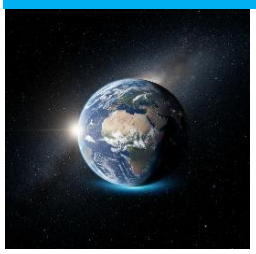
NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ



Journal of Education, Health and Sport. eISSN 2391-8306.

Journal Home Page
https://apcz.umk.pl/JEHS/index

MROZEK, Martyna, BLECHARCZYK, Małgorzata, KAMIŃSKA, Zuzanna Zofia, ZIELIŃSKA, Agnieszka, ZYDLEWSKI, Igor, PACANOWSKA-TRAWNICKA, Martyna, JAKIMOWICZ, Aleksandra and MALCHER, Aleksandra. From Balance to Rupture: Vaginal Microbiome in pPROM. Journal of Education, Health and Sport. 2026;91:70720. eISSN 2391-8306. https://doi.org/10.12775/JEHS.2026.91.70720

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).
Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).
The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 10.04.2026. Revised: 04.05.2026. Accepted: 05.05.2026. Published: 07.05.2026.

From Balance to Rupture: Vaginal Microbiome in pPROM

Martyna Mrozek

Dr Anna Gostynska Wolski Hospital, Independent Public Health Care Institution

Marcina Kasprzaka 17, 01-211 Warsaw, Poland

ORCID: https://orcid.org/0009-0004-1678-4054

E-mail: mrozekmartyna99@gmail.com

Małgorzata Blecharczyk

Brodno Masovian Hospital in Warsaw

Kondratowicza 8, 03-242 Warsaw, Poland

ORCID: https://orcid.org/0009-0009-1146-7662

E-mail: gosia.blech20@gmail.com

Zuzanna Kamińska

Nicolaus Copernicus Hospital in Gdansk

Nowe Ogrody 1-6, 80-803 Gdansk, Poland

ORCID: https://orcid.org/0009-0009-9803-0972

E-mail: kaminska2000@gmail.com

Agnieszka Zielińska

National Medical Institute of the Ministry of the Interior and Administration

Wołoska 137, 02-507 Warsaw, Poland

ORCID: https://orcid.org/0009-0001-4322-064X

E-mail: agzielinska44@gmail.com

Igor Zydlewski

Dr Anna Gostynska Wolski Hospital, Independent Public Health Care Institution

Marcina Kasprzaka 17, 01-211 Warsaw, Poland

ORCID: https://orcid.org/0009-0009-5053-8910

E-mail: igorzydlewski@op.pl

Martyna Pacanowska-Trawnicka

Ministry of the Interior and Administration Hospital in Cracow

Kronikarza Galla 25, 30-053 Cracow, Poland

ORCID: https://orcid.org/0000-0002-5803-548X

E-mail: martyna.pacanowska@gmail.com

Aleksandra Jakimowicz

Medical University of Silesia

Poniatowskiego 15, 40-055 Katowice, Poland

ORCID: https://orcid.org/0009-0002-4490-942X

E-mail: jakimowiczola427@gmail.com

Aleksandra Malcher

Nicolaus Copernicus Hospital in Gdansk

Nowe Ogrody 1-6, 80-803 Gdansk, Poland

ORCID: https://orcid.org/0009-0003-8809-3781

E-mail: olamalcher0@gmail.com

Abstract

PROM and its preterm form, pPROM, are major contributors to preterm birth and remain closely associated with serious maternal and neonatal complications. Growing evidence suggests that disruption of vaginal microbial homeostasis mayis closely linked to the pathogenesis of pPROM. In particular, the loss of protective *Lactobacillus crispatus*, accompanied by a shift toward *Lactobacillus iners*, anaerobic bacteria, and increased microbial diversity, appears to destabilize the vaginal ecosystem, promote local inflammation, activate matrix metalloproteinases, and ultimately compromise the structural integrity of the fetal membranes.

Current empirical antibiotic therapy for pPROM does not account for interindividual variability in the vaginal microbiota and may paradoxically aggravate dysbiosis. In response to these limitations, emerging therapeutic strategies are increasingly focused on microbiota-targeted interventions, including personalized probiotic therapies, immunological and biological approaches, and vaginal microbiota transplantation. In addition, specific microbial signatures may serve as potential biomarkers of pPROM, thereby opening new avenues for personalized prevention and treatment.

