Vaginal microbiome and preterm birth: Composition, mechanisms and microbiota-directed therapies (Review)

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Received May 25, 2025; Accepted August 20, 2025

DOI: 10.3892/ijmm.2025.5644

Abstract. Preterm birth (PTB) is a global maternal and neonatal health challenge, affecting ~15 million infants each year. Despite advances in obstetric and neonatal care, PTB-related morbidity and mortality remain high. Emerging evidence implicates dysbiosis of the vaginal microbiota (VMB) as a key contributor to PTB. A healthy VMB is typically dominated by *Lactobacillus* spp., which maintain an acidic vaginal environment and inhibit pathogen colonization. Conversely, reduced *Lactobacilli* abundance alongside overgrowth of anaerobic taxa such as *Gardnerella*, *Atopobium* and *Mycoplasma* is strongly associated with spontaneous PTB and preterm premature rupture of membranes. Excessive proliferation of vaginal pathogens may lead to ascending infection and intra-amniotic inflammation via activation of host Toll-like receptor signaling and induction of pro-inflammatory cytokines IL-1β, IL-6 and IL-8. Moreover, VMB-derived metabolites such as lactate play important roles in immunomodulation and inflammation. Although antibiotics remain the mainstay for treating bacterial vaginosis, their non-specific effects often disrupt microbial balance and predispose to recurrence. Recently, probiotic therapies and VMB transplantation have emerged as promising alternative or adjunctive strategies for PTB prevention and management. However, variability in probiotic efficacy and lack of standardized intervention protocols remain significant challenges. The present review examined pregnancy-associated VMB dynamics, the mechanisms linking dysbiosis to PTB risk and future microbiome-based intervention strategies, with the aim of informing theoretical and practical approaches to reduce the global burden of preterm birth.

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Key words: preterm birth, vaginal microbiota, probiotics

Contents

1. Introduction

Preterm birth (PTB) is a global maternal and neonatal health problem, with ~15 million infants born preterm each year, 11% of all live births (1). PTB contributes to 15% of under-five childhood mortality and 35% of neonatal mortality worldwide (2). It is associated with a range of serious complications, including bronchopulmonary dysplasia, respiratory distress syndrome, intraventricular hemorrhage, neurodevelopmental impairment, and necrotizing enterocolitis (2), which rank among the leading direct causes of mortality in children under five. Roughly 40-45% of PTB cases occur spontaneously, 25-30% result from preterm premature rupture of the membranes (PPROM), and the remaining 30-35% are due to medically indicated or elective early deliveries (3). Intra-amniotic infection is estimated to underlie ~35% of spontaneous PTB and 50% of PPROM cases (3,4). Studies have isolated specific bacterial species from the amniotic fluid of PTB patients and confirmed that these organisms are common vaginal colonizers, supporting ascending vaginal infection as the primary route of microbial invasion into the amniotic cavity (4,5).

Vaginal microbiota (VMB) plays a critical role in maintaining maternal and fetal health (6). For example, Tabatabaei *et al* (7) reported that *Lactobacillus* spp. may reduce the risk of PTB, whereas community state types (CSTs) associated with bacterial vaginosis (BV) may increase PTB

risk. Moreover, a *Lactobacillus*-depleted CST, accompanied by elevated abundances of *Gardnerella* or *Mycoplasma*, is linked to higher PTB incidence (8). Compared with early gestation, the VMB becomes increasingly stable and *Lactobacillus*-dominated in late pregnancy (9,10), which may represent an evolutionary mechanism to ensure successful pregnancy. By contrast, VMBs dominated by other genera, particularly those associated with BV, are generally considered suboptimal and correlate with greater adverse outcomes (11,12).

While previous reviews, for example Giannella *et al* (13), have characterized shifts in the maternal microbiome during pregnancy, their focus has been largely limited to oral and gastrointestinal communities with minimal consideration of vaginal populations. Bayar *et al* (14) examined the association between the pregnancy microbiome and PTB yet did not address how vaginal microbial communities relate to risk factors such as bacterial vaginosis nor did they explore mechanistic pathways linking vaginal microbes, host immunity, inflammation and microbial metabolites to PTB. In response, the present review summarized the composition and dynamic shifts of the VMB, with emphasis on its pregnancy-specific alterations. It examined the potential mechanisms by which vaginal dysbiosis and BV contribute to PTB, including inflammatory responses, immune modulation and the impact of microbial metabolites on pregnancy outcomes. It further explored molecular pathways driving ascending infection following VMB imbalance, such as Toll-like receptor (TLR)/NF-κB signaling activation and subsequent inflammatory cascades. Finally, it critically analyzed current clinical strategies for restoring vaginal ecosystem homeostasis, through probiotics or VMB transplantation to prevent PTB, highlighting uncertainties in probiotic efficacy and outlining questions for future research. Collectively, these insights aimed to inform novel approaches for vaginal microbiome modulation in PTB prevention.

2. Data collection methods

PubMed and Web of Science were searched from database inception through 31 May 2025. Medical Subject Headings and free text terms 'preterm birth,' 'vaginal microbiota,' 'microbiota,' and 'probiotic' were combined in the search strategy.

Composition and pregnancy-induced dynamics of the vaginal microbiota

Establishment and stability of the vaginal microbial ecosystem. The VMB plays a crucial role in female reproductive tract health and disease (15). A *Lactobacillus*-dominated VMB is generally considered indicative of a healthy vaginal environment, whereas an unhealthy VMB is characterized by high microbial diversity and heterogeneity, with reduced *Lactobacillus* abundance and increased loads of anaerobic bacteria, including *Gardnerella, Atopobium, Mobiluncus, Prevotella, Streptococcus, Chlamydia* and *Mycoplasma* (16). Following menarche, elevated circulating estrogen promotes vaginal epithelial proliferation and glycogen deposition; the breakdown products of this glycogen serve as preferential carbon sources for *Lactobacillus* spp., reinforcing their dominance and lowering vaginal pH (17). This acidic milieu, together with bacteriocins and other antimicrobial compounds produced by *lactobacilli*, establishes a mucosal microenvironment that is inhospitable to most other microorganisms but favorable to *Lactobacillus* spp. Consequently, *Lactobacillus* spp. are regarded as keystone members of the VMB during the reproductive years and contribute to protection against BV, pelvic inflammatory disease, candidiasis, sexually transmitted infections and oncogenic human papillomavirus infection (11,18). Ravel *et al* (18) characterized the VMB of reproductive-age women across four ethnic groups using 16S rRNA gene sequencing and defined five CSTs based on dominant genera and species (as shown in Fig. 1). CSTs I-III and V are LDOM communities (>90% *Lactobacillus* spp.), dominated respectively by *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*. By contrast, CST IV is called the *Lactobacillus*-depleted state and is dominated by strict and facultative anaerobes. It contains almost no *Lactobacillus* and shows high microbial diversity (19).

