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Dynamic changes of cervical microbiome during pregnancy for preterm birth risk prediction: A prospective cohort study

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Abstract

This prospective cohort study investigated the dynamic changes in the cervical microbiome during pregnancy and developed a predictive model for preterm birth risk. Ninety-three singleton pregnant women were enrolled, including 41 with preterm birth and 52 with term delivery. Cervical secretions were collected at four gestational stages and analyzed using 16S rRNA sequencing, alongside ELISA testing for inflammatory markers. The preterm group exhibited significantly lower microbial diversity and a progressively increasing ratio of *Lactobacillus iners* **to** *Lactobacillus crispatus* throughout pregnancy. Early pregnancy IL-6 levels were also significantly elevated in this group. Logistic regression identified the *L. iners/L. crispatus* ratio, IL-6, history of preterm birth, and short cervical length as independent risk factors. The integrated prediction model demonstrated high accuracy (AUC 0.847), with even stronger performance in predicting births before 34 weeks (AUC 0.892). These findings suggest that microbiome patterns and inflammatory markers can effectively predict preterm birth risk, supporting early clinical intervention. *(Afr J Reprod Health 2026; 30 [4]: 50-63).*

Keywords: preterm birth; cervical microbiome; dynamic changes; risk prediction; prospective cohort study

Résumé

Cette étude de cohorte prospective a étudié les changements dynamiques du microbiome cervical pendant la grossesse et a développé un modèle prédictif du risque de naissance prématurée. Quatre-vingt-treize femmes enceintes uniques ont été incluses, dont 41 avec une naissance prématurée et 52 avec un accouchement à terme. Des sécrétions cervicales ont été collectées à quatre stades de la grossesse et analysées par séquençage de l'ARNr 16S, ainsi que par des tests ELISA pour les marqueurs inflammatoires. Le groupe des naissances prématurées a montré une diversité microbienne significativement plus faible et un rapport de plus en plus élevé de Lactobacillus iners par rapport à Lactobacillus crispatus tout au long de la grossesse. Les niveaux d'IL-6 au début de la grossesse étaient également significativement plus élevés dans ce groupe. La régression logistique a identifié le ratio L. iners/ L. crispatus, l'IL-6, les antécédents de naissance prématurée et la longueur cervicale courte comme des facteurs de risque indépendants. Le modèle de prédiction intégré a montré une grande précision (AUC 0,847), avec des performances encore plus fortes pour prédire les naissances avant 34 semaines (AUC 0,892). Ces résultats suggèrent que les motifs du microbiome et les marqueurs inflammatoires peuvent prédire efficacement le risque de naissance prématurée, soutenant ainsi une intervention clinique précoce. *(Afr J Reprod Health 2026; 30 [4]: 50-63).*

Mots-clés: naissance prématurée ; microbiome cervical ; changements dynamiques ; prédiction du risque ; étude de cohorte prospective.

Introduction

Preterm birth represents a major global public health challenge, defined by the World Health Organization as delivery occurring at or after 28 weeks but before 37 weeks (259 days) of gestation [1].According to recent estimates, approximately 13.4 million infants were born preterm globally in 2020, accounting for 10.6% of all live births, with preterm birth rates showing no significant declining trend over the past decade [2]. Preterm birth complications have become the leading cause of death in children under 5 years of age, resulting in approximately 900,000 neonatal deaths annually, with nearly half of survivors facing lifelong disabilities, including learning disabilities, visual and hearing impairments [3]. This problem is particularly severe in low-income countries, where extremely preterm

infants born before 32 weeks have mortality rates reaching 50%, compared to near 0% in high-income countries.[4] The pathogenesis of preterm birth is complex and multifactorial, involving the interaction of multiple pathological processes including infection, inflammation, vascular pathology, uterine overdistension, cervical insufficiency, and maternal-fetal stress.[5] Among these, spontaneous preterm birth accounts for 70-75% of all preterm births, with approximately one-third associated with intrauterine infection.[6] Despite the identification of numerous risk factors, including previous preterm birth history, multiple gestation, uterine malformations, and cervical surgical history, approximately half of preterm birth cases still lack a clear etiology, highlighting the complexity of preterm birth prediction and prevention efforts.[7]