Although further prospective studies are required to validate the safety and efficacy of these emerging therapies, their potential already represents a promising direction for the future of modern perinatal medicine.

Keywords: preterm birth, microbial dysbiosis, infection risk, biomarkers, pregnancy complications

Introduction

In Poland, approximately 8% of all deliveries are preterm, and one of the major risk factors is **premature rupture of membranes (PROM)**. PROM is defined as the complete rupture of the fetal membranes in the absence of uterine contractions, occurring more than one hour before the onset of labor. When membrane rupture develops before 37 weeks of gestation, it is referred to as **preterm premature rupture of membranes (pPROM)** [1].

PROM and pPROM are associated with an increased risk of maternal, fetal, and neonatal complications. The most significant maternal complications include intrauterine infection, sepsis, placental abruption, and peripartum hemorrhage, which in severe cases may even lead to maternal death. In neonates, PROM and pPROM are linked to an increased risk of sepsis and numerous complications of prematurity, including respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, and retinopathy of prematurity. In addition, chronic oligohydramnios may result in pulmonary hypoplasia, limb contractures, and skeletal deformities [2].

Given the significant clinical consequences of PROM and pPROM, understanding the mechanisms responsible for the maintenance and loss of fetal membrane integrity appears to be of critical importance. The fetal membranes are composed of three layers: the amnion, chorion, and decidua. Their proper function depends not only on the presence of collagen, fibronectin, and laminin, but above all on the stability of the extracellular matrix (ECM), whose integrity is maintained by tightly regulated matrix metalloproteinases (MMPs). These enzymes play a central role in both preterm and term rupture of the fetal membranes. Studies have shown that MMP-1 activity decreases before the onset of labor, while MMP-3 and MMP-9 activity increases during labor; in the postpartum period, elevated activity of MMP-1 and MMP-2 has been observed [1, 3].

The integrity of the fetal membranes is also influenced by inflammatory mediators, including tumor necrosis factor alpha (TNF-α), interleukins, hormones, growth factors, and reactive oxygen species (ROS). The latter not only damage cellular structures but also participate in signal transduction, cell differentiation, and the induction of apoptosis [1].

For this reason, infections associated with inflammation, particularly ascending infections originating from the lower genital tract, are considered key contributors to the etiopathogenesis of pPROM. By triggering an inflammatory response, they may lead to weakening of the fetal membranes, their premature rupture, and subsequent leakage of amniotic fluid. Further evidence supporting the important role of infectious factors is provided by the fact that positive microbiological cervical swab results are detected in 25–40% of patients with pPROM [1].

The vaginal microbiome and the role of *Lactobacillus*

The vaginal microbiome, understood as a complex community of bacteria, fungi, and viruses inhabiting the vaginal environment, is under physiological conditions most commonly dominated by bacteria of the *Lactobacillus* genus. These microorganisms are responsible for maintaining the normal, mildly acidic vaginal pH and for preventing colonization by potentially pathogenic organisms. This protective effect is achieved both through inhibition of bacterial adhesion to the vaginal epithelium and through the production of lactic acid, which exerts antimicrobial activity.

Importantly, *Lactobacillus* species contribute to local immune homeostasis without eliciting an inflammatory response, thereby playing a central role in protection against infection and the preservation of reproductive health [4].

How does the vaginal microbiome change during pregnancy?

To date, most studies investigating the vaginal microbiome have focused on non-pregnant women. More recent research, based on sequencing of the variable regions of the 16S rRNA gene, has shown that pregnancy is associated with a marked remodeling of the vaginal ecosystem. Freitas et al. reported that, compared with non-pregnant women, pregnant women exhibit a vaginal microbiome characterized by reduced microbial diversity and a predominance of *Lactobacillus* species [5]. Similar findings were reported by Walther-António et al., who additionally demonstrated a linear relationship between gestational age and microbiome stability, suggesting that the vaginal environment becomes progressively more structured and biologically stable as pregnancy advances [6-7].

The mechanisms underlying these physiological changes have not yet been fully elucidated. However, fluctuations in sex hormone levels during pregnancy are believed to play an important role. Increased estrogen levels promote endometrial proliferation and thickening of the genital

tract epithelium, which in turn leads to glycogen accumulation in the vaginal epithelium. As a source of nutritional substrates for bacteria, glycogen acts as a key metabolic driver of the vaginal microbiota. Its conversion to glucose, followed by fermentation into lactic acid, lowers vaginal pH and creates favorable conditions for the predominance of *Lactobacillus* species. A contrasting pattern is observed during periods of low estrogen levels, such as early childhood and the postmenopausal period, when reduced glycogen content in the vaginal epithelium predisposes to colonization by non-*Lactobacillus* microorganisms, including bacteria of intestinal origin [7].