Throughout a woman's life span, both intrinsic and extrinsic factors modulate VMB composition. Prior to menarche, low estrogen levels correlate with a high-diversity microbiota comprising aerobic, anaerobic and enteric bacteria (20). Although some women maintain VMB stability across the menstrual cycle, the endocrine withdrawal of estrogen and progesterone during menses, alongside endometrial shedding and vaginal bleeding, induces transient shifts marked by decreased *Lactobacillus* abundance that typically rebounds in the follicular phase (21). During pregnancy, hormonal stability, markedly elevated estrogen concentrations and the absence of menses favor a *Lactobacillus* dominated (LDOM) VMB (22). Estrogen is the principal host factor sustaining the VMB, promoting epithelial thickening and intracellular glycogen synthesis (23). Glycogen is hydrolyzed by host α-amylase into glucose and maltose, substrates fermented by *Lactobacillus* spp. into lactic acid, thereby reducing local pH (24). Moreover, various vaginal bacteria encode amylase-like enzymes capable of glycogen metabolism (25,26). Glycogen may also originate from epithelial-cell lysis induced by high lactate concentrations and cytolysins secreted by *Lactobacilli*, a process potentially mediated by hyaluronidase-1 and matrix metalloproteinase (MMP)-8 (27). With the onset of puberty and rising estrogen, vaginal pH acidifies and meta-analyses have demonstrated enhanced VMB stability and *Lactobacillus* predominance (28,29). Similarly, cyclical and pregnancy-related fluctuations in VMB stability parallel estrogen levels; elevated estrogen is consistently associated with increased VMB stability, which is beneficial for vaginal health. Conversely, decreased estrogen post-pregnancy and in menopause is linked to reduced VMB stability; however, estrogen therapy in postmenopausal women restores a pronounced LDOM state (30,31). Notably, estrogen-containing contraceptives increase *Lactobacillus* abundance in reproductive-aged women and decrease BV incidence (32,33), further supporting a causal role for estrogen in VMB regulation.

Pregnancy-induced shifts in microbial composition. During pregnancy, the VMB is characterized by decreased richness and diversity, with *Lactobacillus* spp. emerging as the dominant genus. This maintenance of microbial homeostasis is

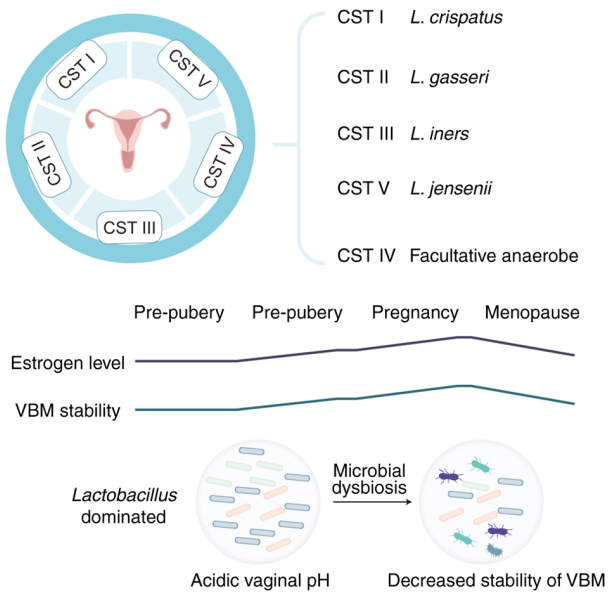

Figure 1. Community state types and stability of the female vaginal microbiota. The vaginal microbiota can be classified into five CSTs (I-V) based on dominant genera and species. CST I, II, III and V are each dominated by *Lactobacillus crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*, respectively, whereas CST IV is characterized by a high diversity of strict and facultative anaerobes. Vaginal microbial stability correlates positively with circulating estrogen: during puberty and pregnancy, rising estrogen levels promote a stable, lactobacilli-dominated community and acidify vaginal pH. Conversely, estrogen withdrawal leads to decreased microbial stability and a shift toward more diverse, non-lactobacilli profiles. CST, community state types; VMB, vaginal microbiota.

closely linked to rising estrogen levels (as shown in Fig. 2), which not only stimulate glycogen synthesis but also promote increased lactic acid production by *Lactobacillus*. As gestation advances into the late trimester, *Lactobacillus* proliferation is often accompanied by a marked decline in dysbiosis-associated taxa such as *G. vaginalis*, *A. vaginae* and *P. bivia* (34). Studies show that during pregnancy, vaginal microbiota communities dominated by *Lactobacillus* usually remain stable or slowly change into other community types still dominated by *Lactobacillus* (8,35,36). Communities showing dysbiosis in mid-gestation often become dominated by *Lactobacillus*. When *Gardnerella vaginalis* dominates, its communities are relatively unstable and usually shift to a *Lactobacillus iners* community (35,36). These dynamic shifts align with the overall trend toward an optimized vaginal microenvironment during pregnancy. As white women generally possess higher baseline *Lactobacillus* levels prior to conception (36), black women demonstrate a more pronounced transition toward *Lactobacillus* dominance and enhanced VMB stability throughout pregnancy (35,36), suggesting a stronger modulatory effect of gestation on the VMB in this population. At parturition, the precipitous decline in estrogen precipitates a significant destabilization of the VMB (37,38), leading to community restructuring: *Lactobacillus* proportions decrease while anaerobic genera such as *Peptoniphilus*, *Prevotella* and *Anaerococcus* proliferate; taxa whose overgrowth is often linked to adverse outcomes (8,37). Moreover, short interpregnancy intervals (<1 year) have been shown to markedly increase the risk of PTB and other obstetric complications (39-41).