Current clinical methods for preterm birth risk assessment primarily include cervical length measurement and fetal fibronectin detection. Transvaginal ultrasound cervical length measurement is considered the gold standard for preterm birth prediction, but its sensitivity is only 25-50%, and it requires skilled technical personnel, making it difficult to implement in resource-limited settings.[8] Fetal fibronectin detection, as a biochemical marker, has a sensitivity of approximately 56%. Although it possesses good negative predictive value, its positive predictive value is relatively low, limiting its clinical application value.[9] More importantly, these traditional prediction methods typically provide effective information only during the mid-trimester (18-22 weeks), leaving a relatively limited time window for clinical intervention.[10]In recent years, with the development of high-throughput sequencing technologies and the refinement of bioinformatics analysis methods, microbiome research has opened new avenues for preterm birth prediction. The vaginal microbiome, as an important regulator of women's reproductive health, plays a crucial role in maintaining vaginal acidic environment, resisting pathogen invasion, and regulating local immune responses.[11] The vaginal microbiome of healthy women of reproductive age is typically dominated by a few *Lactobacillus* species, forming a relatively stable, low-diversity ecosystem. However, significant hormonal changes during pregnancy affect the composition and stability of the vaginal microbiome, which may subsequently influence pregnancy outcomes.[12]The widespread application of machine learning technologies in medicine has provided new analytical tools for preterm birth prediction. Compared to traditional statistical methods, machine learning algorithms can process high-dimensional, nonlinear data, identify complex feature patterns and interactions between variables, thereby improving the accuracy and robustness of prediction models. Previous studies have demonstrated that machine learning-based preterm birth prediction models show superior predictive performance compared to traditional methods across multiple cohorts, with AUC values ranging from 0.69-0.87.[13] These advances have laid the foundation for developing more precise and practical preterm birth risk screening tools. This study employed a prospective cohort design, systematically analyzing the dynamic characteristics of microbiome changes during pregnancy through multi-timepoint collection of cervical secretion specimens from pregnant women.

Simultaneously, by integrating microbiome data, inflammatory marker detection results, and clinical information, machine learning methods were utilized to construct preterm birth risk prediction models, aiming to provide new strategies for early clinical screening and individualized intervention. The innovative aspects of this study include: adopting a longitudinal study design to comprehensively characterize the temporal dynamics of microbiome during pregnancy; integrating multi-omics data to construct comprehensive prediction models; focusing specifically on changes in relative abundance of specific *Lactobacillus* species and their predictive value; exploring the mechanistic association between microbiome changes and local inflammatory responses.

Methods

Study design

This study employed a prospective cohort design and was conducted at Ezhou Central Hospital

obstetrics outpatient clinic from January 2024 to January 2025.

Study participants

The study population consisted of pregnant women receiving regular prenatal care at our obstetrics outpatient clinic. Inclusion criteria included singleton pregnant women aged 18-40 years, gestational age 10-14 weeks, normal fetal ultrasound examination, and ability to complete follow-up throughout the entire pregnancy. Exclusion criteria included multiple gestation; comorbid severe medical or surgical conditions such as diabetes mellitus, hypertension, heart disease, or kidney disease; antibiotic use exceeding 7 days during pregnancy; immunodeficiency diseases or current use of immunosuppressive agents; history of vaginal medication use within the past 3 months; history of cervical conization; history of uterine malformations or cervical insufficiency; and pregnant women unable to complete follow-up.

Sample size was calculated based on an expected preterm birth rate of 15% in the general population, with 80% power to detect a 20% difference in microbiome composition between groups at a significance level of 0.05, yielding a minimum required sample size of 85 participants. Using consecutive sampling methodology, 93 eligible pregnant women were ultimately enrolled. Participants were prospectively followed throughout pregnancy, and group allocation (preterm vs. term) was determined based on actual delivery outcomes. Study participants were divided into two groups based on pregnancy outcomes: the preterm birth group was defined as women delivering before 37 weeks of gestation, and the term birth group was defined as women delivering at or after 37 weeks but before 42 weeks of gestation. Preterm birth was further subdivided into early preterm birth (delivery before 34 weeks) and late preterm birth (delivery at 34-36+6 weeks).

