Review

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Implications of the vaginal microbiome and potential restorative strategies on maternal health: a narrative review

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Abstract: The vaginal microbiome undergoes dramatic shifts before and throughout pregnancy. Although the genetic and environmental factors that regulate the vaginal microbiome have yet to be fully elucidated, high-throughput sequencing has provided an unprecedented opportunity to interrogate the vaginal microbiome as a potential source of next-generation therapeutics. Accumulating data demonstrates that vaginal health during pregnancy includes commensal bacteria such as *Lactobacillus* that serve to reduce pH and prevent pathogenic invasion. Vaginal microbes have been studied as contributors to several conditions occurring before and during pregnancy, and an emerging topic in women's health is finding ways to alter and restore the vaginal microbiome. Among these restorations, perhaps the most significant effect could be preterm labor (PTL) prevention. Since bacterial vaginosis (BV) is known to increase risk of PTL, and vaginal and oral probiotics are effective as supplemental treatments for BV prevention, a potential therapeutic benefit exists for pregnant women at risk of PTL. A new method of restoration, vaginal microbiome transplants (VMTs) involves transfer of one women's cervicovaginal secretions to another. New studies investigating recurrent BV will determine if VMTs can safely establish a healthy *Lactobacillus*-dominant vaginal microbiome. In most cases, caution must be taken in attributing a disease state and vaginal dysbiosis with a causal relationship, since the underlying reason for dysbiosis is usually unknown. This review focuses on the impact of vaginal microflora on maternal outcomes before and during pregnancy, including PTL, gestational diabetes, preeclampsia, and infertility. It then reviews the clinical evidence focused on vaginal restoration strategies, including VMTs.

Keywords: infertility; pregnancy; preterm labor; probiotics; restoration; vaginal microbiome.

Introduction

The vaginal microbiome is a complex community of bacteria that act together to protect the host from disease and maintain vaginal health [1]. The composition of a healthy vaginal microbiota includes commensal bacteria, such as *Lactobacillus*, that serve to reduce pH and prevent pathogenic invasion. Both rapid turnover of the vaginal epithelium and mucinous cervicovaginal secretions help to form the body's first line of innate defense [1]. The vagina and cervix are colonized 99% by *lactobacillus*, while the upper reproductive tract has a much more diverse colonization, with over 23 different species of bacteria with increasingly smaller percentages of *lactobacilli* as you move up the tract to the cervix, endometrium, and fallopian tubes [2]. Most women have a predominance of *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, *Lactobacillus jensenii*, or a mixture of anaerobes [3, 4]. All species of *Lactobacilli* produce both D and L lactic acid, except *L. iners*, which does not produce D-lactate. *L. iners* has been associated with greater

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degrees of vaginal dysbiosis, less protection against sexually transmitted infections, and adverse pregnancy outcomes [5]. D-lactate producing *Lactobacillus* species, conversely, is associated with lower rates of obstetric complications and infections [6, 7]. Among healthy adults, the function of a *Lactobacilli*-dominated vaginal microbiome is to contribute to an acidic environment via glycogen breakdown to L and D-lactic acid, so as to create an unfavorable environment for harmful bacteria by killing or inhibiting their growth [6, 8]. *Lactobacilli* also bind to vaginal epithelial cells and thereby prevent other bacteria from doing so. Lactic acid promotes autophagy in vaginal epithelial cells, which helps degrade viruses and other intracellular pathogens, decreases inflammatory cytokines, and blocks histone deacetylase, which enhances DNA repair and gene transcription [8]. Overall, *lactobacilli* help prevent infection while also reducing inflammation.

