and assisted reproductive technologies.

Conclusion: The assessment of the reproductive tract microbiome adds a new microbiological perspective to human reproduction, pregnancy, and onset of new life, highlighting the importance of considering the evaluation of microbial communities to improve personalized care in reproductive medicine and women's health.

KEYWORDS

dysbiosis, endometrial health, human reproduction, microbiome, reproductive tract bacteria

1 | INTRODUCTION

The female urogenital tract microbiota has long been interrogated using culture to assess the identity of the different microorganisms isolated from this body niche and their impact in reproductive pathophysiology. However, a catalogue of the microbial diversity inhabiting the female reproductive tract has only recently been generated, with the advent of sensitive molecular techniques such as next-generation sequencing (NGS) of bacterial DNA. In the last decade, the Human Microbiome Project has enabled the study of the structure and composition of the microbiome at different body sites, revealing that the female reproductive tract microbiota accounts for approximately 9% of the total bacterial load in humans. This community predominantly comprises *Lactobacilli* in healthy women, although other genera have been identified, namely, *Prevotella*, *Gardnerella*, *Atopobium*, *Sneathia*, *Bifidobacterium*, *Megasphaera*, and *Anaerococcus*.[1-4] This is an interesting finding unique to the human reproductive tract microbiome, as other mammals present a vaginal microbiota not dominated by *Lactobacillus*.[5] Similarly, the male reproductive tract presents an active microbiome, as revealed by the presence of bacteria in seminal fluid samples from infertile men and healthy sperm donors. Interestingly, the bacterial communities found in the seminal samples are associated with semen health. In this regard, *Lactobacillus* may play a protective role; bacteria such as *Anaerococcus*, *Pseudomonas*, or *Prevotella* are mainly found in low-quality sperm.[6,7] These data together suggest that the bacterial communities in the reproductive tract play important roles at different stages of the reproductive process, starting with gamete

formation, fertilization, pregnancy establishment and maintenance, and even the microbial colonization of the newborn.[8] Based on this concept, various studies have sought to define the features and composition of a "normal/healthy" microbiome and establishing the potential shifts leading to a "dysbiotic/abnormal" microbiota.

The aim of this review was to synthesize the current knowledge on the female reproductive tract microbiome and the role of bacteria in human reproduction.

2 | FEMALE REPRODUCTIVE TRACT MICROBIOTA

The majority of studies on the female reproductive tract microbiome are focused on the vagina because of the ease of sampling; yet, several studies have demonstrated the existence of bacterial colonization beyond the vagina, showing that the upper reproductive tract is not sterile. For example, an active uterine microbiome has been characterized in healthy reproductive-age women,[9-11] but bacteria have also been found to inhabit the fallopian tubes and the ovaries,[12] with *Lactobacillus* the most abundant genus throughout the female reproductive tract.[13] Recently, a study surveying the female reproductive tract confirmed the existence of a microbiota continuum starting in the vagina and progressing to the deepest organs in the tract—cervix, uterus, tubes, ovaries, and even colonizing the pouch of Douglas—in women with non-infectious conditions.

2.1 | Lower genital tract

The microbiome of the vagina in healthy reproductive-age women presents a biomass of approximately one billion bacteria per gram of vaginal fluid with low diversity, mainly composed of one or few *Lactobacillus* species, representing 90%-95% of the total bacteria in the reproductive tract.[14,15] However, the vaginal microbiota is not dominated by *Lactobacillus* throughout a woman's lifetime. Indeed, in childhood, anaerobes and *Escherichia coli* predominate.[16,17] After puberty, the estrogen rise leads to the production and accumulation of glycogen, which is essential for *Lactobacillus* growth and the colonization of the vaginal epithelium; the dominance of *Lactobacillus* is maintained during the reproductive years. Finally, after menopause, the proportion of *Lactobacillus* species decreases again due to the drop in endogenous estrogen. Interestingly, the *Lactobacillus* content, as well as a low vaginal pH, is maintained in women receiving hormonal replacement therapy during menopause.[18-20]

The first description of the vaginal microbiome in a set of 396 reproductive-age women using NGS for the 16S rRNA bacterial gene revealed the existence of five distinct community state types (CSTs) depending on the abundance of the bacteria identified. CST-I, -II, -III, and -V are dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii*, respectively.[21] The role of *Lactobacillus* is to maintain vaginal homeostasis by producing lactic acid to lower the vaginal pH.[22,23] This feature, together with the production of hydrogen peroxide, bacteriocins, and other antimicrobial compounds, facilitates the adhesion of *Lactobacilli* to the vaginal epithelial cells and competition for the nutrients in the niche to deter the growth of pathogenic bacteria.[24-28] In contrast, CST-IV is characterized by increased diversity due to polymicrobial colonization with facultative anaerobic bacteria such as *Gardnerella*, *Prevotella*, *Megasphaera*, *Atopobium*, and *Dialister* to the detriment of *Lactobacilli*.[21] Shifts between different CSTs occur in women, sometimes involving acquisition of the CST-IV microbiome. In fact, sexual activity and the transition through CST-IV are risk factors for bacterial vaginosis (BV).[29] However, the CST-IV profile is also common in asymptomatic women depending on their racial background. The percentage of women with a lower genital tract dominated by *Lactobacillus* is 90%, 80%, 60%, and 60%-37% in White, Asian, Hispanic, and Black populations, respectively.[30] The variation of microbiota profiles in these populations may reflect not only racial or genetic predisposition to one or other types of bacteria, but also geographic, social, and/or economic factors.[30] Among the endogenous factors known to contribute to microbiome changes are hormonal changes during the menstrual cycle. These changes are associated with shifts in vaginal bacterial content, with menses representing the phase in which the microbiome is more diverse, while the oestradiol and progesterone peaks are more bacterially stable times.[31] Some external factors can also modulate the vaginal microbiota, such as hygiene habits, sexual exposure, change of sexual partners, and use and type contraceptives.[31-36] The factors influencing the structure and composition of the cervicovaginal microbiota were recently reviewed by Kroon and colleagues (Figure 1).[37]

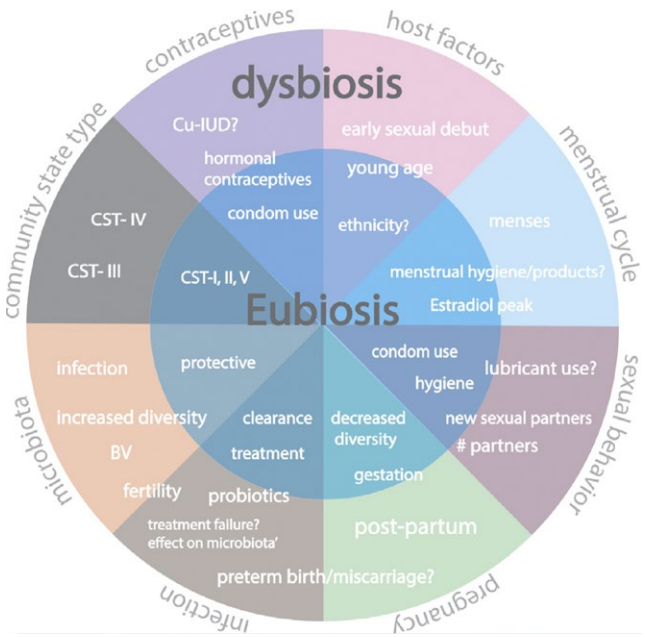

FIGURE 1 Factors influencing the composition of the cervicovaginal microbiota. Reprinted from Kroon et al,[37] with permission from Elsevier