Vaginal microbiota dysbiosis as a risk factor for PTB

Bacterial vaginosis: A prominent dysbiotic state linked to PTB. Intra-amniotic infection is a major trigger of PTB (42), particularly in early gestation (43). Most intra-amniotic infections originate from ascending colonization of the lower genital tract by bacteria present before or during pregnancy (44) and cultures of preterm fetal membranes have confirmed the involvement of BV-associated species (45). Moreover, asymptomatic BV in pregnant women confers more than a two-fold increased risk of PTB compared with BV-negative women (46); BV diagnosed before 20 weeks' gestation is associated with a five-fold higher risk (47) and diagnosis before 16 weeks with a seven-fold increase (48). Clinical trials have investigated antibiotic treatment of asymptomatic BV to prevent PTB (49,50) and several studies suggest efficacy in high-risk populations (51-53). In addition, BV has been identified as an independent risk factor for PTB (54). The global prevalence of BV ranges from 23-29% (55), characterized by replacement of *Lactobacilli* with anaerobic Gram-negative bacteria in the VMB (56). A cross-sectional study of 50 BV patients using denaturing gradient gel electrophoresis fingerprinting detected significant enrichment of *Firmicutes*, *Fusobacteria*, *Bacteroidetes* and *Acinetobacter* (57). In BV-associated VMBs, *Gardnerella, Atopobium, Prevotella, Porphyromonas, Sneathia, Mobiluncus, Mycoplasma* BVAB1, BVAB2, *Mageeibacillus indolicus* (BVAB3) and *Peptostreptococcus* are frequently enriched (56). Moreover, *G. vaginalis* has been reported in the majority of clinically diagnosed BV cases (58). Numerous *G. vaginalis* isolates produce vaginolysin, a cholesterol-dependent cytolysin that disrupts mammalian cell membranes and induces cell death. Amino-acid sequence variations among vaginolysin toxins from different *G. vaginalis* strains underlie differences in pathogenicity (59). *G. vaginalis* comprises four clades, *G. vaginalis*, *G. leopoldii*, *G. piotii* and *G. swidsinskii* (60,61), all of which can occur at low abundance in asymptomatic women, often co-existing within the same VMB (62). Overgrowth of these taxa leads to *Lactobacillus* depletion and disruption of the natural lactic acid defense mechanism, causing vaginal pH to rise above 4.5 (63,64). BV-associated bacteria damage the vaginal mucosal and epithelial barriers by producing biofilms, sialidases and cytolysins, elevating pH and secreting colonization-enhancing enzymes. High expression of cholesterol-dependent cytolysin genes in *L. iners* and *G. vaginalis* correlates with reduced *Lactobacillus* abundance (65). Epithelial cell models indicate that pore forming toxins such as vaginolysin from *G. vaginalis* (66) and putative inerolysin from *L. iners* (67) interact with lipid rafts to lyse epithelial cells, suggesting a mechanism for BV pathogenesis. Vaginal biofilms resist clearance of BV associated bacteria by acidic environments, *lactobacilli* derived antimicrobials, host immunity and antibiotics (56,68). Although their precise role remains unclear, these biofilms are considered critical for BV onset and recurrence (68,69). *In vitro* studies show that initial attachment of *G. vaginalis* may be mediated by *L. iners* or *Peptoniphilus* spp., while biofilm maturation is enhanced by *Atopobium vaginae*, *Prevotella bivia*, *Fusobacterium nucleatum* and *Mobiluncus* spp (68,70), indicating that interspecies interactions promote biofilm formation.

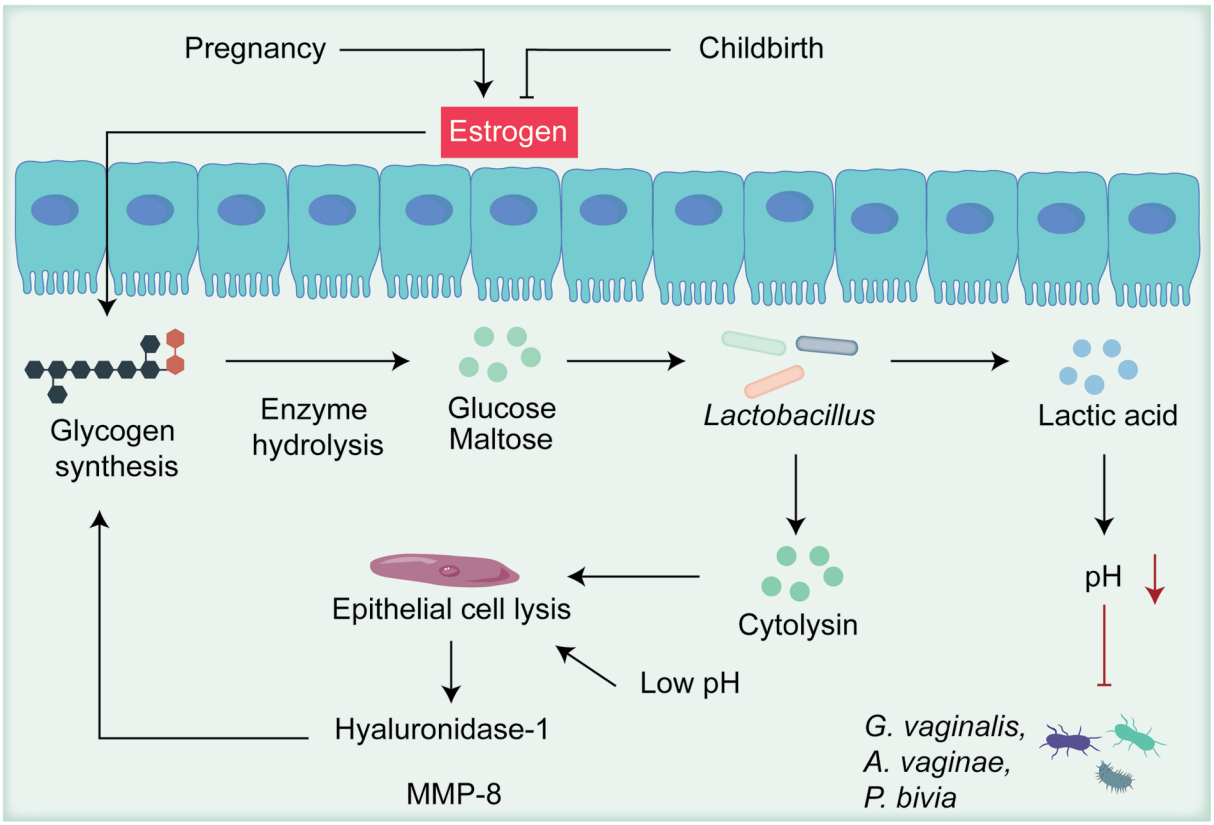

Figure 2. Interplay between pregnancy, glycogen metabolism and vaginal microbial homeostasis. Elevated estrogen during pregnancy stimulates epithelial glycogen synthesis, which vaginal bacterial enzymes hydrolyze into glucose and maltose. These sugars are fermented by lactobacilli to produce lactic acid, lowering vaginal pH and inhibiting dysbiosis-associated taxa (such as *Gardnerella vaginalis*, *Atopobium vaginae* and *Prevotella bivia*). Lactobacilli also secrete cytolysins that induce epithelial cell lysis, triggering the release of hyaluronidase-1 and MMP-8, thereby facilitating glycogen liberation and further supporting lactobacilli growth. After parturition, the sudden decline in estrogen precipitates microbial community restructuring: lactobacilli proportions diminish and dysbiosis-associated organisms expand. MMP, matrix metalloproteinase.