During pregnancy, the composition of the vaginal microbiome undergoes several changes. Rising estrogen levels throughout gestation leads to increased glycogen deposition by vaginal epithelial cells. This results in *Lactobacilli* proliferation in increasing amounts, leading to an increasingly *Lactobacillus* dominated microbiome and an overall increased bacterial load [6]. This leads to decreased diversity overall including a reduction in the levels of non-*Lactobacilli* microbes, such as *Mycoplasma* and *Ureaplasma*. As a result of the increased lactic acid being produced, the vaginal pH is lowered and secretions are increased [9]. At the same time, a woman's general ability to mount a robust immune response is hindered in a healthy pregnancy. This occurs through a decreased innate immune response, with decreased inflammatory mediators like interferon-alpha and toll-like receptor 3 and 7 production occurring in early pregnancy [10]. This is thought to be beneficial for the fetus in that it protects maternal immune rejection against fetal antigens [6].

Despite abundant research on the healthy vaginal microbiome, little is known about why dysbiosis occurs in many unhealthy states. Unfortunately, animal models have limited translational potential due to a neutral vaginal pH and non-*Lactobacillus* dominant microbiomes [6, 11]. Factors such as diet, environment, demographics and sexual practices are not usually taken into consideration when comparing existing studies [12]. In addition, the stability of an individual's microbiome fluctuates throughout one's lifespan [13]. In regards to genetic differences, no studies to date have made direct links to host genetics and vaginal microbiome composition. However, in a study of microbiome characterization in multiple body sites, including the vagina, the composition between donors who were related was more similar than between strangers. However, the study was underpowered for vaginal samples to achieve statistical significance [14].

Most of the research on the vaginal microbiome has focused on healthy individuals [15], bacterial vaginosis (BV) [16, 17], and on preterm labor (PTL) in pregnant women [18, 19]. In addition, in the BV literature especially, therapies to modulate the vaginal microbiome have been explored [20]. When used as an adjunct to standard therapy, both oral and vaginal probiotics have shown to help prevent recurrent BV through stabilization of the vaginal ecosystem [16, 17, 21].

While the data are not as robust for conditions other than BV, changes in the vaginal microbiome have been linked to many other obstetric disease states. This review will approach these outcomes by first focusing on the role of the vaginal microbiome on events preceding pregnancy, such as fertility [22] and *in-vitro* fertilization (IVF) [23, 24]. We will then transition to discussing three conditions in pregnancy including PTL [18, 19], gestational diabetes (GDM) [7, 25–27], and pre-eclampsia (preE) [28, 29]. Finally, we will review existing evidence on restoration strategies including oral and vaginal probiotics as well as vaginal microbiome transplants that seek transfer vaginal microflora from a healthy donor to recipient. Peer-reviewed publications with MeSH terms on PubMed including "vaginal microbiome", "probiotics", "restoration", and "pregnancy" were searched and included if there were interventions or characterization of the vaginal microbiome in women with preE, GDM, PTL, or infertility. Systematic reviews and meta-analyses were included if available. Studies were only included if published in English and based on human data.

The vaginal microbiome and female health outcomes

Infertility and *in-vitro* fertilization (IVF)

Up to 10.5% of women struggle with infertility and while male-factor infertility and other causes are often found; up to 30% of women with infertility remains unexplained [22]. Many couples require multiple IVF cycles, which are both expensive and physically and emotionally taxing on patients [30], and can be unsuccessful in up to 60% of cases [23]. Women with idiopathic infertility and those with unsuccessful IVF cycles have been shown to have higher amounts of anaerobic bacteria and decrease in *Lactobacilli* spp., namely *L. crispatus* and *L. iners* [22]. Although some data suggest alterations in the microbiome are associated with infertility and IVF success, the data are conflicting.

For instance, a randomized-controlled trial measured colonization and pregnancy rates in 117 women who took intravaginal probiotics while undergoing IVF and found no significant difference [24].