Clinical data collection

Standardized questionnaires were used to collect basic demographic information from all study participants, including age, height, weight, educational level, occupation, and place of residence. Detailed obstetric history was recorded, including gravidity, parity, number of abortions, preterm birth history, and term birth history. Information related to the current pregnancy was collected, including last menstrual period, estimated due date, gestational weight gain, and occurrence of pregnancy complications such as gestational diabetes mellitus, gestational hypertensive disorders, and premature rupture of membranes.Vital signs were measured and recorded at each follow-up visit, including blood pressure, pulse, and temperature. Standardized obstetric examinations were performed, including fundal height and abdominal circumference measurements, and fetal heart monitoring. Transvaginal ultrasound examination was performed at 18-22 weeks of gestation to measure cervical length, conducted by professionally trained ultrasonographers, with the average of three measurements taken as the final result.

Specimen collection and processing

All study participants underwent cervical secretion specimen collection at four time points: 10-14 weeks, 18-22 weeks, 28-32 weeks, and 34-36 weeks of gestation. Specimen collection was performed in a dedicated examination room with room temperature maintained at 22-25°C. Pregnant women were positioned in the lithotomy position, and a sterile disposable speculum was used to expose the cervix. The area around the external cervical os was gently cleaned with sterile saline-soaked cotton balls to avoid contamination. A sterile polypropylene swab was gently rotated in the cervical canal for 10-15 seconds at a depth of approximately 1-2 centimeters to ensure adequate collection of cervical secretions. Two parallel specimens were collected from each pregnant woman: one for 16S rRNA gene sequencing and another for inflammatory marker detection.

Collected specimens were immediately placed in sterile EP tubes containing DNA/RNA preservative, temporarily stored at 4°C, and transported to the laboratory within 2 hours. Upon arrival at the laboratory , specimens were

immediately processed or stored in a -80°C ultra-low temperature freezer to ensure DNA integrity and stability.

16S rRNA gene sequencing

Bacterial genomic DNA was extracted using a modified CTAB method. The specific procedure was as follows: specimens were thawed on ice, and 500μL CTAB lysis buffer (containing 2% CTAB, 100mM Tris-HCl pH 8.0, 20mM EDTA, 1.4M NaCl) was added, mixed thoroughly, and incubated in a 65°C water bath for 30 minutes. An equal volume of chloroform-isoamyl alcohol (24:1) was added, vigorously shaken for 5 minutes, then centrifuged at 12,000 rpm for 10 minutes. The supernatant was collected, 0.6 volumes of isopropanol were added, and precipitation was performed overnight at -20°C. After centrifugation at 12,000 rpm for 15 minutes, the supernatant was discarded, the pellet was washed twice with 70% ethanol, air-dried, and dissolved in 50μL sterile water. DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer, with A260/A280 ratios between 1.8-2.0 considered acceptable.

PCR amplification was performed targeting the V3-V4 variable region of the bacterial 16S rRNA gene. The forward primer was 338F (5'-ACTCCTACGGGAGGCAGCA-3'), and the reverse primer was 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reaction system had a total volume of 25μL, containing 2×Taq PCR MasterMix 12.5μL, forward primer (10μM) 1μL, reverse primer (10μM) 1μL, template DNA 2μL, and ddH2O 8.5μL.PCR reaction conditions were 95°C pre-denaturation for 3 minutes, followed by 35 cycles of: 95°C denaturation for 30 seconds, 55°C annealing for 30 seconds, 72°C extension for 45 seconds, and final extension at 72°C for 10 minutes.PCR products were detected by 1.5% agarose gel electrophoresis, with target bands approximately 470bp. PCR products were purified using the AxyPrep DNA Gel Recovery Kit and quantified using a Qubit 2.0 fluorometer. PCR products from each sample were mixed at equimolar concentrations to construct sequencing libraries. Paired-end sequencing was performed using the Illumina MiSeq platform with a sequencing length of 2×300bp.