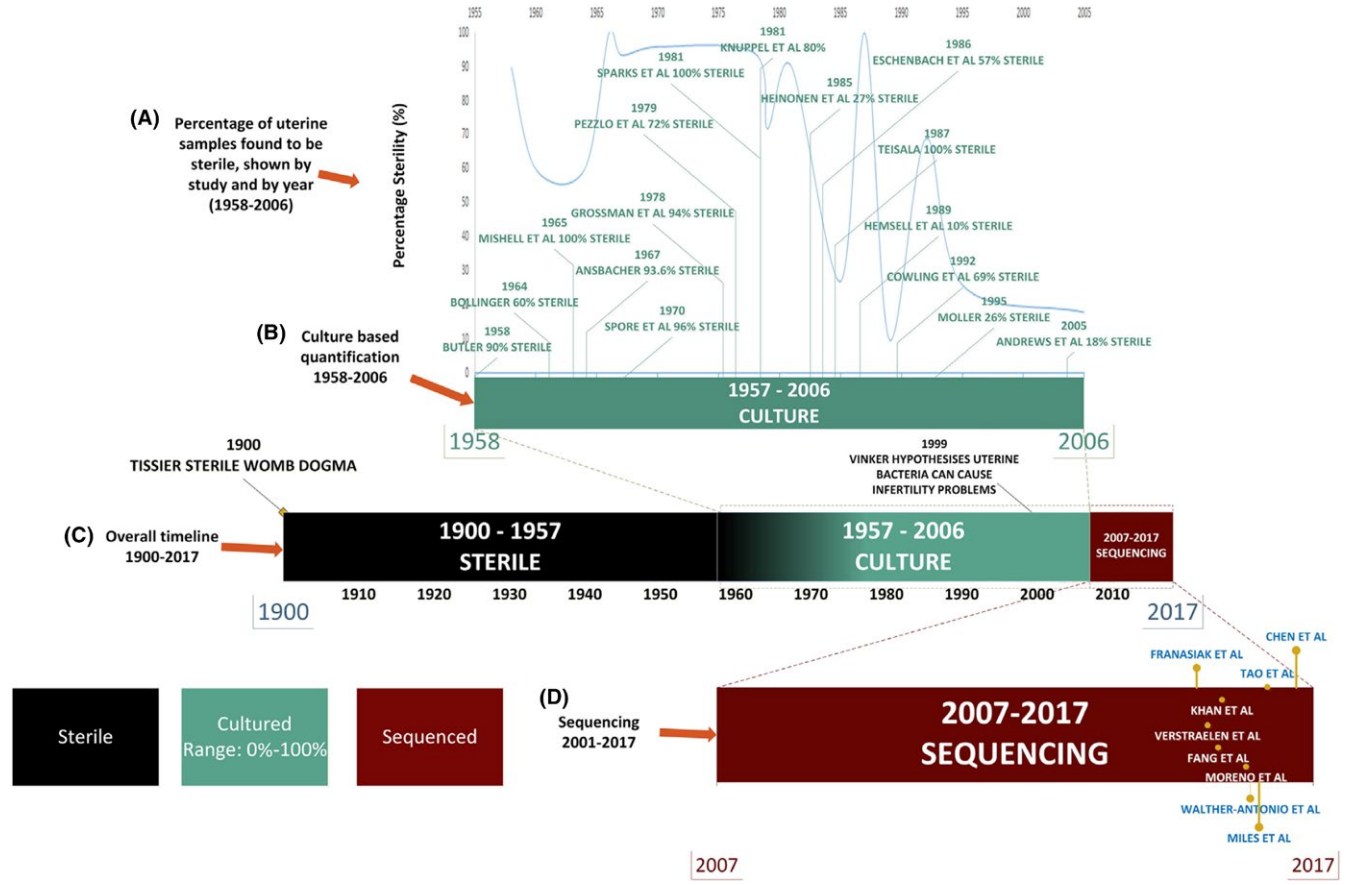

FIGURE 2 History of uterine microbiota investigation. Reprinted from Bakeret al,[100] under the terms of the Creative Commons Attribution License (CC BY)

2.2 | Upper genital tract

Our knowledge about the upper reproductive tract is not as wide, but it is now accepted that the internal organs also harbor a low but active biomass microbiota (Figure 2). The first evidence of bacterial colonization of the human uterine cavity was reported more than 30 years ago following cultivation of endometrial samples obtained either transcervically or after hysterectomy.[38-41] Bacteria (most often *Lactobacillus* spp, *Mycoplasma hominis*, *Gardnerella vaginalis*, and *Enterobacter* spp) were recovered in at least 25%-30% of samples cultured. Later, molecular techniques such as polymerase chain reaction (PCR) were used to demonstrate that the upper genital tract, particularly the uterus, presents bacterial taxa other than those found in paired vaginal samples in both healthy women and women with BV.[9] In this study, 95% of the endometrial samples analyzed tested positive for bacterial DNA, although the overall number of recovered bacteria was significantly lower in endometrial than in vaginal samples. This observation was recently supported by the results of Chen and co-workers, who quantified the bacterial load of samples collected along the reproductive tract using quantitative PCR and 16S rRNA NGS to show that the upper reproductive tract (peritoneal fluid and endometrium) contains 10 000 times less bacteria than the vagina.[42] The quantitative differences in bacterial load observed between the lower tract and the upper tract could be due to the cervical barrier, which may partially inhibit the ascension of bacteria from the vagina. Other hypotheses suggest a specific immune response in the internal organs or differential environmental conditions can lead to differential bacteria growth in both ends of the reproductive tract.[9] Despite bearing an ultralow biomass, this is an active microbiota, as demonstrated by the isolation of *Lactobacillus*—the most abundant bacteria in the reproductive tract—*Actinomyces* and *Staphylococcus*, among other bacterial genera, upon cultivation of fresh peritoneal fluid samples.[42]

Despite some inconsistencies owing to differences in experimental design, several reports to date agree that the most abundant phyla in the uterine cavity are Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria.[10,42-44] Moreover, the genus *Lactobacillus* has been consistently identified as the most represented taxa in the endometrium,[9,10,44,45] while *Gardnerella*, *Streptococcus*, *Staphylococcus*, *Bifidobacterium*, *Prevotella*, *Atopobium*, and *Sneathia* are present in smaller proportions.[10,11,45]

Because it is difficult to obtain samples from the upper genital tract of healthy women, few studies have reported on the "normal" upper reproductive tract microbiome. Using 16S rRNA gene sequencing, the endometrial microbiome of fertile and healthy patients was investigated using minimally invasive methods for the collection of endometrial fluid, and this was compared to vaginal aspirates of the same subjects. This study confirmed that *Lactobacillus*

is the most abundant genus in endometrial samples; *Gardnerella*, *Bifidobacterium*, *Streptococcus*, and *Prevotella* were also detected, all of which were previously identified in the lower reproductive tract.[10] Further, this study helped to classify the endometrial microbiota profile into *Lactobacillus*-dominated (LD) and non-*Lactobacillus*-dominated (NLD) according to the abundance of this bacterial genus (cutoff 90%) and its value to predict reproductive success (see below).