Recently, research has been done to define the microbiome in other parts of the reproductive tract [2]. One study compared endometrial microbial differences in women who had successful implantation during IVF with those who did not. Those with over 90% *Lactobacillus* in their endometrium had higher rates of implantation (p=0.02) and live birth rates (p=0.002), suggesting there is a correlation between endometrial microbiome and fertility. The same study found differing genera between samples collected from vaginal and endometrial fluid within the same women, indicating that the vagina does not accurately reflect microbiota in the endometrium [31]. Another study examined the endometrial microbiome in 91 infertile women undergoing IVF. Samples positive for *Streptococcus viridans* were associated with a 7% live birth rate (p=0.04), while those that grew hydrogen-peroxide producing *Lactobacillus* spp. were associated with an 88% live birth rate (p=0.01), suggesting that the endometrial microbiome may be an important factor in IVF success [32]. However, other studies have found no significant correlation between endometrial *Lactobacillus* dominance and fertility [33–35].

Preterm labor

The area where the vaginal microbiome has been most widely studied in relation to maternal health is with respect to alterations leading to increased preterm premature rupture of membrane (pPROM) and PTL. In the United States, PTL occurs in 10% of all pregnancies and is the leading cause of death in children under age five [18]. While many preterm births are medically indicated, most are due to poorly understood spontaneous processes such as premature cervical changes, premature uterine contractions, or pPROM. There is increasing evidence that alterations in the vaginal microbiome leading to a less *Lactobacillus*-dominated environment are associated with increased risk pPROM and PTL [19], and conclusions from a recent meta-analysis found that using a lack of *Lactobacillus,* in combination with anaerobic bacteria, to be a strong predictor for PTL [36]. One study found that a vaginal microbiome dominated by non-*Lactobacillus* species or by *L. iners* led to increased PTL, regardless of cervical length [37]. Further supporting these results, a study of 20 patients undergoing rescue cerclage noted that precerclage participants had a reduced abundance of *Lactobacillus* compared to that of gestational-matched healthy individuals (p=0.017) and found this reduction in *Lactobacillus* to be associated with premature cervical dilation [38]. A larger study of vaginal microbiomes in 60 women with pPROM found significantly lower total abundance of *Lactobacillus* (pPROM 79% vs. controls 96%, p=0.016), and higher total number of species (pPROM 65 vs. control 10, p=0.009) in women with pPROM [39].

The etiology of the host response to decreasing levels of *Lactobacillus* could be from lower levels of D-lactic acid [6]. Women with PTL have lower concentrations of species of *Lactobacillus* that produce D-lactic acid. When the ratio of D-lactic acid isomer to L-lactic acid is decreased, substances that promote the integrity of the cervical os are lost. Namely, extracellular matrix metalloproteinase inducer (EMMPRIN) and matrix metalloproteinase 8 (MMP-8). EMMPRIN is produced by vaginal epithelial cells, and induces release of MMP-8. The release of MMP-8 leads to loss of integrity of the cervical os and allows for bacterial ascension to the endometrium. Since women with PTL have low levels of D-lactate producing bacteria, EMMPRIN and MMP-8 levels surge and allow for the downstream effects of early labor and increased susceptibility to BV [6]. Lower amounts of *Lactobacillus* thus represent the foundation for hypotheses about the vaginal microbiome's importance in PTL and pPROM.

Gestational diabetes

GDM occurs in 16.5% of pregnancies worldwide and is associated with poor maternal and fetal outcomes, including cardiovascular disease and diabetes, fetal macrosomia, congenital malformations, and fetal hypoglycemia [25]. Cross-sectional studies of the vaginal, oral, and stool microbiome show that there is a significant difference between women with and without GDM [26, 27]. One cross-sectional analysis of 26 women with diet-controlled DM and 42 normal controls demonstrated that the GDM group had significantly higher amounts of *non-Lactobacillus* ($p<0.01$) and decreased levels of both diversity and richness of the *Lactobacillus* spp. ($p<0.01$) [40]. While it has been shown that decreased *Lactobacillus* is associated with increased inflammation [41], it is still unknown if differences in the vaginal microbiome play a causal role in the development of GDM, or if GDM emerges as a result of vaginal dysbiosis. One study has postulated that decreasing amounts of *Lactobacillus* in women with GDM lead to less production of hydrogen peroxide, leaving the host susceptible to infections like vulvovaginal candidiasis [7].