Bioinformatics analysis

Raw sequencing data first underwent quality control, with Trimmomatic software used to remove low-quality sequences and adapter sequences. Quality threshold was set at Q20, with minimum sequence length of 200bp. FLASH software was used to merge paired-end sequences to obtain complete V3-V4 region sequences.Subsequent analysis was performed using the QIIME2 pipeline. The DADA2 algorithm was used for sequence denoising, chimera removal, and ASV (Amplicon Sequence Variants) identification. Taxonomic annotation was performed based on the SILVA database (version 138) with a classification confidence threshold of 0.8. Mitochondrial, chloroplast, and unclassified sequences were removed before subsequent analysis.To eliminate the influence of sequencing depth differences, all samples were rarefied to the same sequencing depth. Alpha diversity of communities was assessed using Shannon index, Simpson index, Chao1 index, and observed OTUs. Beta diversity analysis was performed using Bray-Curtis distance matrices and UniFrac distances, with community structure differences visualized through principal coordinate analysis (PCoA).

Inflammatory marker detection

Enzyme-linked immunosorbent assay (ELISA) was used to detect inflammatory marker levels in cervical secretions. Detection indicators included interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and procalcitonin (PCT). All procedures strictly followed kit instructions, with all detections including standards, quality controls, and blank controls. An enzyme-linked immunosorbent assay reader was used to measure absorbance values at 450nm wavelength, and concentrations of each indicator were calculated according to standard curves. Three replicates were set for each sample, with the average value taken as the final result.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 and R software (version 4.2.0). Continuous variables were tested for normality using the Shapiro-Wilk test. Data following normal distribution were expressed as mean ± standard deviation, with between-group comparisons performed using t-tests. Non-normally distributed data were expressed as median (interquartile range), with between-group comparisons performed using Mann-Whitney U tests. Categorical variables were expressed as frequencies and percentages, with between-group comparisons performed using chi-square tests or Fisher's exact tests.

In microbiome data analysis, between-group comparisons of alpha diversity indices were performed using Wilcoxon rank-sum tests. Beta diversity differences were assessed using PERMANOVA tests. LEfSe (Linear discriminant analysis Effect Size) analysis was used to identify microbial taxa with statistical differences, with LDA score threshold set at 2.0.Time-series analysis methods were used to assess dynamic changes in the microbiome. Generalized linear mixed-effects models (GLMM) were used to analyze temporal trends in microbial communities while controlling for individual differences.Logistic regression models were constructed for preterm birth risk prediction. Univariate analysis was first performed to screen candidate variables ($P<0.10$), followed by forward stepwise regression to establish multivariate models.Random forest algorithms were used to construct machine learning prediction models, with model parameters optimized through cross-validation. Receiver operating characteristic (ROC) curves were used to assess model predictive performance, calculating area under the curve (AUC), sensitivity, specificity, positive predictive value, and negative predictive value. All statistical tests were two-sided, with $P<0.05$ considered statistically significant.

Ethical considerations

The study protocol was reviewed and approved by the Medical Ethics Committee of Ezhou Central Hospital (Ethics Approval Number: L2023-K-13). All participants provided written informed consent. The study strictly adhered to the relevant principles of the Declaration of Helsinki, ensuring that participants' privacy rights and safety were fully protected.

Results

Baseline characteristics of study population

A total of 93 pregnant women were enrolled in this prospective cohort study, with 41 women in the preterm birth group and 52 women in the term birth group. The baseline characteristics of the study population are presented in Table 1. The mean age of participants was 28.7 ± 4.3 years, with no significant difference between groups ($P = 0.412$). The preterm birth group showed a higher proportion of previous preterm birth history (24.4% vs 7.7%, $P = 0.031$) and lower pre-pregnancy BMI (21.8 ± 2.9 vs 23.1 ± 3.2 kg/m², $P = 0.048$). Among the 41 preterm births, 13 cases (31.7%) were classified as early preterm birth (<34 weeks) and 28 cases (68.3%) as late preterm birth (34-36+6 weeks).