Consistent with previous findings, women usually present similar microbiome profiles in the upper and lower genital tract. However, 20% of the women in whom the bacterial taxa were identified in endometrial samples showed significant differences in their paired vaginal aspirates.[10] Moreover, this study showed in 22 fertile and asymptomatic donors that the endometrial microbiota is stable during the 5-day period in which endometrial receptivity—the ability to enable embryo implantation—is achieved, between the pre-receptive state (2 days after the LH surge) and the receptive state (7 days after LH peak).[10] These results were corroborated in a study assessing vaginal and endometrial samples of healthy volunteers, non-infertile patients, and patients receiving in vitro fertilization (IVF) treatment in Japan. This study showed that the healthy volunteers presented LD microbiota (≥90% *Lactobacillus* species), with 25% of the women presenting different taxonomic profiles in endometrial and vaginal samples.[11] When healthy volunteers were sampled again in different phases of the cycle or in the consecutive cycle, the microbial profiles were similar, suggesting that the endometrial microbiome may be stable over time. Importantly, however, the sample size was small, and other studies are needed to support this conclusion.[11] The results of these studies support the idea of a microbiota continuum along the reproductive tract and ascension from the vagina as the most plausible mode of upper genital tract colonization, as proposed after *G. vaginalis* biofilms were found in the fallopian tubes of patients diagnosed with BV.[46] Migration of microorganisms from other organs/tracts to the reproductive tract via hematogenous spreading has also been suggested based on the similarities between the cervical and gastrointestinal tract microbial features in obese patients, or the correspondence between oral and placental microbiota in pregnant women.[47,48]

3 | ROLE OF BACTERIA IN WOMEN'S HEALTH

The upper reproductive tract microbiota is associated with physiology; thus, any unbalance of the microbial composition may impact its functionality, making it a risk factor for many gynecological conditions (reviewed in [[49]]).

For example, pelvic inflammatory disease (PID) is the inflammation of the upper genital tract caused by the infection with *E. coli*, other BV-associated pathogens (*Atopobium vaginae*, *Sneathia sanguinegens*, *Sneathia amnionii*, among others) or sexually transmitted bacteria (*Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Neisseria gonorrhoeae*).[50,51] This disease results in abdominal pain and affects the uterus and the fallopian tubes, leading to infertility.

Additionally, several studies revealed a positive association between the presence of pathogenic bacteria in the uterine cavity and the onset of the disease. The increased isolation of *Actinomyces*, *Corynebacterium*, *Enterococcus*, *E. coli*, *Fusobacterium*, *Gardnerella*, *Prevotella*, *Propionibacterium*, *Staphylococcus*, and *Streptococcus* from endometrial samples and menstrual blood from patients diagnosed with endometriosis has established the evidence for this association.[52-54] Further, the reproductive outcomes of patients with endometriosis are significantly improved after antibiotic therapy, underscoring the impact of pathogens in the disease.[55] Molecular techniques have also isolated bacteria from the Staphylococcaceae and Streptococcaceae families from cystic fluid of endometrioma lesions,[54] and the reproductive tract microbiota of endometriosis patients has proven different to that of women with infertility due to other aetiologies,[42] revealing an altered microbiome as a potential cause of inflammation that may trigger abnormal uterine contractility and the retrograde seeding of endometrial tissue in the peritoneal cavity.[56,57]

Chronic endometritis (CE), the best-studied subclinical inflammation of the endometrial lining, is mainly caused by infection with common bacteria such as Enterobacteria, *Streptococcus* species, and *Enterococcus faecalis*, as well as *Chlamydia*, *Mycoplasma*, and *Ureaplasma* species, although yeast can be also involved,[58] to the detriment of the protective LD microbiota.[59] The reported prevalence of this disease in reproductive-age women varies from 8% to 72%, and it is associated with repeated implantation failure and recurrent pregnancy loss.[58-60] However, because it is often asymptomatic, it is rarely suspected, diagnosed, and treated. One of the challenges of CE management today is the lack of universally standard criteria for the diagnosis of the disease (ie, number of plasma cells identified per area of screened tissue sections). While the current gold-standard method relies on histopathological identification of plasmacyte infiltrations in the endometrial stroma coupled to CD138 immunohistochemistry, other authors promote hysteroscopic examination of the uterine cavity or the classical culture of endometrial specimens.[58,60,61] However, many confounding factors affect the assessment of CE: the method of analysis, the criteria used to discriminate positive and negative samples, the phase of the cycle in which the endometrium is evaluated (ie, the estimated prevalence of CE is approximately 50% higher in endometrial samples collected in the proliferative vs the secretory phase), or even the reagents used in the laboratory developing the test.[60,62] The most objective of the classical methods is microbial culture, which allows for the isolation of the causing bacteria. Yet, interestingly, the bacteria cultured from endometrial biopsies of CE patients are different from those found in the vagina of the same patients.[63] A comparative study of 65 patients subjected to CE evaluation using the three classical methods—histology/CD138, hysteroscopy, and microbial culture—revealed a poor concordance between methods, resulting in only 20% (13 out of 65) of triple-positive or triple-negative results with all the methods used.[59] Thus, diagnosis of CE depends highly

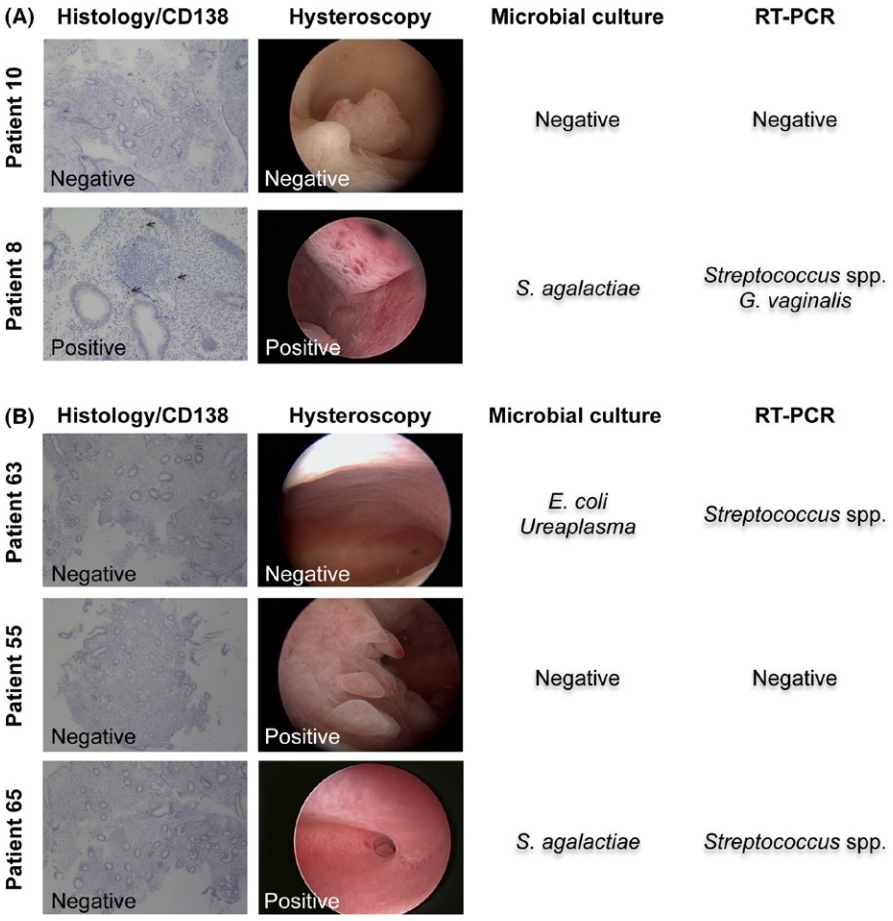

FIGURE 3 Diagnosis of chronic endometritis depends on the method used. A, Examples of concordant cases using evaluation strategies. B, Examples of discordant cases using evaluation strategies. Reprinted from Moreno et al,[59] with permission from Elsevier

on the method used (Figure 3). However, the molecular detection of CE-causing pathogens using targeted PCR or NGS presents a high accuracy (77%) with the results of the three classical methods together and provides information about the identity of those pathogens, even if they are not easy to culture.[59] Thus, the molecular evaluation of CE should be considered in clinical practice because it offers a more objective and reliable diagnostic method to improve clinical management and enable a personalized treatment based on the pathogens identified.