How do microbiome disturbances promote infection? Pathogenic organisms and pPROM

Amniotic fluid, which surrounds the developing fetus, has long been considered sterile. However, more recent studies have demonstrated the presence of microorganisms within the amniotic cavity, particularly in women with pPROM [8], whereas they are generally absent in uncomplicated pregnancies [9]. Pathogens may reach the amniotic cavity through several routes, one of the most important being the ascending route from the lower genital tract [8, 10].

In 2025, Nam et al. presented the results of a comprehensive analysis of the vaginal microbiome in pregnant women with pPROM, comparing samples collected between 32 and 35 weeks of gestation with those obtained from healthy pregnant controls. Their findings revealed a significant depletion of *Lactobacillus crispatus* and increased microbial diversity in patients with pPROM, indicating marked disruption of vaginal microbial homeostasis. The authors further observed that this process was preceded by a shift in microbial dominance toward *Lactobacillus iners* - a transitional lactic acid–producing species whose distinct metabolic profile, including the expression of inerolysin, may promote the proliferation of anaerobic bacteria typically associated with bacterial vaginosis (BV). Through the production of proteolytic enzymes such as sialidases and proteases, these anaerobes can damage epithelial barriers and activate a local inflammatory response involving matrix metalloproteinases (MMPs), thereby contributing to degradation of the structural components of the fetal membranes and increasing the risk of pPROM. The shift in microbial dominance from *L. crispatus* to *L. iners* may therefore represent one of the key early steps in vaginal microbiome destabilization, preceding the development of more complex polymicrobial communities [11].

It should be noted, however, that the findings reported by Nam et al. were based on a small cohort (n = 15), which limits their generalizability and underscores the need for validation in larger prospective studies. Although the cross-sectional nature of the study supports a significant association between dysbiosis and pPROM, further longitudinal investigations are required to characterize the vaginal microbiome from early pregnancy onward, identify predictive biomarkers, and define potential windows for intervention.

Nevertheless, these observations are consistent with the findings of a recent meta-analysis [10], in which pPROM was associated with the loss of protective *Lactobacillus* dominance and the emergence of a more diverse microbiota, including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Streptococcus* spp., and other opportunistic microorganisms [10, 12]. By eliciting a local inflammatory response, these alterations lead to increased levels of proinflammatory cytokines and activation of matrix metalloproteinases (MMPs), which degrade the structural components of the fetal membranes, weaken their integrity, and increase susceptibility to pPROM. In addition, dysbiosis-associated oxidative stress promotes the accumulation of reactive oxygen species (ROS), which can directly damage collagen and extracellular matrix proteins, thereby further amplifying the risk of premature membrane rupture and other complications, including intrauterine infection and chorioamnionitis. Taken together, these findings underscore the critical importance of maintaining a balanced vaginal microbiota for a healthy course of pregnancy [10].

Patients with pPROM	**Patients with term deliveries**
Gardnerella vaginalis	*Lactobacillus crispatus*
Lactobacillus iners	*Lactobacillus gasseri*
Prevotella bivia	
Ochrobactrum spp.	
Prevotella timonensis	
Ureaplasma parvum	

Table 1. Bacterial species isolated from the vaginal microbiota of patients with preterm premature rupture of membranes (pPROM) and women with term deliveries. Species highlighted in bold in the pPROM column represent potential biomarkers.

Chunmei Yan et al. [13] also conducted a study to characterize differences in the vaginal microbiome between pregnant women with pPROM and those delivering at term. The analysis, based on 16S rRNA gene sequencing, confirmed previous observations, revealing higher microbial diversity in women with pPROM compared with controls. In the pPROM cohort, species such as *Lactobacillus iners*, *Gardnerella vaginalis*, *Prevotella bivia*, *Ochrobactrum* spp., *Prevotella timonensis* and *Ureaplasma parvum* predominated, whereas term-delivering women were dominated by *Lactobacillus crispatus* and

Lactobacillus gasseri. The authors further suggested that three species - *Ochrobactrum* sp., *Prevotella timonensis*, and *Gardnerella vaginalis* - could serve as potential biomarkers of pPROM, offering the possibility of early identification of women at risk for premature rupture of membranes [13].