Cervical microbiome diversity analysis

Alpha diversity analysis revealed significant differences between preterm and term birth groups across different gestational periods (Figure 1). At the initial sampling time point (10-14 weeks), the preterm group demonstrated lower microbial diversity compared to the term group, with Shannon index of 2.34 ± 0.67 versus 2.78 ± 0.54 ($P = 0.003$) and Simpson index of 0.76 ± 0.18 versus 0.84 ± 0.12 ($P = 0.018$).

This trend persisted throughout pregnancy, becoming more pronounced in the third trimester. The Chao1 richness estimator also showed consistently lower values in the preterm group at all time points. Beta diversity analysis using Bray-Curtis dissimilarity demonstrated significant clustering differences between groups (PERMANOVA, $P = 0.001$). Principal coordinate analysis (PCoA) revealed distinct microbiome compositions, with the first two principal coordinates explaining 34.7% of the total variance (Figure 2).

Table 1: Baseline characteristics of study participants

Characteristic	Preterm group (n=41)	Term group (n=52)	P-value
Age (years)	28.2 ± 4.8	29.1 ± 3.9	0.412
Nulliparity	24 (58.5%)	32 (61.5%)	0.763
Pre-pregnancy BMI (kg/m²)	21.8 ± 2.9	23.1 ± 3.2	0.048
Educational level (≥College)	26 (63.4%)	35 (67.3%)	0.691
Previous preterm birth	10 (24.4%)	4 (7.7%)	0.031
Previous spontaneous abortion	12 (29.3%)	11 (21.2%)	0.378
Smoking during pregnancy	3 (7.3%)	2 (3.8%)	0.659
Cervical length at 18-22w (mm)	32.4 ± 8.7	38.2 ± 6.3	0.001
Gestational age at delivery (weeks)	34.8 ± 2.3	39.2 ± 1.1	<0.001
Birth weight (g)	2456 ± 467	3298 ± 412	<0.001