4 | ROLE OF BACTERIA IN HUMAN REPRODUCTION

Reproductive tract microbiota at the embryo-maternal interface is receiving increasing attention in human reproduction because it may impact not only the chances of achieving a pregnancy, but also the health status of the mother and the child before and after delivery. The vaginal microbiome of pregnant women who deliver at term is abundant in *Lactobacilli*, with a significant dominance of CST-I, -II, -III, and -V, whereas high levels of *Gardnerella*, *Ureaplasma*, or other bacteria belonging to the CST-IV are often associated with preterm birth and obstetrical complications.[64-69] On the other hand, the postpartum vaginal microbiome is characterized by a shift to CST-IV even in those pregnancies in which a high *Lactobacillus* abundance was maintained throughout pregnancy.[69] The dominance of *Lactobacillus* in the vagina during pregnancy could be explained by the increase in estrogen levels, but it is also hypothesized that this protective microbiome serves as a barrier to prevent the growth of potential pathogens in the fetal membranes and placenta. For this reason, dysbiotic deviations from the LD vaginal profile during pregnancy result in increased risk of miscarriage, premature rupture of membranes, and preterm birth.[70-72] However, these associations have been mainly reported in White women and could be related to the host background, based on the lack of significant association between BV and preterm birth in Black women.[73]

Another upper genital tract infection, deciduitis, is the inflammation of the maternal tissue in the basal plate of the placenta. This infection is proposed to be the consequence of untreated preconception chronic endometritis that produces immune and inflammatory responses at the maternal-fetal interface, leading to preterm labor.[74,75] From the fetal side, infection of the fetal membranes, known as chorioamnionitis, is also an important obstetrical complication caused by bacteria ascending from the vagina and colonizing the choriodecidual space and the amniotic fluid. The most commonly isolated pathogens from amniotic fluid are Bacteroides species, *E. coli*, *G. vaginalis*, *M. hominis*, *Peptostreptococci*, *Streptococci*, and *Ureaplasma urealyticum*, but in some cases, the amniotic inflammation is accompanied by negative microbial cultures, highlighting the importance of high-sensitivity molecular diagnosis of the

microbiome in low biomass environments to detect and prevent severe obstetrical complications.[76,77]

4.1 | Role of microbiota in infertility and assisted reproductive technologies (ART)

Evidence from several groups indicates that infertile patients harbor a differential reproductive tract microbiota (lower and/or upper) compared to healthy and fertile women.[4,78-80] Thus, it is worth considering whether IVF outcomes could be influenced by the microbial taxa present in the reproductive tract during infertility treatment. Indeed, deviations from the low-diversity vaginal microbiome, including BV, have been significantly correlated with decreased pregnancy rates after IVF.[81,82] The association between endometrial dysbiosis and ART failure has long been studied using classical bacterial culture of the tip of the transfer catheter used for embryo transfer. Since the mid-1990s, the isolation of *Enterococci*, Enterobacteriaceae (*E. coli* and *Klebsiella pneumoniae*), *Streptococci*, *Staphylococci*, and/or Gram-negative bacteria has been correlated with lower implantation and pregnancy rates and increased miscarriage rates, while isolation of *Lactobacilli* or samples with negative cultures for the aforementioned pathogens has been correlated with better reproductive results.[83-87] Using the same strategy, the isolation of *Streptococcus viridans* was significantly correlated to decreased live birth rate, with only 7% of successful pregnancies compared to 88% when *Lactobacillus* was isolated from the catheter tip at the time of embryo transfer.[85] These studies have now been confirmed using NGS, which is able to characterize the endometrial microbiota at the molecular level in infertile patients with different ART indications: unexplained infertility, repeated implantation failure (RIF), and recurrent pregnancy loss (RPL).[10,11,43,44] The percent composition of *Lactobacillus* in the endometrium differs between healthy volunteers (85.7%), non-IVF patients (73.9%), and IVF patients (38%), showing that a high percentage of infertile patients subjected to ART present an abnormal endometrial bacterial profile.[11] This is consistent with previous findings showing that 46% (15/32) of IVF patients with a receptive endometrium presented an NLD microbiota.[10] The first report on the endometrial microbiome's influence on ART using 16S rRNA gene sequencing found that *Lactobacillus* and *Flavobacterium* were the most represented genera in the tip of the embryo transfer catheter; however, there was no statistically significant association between the assessed