While some studies have found an association with dysbiosis and GDM, the mechanism is yet to be elucidated.

The gut microbiota has been studied extensively in women with GDM. Low grade inflammation, higher adiposity, and glucose intolerance have all been found to correlate with dysbiosis in the gut microbiome in women with GDM. Higher glucose intolerance has also been linked to higher levels of lipopolysaccharide (LPS), an endotoxin found in gram negative bacterial cell walls, which promotes adhesion to the gut wall [42]. There may be similarities from host–gut microbiota interactions that exist in the vaginal microbiome for women with GDM, but the small amount of *lactobacillus* and a closer interface with diet lead to confounders when drawing comparisons. Despite the high prevalence of GDM is worldwide, there is still a large gap in knowledge on its relationship to the vaginal microbiome.

Preeclampsia

Hypertensive disorders of pregnancy, including preE and eclampsia, occur in up to 8% of women, and while deaths from these conditions have been significantly reduced in the United States, worldwide hypertension and related conditions are a major cause of maternal mortality globally [28]. Currently, no human studies have explicitly evaluated the microbial composition of patients with preE, but several papers have shown an association between increased levels of vaginal and systemic inflammation and earlier onset and more severe preE. The pathogenesis is thought to be due to the increased release of tissue factors in those with more inflammation, although the exact mechanism is not yet known [28, 29]. Researchers at Lund University have found that low levels of alpha-1-microglobulin, a hemoglobin scavenger may lead to increased risk of preE due to the toxicity of free heme [43]. Despite the gap in knowledge, oral probiotic milk has shown reduction in incidence of preE [44], implying a relationship may exist between disease and microbiome. This study will be reviewed in the next section on preE.

In summary, there is increasing evidence that the composition of the vaginal microbiome may be important for several crucial women's health outcomes. Prior to pregnancy, an increased number of anaerobic bacteria and decreased amount of *Lactobacillus* in vaginal and endometrial samples have been associated with infertility and decreased IVF success rate. A similar theme exists for PTL and GDM, where abundance of *Lactobacillus* appears to correlate with a lowered risk. The role the vaginal microbiome has in preE is not as clear, but interventions to restore the vaginal microbiome have started to yield results in preE despite not yet having an identified mechanism.

Vaginal microbiome restoration strategies

Given the increasing evidence that alterations to a *Lactobacillus*-dominated vaginal environment may be associated with a variety of adverse maternal health outcomes, many have begun to explore strategies to restore the vaginal microbiome. This section will discuss the three main methods (Figure 1) that have been explored in the literature.