Specific microbial profiles associated with increased PTB risk. Abnormal VMB is recognized as a potential risk factor for PTB. Clinical studies show that bacteria isolated from the placenta and fetal membranes of preterm deliveries closely match those in the vagina, supporting the idea that ascending vaginal colonization by pathogens frequently leads to intra-amniotic infection and PTB (71) and can be reproduced in various animal models of ascending infection (72,73). Romero *et al* (74) compared the VMB of 18 women who delivered preterm with 70 term controls and found no significant differences in bacterial taxa, relative abundances, or CST distributions when stratified by gestational age. A Peruvian cohort likewise reported no association between vaginal CST and PTB (5). These discrepancies may reflect differences in maternal ethnicity, geographic location, and gestational age at sampling. Fettweis *et al* (35) profiled the VMB and cytokine milieu of 45 African-American women with PTB vs. 90 term-matched controls. Multi-omics analyses showed markedly lower levels of *L. crispatus* and higher abundances of BVAB1, *Sneathia amnii*, TM7-H1 and a subset of *Prevotella* in the PTB group. Term deliveries were more likely to harbor *L. crispatus* and exhibit reduced prevalence of *A. vaginae* and *G. vaginalis*. A small study of nulliparous African-American women noted a nonsignificant trend toward reduced diversity in spontaneous PTB (75), and another black cohort found that although specific *Lactobacillus* abundances were not linked to PTB risk, lower overall diversity associated with PTB among African-American women (76). In a UK study, *L. iners* dominance at 16 weeks was markedly associated with cervical shortening and PTB before 34 weeks, whereas *L. crispatus* dominance strongly predicted term birth; high-diversity communities were not linked to cervical shortening or PTB, but *Lactobacillus* depletion was relatively uncommon in that cohort (77). The role of *L. iners* remains ambiguous, as it appears in both healthy and dysbiotic VMBs. Unlike the exclusionary *L. crispatus*, *L. iners* has a reduced genome lacking key metabolic functions (78), suggesting that *L. iners* has evolved to tolerate other vaginal microbes, including pathogens, and is often found in transitional microbiota states. Consequently, *L. iners* may be unable to prevent pathogen overgrowth during pregnancy, indirectly fostering high-risk VMB profiles. Srinivasan *et al* (79) reported that high levels of *L. iners* can be detected in women who are BV positive or BV negative and across a broad range of vaginal pH values. Although this phenomenon has been extensively documented under BV conditions (80), it does not provide direct evidence that the species is pathogenic during dysbiosis. Of women ~50% are thought to harbor *L. iners* (23), so its persistent detection during BV is unsurprising. Another study showed that the detection rate of *L. iners* is similar among women with normal, intermediate and BV Nugent scores (81); however, relative abundance may be more decisive and further work is

required to quantify both relative and absolute levels under diverse conditions. Shipitsyna *et al* (82) observed a decline in *L. iners* during active BV and therefore suggested that it is unlikely to be a primary pathogen; by contrast, other investigators argue that communities dominated by *L. iners* are more prone to shift toward dysbiosis, whereas those dominated by *L. crispatus* appear more stable (83). A semi-quantitative qPCR study that sampled Belgian women (predominantly Caucasian) throughout the menstrual cycle found that during active BV, menstruation and after intercourse, the previously dominant *L. crispatus* sharply decreased, while *L. iners* became predominant, often accompanied by a concomitant rise in *Gardnerella vaginalis* (84), indicating greater adaptability of *L. iners* to fluctuations in the vaginal environment such as BV. Macklaim *et al* (85) further demonstrated that genes involved in mannose and maltose uptake and glycogen degradation are markedly upregulated in *L. iners* under BV. Additional studies show that both the abundance and gene expression of *L. iners* vary with the phase of the menstrual cycle (86); it is also possible that host physiological changes first trigger BV and that *L. iners* subsequently adapts. Clarifying this temporal sequence will be critical for determining whether, and to what extent, *L. iners* is harmful to the host.

In a cohort of 111 pregnant women, those harboring only a single *Lactobacillus* species exhibited higher PTB risk than women with multiple *Lactobacillus* spp. In addition, this study also indicated that *L. iners* was the dominant species in PTB cases (87). Tabatabaei *et al* (7) similarly reported that *Lactobacillus* spp. may reduce PTB risk, whereas BV-associated CSTs increase it. Another study confirms that the *Lactobacillus* depleted CST (CST IV), marked by elevated *Gardnerella* or *Mycoplasma*, is linked to greater PTB risk (8), underscoring that *Lactobacillus* species diversity is a key determinant of pregnancy outcome. Four studies of PPROM cases revealed that ~50% of women exhibited moderate or low *Lactobacillus* dominance and high diversity (88). In individual PPROM patients, ~50% of those with *Lactobacillus* dominated VMBs prior to membrane rupture developed dysbiosis afterwards and erythromycin treatment exacerbated reductions in *Lactobacillus* alongside enrichment of potential pathogens (89). In a prospective cohort, 25% of women who later experienced PPROM had low *Lactobacillus* abundance and high microbial diversity before membrane rupture, compared with just 3% of women who delivered at term with intact membranes (90). Enhanced vaginal microbial diversity, particularly during the mid-gestational period, demonstrated a significant correlation with PPROM, while vaginal microbiota compositions lacking *Lactobacillus* predominance emerged as a consistent predictive biomarker for PPROM across all gestational age intervals.

Multiple studies have demonstrated significant population specific differences in vaginal microbiome composition and their associations with PTB risk. DiGiulio *et al* (91) identified ethnicity as a strong determinant of VMB composition and PTB risk. In a predominantly white cohort, CSTs characterized by *Lactobacillus* depletion, especially those enriched for *Gardnerella* or *Ureaplasma*, were associated with shorter gestational duration and higher PTB risk. A subsequent comparison of Californian white and Alabaman black women revealed more frequent *Lactobacillus* depletion and *Gardnerella* enrichment among black women, yet these features predicted PTB only in white women; in both groups, *L. crispatus* dominance conferred protection against early delivery (92). Hyman *et al* (93) reported greater bacterial diversity among white women who delivered preterm, while overall species diversity was higher in African-American women. A more recent investigation comparing black and non-black American women confirmed that *Lactobacillus* depletion was more prevalent in black women but increased PTB risk only in white women; it also identified distinct taxa markedly associated with spontaneous PTB, with stronger effects in the black cohort (94). The protective role of *L. crispatus* has also been validated in European, Middle Eastern and Asian populations, where *L. iners* dominance associated with higher PTB risk and *L. crispatus* dominance with term delivery (87). Sun *et al* (95) showed in stratified analyses that African-American women have increased abundance of *Lactobacillus iners* and decreased abundance of *Lactobacillus crispatus* and that lower levels of *L. crispatus* are associated with increased risk of spontaneous PTB. Another study of Asian and Myanmar/Karen minority women combined microbiome profiling with cytokine analysis and found that signatures composed of specific taxa and inflammatory cytokines predict PTB more accurately than single markers, thus providing a framework for biomarker development in resource limited settings (96). Fettweis *et al* (35) employed multi omics approaches to identify a marked reduction in *L. crispatus* and increases in pathogenic taxa such as BVAB1 and *Sneathia amnii* alongside elevated levels of pro inflammatory cytokines in cases of PTB, highlighting these features as candidate biomarkers across populations. These findings indicate that intervention strategies should prioritize probiotic strains that enhance or maintain protective *Lactobacilli* colonization in specific populations and that biomarker discovery should integrate microbial and host inflammatory multi omics signatures to achieve precise cross population applicability.