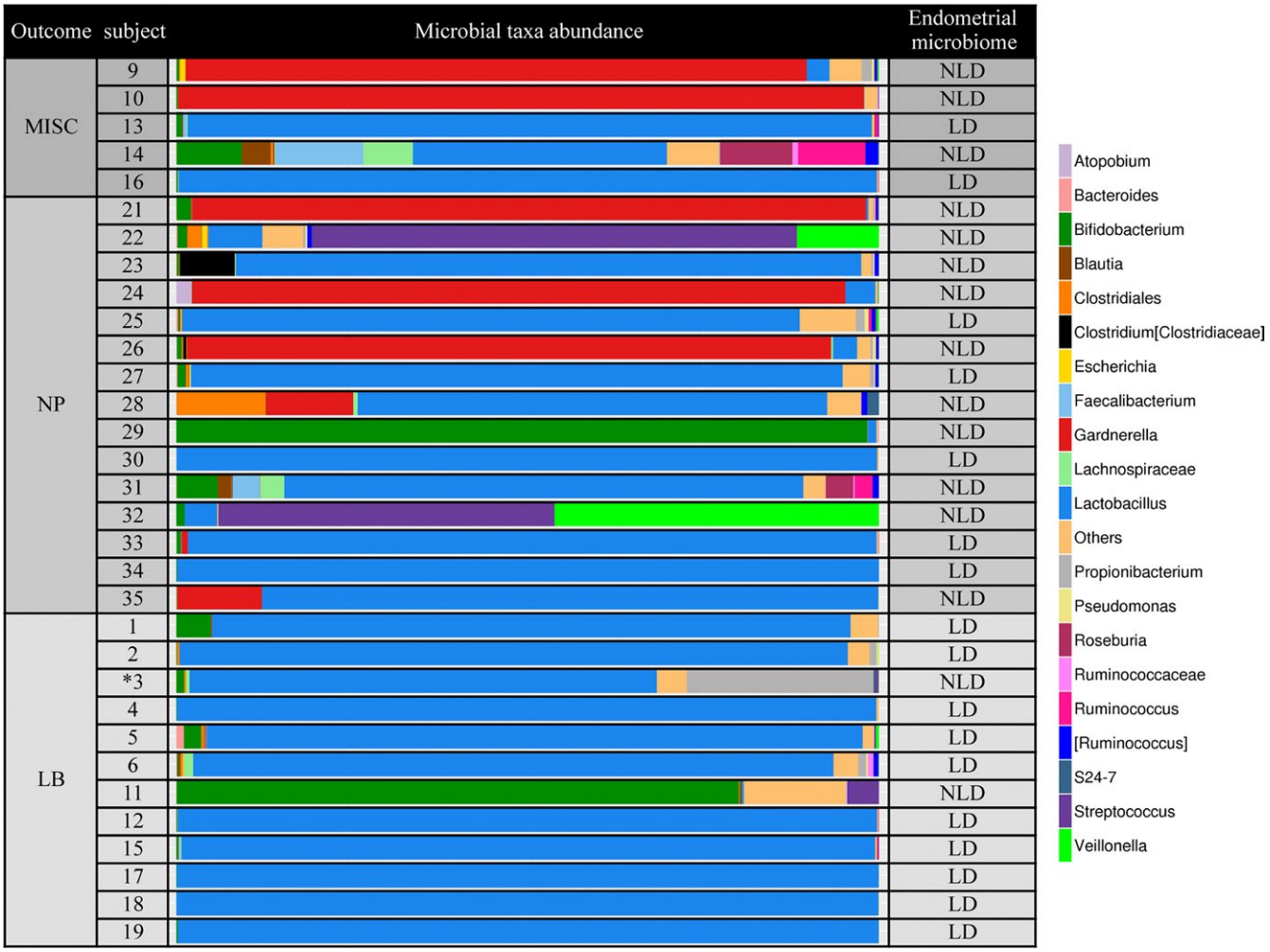

FIGURE 4 The abundance of *Lactobacilli* in endometrial fluid samples is associated with reproductive outcomes in ART patients. Reprinted from Moreno et al,[10] with permission from Elsevier

microbiome and the reproductive outcomes of those patients.[44] Subsequently, the uterine microbiome was investigated by 16S targeted sequencing of endometrial fluid collected from 35 RIF patients with a receptive endometrium. Here, the percentage of *Lactobacilli* was indeed associated with reproductive outcomes (Figure 4). NLD microbiota strongly associated with poor reproductive outcomes, compared to patients with LD microbiota, in terms of implantation rate ($P = 0.02$), pregnancy rate ($P = 0.03$), ongoing pregnancy ($P = 0.02$), and live birth rates ($P = 0.002$).[10] Interestingly, these results have been partially confirmed by a group evaluating the endometrial fluid of 79 IVF patients and its impact on reproductive outcomes. In that study, 62% of infertile patients receiving ART presented an NLD microbiome, and interestingly, patients achieving a pregnancy after IVF presented an average content of *Lactobacillus* of 96.5% ± 33.6%, suggesting that an LD endometrium might favor implantation.[11] Together, these results support the relevance of uterine health, even at the microbiological level, for a successful implantation and pregnancy, and point to endometrial infections (ie, CE) as causes of infertility. The estimated prevalence of CE in infertile patients ranges from 0.2% to 46%.[88] CE is also an additional risk factor for infertility in RIF patients; while RIF patients present an implantation rate of 46% after embryo transfer, this rate falls to 15% in RIF patients with concomitant CE.[89]

4.2 | Strategy to restore microbiota and to improve reproductive outcome

At the end of the 20th century, Egbase et al unequivocally demonstrated the relevance of the reproductive tract microbiota for reproductive success. In this study, the authors compared the bacteria isolated from the transfer catheter tip of a mock transfer performed at oocyte retrieval—when prophylactic antibiotics were administered—and at the time of embryo transfer—performed 48 hours later—with their pregnancy results (Egbase et al[83]). The results of this study showed that isolation of endometrial pathogens (*Enterococcus*, *Staphylococcus*, *E. coli*, and other mixed cultures) at embryo transfer correlated with decreased clinical pregnancy rate per transfer compared to those with negative cultures or those that have responded to prophylactic antibiotics after a positive culture at egg retrieval (18.7% vs 41.3% and 38.1%, $P < 0.01$, respectively).[90]

Following this study, it is logical to hypothesize that decreasing the numbers of bacterial pathogens in the reproductive tract and increasing the proportion of beneficial *Lactobacillus* could improve reproductive outcomes in those patients with an abnormal microbiota. To address this, two different approaches are under study: the use of antibiotics and the use of probiotics.

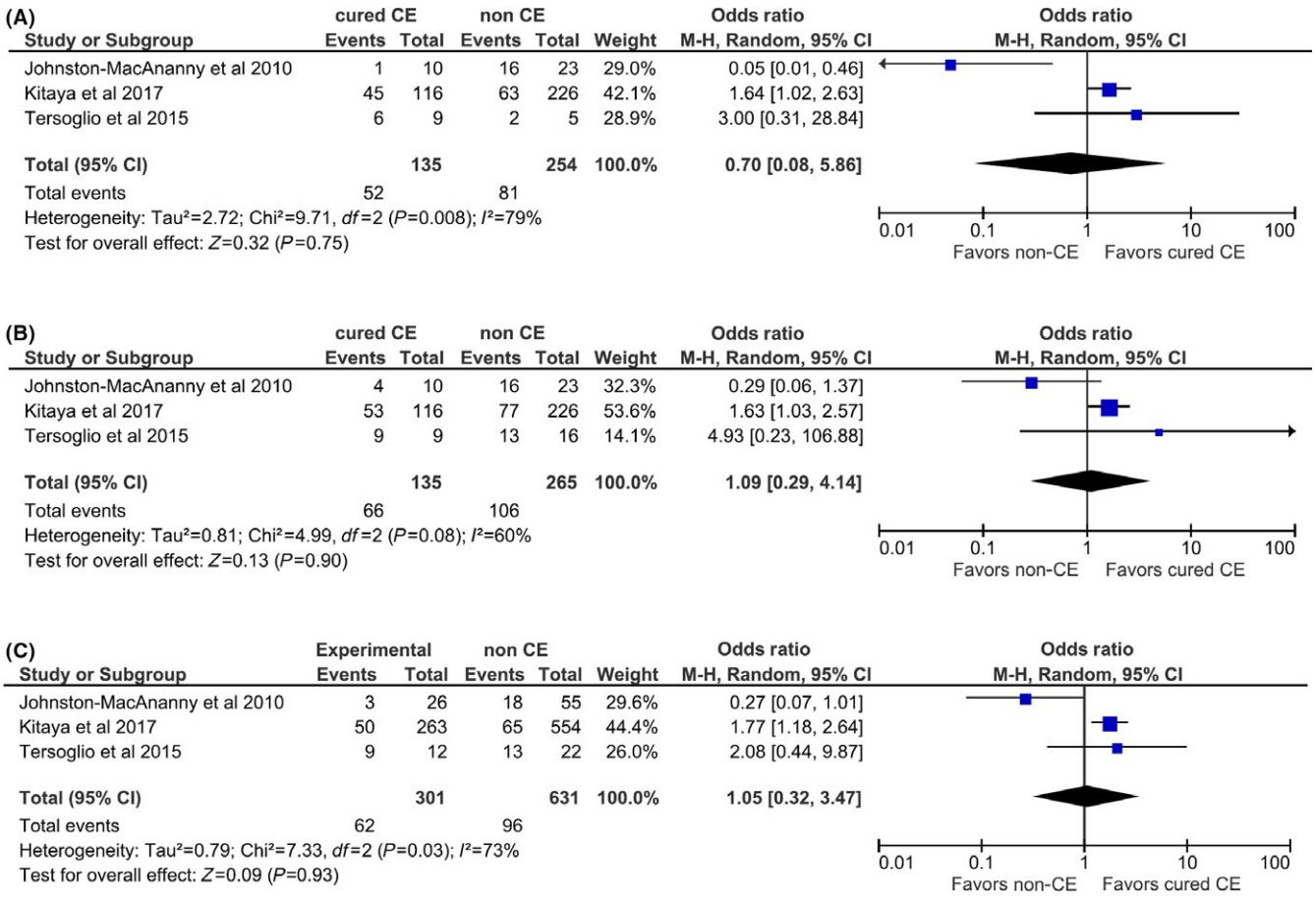

FIGURE 5 Effect of antimicrobial therapy on reproductive outcomes in ART patients. A, Ongoing pregnancy rate and/or live birth rate. B, Clinical pregnancy rate. C, Implantation rates. Reprinted from Vitagliano et al,[92] with permission from Elsevier