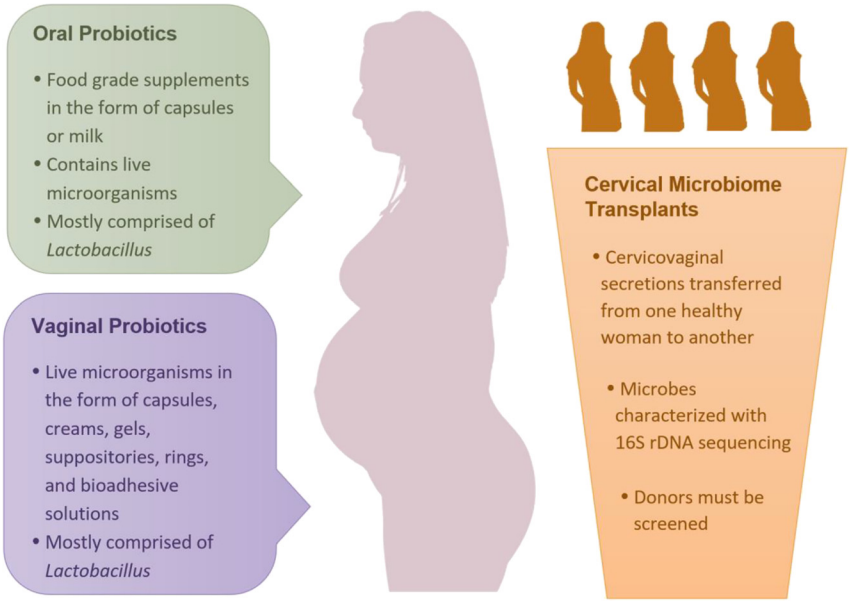

Figure 1: Three methods of vaginal microbiome restoration.

Oral probiotics

Oral probiotics, food-grade supplements consisting of live microorganisms, are thought to replenish the normal flora in the vagina and prevent infection or overgrowth with other microbes. Oral probiotics are thought to alter the vaginal flora via migration of bacteria to the vagina after they are excreted from the rectum [21], and data have shown that strains within oral probiotics colonize the vagina following ingestion [45]. However, recent trials in pregnant women have shown poor success in maintenance of significant compositional changes to the vaginal microbiome with oral probiotic use. A randomized controlled trial including 152 pregnant women who took daily probiotics in the first trimester did not have significant changes to their vaginal microbiota in the second trimester compared to the control group [46]. Similar results were found in another randomized controlled trial of 135 pregnant women who took 60 days of oral probiotics and were followed up with vaginal swabs at 0 and 90 [47]. These trials challenge the utility of oral probiotics as a valuable restoration method for pregnant women. For vaginal microbiome restoration, the currently studied products are composed mainly of *Lactobacillus,* most commonly *L. rhamnosus, L. acidophilus*, and *L. reuteri* [17, 20, 48]. Formulations of probiotics vary between studies, and selection of optimal strains is based on several factors, including: antimicrobial products (e.g. lactic acid, hydrogen peroxide), susceptibility to antibiotics, tolerance to acidic environments, survival during transit in the gastrointestinal tract, and adhesion capacity to vaginal epithelium, among others [49]. Below we have outlined the available data on impact of oral probiotics on previously discussed obstetric outcomes of interest. Of note, there are currently no studies that have evaluated the efficacy of oral probiotics on infertility or IVF outcomes.

Preterm labor

In pregnancy, oral probiotics have been shown to be efficacious in restoring the vaginal flora in patients with PTL, as defined by increased amounts of *Lactobacillus*, and led to a reduction in vaginal discharge [50]. However, a Cochrane meta-analysis found that while probiotics did lead to a reduction in the anti-inflammatory cytokines IL-4 and IL-10 and reduced genital infections during pregnancy by 81%, there is no formulation of oral probiotics that has been found to prevent PTL before 34 weeks (1,017 women) or before 37 weeks (2,484 women) [51].

Gestational diabetes

Oral probiotics alone have unclear benefit in GDM. A randomized-controlled trial of 256 women compared diet counseling combined with oral probiotics composed of *L. rhamnosus* and *Bifidobacterium lactis*, diet counseling alone, and no intervention. The group that received probiotics and diet counseling had a reduced incidence of GDM and reduced birth weight and length compared with diet counseling alone, with a number needed to treat of only four women [40]. Despite these results, information is still needed to show if these changes are due to alteration in the vaginal microbiome and how that relates to GDM. A clinical trial of 50 pregnant obese women, considered higher risk for developing GDM, were given probiotics containing *L. acidophilus* and *L. rhamnoses* daily from weeks 14–20 until delivery. There are no results to date, but the study will measure outcomes of both GDM and preE [52]. Further research on basic differences in vaginal microbiota between healthy women and women with GDM would help in targeting interventions for women with GDM moving forward.

