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Review

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Vaginal flora and pregnancy complications: A narrative literature review

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Abstract

The vaginal microbiota is a dynamic microbial community with a crucial role in the health and homeostasis of the reproductive system, particularly during pregnancy. This literature review investigates the physiological composition and changes of the vaginal microbiome throughout pregnancy, analyzing forms of dysbiosis and their association with complications such as premature rupture of membranes, preterm birth, and preeclampsia. A systematic literature search was conducted in the main databases focusing on studies published between 2010 and 2024. The findings highlight the protective role of Lactobacillus species (especially L. crispatus), the importance of microbial composition stability and a possible predictive value of the microbiome in pregnancy outcomes. Understanding microbial balance during pregnancy can contribute to the improvement of obstetric care through prevention of adverse outcomes and individualized interventions.

Keywords: Vaginal flora, vaginal microbiome, pregnancy, dysbiosis, microbial diversity, Lactobacillus spp, preterm labor, prematurity, adverse obstetric outcomes

Introduction

The human microbial community, the microbiome, is composed of trillions of microorganisms that inhabit various human organs, such as the skin, mouth, gut, and genital tract. The vaginal microbiome plays a critical role in maintaining female reproductive health, especially during pregnancy [1]. In healthy women of reproductive age the vaginal microbiome is dominated by species of the genus Lactobacillus (L. crispatus, L. iners, L. gasseri, L. jensenii, etc) maintaining an acidic environment through the production of lactic acid, which in turn protects against the overgrowth of pathogens [2]. During pregnancy, significant hormonal and immunological changes affect the composition and stability of the vaginal microbiome. Increased estrogen production leads to vaginal epithelial hyperplasia and increased glycogen production, which is metabolized by Lactobacilli to lactic acid, enhancing the acidic environment and protecting the maternal-fetal unit from infections [3]. The stability of the vaginal microbiome during pregnancy is particularly important. The dominance of Lactobacillus and low microbial diversity are associated with favourable pregnancy outcomes, while increased diversity and the presence of anaerobic bacteria (Gardnerella vaginalis, Atopobium vaginae) is related with increased preterm birth risk and other complications [4,5]. Bacterial vaginosis (BV) is the most common form of vaginal dysbiosis, being characterized by a decrease in Lactobacillus and an overgrowth of anaerobic bacteria. Historically, BV has been associated with an increased risk of preterm birth, premature rupture of membranes, and endometritis [6,7]. Vaginal microbiota composition might also influence the success of embryo implantation and pregnancy outcome; the dominance of L. crispatus has been associated with favorable outcomes, while the presence of other species such as L. iners or increased microbial diversity may be associated with an increased risk of miscarriage or preterm birth [1,5]. Understanding the dynamics of the vaginal microbiome during pregnancy is critical for the development of preventive and therapeutic maternal and fetal health strategies. This literature review investigates the composition and changes of the vaginal flora during pregnancy, its relationship with pregnancy complications, and potential interventions maintaining or restoring a healthy microbiome.

Purpose

The literature review emphasises on the observed physiological and pathological vaginal flora alterations during pregnancy, while also highlighting the consequences of microbial dysbiosis on pregnancy complications.

Specifically, the work aims to:

i. Describe the normal composition of the vaginal flora in women of reproductive age and the factors that influence it,

ii. Analyze the changes observed in the vaginal microbiome during the various pregnancy stages,

iii. Correlate microbial imbalance with adverse obstetric outcomes, such as bacterial vaginosis, preterm labor and intrauterine infections,

iv. Present modern diagnostic and therapeutic approaches to maintain or restore the balance of the vaginal microbiome during pregnancy,

v. Identify research gaps and prospects for future study of the microbiome as a risk indicator and/or target of interventions in the field of maternal and neonatal care.

Materials and methods

A systematic approach was adopted to conduct the review, in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and

Meta-Analyses) guidelines, with the aim of ensuring completeness and transparency in the process of selecting and evaluating studies.

Sample

The PICO framework was used to formulate the research question and define the inclusion criteria:

- Population: Pregnant women in any trimester of pregnancy, regardless of gestational age or method of conception (naturally or with assisted reproductive techniques).
- Intervention: Presence of microbial communities with dysbiosis characteristics in the vaginal microbiota, in particular increased microbial diversity and reduced presence of Lactobacillus crispatus.
- Comparison: Normal vaginal flora with a predominance of species of the genus Lactobacillus (in particular L. crispatus and L. jensenii), associated with microbial homeostasis.
- Outcome: Occurrence of adverse obstetric outcomes, such as preterm labor, spontaneous abortion, premature rupture of membranes, preeclampsia, intraamniotic inflammation, increased inflammatory markers and adverse effects on the neonatal microbiota.

Literature Search

The literature search was performed in the main scientific databases (PubMed, Scopus, ScienceDirect, Cochrane Library and Google Scholar) including scientific articles published from 2010 to 2024. The search terms included combinations of the following keywords: "vaginal microbiome", "pregnancy", "vaginal dysbiosis", "preterm birth", "bacterial vaginosis", "lactobacillus", "vaginal flora and pregnancy outcomes".

Study Selection Process

This was accomplished in four stages:

i. Initial search of articles, based on keywords and filters.

ii. Evaluation of titles and abstracts, excluding irrelevant articles.

iii. Reading of full text to confirm relevance to the Review criteria.

iv. Final selection of articles showing positive correlation between the composition of the vaginal flora and pregnancy course or complications.

Results

The selected studies describe both the physiological adaptations of the microbiome during pregnancy and the relevant dysbiotic conditions, highlighting the potential prognostic value of specific microbial markers. The presentation of the results is organized into thematic sections covering the physiological changes in the microbiome, the characteristics of pathological microbial profiles and their connection with pregnancy outcomes.

Physiological changes in the vaginal microbiome during pregnancy

Understanding physiological changes during pregnancy contributes to the development of surveillance and predictive tools as well as potential maternal-fetal unit interventions. Utilizing a metagenomic approach, the study by Aagaard et al. demonstrated that the vaginal microbiome undergoes adaptations during pregnancy, with predominance of species such as Lactobacillus crispatus, L. iners, L. jensenii and L. johnsonii, with significant reduction of the total bacterial diversity, compared to non-pregnant women. This finding suggests a physiological, possibly protective, hormonally induced "restriction" of microbial diversity [8]. Similar results were also observed by MacIntyre et al. while studying consecutive samples from women before and after childbirth [9]. They illustrated that especially in the second and third trimesters, the microbiome is more stable, showing a dominance of Lactobacillus, while in the postpartum period they decline sharply with an increase in microbial diversity. These changes were directly correlated with fluctuations in estrogen and progesterone levels [9]. Estrogens induce hyperplasia of the vaginal epithelium and the deposition of glycogen, which is metabolized by Lactobacillus into lactic acid, creating an acidic protective environment (pH ~3.5) [10,11]. At the same time, increased progesterone enhances microbial stability, reducing the fluctuations observed outside pregnancy [8]. The study by Gupta et al. in 2020 also pointed out that the diversity of microbiome communities (community state types – CSTs) changes during pregnancy with the CST-I (L. crispatus) and CST-III (L. iners) types prevailing in normal pregnancies [11].

The review by Prince et al. suggests that research on viral (virome) and fungal (mycobiome) load should be incorporated into microbiome studies during pregnancy. The complexity of the vaginal ecosystem goes beyond the simple bacterial composition and includes numerous microorganisms maintaining or deregulating the normal microbial balance during pregnancy [10].