The use of antibiotics has been widely studied to treat BV and prevent preterm birth (reviewed in [30,37]). But the usefulness of antibiotic use before embryo transfer remains controversial; while it is efficient in reducing upper genital tract contamination, no beneficial role has been observed in pregnancy outcome.[91] This can be due to the lack of specificity of broad-spectrum antibiotics that could impair not only the growth of dysbiotic bacteria, but also the protective *Lactobacilli*. Other studies describing the impact of prescribed antibiotics to treat CE in infertile patients have yielded dissimilar results. A recent systematic review and meta-analysis evaluated the efficacy of CE antimicrobial therapy to improve pregnancy outcomes in RIF patients.[92] The results show that antibiotic therapy is not only effective at eliminating the cause of infection by targeting specific pathogens, but also is useful to ameliorate infertility in RIF patients with CE. Specifically, effective antibiotic therapy against CE improved implantation, clinical pregnancy, ongoing pregnancy, and live birth rates in a subsequent IVF cycle after CE was resolved. Moreover, there were no statistically significant differences in these outcomes between patients with resolved CE and those without CE (Figure 5). These findings suggest that diagnosing and treating CE in RIF patients before embryo transfer could be a useful intervention to eliminate the source of infection, improve the endometrial microbial health, and increase the live birth rates in these patients.[92] Importantly, antibiotic therapy has been also effective in patients with CE and unexplained infertility to increase their chances to conceive spontaneously and maintain a safe pregnancy to term after CE resolution.[93]

Another possible strategy to modulate the reproductive tract microbiome is the use of probiotics. These are live biotherapeutic products containing one or more strains of the desired bacteria, in this case *Lactobacillus*, that are administered to colonize the niche while displacing potential dysbiotic bacteria. Several oral and vaginal probiotics are currently commercially available, the majority of them including *L. crispatus*, *L. gasseri*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus rhamnosus*. This could offer an interesting approach to restore a healthy LD microbiota while overcoming the disadvantages of antibiotic treatment such as antibiotic resistance, high rate of recurrent infections after treatment, and side effects derived from the clearance of endogenous off-target flora in other body sites.[94,95] However, the efficacy of a probiotic therapy alone in reverting BV and other reproductive tract infections is not certain. For example, treatment with vaginal *L. crispatus* for one cycle results in colonization with this strain in up to 60% of patients.[96] A two-step therapy with vaginal probiotics following antibiotic treatment could be useful to first fight the fastidious bacteria and then repopulate the reproductive tract with *Lactobacillus* strains.[97-99]

5 | CONCLUSION

Mounting evidence demonstrates the importance of reproductive tract microbiota in women's health and reproductive function. This knowledge supports the practice of testing and treating, if required, the microbiota of the reproductive tract as a part of the personalized treatment in IVF settings. This practice is currently possible thanks to technical improvements that enable the study of bacterial communities at the molecular level, offering a new microbiological perspective at an unprecedented resolution. However, a deeper understanding of the microbiome will be useful to improve the gynecological and obstetrical health of every woman, regardless of her age, fertility status, or plans to conceive. In this regard, studies combining the most advanced technologies are required to decipher the interactions between host and microbiome to better define what a normal microbiome is, and the consequences of potential deviations from this normal microbiome.

DISCLOSURES

Inmaculada Moreno is research manager at Igenomix SL, Spain. Carlos Simon is Chief Scientific Officer and own stocks in Igenomix SL. *Human/animal rights*: This article does not contain any studies with human and animal subjects performed by the any of the authors. *Approval by ethics committee*: This review does not contain original research, so approval by a suitable Ethics Committee is not applicable.

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REFERENCES

1. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207-214.
2. Human Microbiome Project Consortium. A framework for human microbiome research. *Nature*. 2012;486:215-221.
3. González A, Vázquez-Baeza Y, Knight R. SnapShot: the human microbiome. *Cell*. 2014;158(3):690-690.e1
4. Sirota I, Zarek SM, Segars JH. Potential influence of the microbiome on infertility and assisted reproductive technology. *Semin Reprod Med*. 2014;32(1):35-42.
5. Miller EA, Beasley DE, Dunn RR, Archie EA. Lactobacilli dominance and vaginal pH: why is the human vaginal microbiome unique? *Front Microbiol*. 2016;7:1936.
6. Hou D, Zhou X, Zhong X, et al. Microbiota of the seminal fluid from healthy and infertile men. *Fertil Steril*. 2013;100:1261-1269.
7. Weng SL, Chiu CM, Lin FM, et al. Bacterial communities in semen from men of infertile couples: metagenomic sequencing reveals relationships of seminal microbiota to semen quality. *PLoS One*. 2014;9:e110152.
8. Franasiak JM, Scott RT. Introduction: Microbiome in human reproduction. *Fertil Steril*. 2015;104(6):1341-1343.
9. Mitchell CM, Haick A, Nkwopara E, et al. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. *Am J Obstet Gynecol*. 2015;212(5):611.e1-619.
10. Moreno I, Codoñer FM, Vilella F, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. *Am J Obstet Gynecol*. 2016;215(6):684-703.
11. Kyono K, Hashimoto T, Nagai Y, Sakuraba Y. Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among