Preeclampsia

Preliminary studies investigating oral probiotics and hypertensive disorders in pregnancy have found probiotics to be protective. A prospective cohort study of more than 34,000 nulliparous women in Norway found that intake of probiotic milk containing *L. acidophilus*, *L. rhamnosus*, and *B. lactis*, specifically during late pregnancy, reduced the risk of preE by 20% and severe preE by 40% in a dose-dependent fashion. A follow-up study looked at the timing of probiotic milk intake in more than 70,000 pregnancies and found that late milk intake reduced the risk of preE ($p=0.007$), while early milk intake reduced risk of preterm delivery ($p=0.03$). The authors proposed that the probiotics in milk may be reducing systemic inflammation by altering the vaginal microbiome to decrease risk of genital infections, thereby preventing the release of tissue factor and prostaglandins that can increase the risk for preE and PTL respectively [44, 48]. However, it should be noted that this study did not specifically evaluate the vaginal microbiome, so it is possible that the mechanism here is unrelated to the vaginal microbiome. Nevertheless, this preliminary evidence shows probiotic milk to be a potentially useful concept, especially given that the cost to the patient of simply drinking more supplemented milk during pregnancy may be minimal.

In summary, oral probiotics have few studies showing benefit in GDM when combined with diet counseling, and in preE when taken daily as probiotic milk. Data on oral probiotics in PTL is inconclusive, and

they have not been tested in women with infertility, indicating potential areas of study. Importantly, oral probiotics have shown potential in restoring *Lactobacillus*-dominant vaginal flora [45], which provides rationale for future studies of women who have pathology related to deficient *Lactobacilli*.

Vaginal probiotics

Vaginal probiotics are another restoration strategy with emerging evidence in women's health. Current methods of delivery include creams, gels, suppositories, rings, and bioadhesive solutions [53]. The use of the vagina as a drug delivery system is known to have unique benefits, due to the vagina's increased vascularization and innervation, the adhesion properties of the vaginal mucosa, and bypassing of first pass metabolism [54]. For example, drugs like propranolol, the contraceptive vaginal ring, antifungals, and misoprostol have been effectively used as vaginally administered drugs [53, 54]. However, the mucoadhesive properties of the vaginal epithelium, volume and viscosity of mucous, and local irritation to mucosa introduce challenges to using the vagina as a route of drug delivery. Alterations by the existing microbiota or biofilms pose a challenge for vaginally-administered drugs, especially for vaginal probiotics [53]. There is also concern that colonization weeks after discontinuing the vaginal probiotic diminishes with time, based on a recent systematic review. Mean survival of probiotic strains is difficult to determine due to differing follow-up points between studies [55]. Of note, there are currently no studies that have evaluated the impact of vaginal probiotics on GDM or preE.

Infertility and IVF

Unlike with oral probiotics, there is one study that has tested the impact of vaginal probiotics on IVF. In this study, 50 women who underwent ovarian stimulation were given a *Lactobacillus* vaginal probiotic immediately after oocyte retrieval. Pregnancy rates did not differ between these women and the control group of 67 women [24]. No studies have looked at infertility and vaginal probiotics outside of the setting of IVF; however, ongoing trials are investigating this relationship [24].

Preterm labor

Dysbiosis within the vaginal microbiome, such as in BV, is well known to be associated with PTL. In non-pregnant women, many studies have found benefit of a vaginally-inserted probiotic as adjuvant therapy to metronidazole in prevention of recurrent BV [16, 17]. Therefore, prevention of antepartum infections with BV has been considered as a strategy to reduce adverse neonatal outcomes, due to its relationship with PTL and pPROM. In a study of 40 women who presented with pPROM, participants were grouped either into ampicillin alone or ampicillin plus vaginal capsules containing *Lactobacillus casei rhamnosus*. The group with ampicillin and probiotics had increased latency periods in pPROM (12.3 vs. 41.4 days) and increased gestational age at delivery (28.1 vs. 31.5 weeks), which was associated with higher APGAR scores and birth weights in the treatment cohort [56]. In another study, pregnant women treated with vaginal capsules of *L. rhamnosus* once weekly for 12 weeks had longer cervical lengths, less dilation, and higher fetal station in serial visits. The untreated group had higher occurrences of a positive "whiff test", without microscopic evidence of confirmed BV infection [50]. These studies are some of the first to report on the impact of vaginal probiotic use on clinically meaningful outcomes during pregnancy. An ongoing clinical trial is evaluating the effect of vaginal *Lactobacillus*-containing powder in prevention of preterm labor in high risk women [57].