3. Mechanistic pathways linking vaginal microbial dysbiosis to PTB

Inflammatory pathways mediated by vaginal microbial dysbiosis. Elevated diversity within vaginal microbial communities and the presence of bacterial vaginosis-associated taxa, including *Gardnerella vaginalis*, *Atopobium vaginae* and representatives of the Veillonellaceae family, demonstrate a robust association with heightened risk of PTB prior to 34 gestational weeks (7). A study (97) indicates that one pathway by which a diverse VMB elevates PTB risk is through premature activation of parturition-associated inflammatory cascades. In one early clinical investigation, women undergoing cervical cerclage with braided sutures exhibited persistent VMB dysbiosis and local inflammation, accompanied by ultrasound evidence of premature cervical remodeling (97). A UK multicenter retrospective analysis further showed that braided-suture cerclage was associated with a threefold increase in intra-amniotic fetal death and a twofold rise in PTB, whereas monofilament cerclage had no significant impact on the VMB, cytokine concentrations or cervical anatomy. These findings suggest that microbiota-triggered inflammation may act directly on the cervix as pregnancy advances (14). Activation of TLRs

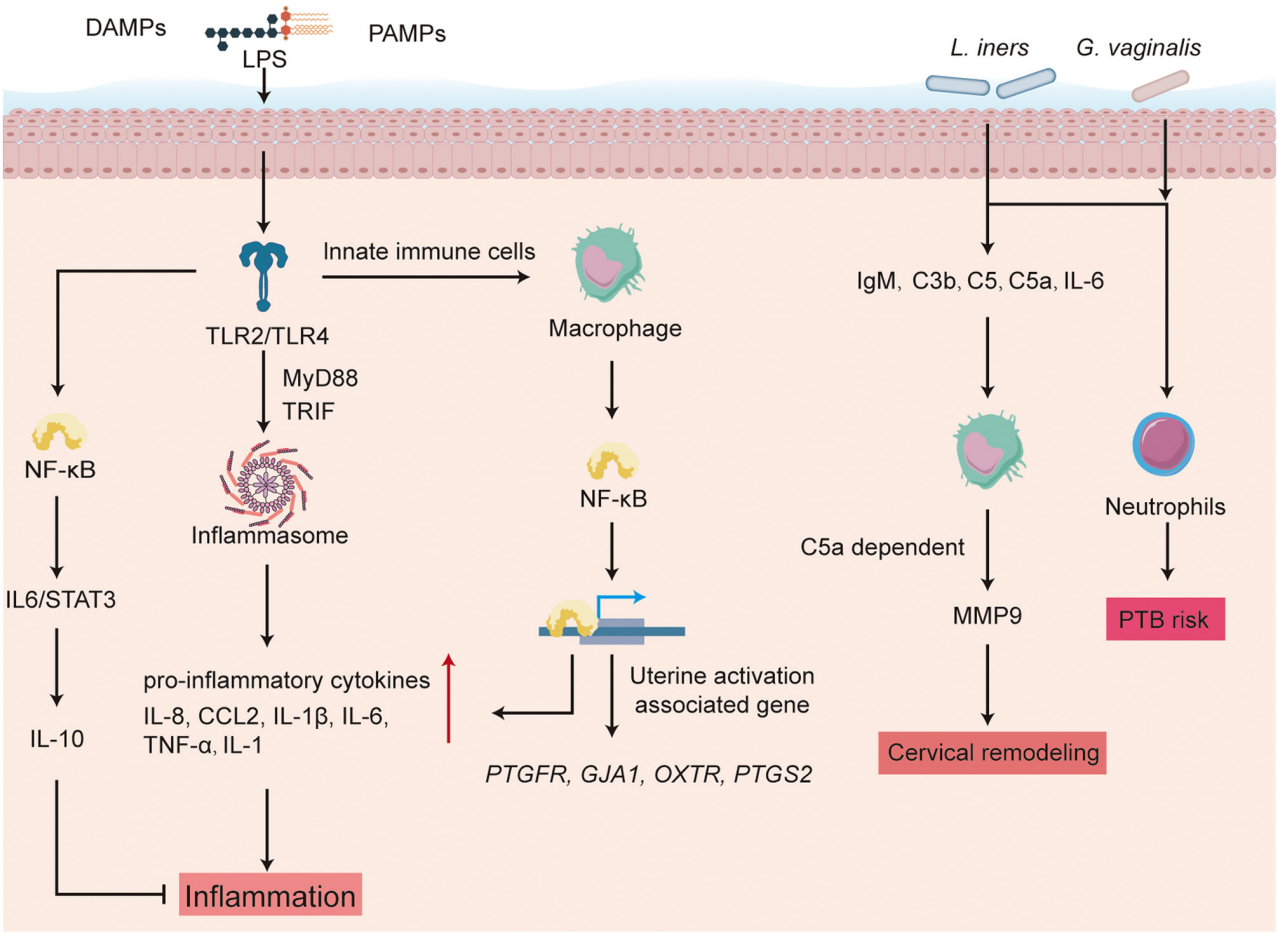

Figure 3. Vaginal microbiota-mediated inflammatory and immune pathways in preterm birth. Pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) activate TLRs, which signal via MyD88- and TRIF-dependent pathways to assemble inflammasomes and release proinflammatory cytokines. TLR activation also recruits macrophages, whose NF-κB signaling upregulates both proinflammatory mediators and contraction-associated genes. Additionally, NF-κB-driven IL-6/STAT3 activation in perivascular stromal cells induces IL-10 production, maintaining immune homeostasis under inflammatory stress. Elevated *L. iners* abundance has been associated with increased vaginal IgM, C3b, C5/C5a and IL-6; C5a promotes macrophage MMP-9 secretion, driving cervical remodeling. *L. iners* and *G. vaginalis* have both been linked to higher neutrophil counts in the vagina, which contribute to cervical shortening during labor and elevate preterm birth risk. PAMPs, pathogen associated molecular patterns; DAMPs, damage associated molecular patterns; TLRs, Toll-like receptors; LPS, lipopolysaccharide.

is regarded as a critical initiating event for inflammation in both term and preterm labor (PTL). TLRs are evolutionarily conserved pattern recognition receptors that orchestrate innate immune responses by sensing pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs), then triggering downstream signaling to induce cytokine and chemokine release (as illustrated in Fig. 3) (98). Among TLR family members, the functional role of TLR4 is best characterized. TLR4 binds lipopolysaccharide (LPS) and other PAMP/DAMP ligands and its activation state in the uterus correlates with both term and PTL. In mice, TLR4 knockout delays parturition and markedly reduces neonatal survival (99). Pharmacologic blockade of TLR4 signaling with the antagonist (+)-naloxone inhibits *Escherichia coli* induced inflammatory cascades and prevents PTB (100). More recently, decidual endothelial cell specific TLR4 expression has been shown to be essential: Mice lacking endothelial TLR4 resist LPS-induced PTB, indicating a non-immune-cell locus of the action of TLR4 (101). Clinically, maternal TLR4 single-nucleotide polymorphisms are markedly associated with extreme preterm delivery (<32 weeks) (102). TLR4 and its co-receptor CD14 signal via both MyD88-dependent and TRIF-dependent pathways to regulate distinct inflammatory gene programs. MyD88-deficient mice are completely protected against *E. coli* induced PTB, underscoring the dominant role of the MyD88 axis in preterm parturition (103). Other TLRs, such as TLR2, also contribute to labor timing (104,105), and fetal TLR4/TLR2 polymorphisms further modulate PTB risk (106,107).