The study by Freitas et al. compared the composition of the vaginal microbiome between 182 pregnant and 310 non-pregnant women, using the cpn60 pyrosequencing technique, which offers superior taxonomic resolution than traditional 16S rRNA. They illustrated significantly lower diversity and richness of the microbiome in pregnant women (Shannon Index 1.3 vs. 1.6, $p < 0.001$), indicating increased microbial monotypy and stability [5]. Most pregnant women showed a dominance of Lactobacillus species (over 80%), with L. crispatus, L. iners, L. gasseri and L. jensenii species defining CST community types I to V. Overall, 62% of pregnant women were included in communities dominated by Lactobacillus, confirming that normal pregnancy promotes microbial compositions of low diversity but high stability [5]. In addition, the study showed that pregnant women showed significantly lower rates of colonization by anaerobic and potentially pathogenic bacteria, such as Gardnerella vaginalis, Mycoplasma hominis and Ureaplasma urealyticum. The microbial load, however, was higher in pregnant women (log 7.7 ± 0.9) compared to non-pregnant women (log 6.8 ± 1.5), possibly attributed to the enhanced metabolism of protective Lactobacilli [5].

The work of Walther-Antonio et al. was among the first studies demonstrating that pregnancy is characterized by a microbiome of high stability and limited diversity, mainly due to the dominance of the species Lactobacillus crispatus and Lactobacillus iners. In this study, L. crispatus dominated most (8 out of 12) samples throughout the course of pregnancy, while only two showed a change to dominance of L. iners, reinforcing the hypothesis that normal pregnancy facilitates the maintenance of a protective, acidic vaginal environment [12].

Similar findings were observed by Li et al; by sampling at different pregnancy stages (pre-conception, 8–12, 24–28 and 37–38 weeks, as well as in the puerperium) these authors identified that Lactobacillus crispatus was the dominant species during pregnancy, while after delivery a significant decrease in Lactobacillus species and increased microbial diversity were documented. Their PCoA analysis (Principal Coordinate Analysis) strengthened the hypothesis of a distinct bacterial signature during pregnancy compared to the puerperium [13].

The large study by Zhang et al. including 454 Chinese women, also concluded that species of the genus Lactobacillus, mainly L. crispatus and L. iners, prevailed during pregnancy. In this study the microbial profile varied depending on the mode of delivery and the mother's age, while after delivery the diversity of the microbiome increased, which was considered a normal evolution [14].

The study by Nunn et al. confirms the decline in Lactobacillus species dominance in the puerperium and their replacement by diverse anaerobic species, such as Streptococcus anginosus and Prevotella bivia, leading to increased diversity and changes in the biochemical composition of vaginal secretions which could be attributed to decreases in the levels of lactic acid isomers and increases in hyaluronic acid and heat shock protein 70 (Hsp70) molecules, affecting the integrity and inflammatory response of the vaginal microenvironment [15].

The study by Li et al. enrolled South African women, monitoring changes in the microbiome during pregnancy and puerperium. A shift towards a Lactobacillus-dominant profile was observed during pregnancy, mainly in women who initially harboured Gardnerella-dominant communities. However, after delivery, a reversal towards diverse anaerobic communities was observed, associated with increased risk of HIV infection or transmission. This work highlights the dynamic nature of the microbiome and the importance of monitoring its changes throughout the reproductive course [16].

Normal pregnancy is accompanied by complex interactions between the maternal microbiome and the immune system, protecting the fetus from pathogens and preventing adverse outcomes. Mei et al. underscore that normal pregnancy requires the maintenance of a stable but delicate balance between immune tolerance and defence. The vagina and endometrium, possess innate safeguard mechanisms against microbes, such as tight junctions of epithelial cells, mucus production, secretion of antimicrobial peptides, and the presence of natural killer (NK) cells and macrophages. Lactobacillus spp. produce lactic acid and hydrogen peroxide (H_2O_2), contributing to the low pH, preventing the establishment of pathogenic bacteria [17].

Severgnini et al. monitored the evolution of the vaginal microbiome during pregnancy and the puerperium. During pregnancy, stability and reduced diversity were observed, with a predominance of Lactobacillus spp., especially L. crispatus and L. jensenii. In the puerperium, however, a significant decrease in Lactobacillus and an increase in Gardnerella, Prevotella, Atopobium and Streptococcus were recorded, especially in women who received intrauterine antibiotic prophylaxis for GBS [18].

The study by Romero et al. is a hallmark extensive longitudinal study of the vaginal microbiome in normally pregnant women through 16S rRNA gene pyrosequencing, illustrating the extreme stability of the pregnant microbiome, compared to non-pregnant women. Pregnant women showed dominance of four Lactobacillus species (L. crispatus, L. jensenii, L. gasseri, and L. vaginalis), while microbial community phenotypes characterized by a high presence of anaerobic bacteria (Gardnerella, Prevotella, Mobiluncus, Atopobium) and associated with bacterial vaginosis (CST IV-A and IV-B), were rarely found in normal pregnancies (Romero et al., 2014). Another key finding was the shift of microbial communities between different types of Lactobacillus-dominated CSTs during pregnancy, without a transition to dysbiotic states, which was interpreted as an adaptive mechanism, protecting pregnancy course [19].

During pregnancy, the female body undergoes profound physiological and metabolic adaptations, also reflected in the composition of the microbiome in several cavities such as the intestine, vagina and oral cavity. Zakaria et al. consider these changes being the result of interactions between hormones, immune response and endogenous microbial flora, maintaining normal pregnancy and promoting fetal development [20]. Increased estrogen and progesterone levels, from the onset of pregnancy, have decisive effects on microbiome composition. Progesterone contributes to the inhibition of uterine contractions and endometrial preparation for implantation, while at the same time affecting the gastrointestinal microbiota, inducing changes related to energy intake and immunoregulation. The production of glycogen and the redistribution of metabolism towards glycolytic and lipogenic mechanisms also affect the microbial composition of the gut, creating an environment rich in short-chain fatty acids [20].

The article by Di Simone et al. emphasizes that physiological changes in gut microbiota during pregnancy – especially in the third trimester – induce a metabolic syndrome, with increased glucose intake, fatty acids and pro-inflammatory markers. Fecal transplants from third-trimester women into mice resulted in increased body weight and insulin resistance, confirming the functional impact of the gut microbiota [21]. Vaginal microbial flora with lactobacilli predominance, contributes to maintaining an acidic pH and prevents pathogens overgrowth. Disruption of this balance, also known as dysbiosis, has been associated with an increased risk of preterm labor, miscarriage, and inflammatory complications. The study underscores improved obstetric prognosis for women with lower bacterial diversity and increased Lactobacillus crispatus prevalence, in contrast to those with a predominance of Gardnerella vaginalis and Mycoplasma [21].

Previous studies of our group have demonstrated increased prevalence of lower genital tract infections (STIs), more specifically bacterial pathogens such as Mycoplasma spp, Ureaplasma spp and chlamydia Trachomatis in women with abnormal cytology and cervical dysplasias, suggesting further higher risk for subsequent obstetric and neonatal morbidity, the extent of which remains to be determined [22-25].

Finally, Amir et al. provide a comprehensive overview of microbial changes in the gastrointestinal, oral, and vaginal microbiota and their association with pregnancy complications, such as preeclampsia, preterm labor, and gestational diabetes mellitus. They emphasize that increased progesterone levels increase Bifidobacterium levels in the gut, which might have a protective role. Furthermore, they report that increased intestinal permeability in experimental pregnancy models is associated with lipopolysaccharide transport and inflammatory response, with potential effects on placental and fetal development [26].

Vaginal dysbiosis and abnormal microbial profiles during pregnancy

Maintaining a favorable vaginal microbial environment represents a critical factor for a smooth pregnancy outcome. During pregnancy, the presence of microbial communities dominated by Lactobacillus species is associated with the maintenance of local homeostasis and the prevention of complications. Occasionally, dysbiosis, a disturbance of the microbial balance, is observed, characterized by increased diversity and predominance of anaerobic or potentially pathogenic microorganisms.