In conclusion, vaginal probiotics are an emerging new method being tested as a therapeutic for pROM and PTL, but more data is needed. In addition, it has not been studied widely enough to make conclusions about its potential efficacy in infertility, IVF, GDM, or preE.

Vaginal microbiome transplants

Successful transfer of fecal matter has proven therapeutic benefits in altering the gut microbiome [58], and thus investigators are now beginning to test the effects of transferring the vaginal microbiome. Components of the vaginal microbiome have been noted to transfer between women who have sex with women, as evidenced by a study of 31 monogamous female couples, in which 23 had identical rep-PCR fingerprints of *Lactobacillus* spp. [59]. The safety and efficacy of transferring one person's cervico-vaginal secretions (CVS) to another, however, is unknown, and barriers such as immunologic rejection [9] and infectious disease transmission have been cited as a concern [60]. Investigators at Johns Hopkins published a detailed proposal of a donor screening system for CVS to expedite future research along with some preliminary data on the use of this system [61]. They described how to collect and characterize the samples and tested the protocol in 20 women. Blood, urine, vaginal swabs, and CVS were

collected and underwent thorough evaluation for infectious diseases. The abundance of *Lactobacillus* spp. was characterized by 16S rDNA sequencing. Based on FDA regulations and other eligibility criteria, seven out of the 20 participants met the standards for CVS donation [61]. One study to date has been published as a case series in Israel of five recipients with intractable BV (defined as more than four episodes per year). Participants underwent intravaginal antibiotic treatment with CVS transfer seven days later. Four out of five participants reported clearance of BV symptoms subjectively and based on the Amsel criteria were objectively cleared of BV at follow up (between 5 and 12 months later). The fifth study participant reported partial improvement of symptoms, and all five were found to have expansion in species of *Lactobacillus* [62].

The Food and Drug Administration (FDA), the US organization which oversees the safety and efficacy of foods, drug, and biologics, has not made an official statement about regulation of VMTs. With respect to FMTs, the FDA initially stated that the stool in FMTs fall into a subcategory of drugs, which would require an Investigational New Drug application (IND) from physicians performing FMTs [63]. After much opposition, the FDA stated in 2016 that FMTs did not require an IND for the use of FMTs for *Clostridium difficile* not responding to standard therapies as long as an informed consent stated FMTs were investigational, they were not obtained from a stool bank, and the donor and stool underwent screening and testing with supervision of a health care provider [64]. There is debate surrounding how the FDA will regulate VMTs. If the CVS collected from donors is stored in a CVS bank or sold to healthcare providers, or transported across state lines, they will be subject to FDA rules and regulations. If VMTs are intended to cure disease, such as recurrent BV, they will be classified as a drug or biologic and require FDA regulatory oversight. As such, despite no formal guidance on VMTs, the FDA will likely require an IND to be submitted for clinical trials in human subjects [63].

If VMTs were to be eventually approved as a therapy for treatment resistant BV, the regulations and process of approval could make the final product expensive. The approval process on average takes 10 years. If women wanted to forgo the safety net of FDA approval and "do-it-yourself" (DIY) for transfer of CVS, self-collection of CVS is minimally invasive, comparable to insertion and removal of a tampon or menstrual cup [63]. Again, transfer of bodily fluids poses the risk of immune rejection or transfer of infectious disease, which would be considered less safe in the DIY method.