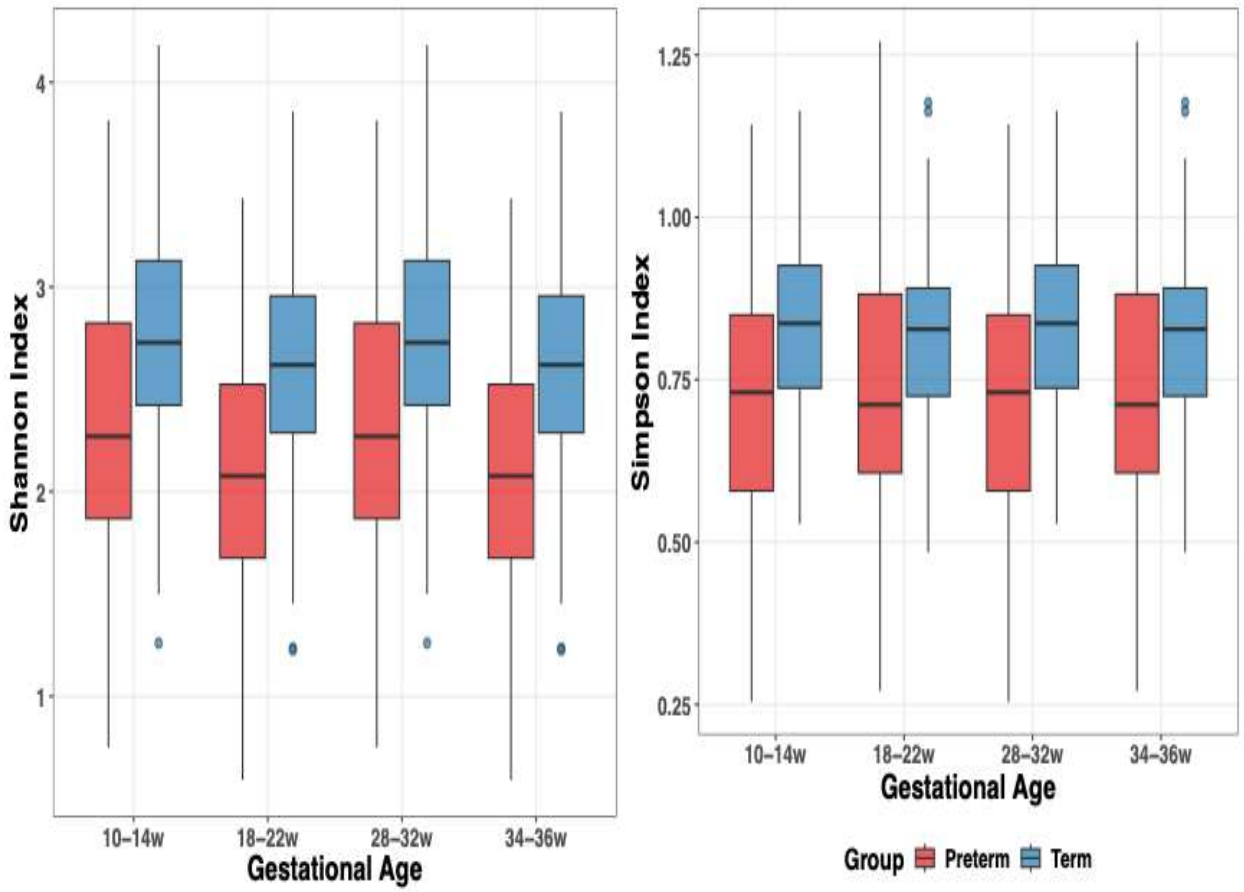

Figure 1: Alpha diversity comparison between preterm and term groups across gestational periods. A: Shannon diversity index; B: Simpson diversity index.

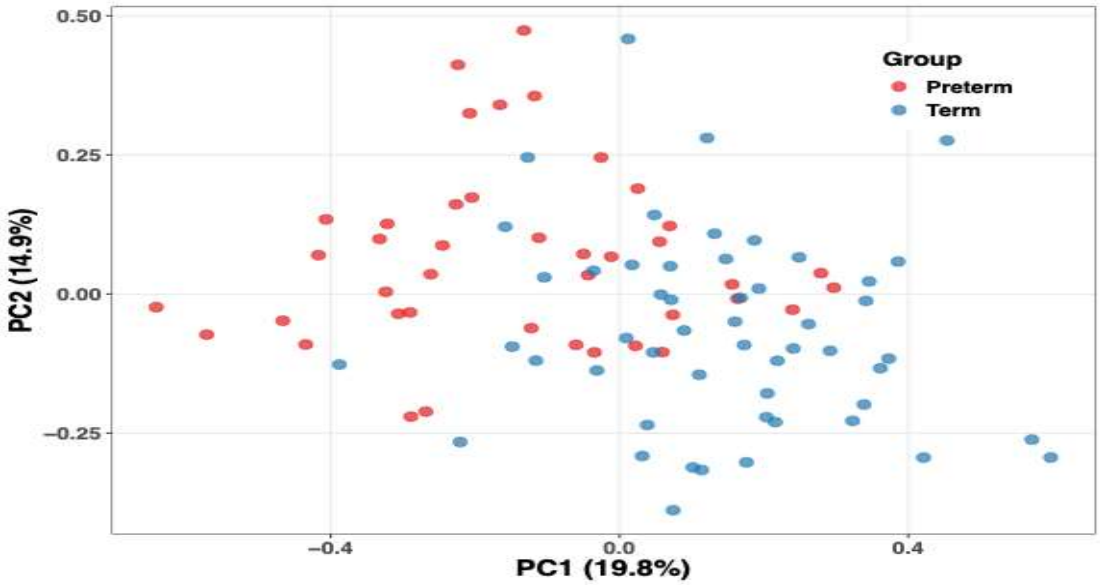

Figure 2: Principal coordinate analysis (PCoA) showing microbiome composition differences between preterm and term birth groups