Beyond compositional changes, the study highlighted a profound remodeling of microbial interactions. In the control group, the microbial network was centered around beneficial *Lactobacillus* species, which acted as a stable core of the ecosystem and exhibited antagonistic effects against anaerobic bacteria. In contrast, the pPROM cohort displayed disruption of this structure, replaced by a densely interconnected network dominated by anaerobic bacteria. The central role of *Gardnerella vaginalis* within this pPROM-associated network may facilitate the proliferation and pathogenic activity of other anaerobes, further exacerbating vaginal dysbiosis and the accompanying inflammatory processes [11].

The predominance of anaerobic bacteria in the vaginal microbiome is also associated with a shift in metabolic activity toward a proinflammatory profile. Metabolites produced by these microbes, such as amines and selected short-chain fatty acids, may contribute to cervical weakening and local inflammation, potentially affecting the intrauterine environment and fetal development. Under physiological conditions, the vaginal microbiome primarily serves a protective role, supporting the maintenance of a stable environment critical for normal pregnancy progression [11].

An additional intriguing observation concerns the relationship between vitamin D, the vaginal microbiome, and the risk of preterm birth. Vitamin D participates in the regulation of glycogen metabolism, promoting its synthesis and storage, thereby increasing nutrient availability for *Lactobacillus* species. Concurrently, it induces the expression of LL-37, an antimicrobial peptide involved in innate immune defense. Consequently, vitamin D may support both the maintenance of a healthy vaginal microbial composition and the integrity of the genital epithelium. Vitamin D deficiency during pregnancy could therefore represent a factor contributing to dysbiosis and spontaneous preterm birth [7].

Therapeutic strategies in pPROM

In light of the growing evidence implicating vaginal dysbiosis in the pathogenesis of preterm premature rupture of membranes, recent years have seen the development of potential therapeutic strategies aimed at the prevention of PROM, particularly pPROM. These interventions are designed to reduce the risk of membrane rupture and to mitigate the associated maternal and neonatal complications.

Antibiotic therapy in pPROM

A landmark study, ORACLE I, demonstrated significant neonatal benefits associated with a 10-day course of erythromycin in women with pPROM, although the optimal dosing regimen remains a matter of debate [14]. Current guidelines from the American College of Obstetricians and Gynecologists (ACOG) recommend a 7-day sequential therapy, initially involving intravenous administration of ampicillin and erythromycin, followed by oral amoxicillin and erythromycin in pregnant women before 34 weeks of gestation. ACOG also allows substitution of erythromycin with azithromycin in cases of unavailability or intolerance. Importantly, the use of amoxicillin-clavulanate is discouraged in the context of pPROM due to its potential association with an increased risk of necrotizing enterocolitis in neonates [15-16].

It should be emphasized, however, that this therapeutic approach is empirical and not targeted to the specific pathogens associated with vaginal dysbiosis in individual patients. Moreover, it may negatively impact the beneficial *Lactobacillus* population. Emerging evidence suggests that erythromycin therapy may even exacerbate vaginal dysbiosis [17], underscoring the need for further research into more targeted prophylactic strategies, tailored to the patient's individual vaginal microbiome [16].

Probiotics in pPROM

Analyses of the vaginal microbiome in women with uncomplicated pregnancies consistently demonstrate a dominance of *Lactobacillus* species, suggesting that modulation of the microbiome through probiotics containing these strains could support the maintenance of a favorable microbial environment and represent a promising preventive strategy. However, current clinical evidence supporting the efficacy of probiotics in preventing pPROM remains limited. A comprehensive review and meta-analysis conducted by Jarde et al. [18] in 2018

found no significant effect of probiotic supplementation during pregnancy on reducing the risk of pPROM or other maternal and neonatal complications [16].

In recent years, increasing attention has been given to personalized probiotic therapies, tailored to the individual microbiome of each patient. However, this approach is challenging - requiring precise identification of strains in each woman due to the high variability of the vaginal microbiome. Moreover, certain species, such as *Lactobacillus iners*, through their metabolites, may inhibit the proliferation of exogenous probiotic strains, limiting the durability and effectiveness of therapy. Host responses, which are influenced by age, local inflammatory reactions, immune status, vaginal pH, and cervical mucus composition, further complicate the predictability and standardization of probiotic interventions [19]. In light of these observations, while probiotics are generally considered safe during pregnancy, their use as a standalone preventive strategy for pPROM appears insufficient, highlighting the need for further investigation [16].