In March of 2020, the FDA issued a Safety Alert regarding the potential for transmission of SARS-CoV2, or COVID-19, via fecal matter in FMTs. They recommended testing donors and their stool for COVID-19 as feasible and obtaining informed consent about the possibility of transmission [65]. This idea carries over into potential of COVID-19 transmission in VMTs.

Currently, there are no other published studies on use of VMTs except for the previously mentioned case series from Israel [62]. However, there are two ongoing clinical trials investigating the use of vaginal microbiome transplants (VMTs) for prevention of recurrent BV. One with estimated completion in 2022 at Massachusetts General Hospital is comparing oral metronidazole with VMT to oral metronidazole with sterile saline as a placebo [66]. Another study at Johns Hopkins with estimated completion in 2025 is comparing metronidazole gel with VMT to metronidazole gel with sterile saline. Participants in this study have behavioral restrictions that may limit the applicability of the data, including abstaining from sex, use of tampons, swimming, or taking baths [67].

Conclusions

There is a growing body of literature on the importance of the vaginal microbiome for reproductive outcomes along a wide spectrum, from achieving pregnancy through natural means or with assisted reproduction technology, to conditions within pregnancy like GDM, preE, and PTL. Specifically, there is early data to suggest reduced incidence of preE and PTL with probiotic milk, and reduced incidence of GDM with oral probiotics and counseling [44, 48]. Vaginal probiotics have been shown to increase the latency period in women with pROM and help to prevent progression to PTL [56]. Finally, the concept that women can transfer their vaginal microbiomes to one another via intercourse supports the hypothesis about the expected efficacy of VMTs [67], and early data on clinical outcomes and safety protocols are being reported [61, 62].

Our review is limited by a relatively small number of studies on mentioned disease states, deficiency of large clinical trials, and heterogeneity between available studies. Furthermore, many of these studies are case–control or cohort studies where confounding factors are not controlled. While many do show a correlation between microbiome changes and pregnancy outcomes, no causation can be established based on their evidence. Our inclusion criteria for referenced studies were imprecise and lead to the possibility of potentially relevant studies being excluded. Furthermore, our review highlights several other areas that also merit future study. Most of the studies we reviewed have been conducted at single institutions with

small sample sizes. Larger studies, especially of women with GDM and infertility, will allow more conclusions to be drawn in restoration strategies. In addition, more mechanistic data is needed. One critique on oral probiotic studies is whether the existing documented impact on clinical outcomes is due to restoration of the vaginal microbiome or other mechanisms. Another limitation is the variation in collection of samples in studies cited throughout the paper. Most methods sections include a description that swabs were collected from the posterior fornix of the vagina, but there may be differences in collection methods from studies who do not specify location, and that could lead to variations in the composition of the vaginal microbiomes between studies. A potential barrier for vaginal probiotics is the rate of decrease in strain concentration after probiotic administration over time [55]. Another critique surrounds the selection of strains in probiotics. In most studies, while a combination of *Lactobacillus* and sometimes other vaginal genera are used in formulations of probiotics, there is minimal explanation of the methods used by investigators to select the strains used in trials. Unfortunately, several studies are sponsored by the manufacturer of the probiotic, adding an additional layer of uncertainty in the rationale for composition in many products because of commercial interest [68]. The VMT field is particularly ripe for study although there continues to be several potential risks, including the transfer of infectious diseases, although this is largely avoidable with extensive screening and testing of samples [61]. Finally, it may be challenging for patients to accept the vaginal microbiota of an anonymous individual in real practice. Future studies could investigate participants' willingness to accept such therapeutic strategies.

The vaginal microbiome represents an exciting area of study in maternal health. The many ongoing studies that will help us assess if VMTs can establish a healthy *Lactobacillus*-dominant vaginal microbiome have important implications for many disease states shown to be impacted by vaginal dysbiosis. Ultimately, the efficacy on clinically meaningful outcomes among pregnant and nonpregnant women should continue to be a focus.

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