Table 2: Alpha diversity indices across gestational periods

Time Point	Group	Shannon index	Simpson index	Chao1 index	Observed ASVs
10-14 weeks	Preterm	2.34 ± 0.67	0.76 ± 0.18	89.3 ± 31.2	67.8 ± 24.1
10-14 weeks	Term	2.78 ± 0.54	0.84 ± 0.12	112.7 ± 28.9	84.2 ± 22.6
18-22 weeks	Preterm	2.18 ± 0.71	0.73 ± 0.21	82.6 ± 29.4	61.3 ± 26.8
18-22 weeks	Term	2.69 ± 0.58	0.82 ± 0.14	108.4 ± 31.7	79.7 ± 25.3
28-32 weeks	Preterm	2.08 ± 0.69	0.71 ± 0.19	78.9 ± 27.8	58.4 ± 23.7
28-32 weeks	Term	2.61 ± 0.52	0.81 ± 0.13	105.2 ± 29.6	77.8 ± 24.1
34-36 weeks	Preterm	1.97 ± 0.74	0.68 ± 0.22	73.1 ± 25.6	54.9 ± 21.8
34-36 weeks	Term	2.55 ± 0.49	0.79 ± 0.15	101.8 ± 27.3	75.2 ± 23.4

Taxonomic composition and differential abundance analysis

The cervical microbiome was dominated by Lactobacillus species across all samples, accounting for 67.8% of the total microbial community. However, significant differences in species composition were observed between groups. LEfSe analysis identified key discriminatory taxa associated with preterm birth risk (Figure 3). Lactobacillus iners showed significantly higher relative abundance in the preterm group compared to the term group (42.3% vs 28.7%, $P < 0.001$), while Lactobacillus crispatus demonstrated the opposite pattern (18.6% vs 35.4%, $P < 0.001$).

Temporal dynamics of cervical microbiome

Longitudinal analysis revealed distinct temporal patterns in microbiome composition between groups.

The preterm group demonstrated greater microbiome instability, with higher inter-individual variation coefficients throughout pregnancy. The L. iners/L. crispatus ratio showed a progressive increase in the preterm group from 2.84 ± 1.67 at 10-14 weeks to 4.12 ± 2.31 at 34-36 weeks (P for trend < 0.001), while remaining relatively stable in the term group (0.89 ± 0.74 to 0.96 ± 0.83, P for trend = 0.634).Generalized linear mixed-effects models revealed significant time-dependent changes in key bacterial taxa.