In response to the limited efficacy of probiotics alone, Deng et al. proposed a combined antibiotic-probiotic approach to modulate the vaginal microbiome. This strategy simultaneously eliminates pathogenic organisms while supporting the restoration of a beneficial microbial community. Such an approach not only addresses infections in the short term but also reduces the risk of recurrence and exacerbation by maintaining long-term microbial stability [19].

Modulation of the inflammatory response

One of the key mechanisms linking vaginal dysbiosis to preterm premature rupture of membranes (pPROM) is the activation of local and systemic inflammatory responses. Through Toll-like receptors (TLRs), the production of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α is upregulated. This process promotes cervical maturation and the activation of extracellular matrix metalloproteinases (MMPs), leading to collagen degradation in the fetal membranes. Concurrently, oxidative stress and the associated increase in reactive oxygen species (ROS) damage structural proteins, compromising membrane integrity and increasing susceptibility to rupture. In a 2021 case-control study, Major et al. reported significantly higher mRNA expression levels of TNF-α, IL-6, and ADAMTS9 in patients with PROM and pPROM [20]. These mechanisms suggest that targeting inflammatory modulation, MMP activity, and oxidative stress may represent viable strategies for PROM prevention [19].

Consequently, TNF-α inhibitors demonstrate promising therapeutic potential. Additionally, preclinical studies are exploring the use of biologics that modulate MMP activity [21]. Antioxidants, such as vitamins C and E, may also confer protective effects by limiting ROS production and reducing oxidative stress-induced structural damage to fetal membranes [22]. Collectively, multifaceted therapeutic strategies that combine inflammation modulation, oxidative stress reduction, and restoration of vaginal microbiota homeostasis could form the basis for a more comprehensive and individualized approach to PROM prophylaxis [19].

Innovative therapies

The growing body of evidence implicating vaginal dysbiosis in the pathogenesis of pPROM has stimulated interest in therapeutic strategies aimed at restoring a healthy vaginal microbiome. One such approach is vaginal microbiota transplantation (VMT) [24]. This therapy has already been applied in the management of recurrent bacterial vaginosis (BV), but its implementation requires rigorous donor screening to minimize the risk of pathogenic microorganism transmission. A critical criterion for donor selection is the composition of the vaginal microbiota, with a predominance of Lactobacillus species, particularly *L. crispatus*, which should constitute at least 70% of the microbial community [25]. Further studies are needed to verify the long-term safety of this therapy, to standardize transplantation procedures, and to address ethical considerations [19].

A major limitation in treating vaginal dysbiosis remains the increasing antimicrobial resistance of pathogens. Accordingly, there is growing interest in developing novel interventions targeting bacterial biofilms, which contribute to persistent infections and reduce therapeutic efficacy. Promising approaches include bioactive membranes [26], equipped with enzymes capable of degrading extracellular biofilm polymers, and bacteriophages [27], which allow selective elimination of pathogenic bacteria while preserving the commensal microbiota [19].

Conclusions

The occurrence of PROM or pPROM carries a significant risk of perinatal complications, affecting both the mother - such as placental abruption, intrauterine infections, sepsis, peripartum hemorrhage, and, in severe cases, death - and the neonate, who faces an increased

risk of sepsis and prematurity-related complications, including respiratory distress, intraventricular hemorrhage, and necrotizing enterocolitis.

Available evidence suggests that disturbances in the vaginal microbiota are not merely an associated phenomenon but may actively contribute to the development of pPROM. Increased microbial diversity, coupled with a reduction in protective Lactobacillus species, particularly *Lactobacillus crispatus*, predisposes the vaginal ecosystem to destabilization and promotes processes leading to the weakening of fetal membranes.

This destabilization is mediated by the activation of both local and systemic inflammatory responses to pathogens, with elevated levels of pro-inflammatory cytokines, increased extracellular matrix metalloproteinase (MMP) activity, and enhanced oxidative stress. Consequently, degradation of collagen and other structural components of the membranes occurs, compromising their integrity and increasing the risk of premature rupture.