TLR activation can subsequently drive inflammasome assembly, sustaining secretion of IL-8, CCL2, IL-1β and IL-6 by placental and decidual tissues (108). These mediators recruit immune cells and induce prostaglandin and MMP production, collectively promoting cervical ripening and uterine contractility. Amniotic cavity macrophage infiltration constitutes a critical pathophysiological mechanism for spontaneous labor initiation, functionally mediated through NF-κB-driven transcriptional activation of uterine activation-associated gene networks (such as *PTGFR*, *GJA1*, *OXTR* and *PTGS2*) and pro-inflammatory cytokines TNFα, IL-1β, IL-6 and IL-8 (109). Thus, pattern-recognition receptors and downstream effectors orchestrate both physiological and pathological labor initiation.

Immune modulation by the vaginal microbiota in PTB pathogenesis. Various approaches are used to induce PTL and parturition in mice, including immunological, hormonal and environmental triggers. Immunomodulators activate or amplify physiological inflammatory pathways that drive labor onset, recapitulating clinical features of infection and inflammation-associated parturition. The most common murine PTL models involve intrauterine or intraperitoneal administration of LPS or heat-killed whole *Escherichia coli* cells (110). Although LPS is widely employed to induce PTB via pro-inflammatory responses in mice (72), its role in the human female reproductive tract remains unclear. Clinical studies (35,96,111) report elevated concentrations of pro-inflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-8, IL-10, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein-1β (MIP-1β) and eosinophil chemotactic factor, in the amniotic fluid, cervicovaginal fluid and plasma of women who subsequently experienced spontaneous PTB. Moreover, cervical levels of IL-1β, IL-6, IL-8, IL-10, eotaxin, MIP-1β and GM-CSF are associated with VMB composition (112). Animal models demonstrate a central role for macrophages in both term and PTL. Macrophage depletion via anti-F4/80 antibody markedly reduces susceptibility to LPS-induced PTB in mice (113). Mechanistically, macrophages contribute to PTB pathogenesis by secreting TNF-α, IL-1, IL-6, IL-8 and by regulating expression of contraction-associated genes such as MMPs (109). Specifically, IL-1 is critical in inflammatory PTB; exogenous IL-1 induces PTL in mice, whereas blockade of the IL-1 receptor inhibits labor initiation (114), probably through activation of the NF-κB signaling pathway (115). Research indicates that IL-6 deficient mice exhibit delays parturition and resistance to LPS-induced PTB (116). Dominance of *L. iners* is associated with elevated levels of immunoglobulin M, complement components C3b, C5, C5a, and IL-6, collectively leading to cervical shortening, reduced endometrial receptivity, decreased implantation rates and increased PTB risk (117,118). Complement activation also plays a pivotal role: Clinical data show raised terminal complement fragments C3a, C4a and C5a in women undergoing infection-associated spontaneous PTB (113). In mouse models, C5a receptor (C5aR)-deficient animals are protected from both LPS- and RU486-induced PTB; this protection involves aberrant complement deposition in the cervical epithelium and C5a-dependent macrophage MMP-9 release that drives cervical remodeling (119). IL-10 has a protective effect against PTB. IL-10 knockout or neutralization markedly increases susceptibility to PTB in mice (120). IL-10 deficiency leads to downregulation of inflammatory cytokine gene expression in uterine and placental tissues, suggesting its role in tempering excessive inflammation during labor. Decidual endothelial cell TLR4 signaling at term may activate the IL-6/STAT3 axis via NF-κB, promoting perivascular stromal cell production of IL-10 (101). This mechanism probably maintains immune homeostasis under inflammatory conditions, a balance that is disrupted in PTB. Neutrophils, which contribute to cervical remodeling, may infiltrate the myometrium and facilitate cervical shortening during labor, thereby increasing PTB risk (121,122). Limited data suggest that neutrophil numbers in cervicovaginal fluid decline as gestation advances (123). Notably, women with a low-diversity VMB (CST III, dominated by *L. iners*) often exhibit elevated vaginal neutrophil counts during pregnancy (123). One study reported a positive association between *G. vaginalis* (CST IV) abundance and neutrophil levels in samples from women who subsequently delivered preterm (123).

Cezar-de-Mello *et al* (124) analyzed miRNA cargo in extracellular vesicles secreted by *in vitro* human VMB models under BV and *Lactobacillus crispatus* conditions, identifying upregulation of miRNAs targeting the glucocorticoid receptor in the BV environment. The glucocorticoid receptor (GR) pathway mediates canonical anti-inflammatory effects across diverse immune cell types (125). Macrophages expressing pattern recognition receptors, including TLRs, detect danger-associated molecular patterns and initiate inflammasome activation pathways that orchestrate proinflammatory cytokine secretion (126). Glucocorticoids can upregulate NLRP3 inflammasome components to enhance inflammatory responses (126) and GR, in concert with TNF-α, induces TLR2 expression to stimulate innate immunity (127). Conversely, GR can suppress inflammation by promoting macrophage clearance of neutrophils (128). Glucocorticoids also modulate B cell activation, survival, proliferation and differentiation in a dose-dependent manner, reducing B cell counts, progenitor proliferation, and IgG production (129), and they downregulate the anti-apoptotic protein Bcl-2, sensitizing B cells to glucocorticoid-induced apoptosis (125). These findings suggest that vaginal dysbiosis may trigger compensatory local activation of the GR signaling axis to suppress inflammation.

Microbial metabolites as modulators of pregnancy outcomes

Lactate: A protective immunoregulatory metabolite. In vaginal epithelial-cell models, both D-lactic and L-lactic acid, alone or in combination with a spectrum of VMB-associated metabolites, induce the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1RA) while suppressing pro-inflammatory mediators such as IL-6, IL-8, TNF-α, RANTES and macrophage inflammatory protein-3α (130,131). Organotypic models of the female reproductive-tract epithelium confirm that L-lactic acid upregulates IL-1RA and downregulates IL-8 (130). Although certain *in vitro* studies have reported pro-inflammatory responses to specific *Lactobacillus* strains (132,133), these strains are not dominant members of the VMB. *In vivo* experiments and cervical-epithelial models both demonstrate that lactate production by *Lactobacillus* spp. and the resulting acidic vaginal pH reinforce epithelial-barrier integrity, thereby inhibiting colonization by anaerobes and pathogens (134,135). Thus, dominance of protective *lactobacilli* is associated with reduced risk of suboptimal vaginal health. However, excessive *lactobacilli* proliferation can give rise to cytolytic vaginosis, a condition marked by epithelial-cell lysis, dissolution and shedding attributed to overproduction of lactic acid (136). Although cytolytic vaginosis is less prevalent than BV, its occurrence underscores the clinical importance of quantitatively assessing the VMB.