Significant research has focused on the emergence of abnormal microbial profiles during pregnancy, being associated with inflammatory responses, immune dysregulation and increased risk of complications. Understanding the forms of dysbiosis and their biological mechanism is essential for the development of prevention and treatment strategies aimed at maintaining a protective microbial environment during pregnancy.

An Indian study by Talukdar et al. observed increased diversity and presence of anaerobic dysbiotic bacteria such as Gardnerella, Atopobium, Prevotella in women who developed complications or had preterm labor history. According to Gupta et al, despite CST-I (L. crispatus) and CST-III (L. iners) types predominate in normal pregnancies, the presence of CST-IV (increased diversity, lack of Lactobacillus) is associated with adverse outcomes, such as premature rupture of membranes and cervical inflammation [11,27].

In the same context, Prince et al. point out that pathogenic viruses, such as herpes or human papillomaviruses, might interact with vaginal microbiome bacteria, fuelling the inflammatory response. These interactions might predispose to obstetric complications, making interdisciplinary studies of microbial communities important [10]. At the same time, Freitas et al. report that the presence of CST types without Lactobacillus dominance (CST-IVC and CST-IVD) correlate with microbial imbalance indicators, such as a higher Nugent score and Ureaplasma spp. detection, reinforcing the notion that Lactobacillus dominance is a normal pregnancy feature [5].

The study by Severgnini et al. focused on the different genetic subgroups (clades) of Gardnerella vaginalis and their role BV pathogenesis, examining the presence of four clades of G. vaginalis, as well as the gene expression of sialidase in 61 pregnant women. The presence of clade 2 was associated with dysbiosis and low Lactobacillus levels, while the simultaneous presence of ≥2 clades was associated with increased microbial diversity, with a predominance of pro-inflammatory bacteria such as Prevotella, Sneathia, Dialister and Atopobium vaginae. The authors suggested that these clades probably cooperate in suppressing protective Lactobacillus and in the formation of biofilms, thus degrading local defence [28].

Zhang et al. found that women with a history of miscarriage or gestational hypertension had a diverse microbiota. Following delivery, an increase in pathogenic microorganisms such as Gardnerella, Atopobium, Prevotella, and Streptococcus was observed, which has been associated with inflammatory conditions and other morbidities [14].

Studying pregnant women in Uganda, Bayigga et al. (2020) documented a clear association between microbial diversity and inflammatory vaginal status. Only 19% of the samples (n = 179) illustrated Lactobacillus dominance, while the remaining profiles were Gardnerella, Atopobium, and Prevotella dominated. Women with mixed or non-Lactobacillus communities (CT3 and CT4) had significantly elevated levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-8), indicating microbial signatures favouring increased susceptibility to HIV infection and other potential pregnancy complications [29].

The multivariate approach of Oliver et al. combined metabolomic data with characteristics of microbiome composition. Communities dominated by L. crispatus were associated with high mannitol levels, a metabolic signature indicating a potential metabolic stability and protective environment. In contrast, communities rich in Gardnerella were associated with inflammatory and dysbiotic metabolic profiles. This study confirms the importance of a functional (rather than simply taxonomic) approach to appreciate the influence of the microbiome on pregnancy [30].

A recent study by Shabayek et al. in 2022, focused on Streptococcus agalactiae (GBS) colonization effects on the composition of the vaginal microbiome of gravidas in the third trimester. Vaginal microbiome of GBS-positive women was significantly more heterogeneous with increased relative abundance of pathogens such as Gardnerella, Ureaplasma, Corynebacterium and Peptostreptococcus, while showing a significant decrease in the genus Lactobacillus compared to GBS-negative women [31]. This differentiation was associated with reduced functional capacity in GBS-positive communities, especially in pathways such as fatty acid biosynthesis and pyruvate metabolic activity, related to lactic acid production by Lactobacillus spp. The study highlighted the protective function of the acidic environment created by Lactobacillus, inhibiting the growth of pathogens, but also the activation of antioxidant mechanisms by saturated and unsaturated fatty acids [31].

Zierden et al. investigated the physico-chemical properties of cervical mucus (cervicovaginal mucus – CVM) during pregnancy and its relationship with vaginal microbiome. They showed that the composition of the microbiota, especially the Lactobacillus crispatus (CST I) dominance influences the ability of particles to penetrate the mucus. Porosity and particle mobility indices were significantly more limited in samples with CST I, indicating a tighter and more protective barrier. The authors concluded that the composition of the microbiome might determine the efficacy of medicinal vaginal preparations during pregnancy [32].

The study by Chen et al. analyzed the vaginal microbiome in pregnant and non-pregnant women with and without HPV infection. Distinct community status types (CSTs) were identified, with CST I (L. crispatus dominance) occurring more frequently in healthy women, while CST IV (polymicrobial, rich in Gardnerella, Atopobium, etc.) were associated with increased HPV presence and pathological findings. The study highlights the protective effect of Lactobacillus spp. against infections and highlights the importance of the microbiome in maintaining vaginal homeostasis [33].

The study by Nasioudis et al. is one of the few studies to examine the influence of the composition of the vaginal microbiota on intracellular functions of the vaginal epithelium, specifically autophagy and stress response, during the first trimester of pregnancy. Given the importance of the vaginal epithelial surface in maintaining local immunity and homeostasis, the authors investigated whether the prevalence of different bacterial genera is associated with alterations in basic cellular processes [34]. The presence of L. iners, L. gasseri and L. jensenii was associated with intermediate levels of p62 and hsp70, indicating a gradual shift from the favorable state of L. crispatus towards a more "unbalanced" microbial state. Indicatively, L. iners was associated with 4.3 ng/ml p62 and 14.5 ng/ml hsp70, values significantly higher than L. crispatus, but lower than pathogenic microorganisms such as Streptococcus (Nasioudis et al., 2017). Another interesting finding was the indolent behaviour of Gardnerella: despite a bacterium associated with dysbiosis, the levels of p62 (2.3 ng/ml) and hsp70 (29.5 ng/ml) were lower than other non-lactobacilli, which is probably due to different Gardnerella strains or the absence of coexisting bacterial vaginosis. The authors emphasize that none of the enrolled women harboring Gardnerella had symptoms or were diagnosed with vaginitis, suggesting that the "presence" of Gardnerella does not always imply a pathological environment (Nasioudis et al., 2017). Overall, the study reinforces the stance that L. crispatus is the most favorable cellular homeostasis microbial factor during pregnancy, supporting a tolerant, low-inflammatory environment and favoring the induction of autophagy. The presence of other lactobacilli or potential pathogens was associated with a more intense cellular reaction and possible disruption of the immune microenvironment of the vagina (Nasioudis et al., 2017).

Geldenhuys et al. examined the differentiation of the microbiome in women with preeclampsia versus healthy pregnant women. Women with preeclampsia had increased alpha diversity in the vaginal microbiota and a concomitant decrease in Lactobacillus spp., especially L. crispatus, while a relative prevalence of L. iners was observed, which may reflect microbial instability with a potential role in the pathogenesis of preeclampsia [35].

Correspondingly, the study by Gryaznova et al. identified a significant presence of Gardnerella vaginalis and Gram-negative anaerobic bacteria in women with recurrent miscarriages and increased levels of NK cells, indicating a correlation between dysbiosis and inflammation or immunological dysregulation [36].

Association of the microbiome with obstetric outcomes and prognostic value

During pregnancy, the stability and balance of the vaginal microbiome are critical factors for maintaining favorable endometrial environment and the smooth pregnancy course. The microbial composition of the vagina, beyond its local importance, is closely linked to the gravida's immune status and the prevention of detrimental inflammatory pathways.