In recent years, substantial progress has been made in understanding the relationship between vaginal dysbiosis and pPROM. Emerging data indicate that specific microbial profiles may have predictive value. A decrease in *L. crispatus*, dominance of *L. iners*, and the presence of pathogens such as *Gardnerella vaginalis*, *Prevotella* spp., and *Ureaplasma*s pp. may serve as potential biomarkers for pPROM risk, facilitating the early identification of patients who may benefit from targeted preventive interventions.

Current therapeutic approaches, however, remain limited. Empirical antibiotic regimens are not tailored to individual vaginal microbiota profiles, may disrupt beneficial flora, and can contribute to the development of antimicrobial resistance. Moreover, vaginal microbiota is influenced by genetic, environmental, and lifestyle factors, which adds variability and complicates the standardization of study outcomes and the development of universal therapeutic strategies.

Personalized and multidirectional interventions appear most promising, encompassing the restoration of a healthy vaginal microbiota, modulation of the inflammatory response, reduction of oxidative stress, and biofilm-targeted strategies. Combined approaches are of particular interest, including: antibiotics with probiotics, immunomodulatory therapies, vaginal microbiota transplantation, and innovative biological and phage-based therapies.

The vaginal microbiota may thus serve not only as a marker of pPROM risk but also as a key target for modern preventive and therapeutic interventions. Nevertheless, further prospective studies are required to validate potential microbial biomarkers and assess the safety and efficacy of emerging treatment strategies.

Disclosure

Author's contribution:

Conceptualization: Martyna Mrozek

Methodology: Małgorzata Blecharczyk, Martyna Pacanowska-Trawnicka

Software: Zuzanna Kamińska

Check: Agnieszka Zielińska, Aleksandra Jakimowicz

Formal analysis: Igor Zydlewski

Investigation: Martyna Pacanowska-Trawnicka

Resources: Aleksandra Jakimowicz

Data curation: Aleksandra Malcher

Writing - rough preparation: Zuzanna Kamińska, Agnieszka Zielińska

Writing - review and editing: Martyna Mrozek, Igor Zydlewski

Visualization: Małgorzata Blecharczyk, Aleksandra Malcher

Supervision: Martyna Mrozek

Project administration: Martyna Mrozek

All authors have read and agreed with the published version of the manuscript.

Financing statement

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflict of interest

The authors deny any conflict of interest.

References

1. Huras H. Przedwczesne odpływanie płynu owodniowego - aktualne standardy. In: Huras H, red. *Położnictwo Yearbook 2025*. Gdańsk: Via Medica; 2025:94-107.
2. Dayal S, Jenkins SM, Hong PL. Preterm and term prelabor rupture of membranes (PPROM and PROM). In: *StatPearls* [Internet]. Treasure Island, FL: StatPearls Publishing; 2026. Accessed April 8, 2026. https://www.ncbi.nlm.nih.gov/books/NBK532888/
3. Woessner JF Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J*. 1991;5(8):2145-2154. doi:10.1096/fasebj.5.8.1850705
4. Witkin SS, Linhares IM. Why do lactobacilli dominate the human vaginal microbiota? *BJOG*. 2017;124(4):606-611. doi:10.1111/1471-0528.14390
5. Freitas AC, Chaban B, Bocking A, et al; VOGUE Research Group. The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Sci Rep*. 2017;7(1):9212. doi:10.1038/s41598-017-07790-9
6. Walther-António MR, Jeraldo P, Berg Miller ME, et al. Pregnancy's stronghold on the vaginal microbiome. *PLoS One*. 2014;9(6):e98514. doi:10.1371/journal.pone.0098514
7. Gupta P, Singh MP, Goyal K. Diversity of vaginal microbiome in pregnancy: deciphering the obscurity. *Front Public Health*. 2020;8:326. doi:10.3389/fpubh.2020.00326
8. Kacerovsky M, Stranik J, Matulova J, et al. Clinical characteristics of colonization of the amniotic cavity in women with preterm prelabor rupture of membranes: a retrospective study. *Sci Rep*. 2022;12:5062. doi:10.1038/s41598-022-09042-x
9. Lim ES, Rodriguez C, Holtz LR. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome*. 2018;6:87. doi:10.1186/s40168-018-0475-7
10. Feduniw S, Zeber-Lubecka N, Pruc M, Gaca Z, Szarpak Ł, Ciebiera M. Does the vaginal microbiota influence the incidence of the preterm premature rupture of membranes? *J Clin Med*. 2025;14(18):6577. doi:10.3390/jcm14186577
11. Nam S, Hong S, Park IY, Shin S. Vaginal microbiome and functional pathway alterations in preterm premature rupture of membranes revealed by 16S rRNA sequencing. *Life (Basel)*. 2025;15(10):1604. doi:10.3390/life15101604