Short-chain fatty acids (SCFAs): immunological consequences in vaginal health. Beyond the presence or absence of key taxa, the VMB influences immune responses and pregnancy outcomes via production of SCFAs. While SCFAs enhance intestinal-barrier integrity (137), their effects in the vaginal milieu appear deleterious. SCFAs are organic

fatty acids typically containing fewer than six carbon atoms; common examples include acetate, propionate and butyrate (138). Elevated levels of SCFAs, such as propionate, succinate, acetate and butyrate, are associated with increased concentrations of IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, IFN-γ and RANTES (139). For instance, in early gestation, elevated acetate is associated with PTB under conditions of weakened *L. crispatus* dominance (139,140). High SCFA concentrations also inhibit expression of neutrophil gelatinase-associated lipocalin, an innate-antimicrobial factor, potentially facilitating overgrowth of BV-associated communities and further contributing to PTB (141). In BV settings, high concentrations of acetate (100 mM) and butyrate (20 mM) selectively activate TLR1/2/3 in cervicovaginal epithelial cells, resulting in increased TNF-α secretion and concomitant suppression of IL-6, RANTES and IP-10 production (131).

Other bioactive metabolites and their effect on parturition. Metabolomic analyses of PTB patients reveal upregulation of glycerophospholipid-metabolism-related metabolites. Glycerophospholipids are fundamental membrane constituents and precursors for bioactive lipids such as arachidonic acid (AA) and lysobisphosphatidic acid (LPA). These bioactive lipids are generated by phospholipase A2 (PLA2) and, in the case of AA, further converted by cyclooxygenase-2 (COX-2) into prostaglandins (PGs) (142,143). LPA3, a G protein-coupled receptor expressed in uterine epithelium, modulates COX-2 activity and PG levels (144). Collectively, these molecules serve as key regulators of inflammation. Increased phosphoinositol, a glycerophospholipid metabolite, depletes glycerophospholipid pools and downstream mediators (LPA and PG), potentially impairing implantation; additionally, elevated phosphoinositol promotes Ca^{2+} influx and uterine contractility, which may precipitate premature labor (145).

Enhanced primary bile-acid and steroid-hormone metabolism pathways have also been observed in PTB cohorts, consistent with findings by Menon *et al* (146) and Lizewska *et al* (147). Bile acids, synthesized from cholesterol in the liver, are modified by the gut microbiota and facilitate dietary-fat absorption (148). Pregnancy-related hormonal changes (such as cholestasis) can disrupt bile acid clearance, leading to elevated circulating levels that induce fetal stress (148). Steroid hormones, also derived from cholesterol, govern development, osmoregulation, metabolism and stress responses. Maturation of the fetal hypothalamic-pituitary-adrenal axis and increased fetal adrenal production of C19 steroids (dehydroepiandrosterone sulfate) and cortisol are pivotal for labor initiation, whereas placental progesterone maintains uterine quiescence during gestation (149). Perturbations in steroid synthesis, metabolism or transport may likewise trigger labor onset.

Hydrogen peroxide (H_2O_2) produced by H_2O_2-generating *Lactobacillus* spp., notably *L. crispatus*, *L. jensenii*, *L. gasseri* and *L. vaginalis* (150), inhibits pathogens such as *G. vaginalis* and *Candida albicans* via oxidation of proteins, essential thiol groups in enzymes, DNA and other cellular components (151). This activity diminishes pathogen adhesion to the vaginal epithelium and ascension into the uterus (150). H_2O_2 also modulates immune responses by suppressing pro-inflammatory cytokines, including IL-1β (152).

4. Probiotic interventions targeting vaginal microbial homeostasis for PTB prevention

The VMB can be modulated by various interventions, including probiotics, prebiotics, antibiotics and pH modulators (153). When treating infectious diseases related to BV, the most commonly used and fastest-acting method is antibiotic therapy. Although antibiotics are effective against infection, they also disrupt the overall microbial balance, including beneficial lactobacilli, leading to high recurrence rates (154) and increased risk of spontaneous PTB (12). As an alternative or adjunct to antibiotics, several approaches have been proposed to restore a *Lactobacillus*-dominated vaginal community. Oral probiotics, leveraging cross-talk between the gut and vaginal microbiomes, are considered a promising method. Probiotics are live, beneficial strains capable of colonizing the vagina and improving its microbial composition, such as *Lactobacillus crispatus*, *L. jensenii* and *L. gasseri* (12,155). These probiotics can be administered orally or vaginally, in formulations including capsules, suppositories or creams (156,157). Most intervention studies have focused on using *Lactobacilli* to alleviate BV and aerobic vaginitis, both of which are dysbiotic states associated with increased risk of spontaneous PTB. Results have been mixed: some studies report efficacy, while others find no significant effect. Probiotic studies are summarized in Table I. For example, one study (158) showed that oral capsules containing *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 reduced vaginal yeast and *E. coli* counts, increased lactobacilli levels and restored an asymptomatic BV microbiota to a healthy *Lactobacilli*-dominated state. Ho *et al*'s (159) study of group B *Streptococcus* (GBS)-positive pregnant women at 35-37 weeks demonstrated that daily oral GR-1 + RC-14 until delivery markedly lowered GBS positivity compared with placebo. In 40 BV-afflicted black women, GR-1 + RC-14 achieved higher cure rates than metronidazole (160). Maternal oral supplementation with *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 probiotic capsules markedly reduced vaginal microbial α diversity and species richness, while concurrently suppressing *Gardnerella vaginalis* colonization. The probiotic cohort exhibited enrichment of *Lactobacillus crispatus*, *Lactobacillus iners* and the gut-associated species *Prevotella copri*, taxa positively associated with VMB stability (157). Other studies have shown that probiotic intervention reduces expression of inflammatory factors, a known risk factor for PTB. In a double-blind trial of 159 volunteers, vaginal tablets containing *Lactobacillus brevis* CD2, *L. salivarius* subsp. *salicinius* and *L. plantarum* achieved clinical cure in nearly 80% of BV patients, with 32% restoring a normal VMB (161). Post-treatment vaginal lavage fluids showed significant decreases in IL-1β and IL-6, whereas the control group showed no change in local pro-inflammatory cytokines (161). Bayar *et al* (155) reported that administering LACTIN-V to high-risk pregnant women markedly reduced early PTB incidence. The protective effect of LACTIN-V may be attributed to its inhibition of pro-inflammatory responses to uropathogens. However, in a randomized, double-blind trial, Husain *et al* (162) demonstrated that daily oral administration of a probiotic preparation comprising *Lactobacillus*

Table I. Effects of probiotic interventions on the female reproductive tract microbiota, bacterial vaginosis, and pregnancy outcomes.