Recently, evolution in metagenomic technology has allowed in-depth analysis of microbial diversity and the identification of specific patterns that may be associated with adverse obstetric outcomes. The possibility of evaluating the microbiome as a diagnostic or prognostic indicator gathers intense research interest, aiming at risk early identification and potential prevention of complications. Several studies focus on the association between microbial composition and obstetric outcomes, as well as the microbiome's prognostic value during pregnancy.

The review by Gerede et al., focused on possible associations between the vaginal microbiome and pregnancy complications, highlighting pathological changes in the microbial community as prognostic factors. They concluded that the loss of dominance of Lactobacillus spp. and increased microbial diversity (especially the presence of anaerobic bacteria such as Gardnerella, Prevotella, Atopobium, Sneathia and BVAB-2) are associated with a higher preterm birth risk, premature rupture of membranes, spontaneous abortion, preeclampsia and gestational diabetes [37]. The presence of CST-IV communities (lack of Lactobacillus and anaerobes predominance) has been repeatedly associated with adverse outcomes. Lactobacillus crispatus, appears protective, as women with this species predominance had a significantly lower rate of early complications. In contrast, Lactobacillus iners, despite essentially a Lactobacillus, does not offer the same stability and has been occasionally associated with preterm birth and infections. Another crucial finding was that early microbiome changes (even from the first trimester) seem to accurately predict the risk of future complications. This supports the need for monitoring and possible diagnostic use of the microbiome composition from conception onwards [37].

The study by Fettweis et al. focuses mainly on the pathophysiological association of the vaginal microbiome with preterm labor, providing valuable evidence for the predictive value of the microbial composition. In this study, women who experienced preterm labor had lower L. crispatus levels and increased presence of potential pathogens, such as BVAB1, Sneathia amnii, and species of the genus Prevotella, which were associated with increased levels of proinflammatory cytokines in the vaginal biochemical environment [38]. These findings also highlight the importance of microbial homeostasis in preventing pregnancy complications.

Silvano et al. examined the composition of the vaginal microbiota in pregnant women with and without cervical shortening, an established preterm birth predictor, by comparing 68 women with a cervical length ≤25 mm versus 29 women with a normal cervical length. Women with cervical shortening had higher relative abundance of the phyla Proteobacteria and Actinobacteriota, while overall microbial diversity was reduced. In particular, the microorganisms Staphylococcus, Pseudomonas and Brevundimonas were significantly increased in women with a short cervix – these species are associated with aerobic vaginitis, which has been linked to preterm birth and miscarriage. In contrast, the presence of Lactobacillus iners and Bifidobacterium were associated with normal cervical length [39].

Dunlop et al. investigated the association of early pregnancy vaginal microbiota with the likelihood of spontaneous preterm or early birth (sPTB and sETB, respectively) in African-American women. The presence of CST-III (L. iners) or CST-IV (without Lactobacillus dominance) was associated with an increased risk of sPTB. The abundance of bacteria such as Gardnerella vaginalis, Atopobium vaginae, Prevotella amnii, and Dialister microaerophilis was associated with the occurrence of sPTB, highlighting the potential role of specific pathogenic species as biomarkers of preterm birth [40].

The review by Mendz summarizes findings on vaginal microbiota during pregnancy. The presence of L. crispatus and L. jensenii is an indicator of a favorable microbial profile, while the dominance of mixed anaerobic colonies with Gardnerella, Atopobium and Megasphaera is associated with adverse outcomes (preeclampsia, miscarriage and preterm birth). Furthermore, the study highlighted the potential importance of individualized monitoring of the vaginal microbiota in predicting obstetric outcomes [41].

Saadaoui et al. discuss the role of vaginal dysbiosis in miscarriage, a phenomenon affecting up to 25% of pregnancies. The dominance of Lactobacillus appears to be protective, while dysbiosis – especially with proinflammatory or potentially pathogenic species such as Gardnerella vaginalis and Atopobium vaginae – is associated with increased miscarriage risk. The importance of microbiome control and monitoring during pregnancy is also highlighted, especially in cases of recurrent miscarriage or threatened miscarriage [42].

The recent work by Liao et al. focuses on the genetic diversity of the vaginal microbiome and its association with preterm birth. Using metagenomic data from 705 samples, the authors found that women who subsequently had preterm birth had significantly higher microdiversity, particularly in Gardnerella species, which showed more genetic mutations, recombinations and evidence of evolutionary pressure in lipid metabolism genes. The presence of antimicrobial resistance genes was also increased, indicating a possible evolutionary advantage of pathogenic microorganisms in these cases [43].

In the study by Hocevar et al., 155 women were compared based on pregnancy outcome (full-term vs. preterm birth). Women who delivered preterm had higher diversity (richness and Shannon diversity) showing increased presence of Gardnerella, Atopobium, Sneathia, Megasphaera and Streptococcus, while the presence of Lactobacillus was significantly reduced. The inflammatory response induced by these communities is proposed as a mechanism for triggering preterm birth, possibly through production of pro-inflammatory cytokines and subsequent fetal membranes degradation [44].

Pace et al. used metagenomic data for in-depth study of the vaginal microbiome evolution from pregnancy to the puerperium. The presence of L. iners and G. vaginalis was associated with microbiome instability and increased preterm birth risk. Low L. crispatus abundance, combined with increased Prevotella presence, emerged as a preterm birth predictor. Furthermore, the use of WGS allowed the detection of distinct strains with pathogenic potential, highlighting the importance of strain-level analysis [45].

The review by Fox et al. focused on the association between the maternal microbiome (vaginal, intestinal and placental) and PTB. New data from metagenomics technologies, such as 16S rRNA and

Whole Genome Sequencing (WGS), allow the discovery of microbial signatures that may act as predictive markers of risk for preterm birth. This review highlights the future prospect of personalized interventions, such as the administration of probiotics or acidifying preparations (e.g. lactic acid) for PTB prevention [46].

Lledó et al. compared the microbiome of pregnant women after natural conception, with those who successfully underwent assisted reproduction (IVF). Women in the IVF group had a significantly greater diversity of microbial genera and an increased presence of Gardnerella, Prevotella, Neisseria and Staphylococcus, compared to the natural conception group. L. iners was found to be increased in IVF women, while L. gasseri appeared to be decreased, indicating a possible association of the microbiome composition with the increased risk of preterm birth observed in IVF pregnancies [47].

The study by Tabatabaei et al. identified different patterns of vaginal microbiome in pregnant women depending on the presence of urogenital infections. Women with a history of infections had increased relative abundance of pathogens such as Gardnerella and Atopobium, while healthy women had a predominance of L. crispatus. This variation was associated with an increased bacterial vaginosis and preterm labor risk [48].

Nasioudis et al. investigated how pregnancy history affects the composition of the microbiome during the first trimester of pregnancy. Primigravidas showed L. crispatus predominance (76.4%), while parous women had significantly lower rates (22.1%) and an increased presence of L. iners and Gardnerella [49]. The findings suggest that parity and gravidity alter the vaginal microbial composition in the long term, possibly affecting the course and outcome of pregnancy.

The study by Eslami et al. highlights the importance of the vaginal microbiome in female reproductive health, focusing on its potential impact on spontaneous abortion. Women who suffered abortions had a disturbed vaginal microbiome, with a decrease in the population of Lactobacillus spp. and an increase in potentially pathogenic microorganisms. In fact, 77.3% of women with a history of abortion were classified as suboptimal microbial profiles (CSTs), reinforcing the hypothesis that disruption of the microbial balance may be a predictor of increased pregnancy loss risk [50].