infertile patients: a single-center pilot study. *Reprod Med Biol*. 2018;17(3):297-306.
12. Miles SM, Hardy BL, Merrell DS. Investigation of the microbiota of the reproductive tract in women undergoing a total hysterectomy and bilateral salpingo-oopherectomy. *Fertil Steril*. 2017;107(3):813-820.e1.
13. Franasiak JM, Scott RT. Reproductive tract microbiome in assisted reproductive technologies. *Fertil Steril*. 2015;104(6):1364-1371.
14. Delaney ML, Onderdonk AB. Nugent score related to vaginal culture in pregnant women. *Obstet Gynecol*. 2001;98:79-84.
15. Srinivasan S, Liu C, Mitchell CM, et al. Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. *PLoS One*. 2010;5:e10197.
16. Hammerschlag MR, Alpert S, Onderdonk AB, et al. Anaerobic microflora of the vagina in children. *Am J Obstet Gynecol*. 1978;131:853-856.
17. Hammerschlag MR, Alpert S, Rosner I, et al. Microbiology of the vagina in children: normal and potentially pathogenic organisms. *Pediatrics*. 1978;62:57-62.
18. Hillier SL, Lau RJ. Vaginal microflora in postmenopausal women who have not received estrogen replacement therapy. *Clin Infect Dis*. 1997;25:S123-S126.
19. Galhardo CL, Soares JM, Simoes RS, Haidar MA, Rodrigues de Lima G, Baracat EC. Estrogen effects on the vaginal pH, flora and cytology in late postmenopause after a long period without hormone therapy. *Clin Exp Obstet Gynecol*. 2006;33:85-89.
20. Brotman RM, Shardell MD, Gajer P, et al. Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause*. 2013;21:450-458.
21. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA*. 2011;108(Suppl 1):4680-4687.
22. Ronnqvist PD, Forsgren-Brusk UB, Grahn-Hakansson EE. Lactobacilli in the female genital tract in relation to other genital microbes and vaginal pH. *Acta Obstet Gynecol Scand*. 2006;85:726-735.
23. Aldunate M, Srbinovski D, Hearps AC, et al. Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. *Front Physiol*. 2015;6:164.
24. Tachedjian G, O'Hanlon DE, Ravel J. The implausible ''in vivo'' role of hydrogen peroxide as an antimicrobial factor produced by vaginal microbiota. *Microbiome*. 2018;6:29.
25. Barbes C, Boris S. Potential role of lactobacilli as prophylactic agents against genital pathogens. *AIDS Patient Care STDS*. 1999;13(12):747-751.
26. Eschenbach DA, Davick PR, Williams BL, et al. Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. *J Clin Microbiol*. 1989;27:251-256.
27. Ocana VS, De Ruiz P, Holgado AA, Nader-Macias ME. Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain. *Appl Environ Microbiol*. 1999;65:5631-5635.
28. Stoyancheva G, Marzotto M, Dellaglio F, Torriani S. Bacteriocin production and gene sequencing analysis from vaginal Lactobacillus strains. *Arch Microbiol*. 2014;196:645-653.
29. Bradshaw CS, Walker J, Fairley CK, et al. Prevalent and incident bacterial vaginosis are associated with sexual and contraceptive behaviours in young Australian women. *PLoS One*. 2013;8:e57688.
30. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal microbiota and reproductive health: the virtue of simplicity. *Cell Host Microbe*. 2018;23(2):159-168.
31. Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. *Sci Transl Med*. 2012;4(132):132ra52-132ra52.
32. Ma L, Lv Z, Su J, et al. Consistent condom use increases the colonization of *Lactobacillus crispatus* in the vagina. *PLoS ONE*. 2013;8:e70716.
33. Brooks JP, Edwards DJ, Blithe DL, et al. Effects of combined oral contraceptives, depot medroxyprogesterone acetate and the levonorgestrel-releasing intrauterine system on the vaginal microbiome. *Contraception*. 2017;95:405-413.
34. Bassis CM, Allsworth JE, Wahl HN, Sack DE, Young VB, Bell JD. Effects of intrauterine contraception on the vaginal microbiota. *Contraception*. 2017;96:189-195.
35. Noyes N, Cho KC, Ravel J, Forney LJ, Abdo Z. Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PLoS One*. 2018;13:e0191625.
36. Achilles SL, Austin MN, Meyn LA, Mhlanga F, Chirenje ZM, Hillier SL. Impact of contraceptive initiation on vaginal microbiota. *Am J Obstet Gynecol*. 2018;218(6):622.e1-622.e10.
37. Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and reproductive outcomes. *Fertil Steril*. 2018;110(3):327-336.
38. Eschenbach DA, Rosene K, Tompkins LS, Watkins H, Gravett MG. Endometrial cultures obtained by a triple-lumen method from afebrile and febrile postpartum women. *J Infect Dis*. 1986;153(6):1038-1045.
39. Hemsell DL, Obregon VL, Heard MC, Nobles BJ. Endometrial bacteria in asymptomatic, nonpregnant women. *J Reprod Med*. 1989;34(11):872-874.
40. Moller BR, Kristiansen FV, Thorsen P, Frost L, Mogensen SC. Sterility of the uterine cavity. *Acta Obstet Gynecol Scand*. 1995;74(3):216-219.
41. Cowling P, McCoy DR, Marshall RJ, Padfield CJ, Reeves DS. Bacterial colonization of the nonpregnant uterus: a study of pre-menopausal abdominal hysterectomy specimens. *Eur J Clin Microbiol Infect Dis*. 1992;11:204-205.
42. Chen C, Song X, Wei W, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat Commu*. 2017;8(1):875.
43. Verstraelen H, Vilchez-Vargas R, Desimpel F, et al. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1–2 region of the 16S rRNA gene. *PeerJ*. 2016;4:e1602.
44. Franasiak JM, Werner MD, Juneau CR, et al. Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. *J Assist Reprod Genet*. 2016;33(1):129-136.
45. Tao X, Franasiak JM, Zhan Y, et al. Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. *Hum Microb J*. 2017;3:15-21.
46. Swidsinski A, Verstraelen H, Loening-Baucke V, Swidsinski S, Mendling W, Halwani Z. Presence of a polymicrobial endometrial biofilm in patients with bacterial vaginosis. *PLoS One*. 2013;8(1):e53997.
47. Solt I. The human microbiome and the great obstetrical syndromes: a new frontier in maternal-fetal medicine. *Best Pract Res Clin Obstet Gynaecol*. 2015;29:165-175.
48. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med*. 2014;6(237):237ra65-237ra65.
49. Moreno I, Franasiak JM. Endometrial microbiota—new player in town. *Fertil Steril*. 2017;108(1):32-39.
50. Sharma H, Tal R, Clark NA, Segars JH. Microbiota and pelvic inflammatory disease. *Semin Reprod Med*. 2014;32:43-49.