Authors, year	Study population	Intervention protocol	Outcome	(Refs.)
Smith *et al*, 2020	64 healthy women	Oral administration of freeze-dried capsules (containing *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14), once daily for 60 days.	Compared with placebo, oral probiotic therapy markedly increased vaginal *Lactobacilli* counts (logarithmic increase, P=0.01) within 4 weeks, while markedly decreasing yeast and *E. coli* counts. Vaginal health improvement was perceived by 30% in the probiotic group vs. 12% in the placebo group (P=0.17).	(148)
Tuckey *et al*, 2005	110 pregnant women (35-37 weeks), vaginal GBS-positive	Two probiotic capsules daily at bedtime, each capsule containing $1x10^9$ CFU live *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14.	Oral probiotics containing *L. rhamnosus* GR-1 and *L. fermentum* RC-14 markedly reduced vaginal and rectal GBS colonization. GBS negativity rate was 42.9% in probiotic group vs. 18.0% in placebo group.	(149)
Wilks *et al*, 2004	40 women with BV	Two probiotic capsules daily at bedtime (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14).	Probiotic treatment achieved markedly higher BV cure rates compared with metronidazole treatment.	(150)
Lizewska *et al*, 2018	140 BV-positive pregnant women	Topical clindamycin treatment (2% gel, 5 g, intravaginally at bedtime for 7 days), followed by randomization to oral probiotics or placebo, one capsule daily for 30 days.	The probiotic group showed significant reduction in vaginal α-diversity and species richness, decreased *Gardnerella vaginalis* abundance, and increased abundance of *L. crispatus*, *L. iners*, and the gut-associated species *Prevotella copri*, indicative of a healthy vaginal microbiota.	(147)
Srinivasan *et al*, 2015	61 pregnant women at risk for preterm birth	LACTIN-V administration ($2x10^9$ CFU) starting at 14 weeks gestation, daily for 5 consecutive days, then once weekly for 6 weeks.	The probiotic group exhibited a PTB rate of 3.3% before 34 weeks, markedly lower than the 7% rate observed in a background population of 2190 women with similar PTB risk.	(145)
O'Hanlon *et al*, 2011	67 BV patients	Intravaginal probiotic tablets containing a mixture of *Lactobacillus brevis* CD2, *L. salivarius* subsp. *salicinius*, and *L. plantarum* (≥$1x10^9$ CFU/tablet), administered nightly for 8 consecutive days.	Clinical cure was achieved in nearly 80% of the BV patients receiving probiotics, with 32% restoring a normal vaginal microbiota. Levels of IL-1β and IL-6 in vaginal lavage fluid markedly decreased.	(151)

GBS, group B *Streptococcus*.

rhamnosus GR-1 and *Lactobacillus reuteri* RC-14 from early pregnancy did not modify the vaginal microbiota of pregnant women nor prevent bacterial vaginosis. In a similar study, Gille *et al* observed that eight weeks of daily oral administration of *L. rhamnosus* GR-1 or *L. reuteri* RC-14, compared with placebo, yielded no significant difference in the incidence of BV as assessed by Nugent score before and after intervention (163). These divergent findings may reflect limitations in study design. In particular, the proportion of women with abnormal vaginal microbiota in Gille *et al*'s (163) intervention arm was substantially lower than that reported in general populations, warranting validation in larger and more representative cohorts. Moreover, an unexpectedly high loss to follow up may have reduced the statistical power to detect intergroup differences. The two trials employed oral delivery, which may not ensure

sufficient vaginal colonization or confer protection against spontaneous PTB. Consequently, the investigators recommend that future research prioritize the identification of probiotic strains and delivery modalities capable of beneficially modulating the vaginal microbiota during pregnancy before assessing their efficacy in preventing PTB (162). VMB transplantation (VMT) is another strategy under consideration. A recent case series reported successful engraftment of healthy vaginal communities into patients with refractory BV, with four out of five cases achieving sustained remission for up to 21 months (164). Although limited by sample size, these findings underscore the need to assess the long-term efficacy of both probiotic and VMT approaches in pregnant populations.

5. Conclusions and future perspectives

Conclusions. PTB remains a major global health challenge, with 35-50% of spontaneous PTB and PPROM cases attributed to ascending vaginal infections. A growing body of evidence indicates that dynamic shifts in the VMB critically influence pregnancy outcomes. Vaginal microbiota dominated by *Lactobacillus* species, particularly *L. crispatus*, confer protective effects through three synergistic mechanisms: Lactic acid-driven pH homeostasis, bacteriocin-mediated pathogen suppression, and immunoregulatory pathways that attenuate proinflammatory cytokine signaling, including IL-6, IL-8, and TNF-α. By contrast, high-diversity, *Lactobacillus*-depleted (CST IV) vaginal microbiota enriched with opportunistic pathogens such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Prevotella* spp. demonstrate a strong association with increased PTB risk. These pathogens compromise epithelial integrity through biofilm formation, protease secretion and production of SCFAs, exacerbating local inflammation and activating TLR/NF-κB signaling cascades.

Future perspectives. The field continues to confront several persistent challenges despite recent advancements. While observational studies have reproducibly linked vaginal microbial dysbiosis to PTB risk, establishing definitive causal relationships requires longitudinal investigations capable of tracing microbial community trajectories across pregnancy trimesters. Such studies would clarify whether specific pathogen colonization patterns precede cervical remodeling events and identify predictive microbial signatures. A parallel knowledge gap exists in understanding functional differences between *Lactobacillus* species, particularly the clinically relevant dichotomy between *L. crispatus*' protective mucosal interactions and *L. iners*' ambiguous ecological role, which demands comparative multi-omic analyses spanning genomic, proteomic and metabolomic dimensions. Current microbiome profiling techniques have limitations; for example, 16S rRNA sequencing lacks resolution at the species level and does not capture functional information. Future studies should integrate metagenomic and multi-omics approaches to achieve a more comprehensive characterization of both microbial communities and their metabolite profiles. Current therapeutic development efforts face dual limitations: heterogeneous probiotic formulations lacking strain-specific rationale and clinical trials inadequately powered to detect ethnic-geographic variations in microbial-host interactions. Progress necessitates standardized intervention protocols paired with mechanistic studies decoding how microbial metabolites interface with cervical tissue immune networks at single-cell resolution. Addressing these interconnected biological and methodological complexities could enable microbiota-informed risk stratification systems and next-generation therapeutic strategies tailored to individual microbial ecologies.

6. Limitations

Although the present review was based on a comprehensive search and synthesis of PubMed and Web of Science from inception to May 2025, it still has several limitations. First, by restricting its analysis to English-language publications, it may have overlooked important studies in other languages. Second, the included studies vary in their definitions of bacterial vaginosis, sampling time points and sequencing platforms, making direct comparisons difficult. Third, as a narrative review, it did not conduct a formal risk of bias assessment or quantitative meta-analysis, so it cannot rule out the influence of publication bias. Future research would benefit from standardized protocols, broader inclusion criteria, and multicenter collaboration to validate and build upon these findings.

Acknowledgements

Not applicable.

Funding

The present review was supported by the Science and Technology Development Program of Jinan Municipal Health Commission (grant no. 2024203001).

Availability of data and materials

Not applicable.

Authors' contributions

DC, FG and KW reviewed literature and wrote the manuscript. NL and QS collected data and prepared figures. Data authentication is not applicable. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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