Marangoni et al. delve into the metabolic and microbial properties of the vagina in women with normal pregnancies and those who have experienced spontaneous abortion. They illustrate that in normal pregnancies vaginal flora is dominated by Lactobacillus species, accompanied by a metabolic profile that includes high levels of lactic acid, glycine and other amino acids. In contrast, in women with miscarriage, an increase in metabolites such as inosine, fumarate and xanthine was recorded, which are associated with pathogenic microorganisms and possibly with dysregulation of the local immune environment. These findings highlight potential biomarkers for early pregnancy loss prediction [51].

Haque et al. performed a meta-analysis of microbiome data from first trimester gravidas to predict preterm birth risk. Microbiome diversity was significantly lower in women who delivered preterm, suggesting the use of new diversity markers (such as TCS) with strong diagnostic capacity for early detection of preterm birth risk. This work foresees the development of early intervention tools to reduce neonatal mortality [52].

Pruski et al. introduce the innovative DESI-MS technique for the direct metabolic characterization of the vaginal microbiome and the inflammatory response of the host immune system during pregnancy. They demonstrated that microbiome instability and activation of the innate immune system are associated with an increased risk of preterm birth, even in cases with preventive cervical cerclage. Of particular importance is the potential of this method to provide rapid prognostic information in clinical practice, paving the way for targeted interventions [53].

In the study by Witkin et al. the authors examined the association between specific biomarkers in the vaginal membrane and microbial composition, as well as cervical length. High levels of TIMP-1 and low levels of D-lactic acid were found to be associated with shorter cervical length and a microbiome profile not dominated by Lactobacillus crispatus or jensenii. The authors' causal model suggests that biomarkers can predict microbiome composition and the risk of preterm birth, making their measurement a useful tool in poor settings where sophisticated molecular techniques are not available [3].

Tabatabaei et al. analyzed vaginal microbial colonization in early pregnancy and associated specific microbiome profiles with the risk of preterm birth. Women with colonization by L. crispatus, L. gasseri, and L. johnsonii had a reduced risk of early preterm birth, while the presence of CST IV (Gardnerella, Atopobium, Veillonellaceae) microbial communities was associated with an increased risk [48].

The study by Cobo et al. investigated the association of vaginal microbiome composition with intra-amniotic inflammation (IAI) in 64 women with preterm birth (<34 weeks). The results showed that women with IAI had higher microbial diversity and significantly reduced Lactobacillus spp. load, compared to women without IAI [54]. The loss of Lactobacillus, especially L. crispatus, was associated with increased levels of IL-6 in amniotic fluid ($p = 0.008$), increased risk of chorioamnionitis, and shorter latency period to delivery. In contrast, L. iners did not appear to protect against inflammatory conditions. The vaginal environment of women with IAI was enriched in pathogens such as Gardnerella, Peptoniphilus, Haemophilus, and Ureaplasma [54]. Furthermore, microorganisms detected in the amniotic fluid by 16S rRNA sequencing (such as Mycoplasma hominis, Fusobacterium, Prevotella) were identical to those in the vagina, suggesting a possible ascendance of pathogens from the vagina to the endometrium. The study reinforces the importance of microbial homeostasis and Lactobacillus spp. dominance in the prevention of intrauterine inflammation and preterm labor [54].

The study by Baud et al., with a sample of 749 third trimester gravidas, confirmed that the normal, healthy vaginal microbiota is characterized by low microbial diversity and dominance of Lactobacillus species, especially L. crispatus. In this study again, the lack of this dominance, as well as increased species diversity – including Gardnerella vaginalis and Ureaplasma, was associated with an increased risk of preterm labor. The authors stressed that it was the overall diversity of the microbiome, and not necessarily the presence of a specific species, which emerged as the best predictor of preterm birth. Using a Random Forest algorithm, twelve bacterial species were identified as the most predictive indicators for preterm birth, with typical examples being Mageeibacillus indolicus, Streptococcus mitis, Fusobacterium nucleatum, but also non-traditional Lactobacillus species such as L. delbrueckii and L. reuteri [55].

DiGiulio et al. focused on the temporal and spatial dynamics of the microbiome at multiple sites of pregnant women cavities (vagina, mouth, intestine) during pregnancy and up to one year postpartum. The authors observed significant stability in the composition of microbial communities, especially in the vagina, throughout pregnancy. However, women who had microbial communities poor in Lactobacillus and rich in Gardnerella and Ureaplasma – corresponding to CST IV – had significantly increased risk of preterm birth. A shift towards such a profile can be detected early in pregnancy and used prognostically [4].

Summarizing the findings of the studies reviewed, it is evident that the composition and stability of the microbiome during pregnancy –especially of the vaginal microbiome – are influenced by a multitude of factors, with physiological and pathological changes having significant consequences on obstetric outcome. The dominance of the genus Lactobacillus, especially specific species such as L. crispatus, appears to be associated with favorable prognosis, while the presence of potentially pathogenic microorganisms and increased microbial diversity are repeatedly associated with complications, such as premature birth and miscarriage. The increasing understanding of microbial profiles and their changes during pregnancy highlights the importance of microbial homeostasis for reproductive health and lays the foundation for future diagnostic and therapeutic interventions.

Discussion

The importance of the vaginal microbiome role in pregnancy is based in maintaining the normal course of pregnancy and as a potential predictor of obstetric complications. The healthy microbiome is mainly characterized by low diversity and dominance of Lactobacillus species, especially L. crispatus, which maintains an acidic environment protecting against the colonization of pathogenic microorganisms [1,2,55].

The presence of L. iners and the increased abundance of species such as Gardnerella vaginalis, Atopobium vaginae, Prevotella spp. and Ureaplasma is associated with microbiome imbalance (CST-IV), which in turn has been linked to higher rates of preterm birth, miscarriage, and bacterial vaginosis [40,42]. This association is also confirmed by metagenomic studies, which revealed that genetic variation and microdiversity in species such as Gardnerella lead to increased inflammatory response and expression of genes related to pathogenesis [43].

The use of newer technologies such as metagenomic sequencing and artificial intelligence algorithms (e.g. Random Forest) allowed the detection of bacterial species that act as biomarkers of preterm labor, including Mageeibacillus indolicus, Fusobacterium nucleatum and Streptococcus mitis [55]. This finding highlights the value of strain-level analysis and the need for sophisticated diagnostic strategies.

Furthermore, it was observed that pregnancy history significantly influences the microbial profile. Primiparity is associated with a higher presence of L. crispatus, while multiparity with a higher probability of dysbiosis and the presence of pathogenic microorganisms. This finding may be crucial for personalized care of pregnant women, with risk stratification according to reproductive history [34,49].

The association of microbial imbalance with spontaneous abortions is reinforced by the study of Eslami et al., who found that 77.3% of women with a history of miscarriages were categorized as suboptimal microbial profiles (CSTs). These data support the hypothesis that the vaginal microbiome can be used as an early biomarker of pregnancy loss risk, with important implications for prevention [50]. However, questions remain regarding the direction of causality: is dysbiosis a cause or a consequence of inflammatory and immune responses that lead to complications? Longitudinal follow-up of pregnant women using variables such as mode of delivery, age, diet, and ethnicity could further elucidate these relationships [14].

This review highlights the need to incorporate vaginal microbiota screening into prenatal care, particularly in high-risk groups. Assessment of microbial communities through non-invasive techniques (e.g., PCR, 16S rRNA) could become routine in women with a history of preterm labor or miscarriage, allowing for early intervention with probiotics, antimicrobials or even targeted immunomodulation [17]. Furthermore, a revision of obstetric care guidelines emerges, with an emphasis on training healthcare professionals – and especially obstetricians and midwives to recognize microbial profiles and understand their implications for pregnancy. A personalized, microbiome-based approach is the next step in preventing adverse outcomes.