51. Brunham RC, Gottlieb SL, Paavonen J. Pelvic inflammatory disease. *N Engl J Med*. 2015;372(21):2039-2048.e48.
52. Khan KN, Kitajima M, Hiraki K, et al. *Escherichia coli* contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. *Fertil Steril*. 2010;94(7):2860-2863.
53. Khan KN, Fujishita A, Kitajima M, Hiraki K. NakashimaM, Masuzaki H. Intrauterine microbial colonization and occurrence of endometritis in women with endometriosis. *Hum Reprod*. 2014;29:2446-2456.
54. Khan KN, Fujishita A, Masumoto H, et al. Molecular detection of intrauterine microbial colonization in women with endometriosis. *Eur J Obstet Gynecol Reprod Biol*. 2016;199:69-75.
55. Cicinelli E, Matteo M, Tinelli R, et al. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci*. 2014;21(5):640-647.
56. Takebayashi A, Kimura F, Kishi Y, et al. The association between endometriosis and chronic endometritis. *PLoS One*. 2014;9:e88354.
57. Pinto V, Matteo M, Tinelli R, Mitola PC, De Ziegler D, Cicinelli E. Altered uterine contractility in women with chronic endometritis. *Fertil Steril*. 2015;103:1049-1052.
58. Kitaya K, Matsubayashi H, Yamaguchi K, et al. Chronic endometritis: potential cause of infertility and obstetric and neonatal complications. *Am J Reprod Immunol*. 2016;75(1):13-22.
59. Moreno I, Cicinelli E, Garcia-Grau I, et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. *Am J Obstet Gynecol*. 2018;218(6):602.e1-602.e16.
60. Kitaya K, Takeuchi T, Mizuta S, Matsubayashi H, Ishikawa T. Endometritis: new time, new concepts. *Fertil Steril*. 2018;110(3):344-350.
61. Moreno I, Simon C. Microbiological diagnosis: the human endometrial microbiome – endometritis. In: Simon C, Giudice LC, eds. *The Endometrial Factor: A Reproductive Precision Medicine Approach*. Boca Raton, FL: CRC Press; 2017:65-77.
62. Song D, Feng X, Zhang Q, et al. Prevalence and confounders of chronic endometritis in premenopausal women with abnormal bleeding or reproductive failure. *Reprod Biomed Online*. 2018;36:78-83.
63. Cicinelli E, De Ziegler D, Nicoletti R, et al. Poor reliability of vaginal and endocervical cultures for evaluating microbiology of endometrial cavity in women with chronic endometritis. *Gynecol Obstet Invest*. 2009;68:108-115.
64. Aagaard K, Riehle K, Ma J, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One*. 2012;7(6):e36466.
65. Romero R, Hassan SS, Gajer P, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome*. 2014;2(1):4.
66. Walther-Antonio MR, Jeraldo P, Berg Miller ME, et al. Pregnancy's stronghold on the vaginal microbiome. *PLoS One*. 2014;9:e98514.
67. Hyman RW, Fukushima M, Jiang H, et al. Diversity of the vaginal microbiome correlates with preterm birth. *Reprod Sci*. 2014;21(1):32-40.
68. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. *N Engl J Med*. 1995;333(26):1737-1742.
69. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci USA*. 2015;112(25):11060-11065.
70. McDonald HM, Chambers HM. Intrauterine infection and spontaneous midgestation abortion: is the spectrum of microorganisms similar to that in preterm labor? *Infect Dis Obstet Gynecol*. 2000;8:220-227.
71. Stout MJ, Zhou Y, Wylie KM, Tarr PI, Macones GA, Tuuli MG. Early pregnancy vaginal microbiome trends and preterm birth. *Am J Obstet Gynecol*. 2017;217(356):e1–e18.
72. Brown RG, Marchesi JR, Lee YS, et al. Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. *BMC Med*. 2018;16(1):9.
73. Romero R, Hassan SS, Gajer P, et al. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. *Microbiome*. 2014;2:18.
74. Edmondson N, Bocking A, Machin G, Rizek R, Watson C, Keating S. The prevalence of chronic deciduitis in cases of preterm labor without clinical chorioamnionitis. *Pediatr Dev Pathol*. 2009;12:16-21.
75. Kim CJ, Romero R, Chaemsaithong P, Kim JS. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. *Am J Obstet Gynecol*. 2015;213(4 Suppl):S53-S69.
76. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. *N Engl J Med*. 1988;319:972-978.
77. DiGiulio DB, Romero R, Kusanovic JP, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. *Am J Reprod Immunol*. 2010;64:38-57.
78. Wilson JD, Ralph SG, Rutherford AJ. Rates of bacterial vaginosis in women undergoing in vitro fertilisation for different types of infertility. *BJOG*. 2002;109(6):714-717.
79. Campisciano G, Florian F, D'Eustacchio A, et al. Subclinical alteration of the cervical-vaginal microbiome in women with idiopathic infertility. *J Cell Physiol*. 2017;232:1681-1688.
80. Wee BA, Thomas M, Sweeney EL, et al. A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Aust N Z J Obstet Gynaecol*. 2018;58(3):341-348.
81. Hyman RW, Herndon CN, Jiang H, et al. The dynamics of the vaginal microbiome during infertility therapy with in vitro fertilization-embryo transfer. *J Assist Reprod Genet*. 2012;29:105-115.
82. Haahr T, Jensen JS, Thomsen L, Duus L, Rygaard K, Humaidan P. Abnormal vaginal microbiota may be associated with poor reproductive outcomes: a prospective study in IVF patients. *Hum Reprod*. 2016;31:795-803.
83. Egbase PE, al-Sharhan M, al-Othman S, al-Mutawa M, Udo EE, Grudzinskas JG. Incidence of microbial growth from the tip of the embryo transfer catheter after embryo transfer in relation to clinical pregnancy rate following in-vitro fertilization and embryo transfer. *Hum Reprod*. 1996;11(8):1687-1689.
84. Fanchin R, Harmas A, Benaoudia F, Lundkvist U, Olivennes F, Frydman R. Microbial flora of the cervix assessed at the time of embryo transfer adversely affects in vitro fertilization outcome. *Fertil Steril*. 1998;70(5):866-870.
85. Moore DE, Soules MR, Klein NA, Fujimoto VY, Agnew KJ, Eschenbach DA. Bacteria in the transfer catheter tip influence the live-birth rate after in vitro fertilization. *Fertil Steril*. 2000;74:1118-1124.
86. Salim R, Ben-Shlomo I, Colodner R, Keness Y, Shalev E. Bacterial colonization of the uterine cervix and success rate in assisted reproduction: Results of a prospective survey. *Hum Reprod*. 2002;17(2):337-340.
87. Selman H, Mariani M, Barnocchi N, et al. Examination of bacterial contamination at the time of embryo transfer, and its impact on the IVF/pregnancy outcome. *J Assist Reprod Genet*. 2007;24(9):395-399.
88. Peymani R, DeCherney A. Microbiome, infection and inflammation in infertility. In: Darwish A (Ed.). Chapter 8, InTech; 2016:99-133. https://www.intechopen.com/books/

genital-infections-and-infertility/microbiome-infection-and-inflammation-in-infertility. Accessed June 29, 2016.
89. Johnston-MacAnanny EB, Hartnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. *Fertil Steril*. 2010;93:437-441.
90. Egbase PE, Udo EE, al-Sharhan M, Grudzinskas JG. Prophylactic antibiotics and endocervical microbial inoculation of the endometrium at embryo transfer. *Lancet*. 1999;354(9179):651-652.
91. Kroon B, Hart RJ, Wong BM, Ford E, Yazdani A. Antibiotics prior to embryo transfer in ART. *Cochrane Database Syst Rev*. 2012;(3):CD008995.
92. Vitagliano A, Saccardi C, Noventa M, et al. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. *Fertil Steril*. 2018;110(1):103-112.e1.
93. Cicinelli E, Matteo M, Trojano G, et al. Chronic endometritis in patients with unexplained infertility: prevalence and effects of antibiotic treatment on spontaneous conception. *Am J Reprod Immunol*. 2018;79(1):e12782.
94. Sun X, Fiala JL, Lowery D. Patent watch: Modulating the human microbiome with live biotherapeutic products: intellectual property landscape. *Nat Rev Drug Discov*. 2016;15:224-225.
95. Bradshaw CS, Morton AN, Hocking J, et al. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *J Infect Dis*. 2006;193:1478-1486.
96. Hemmerling A, Harrison W, Schroeder A, et al. Phase 2a study assessing colonization efficiency, safety, and acceptability of *Lactobacillus crispatus* CTV-05 in women with bacterial vaginosis. *Sex Transm Dis*. 2010;37:745-750.
97. Marcone V, Calzolari E, Bertini M. Effectiveness of vaginal administration of *Lactobacillus rhamnosus* following conventional metronidazole therapy: how to lower the rate of bacterial vaginosis recurrences. *New Microbiol*. 2008;31:429-433.
98. Mastromarino P, Macchia S, Meggiorini L, et al. Effectiveness of Lactobacillus-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis. *Clin Microbiol Infect*. 2009;15:67-74.
99. Bradshaw CS, Pirotta M, De Guingand D, et al. Efficacy of oral metronidazole with vaginal clindamycin or vaginal probiotic for bacterial vaginosis: randomised placebo-controlled double-blind trial. *PLoS One*. 2012;7:e34540.
100. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine Microbiota: Residents, Tourists, or Invaders? *Front Immunol*. 2018;9:208.

How to cite this article: Moreno I, Simon C. Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol*. 2019;18:40–50. https://doi.org/10.1002/rmb2.12249