12. Noda-Nicolau NM, Tantengco OAG, Polettini J, et al. Genital mycoplasmas and biomarkers of inflammation and their association with spontaneous preterm birth and preterm prelabor rupture of membranes: a systematic review and meta-analysis. *Front Microbiol*. 2022;13:859732. doi:10.3389/fmicb.2022.859732
13. Yan C, Hong F, Xin G, Duan S, Deng X, Xu Y. Alterations in the vaginal microbiota of patients with preterm premature rupture of membranes. *Front Cell Infect Microbiol*. 2022;12:858732. doi:10.3389/fcimb.2022.858732
14. Kenyon SL, Taylor DJ, Tarnow-Mordi W; ORACLE Collaborative Group. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. *Lancet*. 2001;357(9261):979-988. doi:10.1016/S0140-6736(00)04233-1
15. American College of Obstetricians and Gynecologists. Prelabor rupture of membranes. *ACOG Practice Bulletin*. 2020;(217). Accessed April 8, 2026. https://www.acog.org/clinical/clinical-guidance/practice-bulletin/articles/2020/03/prelabor-rupture-of-membranes
16. Alikamali M, Mohammad-Alizadeh-Charandabi S, Ahmadi S, Memar MY, Shahnazi M. The role of the vaginal microbiome in preterm premature rupture of membranes: a comprehensive review of mechanisms and clinical implications. *Health Sci Rep*. 2025;8(12):e71484. doi:10.1002/hsr2.71484
17. Brown RG, Marchesi JR, Lee YS, et al. Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. *BMC Med*. 2018;16(1):9. doi:10.1186/s12916-017-0999-x
18. Jarde A, Lewis-Mikhael AM, Moayyedi P, et al. Pregnancy outcomes in women taking probiotics or prebiotics: a systematic review and meta-analysis. *BMC Pregnancy Childbirth*. 2018;18(1):14. doi:10.1186/s12884-017-1629-5
19. Deng Y, Li Y, Liu T, Wu F. Interaction of vaginal microbiota and biomarkers in premature rupture of membranes: from bench to bedside. *Front Immunol*. 2025;16:1642942. doi:10.3389/fimmu.2025.1642942
20. Kumar M, Murugesan S, Singh P, et al. Vaginal microbiota and cytokine levels predict preterm delivery in Asian women. *Front Cell Infect Microbiol*. 2021;11:639665. doi:10.3389/fcimb.2021.639665
21. Gomez-Lopez N, Galaz J, Miller D, et al. The immunobiology of preterm labor and birth: intra-amniotic inflammation or breakdown of maternal-fetal homeostasis. *Reproduction*. 2022;164(2):R11-R45. doi:10.1530/REP-22-0046

22. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020;21(24):9739. doi:10.3390/ijms21249739
23. Sebastiani G, Navarro-Tapia E, Almeida-Toledano L, et al. Effects of antioxidant intake on fetal development and maternal/neonatal health during pregnancy. *Antioxidants (Basel)*. 2022;11(4):648. doi:10.3390/antiox11040648
24. Moumne O, Hampe ME, Montoya-Williams D, et al. Implications of the vaginal microbiome and potential restorative strategies on maternal health: a narrative review. *J Perinat Med*. 2021;49(4):402-411. doi:10.1515/jpm-2020-0367
25. Lev-Sagie A, Goldman-Wohl D, Cohen Y, et al. Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nat Med*. 2019;25(10):1500-1504. doi:10.1038/s41591-019-0600-6
26. Al-Madboly LA, Aboulmagd A, El-Salam MA, Kushkevych I, El-Morsi RM. Microbial enzymes as powerful natural anti-biofilm candidates. *Microb Cell Fact*. 2024;23(1):343. doi:10.1186/s12934-024-02610-y
27. Liu S, Lu H, Zhang S, Shi Y, Chen Q. Phages against pathogenic bacterial biofilms and biofilm-based infections: a review. *Pharmaceutics*. 2022;14(2):427. doi:10.3390/pharmaceutics14020427