Despite the vast progress in understanding the relationship between the vaginal microbiome and obstetric outcomes, significant knowledge gaps remain, necessitating further research. A key emerging question focuses on the long-term effects of microbial dysbiosis on fertility, as well as the risk of complications in subsequent pregnancies. The investigation of whether the adverse effects of microbial disturbance persist beyond the puerperium or are spontaneously reversed is a critical research area. Besides, it is necessary to clarify the nature of the interaction between the microbiome, the hormonal substrate and the immune response during pregnancy. Although there is evidence for the influence of progesterone and estrogens on the composition of the microbiome, the exact molecular pathways that mediate this relationship remain largely unclear.

Furthermore, uncertainty concerns the role of geographical origin, genetic polymorphisms, ethnic identity and socioeconomic context in the formation of microbial communities. The differences observed between populations, such as African and European groups, indicate that the creation of universal criteria for a "healthy" microbiome may be unrealistic without adjustments to demographic data. At the same time, questions arise about the efficacy, duration of action and safety of interventions such as probiotics in pregnant women with microbial imbalance. Studies to date are limited, short-term and heterogeneous in terms of strains and dosages, making it imperative for well-conducted randomized, multicenter trials with strict inclusion criteria. Finally, in a broader context, the development of a "pregnancy microbial passport" could be considered – a predictive tool based on artificial intelligence algorithms and metagenomics analyses, which would allow the classification of pregnant women into risk groups based on the composition of their microbiome. Such a tool could support clinical decision-making and the early prevention of adverse outcomes, enhancing the transition towards highly individualized prenatal care.

Strengths and limitations of this work

This literature review presents important virtues enhancing its scientific validity and practical value. Recent studies of high methodological quality were systematically identified and analyzed, utilizing sources from both metagenomic and traditional microbiome analysis techniques, emphasizing on pregnancy. The multi-level approach, including examining the relationship of the vaginal microbiome with several obstetric outcomes (preterm labor, miscarriage, preeclampsia and inflammation) makes this review comprehensive and clinically useful. Furthermore, the work highlights not only the pathogenetic associations but also the potential prognostic and diagnostic value of the microbial composition, contributing to the formulation of proposals for future applications in daily obstetric practice.

However, there are several limitations, related to the nature of the literature review and the characteristics of the primary studies themselves. Many of the included studies were heterogeneous in terms of design (cross-sectional, prospective, meta-analyses), population (geographical and ethnic differences), and microbiome analysis techniques (e.g., use of 16S rRNA versus WGS). This heterogeneity impedes comparisons and generalization of the findings. In addition, most studies focus on the vaginal microbiome, with limited research on the interaction with the intestinal or placental microbiome, despite evidence of a systemic effect. Finally, most included studies were based on correlations, rather than evidence of causality, which limits the drawing of clear conclusions regarding the pathogenicity or protective effect of specific microbial species.

Conclusion

The study of the vaginal microbiome during pregnancy is a rapidly evolving field of research, highlighting important aspects of the interaction between microbial ecology and human reproductive health. Maintenance of the dominance of species of the genus Lactobacillus, and mainly L. crispatus, is associated with a normal course of pregnancy and favorable obstetric outcomes. In contrast, the presence of highly diverse microbial communities, with a predominance of anaerobic species such as Gardnerella, Atopobium and Sneathia, is constantly associated with an increased risk of preterm birth, spontaneous abortion, inflammatory complications and intrauterine infections.

Evidently, microbial changes are not simply a result of the normal hormonal and immunological pregnancy alterations but potentially pre-exist or emerge early in the 1st trimester, exerting a prognostic impact on the course of pregnancy. The ability to identify specific microbial "signatures" through technologies such as 16S rRNA sequencing or Whole Genome Sequencing (WGS) enhances the prospect of developing reliable risk biomarkers, with application in early prevention and/or intervention. Despite the methodological limitations of existing studies, this review underscores that monitoring the composition of the vaginal microbiome can be a valuable tool in modern prenatal care. The introduction of predictive models based on microbial diversity, as well as the evaluation of interventions such as the administration of personalized probiotic formulations, are potentially revolutionary strategies for reducing maternal and perinatal morbidity. Overall, the present work confirms the importance of microbial balance in the female reproductive system and highlights the need for further research, as well as a practically applicable framework for the prognosis and management of the risks associated with dysbiosis during pregnancy.

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Abbreviations

BV - Bacterial Vaginosis
CSTs - Community State Types
DESI-MS - Desorption Electrospray Ionization Mass Spectrometry
Hsp - Heat shock protein
IAI - Intra-Amniotic Inflammation
NK - Natural Killer
PCoA analysis - Principal Coordinate Analysis
PICO framework – Acronym for: Population/Patient/Problem, Intervention, Comparison, and Outcome
PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PTB - Preterm Birth
sPTB - spontaneous Preterm Birth
sETB - spontaneous Early Term Birth
STIs – Sexually Transmitted Infectios
TIMP-1 - Tissue Inhibitor of Metalloproteinase-1
WGS - Whole Genome Sequencing

References

1. Petrova, M.I., Lievens, E., Malik, S., Imholz, N., Lebeer, S. Lactobacillus species as biomarkers and agents that can promote various aspects of vaginal health. Front Physiol 2015, 6, 81, doi: 10.3389/fphys.2015.00081.
2. Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., et al. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences 2011, 108, 4680-4687, doi:10.1073/pnas.1002611107.
3. Witkin, S.S., Moron, A.F., Ridenhour, B.J., Minis, E., Hatanaka, A., Sarmento, S.G.P., Franca, M.S., Carvalho, F.H.C., Hamamoto, T.K., Mattar, R., et al. Vaginal Biomarkers That Predict Cervical Length and Dominant Bacteria in the Vaginal Microbiomes of Pregnant Women. mBio 2019, 10, doi:10.1128/mbio.02242-02219.
4. DiGiulio, D.B., Callahan, B.J., McMurdie, P.J., Costello, E.K., Lyell, D.J., Robaczewska, A., Sun, C.L., Goltsman, D.S., Wong, R.J., Shaw, G., et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A 2015, 112, 11060-11065, doi:10.1073/pnas.1502875112.
5. Freitas, A.C., Bocking, A., Hill, J.E., Money, D.M., Group, V.R. Increased richness and diversity of the vaginal microbiota and spontaneous preterm birth. Microbiome 2018, 6, 117, doi:10.1186/s40168-018-0502-8.
6. Klebanoff, M.A., Nansel, T.R., Brotman, R.M., Zhang, J., Yu, K.F., Schwebke, J.R., Andrews, W.W. Personal hygienic behaviors and bacterial vaginosis. Sexually transmitted diseases 2010, 37, 94-99, doi:10.1097/OLQ.0b013e3181bc063c.
7. Stout, M.J., Zhou, Y., Wylie, K.M., Tarr, P.I., Macones, G.A., Tuuli, M.G. Early pregnancy vaginal microbiome trends and preterm birth. American Journal of Obstetrics and Gynecology 2017, 217, 356.e351-356.e318, doi:10.1016/j.ajog.2017.05.030.
8. Aagaard, K., Riehle, K., Ma, J., Segata, N., Mistretta, T.A., Coarfa, C., Raza, S., Rosenbaum, S., Van den Veyver, I., Milosavljevic, A., et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One 2012, 7, e36466, doi:10.1371/journal.pone.0036466.
9. MacIntyre, D.A., Chandiramani, M., Lee, Y.S., Kindinger, L., Smith, A., Angelopoulos, N., Lehne, B., Arulkumaran, S., Brown, R., Teoh, T.G., et al. The vaginal microbiome during pregnancy and the postpartum period in a European population. Sci Rep 2015, 5, 8988, doi:10.1038/srep08988.
10. Prince, A.L., Chu, D.M., Seferovic, M.D., Antony, K.M., Ma, J., Aagaard, K.M. The perinatal microbiome and pregnancy: moving beyond the vaginal microbiome. Cold Spring Harbor perspectives in medicine 2015, 5, a023051.
11. Gupta, P., Singh, M.P., Goyal, K. Diversity of Vaginal Microbiome in Pregnancy: Deciphering the Obscurity. Front Public Health 2020, 8, 326, doi: 10.3389/fpubh.2020.00326.
12. Walther-António, M.R., Jeraldo, P., Berg Miller, M.E., Yeoman, C.J., Nelson, K.E., Wilson, B.A., White, B.A., Chia, N., Creedon, D.J. Pregnancy's stronghold on the vaginal microbiome. PLoS One 2014, 9, e98514, doi:10.1371/journal.pone.0098514.
13. Li, D., Chi, X.Z., Zhang, L., Chen, R., Cao, J.R., Sun, X.Y., Yang, H.Q., Liao, Q.P. Vaginal microbiome analysis of healthy women during different periods of gestation. Biosci Rep 2020, 40, doi: 10.1042/BSR20201766.
14. Zhang, X., Zhai, Q., Wang, J., Ma, X., Xing, B., Fan, H.,

Gao, Z., Zhao, F., Liu, W. Variation of the Vaginal Microbiome During and After Pregnancy in Chinese Women. Genomics, Proteomics & Bioinformatics 2022, 20, 322-333, doi:10.1016/j.gpb.2021.08.013.
15. Nunn, K.L., Witkin, S.S., Schneider, G.M., Boester, A., Nasioudis, D., Minis, E., Gliniewicz, K., Forney, L.J. Changes in the Vaginal Microbiome during the Pregnancy to Postpartum Transition. Reproductive sciences 2021, 28, 1996-2005, doi: 10.1007/s43032-020-00438-6.
16. Li, K., Li, F., Jaspan, H., Nyemba, D., Myer, L., Aldrovandi, G., Joseph-Davey, D. Changes in the vaginal microbiome during pregnancy and the postpartum period in South African women: a longitudinal study. Res Sq 2023, doi:10.21203/rs.3.rs-2617869/v1.
17. Mei, C., Yang, W., Wei, X., Wu, K., Huang, D. The Unique Microbiome and Innate Immunity During Pregnancy. Front Immunol 2019, 10, 2886, doi:10.3389/fimmu.2019.02886.
18. Severgnini, M., Morselli, S., Camboni, T., Ceccarani, C., Laghi, L., Zagonari, S., Patuelli, G., Pedna, M.F., Sambri, V., Foschi, C., et al. A Deep Look at the Vaginal Environment During Pregnancy and Puerperium. Front Cell Infect Microbiol 2022, 12, 838405, doi:10.3389/fcimb.2022.838405.
19. Romero, R., Hassan, S.S., Gajer, P., Tarca, A.L., Fadrosh, D.W., Nikita, L., Galuppi, M., Lamont, R.F., Chaemsaithong, P., Miranda, J., 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, 4, doi:10.1186/2049-2618-2-4.
20. Zakaria, Z.Z., Al-Rumaihi, S., Al-Absi, R.S., Farah, H., Elamin, M., Nader, R., Bouabidi, S., Suleiman, S.E., Nasr, S., Al-Asmakh, M. Physiological Changes and Interactions Between Microbiome and the Host During Pregnancy. Front Cell Infect Microbiol 2022, 12, 824925, doi:10.3389/fcimb.2022.824925.
21. Di Simone, N., Santamaria Ortiz, A., Specchia, M., Tersigni, C., Villa, P., Gasbarrini, A., Scambia, G., D'Ippolito, S. Recent Insights on the Maternal Microbiota: Impact on Pregnancy Outcomes. Front Immunol 2020, 11, 528202, doi:10.3389/fimmu.2020.528202.
22. Valasoulis, G., Pouliakis, A., Michail, G., Magaliou, I., Parthenis, C., Margari, N., Kottaridi, C., Spathis, A., Leventakou, D., Ieronimaki, A.I., et al. Cervical HPV Infections, Sexually Transmitted Bacterial Pathogens and Cytology Findings-A Molecular Epidemiology Study. Pathogens 2023, 12, doi:10.3390/pathogens12111347.
23. Valasoulis, G., Pouliakis, A., Michail, G., Daponte, A.I., Galazios, G., Panayiotides, I.G., Daponte, A. The Influence of Sexual Behavior and Demographic Characteristics in the Expression of HPV-Related Biomarkers in a Colposcopy Population of Reproductive Age Greek Women. Biology (Basel) 2021, 10, doi:10.3390/biology10080713.
24. Valasoulis, G., Pouliakis, A., Magaliou, I., Papoutsis, D., Daponte, N., Margioula-Siarkou, C., Androutsopoulos, G., Daponte, A., Michail, G. Could APTIMA mRNA Assay Contribute to Predicting Cervical Bacterial Sexually Transmitted Co-Infections? A Colposcopy Population Study. International journal of molecular sciences 2024, 25, doi:10.3390/ijms252313146.
25. Magaliou, I., Michail, G., Papoutsis, D., Daponte, N., Truva, T., Pouliakis, A., Daponte, A., Valasoulis, G. Pharmacogenomics and Perinatal Morbidity: A Comprehensive Review of Bacterial Sexually Transmitted Infections. In Convergence of Population Health Management, Pharmacogenomics, and Patient-Centered Care, Moumtzoglou, A.S., Ed., IGI Global Scientific Publishing: Hershey, PA, USA, 2025, pp. 205-238.
26. Amir, M., Brown, J.A., Rager, S.L., Sanidad, K.Z., Ananthanarayanan, A., Zeng, M.Y. Maternal Mi-

crobiome and Infections in Pregnancy. Microorganisms 2020, 8, doi:10.3390/microorganisms8121996.

27. Talukdar, D., Sarkar, M., Ahrodia, T., Kumar, S., De, D., Nath, S., Jana, P., Verma, J., Mehta, O., Kothidar, A., et al. Previse preterm birth in early pregnancy through vaginal microbiome signatures using metagenomics and dipstick assays. iScience 2024, 27, 111238, doi:10.1016/j.isci.2024.111238.
28. Severgnini, M., Morselli, S., Camboni, T., Ceccarani, C., Salvo, M., Zagonari, S., Patuelli, G., Pedna, M.F., Sambri, V., Foschi, C., et al. Gardnerella vaginalis clades in pregnancy: New insights into the interactions with the vaginal microbiome. PLoS One 2022, 17, e0269590, doi:10.1371/journal.pone.0269590.
29. Bayigga, L., Nabatanzi, R., Ssekagiri, A., Kateete, D.P., Sekikubo, M., Anderson, D.J., Xu, J., Kwon, D.S., Nakanjako, D. Diverse vaginal microbiome was associated with pro-inflammatory vaginal milieu among pregnant women in Uganda. Human Microbiome Journal 2020, 18, 100076, doi:10.1016/j.humic.2020.100076.
30. Oliver, A., LaMere, B., Weihe, C., Wandro, S., Lindsay, K.L., Wadhwa, P.D., Mills, D.A., Pride, D.T., Fiehn, O., Northen, T., et al. Cervicovaginal Microbiome Composition Is Associated with Metabolic Profiles in Healthy Pregnancy. mBio 2020, 11, doi:10.1128/mbio.01851-01820.
31. Shabayek, S., Abdellah, A.M., Salah, M., Ramadan, M., Fahmy, N. Alterations of the vaginal microbiome in healthy pregnant women positive for group B Streptococcus colonization during the third trimester. BMC Microbiology 2022, 22, 313, doi:10.1186/s12866-022-02730-8.
32. Zierden, H.C., DeLong, K., Zulfiqar, F., Ortiz, J.O., Laney, V., Bensouda, S., Hernández, N., Hoang, T.M., Lai, S.K., Hanes, J., et al. Cervicovaginal mucus barrier properties during pregnancy are impacted by the vaginal microbiome. Front Cell Infect Microbiol 2023, 13, 1015625, doi:10.3389/fcimb.2023.1015625.
33. Chen, Y., Hong, Z., Wang, W., Gu, L., Gao, H., Qiu, L., Di, W. Association between the vaginal microbiome and high-risk human papillomavirus infection in pregnant Chinese women. BMC Infectious Diseases 2019, 19, 677, doi:10.1186/s12879-019-4279-6.
34. Nasioudis, D., Forney, L.J., Schneider, G.M., Gliniewicz, K., France, M.T., Boester, A., Sawai, M., Scholl, J., Witkin, S.S. The composition of the vaginal microbiome in first trimester pregnant women influences the level of autophagy and stress in vaginal epithelial cells. J Reprod Immunol 2017, 123, 35-39, doi:10.1016/j.jri.2017.08.009.
35. Geldenhuys, J., Redelinghuys, M.J., Lombaard, H.A., Ehlers, M.M., Cowan, D., Kock, M.M. Diversity of the gut, vaginal and oral microbiome among pregnant women in South Africa with and without pre-eclampsia. Front Glob Womens Health 2022, 3, 810673, doi:10.3389/fgwh.2022.810673.
36. Gryaznova, M., Lebedeva, O., Kozarenko, O., Smirnova, Y., Burakova, I., Syromyatnikov, M., Maslov, A., Popov, V. Lower Genital Tract Microbiome in Early Pregnancy in the Eastern European Population. Microorganisms 2022, 10, 2368.
37. Gerede, A., Nikolettos, K., Vavoulidis, E., Margioula-Siarkou, C., Petousis, S., Giourga, M., Fotinopoulos, P., Salagianni, M., Stavros, S., Dinas, K., et al. Vaginal Microbiome and Pregnancy Complications: A Review. J Clin Med 2024, 13, doi:10.3390/jcm13133875.
38. Fettweis, J.M., Serrano, M.G., Brooks, J.P., Edwards, D.J., Girerd, P.H., Parikh, H.I., Huang, B., Arodz, T.J., Edupuganti, L., Glascock, A.L., et al. The vaginal microbiome and preterm birth. Nat Med 2019, 25, 1012-1021, doi:10.1038/s41591-019-0450-2.
39. Silvano, A., Meriggi, N., Renzi, S., Seravalli, V.,

Torcia, M.G., Cavalieri, D., Di Tommaso, M. Vaginal Microbiome in Pregnant Women with and without Short Cervix. Nutrients 2023, 15, 2173.

40. Dunlop, A.L., Satten, G.A., Hu, Y.J., Knight, A.K., Hill, C.C., Wright, M.L., Smith, A.K., Read, T.D., Pearce, B.D., Corwin, E.J. Vaginal Microbiome Composition in Early Pregnancy and Risk of Spontaneous Preterm and Early Term Birth Among African American Women. Front Cell Infect Microbiol 2021, 11, 641005, doi:10.3389/fcimb.2021.641005.
41. Mendz, G.L. The Vaginal Microbiome during Pregnancy in Health and Disease. Applied Microbiology 2023, 3, 1302-1338.
42. Saadaoui, M., Singh, P., Ortashi, O., Al Khodor, S. Role of the vaginal microbiome in miscarriage: exploring the relationship. Front Cell Infect Microbiol 2023, 13, 1232825, doi:10.3389/fcimb.2023.1232825.
43. Liao, J., Shenhav, L., Urban, J.A., Serrano, M., Zhu, B., Buck, G.A., Korem, T. Microdiversity of the vaginal microbiome is associated with preterm birth. Nature Communications 2023, 14, 4997, doi:10.1038/s41467-023-40719-7.
44. Hočevar, K., Maver, A., Vidmar Šimic, M., Hodžić, A., Haslberger, A., Premru Seršen, T., Peterlin, B. Vaginal Microbiome Signature Is Associated With Spontaneous Preterm Delivery. Front Med (Lausanne) 2019, 6, 201, doi:10.3389/fmed.2019.00201.
45. Pace, R.M., Chu, D.M., Prince, A.L., Ma, J., Seferovic, M.D., Aagaard, K.M. Complex species and strain ecology of the vaginal microbiome from pregnancy to postpartum and association with preterm birth. Med 2021, 2, 1027-1049, doi: 10.1016/j.medj.2021.06.001.
46. Fox, C., Eichelberger, K. Maternal microbiome and pregnancy outcomes. Fertility and Sterility 2015, 104, 1358-1363, doi:10.1016/j.fertnstert.2015.09.037.
47. Lledo, B., Fuentes, A., Lozano, F.M., Cascales, A., Morales, R., Hortal, M., Sellers, F., Palacios-Marques, A., Bermejo, R., Quereda, F., et al. Identification of vaginal microbiome associated with IVF pregnancy. Scientific Reports 2022, 12, 6807, doi:10.1038/s41598-022-10933-2.
48. Tabatabaei, N., Eren, A.M., Barreiro, L.B., Yotova, V., Dumaine, A., Allard, C., Fraser, W.D. Vaginal microbiome in early pregnancy and subsequent risk of spontaneous preterm birth: a case-control study. BJOG 2019, 126, 349-358, doi:10.1111/1471-0528.15299.
49. Nasioudis, D., Forney, L.J., Schneider, G.M., Gliniewicz, K., France, M., Boester, A., Sawai, M., Scholl, J., Witkin, S.S. Influence of Pregnancy History on the Vaginal Microbiome of Pregnant Women in their First Trimester. Scientific Reports 2017, 7, 10201, doi:10.1038/s41598-017-09857-z.
50. Eslami, M., Naderian, R., Ahmadpour, A., Shushtari, A., Maleki, S., Mohammadian, P., Amiri, A., Janbazi, M., Memarian, M., Yousefi, B. Microbiome structure in healthy and pregnant women and importance of vaginal dysbiosis in spontaneous abortion. Front Cell Infect Microbiol 2024, 14, 1401610, doi:10.3389/fcimb.2024.1401610.
51. Marangoni, A., Laghi, L., Zagonari, S., Patuelli, G., Zhu, C., Foschi, C., Morselli, S., Pedna, M.F., Sambri, V. New Insights into Vaginal Environment During Pregnancy. Front Mol Biosci 2021, 8, 656844, doi:10.3389/fmolb.2021.656844.
52. Haque, M.M., Merchant, M., Kumar, P.N., Dutta, A., Mande, S.S. First-trimester vaginal microbiome diversity: A potential indicator of preterm delivery risk. Sci Rep 2017, 7, 16145, doi:10.1038/s41598-017-16352-y.
53. Pruski, P., Correia, G.D.S., Lewis, H.V., Capuccini, K., Inglese, P., Chan, D., Brown, R.G., Kindinger, L., Lee, Y.S., Smith, A., et al. Direct on-swab metabolic profiling of vaginal microbiome host

interactions during pregnancy and preterm birth. Nature Communications 2021, 12, 5967, doi:10.1038/s41467-021-26215-w.

54. Cobo, T., Vergara, A., Collado, M.C., Casals-Pascual, C., Herreros, E., Bosch, J., Sanchez-Garcia, A.B., Lopez-Parellada, R., Ponce, J., Gratacos, E. Characterization of vaginal microbiota in women with preterm labor with intra-amniotic inflammation. Sci Rep 2019, 9, 18963, doi: 10.1038/s41598-019-55611-y.
55. Baud, A., Hillion, K.H., Plainvert, C., Tessier, V., Tazi, A., Mandelbrot, L., Poyart, C., Kennedy, S.P. Microbial diversity in the vaginal microbiota and its link to pregnancy outcomes. Sci Rep 2023, 13, 9061, doi:10.1038/s41598-023-36126-z.

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