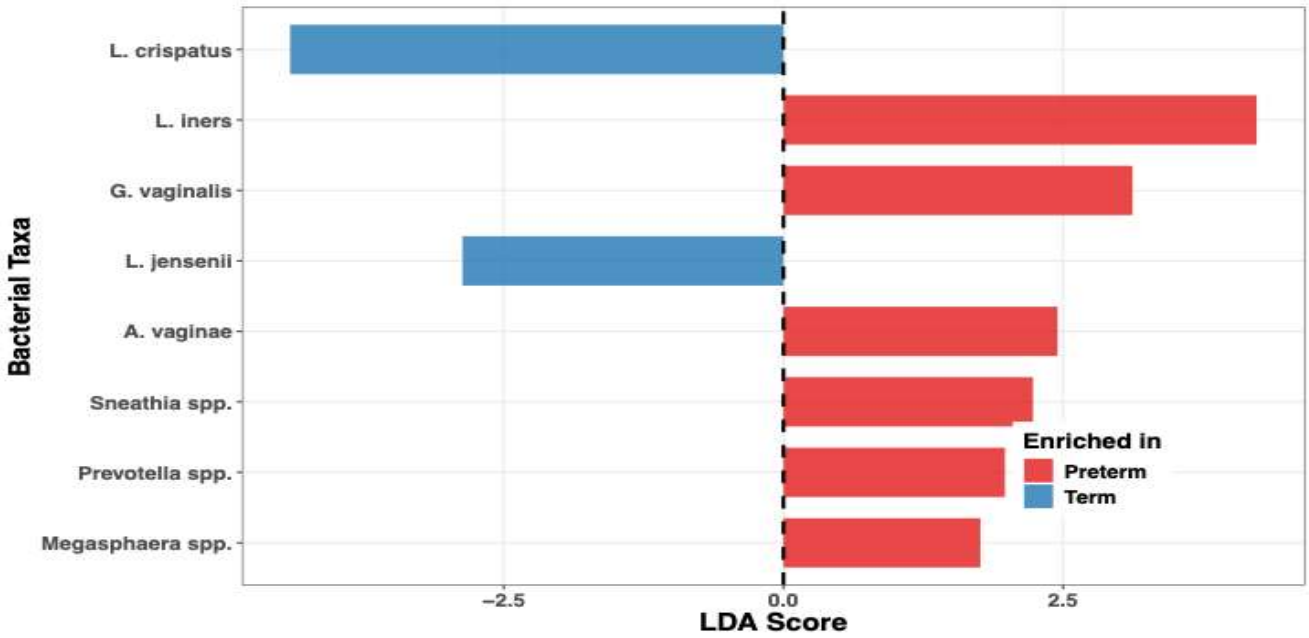

Figure 3: LEfSe analysis identifying differentially abundant bacterial taxa between groups.

Table 3: Relative abundance of dominant bacterial taxa (%)

Taxonomic group	Preterm group	Term group	P-value	LDA Score
Lactobacillus iners	42.3 ± 21.8	28.7 ± 19.4	<0.001	4.23
Lactobacillus crispatus	18.6 ± 16.2	35.4 ± 20.1	<0.001	-4.41
Lactobacillus gasseri	8.7 ± 11.3	12.4 ± 13.6	0.182	-2.14
Lactobacillus jensenii	3.4 ± 6.8	7.2 ± 9.1	0.045	-2.87
Gardnerella vaginalis	12.8 ± 15.6	7.3 ± 9.8	0.037	3.12
Atopobium vaginae	6.2 ± 8.9	3.4 ± 5.7	0.089	2.45
Prevotella spp.	2.8 ± 4.2	1.6 ± 2.9	0.127	1.98
Sneathia spp.	1.9 ± 3.4	0.8 ± 1.6	0.043	2.23
Megasphaera spp.	1.7 ± 2.9	0.9 ± 1.8	0.159	1.76
Others	1.6 ± 2.1	2.3 ± 3.4	0.298	-1.34

L. iners abundance increased significantly over time in the preterm group (β = 0.124, 95% CI: 0.067-0.181, P < 0.001), while L. crispatus showed a declining trend (β = -0.089, 95% CI: -0.143 to -0.035, P = 0.002).

Inflammatory markers analysis

Cervical inflammatory markers showed significant differences between groups at early pregnancy (Table 4). IL-6 levels were consistently elevated in the preterm group across all time points, with the most pronounced difference observed at 10-14 weeks (24.8 ± 18.7 vs 14.2 ± 11.3 pg/mL, P = 0.002). TNF-α and IL-1β also demonstrated higher levels in the preterm group during early pregnancy.

Predictive model development and validation

Multiple logistic regression analysis identified several independent risk factors for preterm birth (Table 5). The model incorporating L. iners/L. crispatus ratio, IL-6 levels, previous preterm birth history, and cervical length at 18-22 weeks demonstrated the best predictive performance. The final model showed an AUC of 0.847 (95% CI: 0.763-0.931) for overall preterm birth prediction, with sensitivity of 82.9% and specificity of 80.8%.

Table 4: Cervical inflammatory markers at 10-14 weeks gestation

Inflammatory Marker	Preterm group (n=41)	Term group (n=52)	P-value
IL-6 (pg/mL)	24.8 ± 18.7	14.2 ± 11.3	0.002
TNF-α (pg/mL)	8.9 ± 6.4	5.7 ± 4.2	0.012
IL-1β (pg/mL)	15.3 ± 12.8	9.8 ± 8.6	0.024
CRP (mg/L)	3.2 ± 2.1	2.4 ± 1.8	0.067
PCT (ng/mL)	0.18 ± 0.14	0.13 ± 0.09	0.058

Table 5: Multivariate logistic regression analysis for preterm birth prediction

Variable	Odds Ratio	95% CI	P-value
***L. iners*/*L. crispatus* ratio (10–14w)**	2.34	1.47–3.72	<0.001
IL-6 level (pg/mL)	1.08	1.03–1.14	0.003
Previous preterm birth	4.67	1.89–11.54	0.001
Cervical length <25mm	3.21	1.32–7.81	0.011
Pre-pregnancy BMI	0.89	0.78–1.01	0.074
	1.02	0.94–1.11	0.634

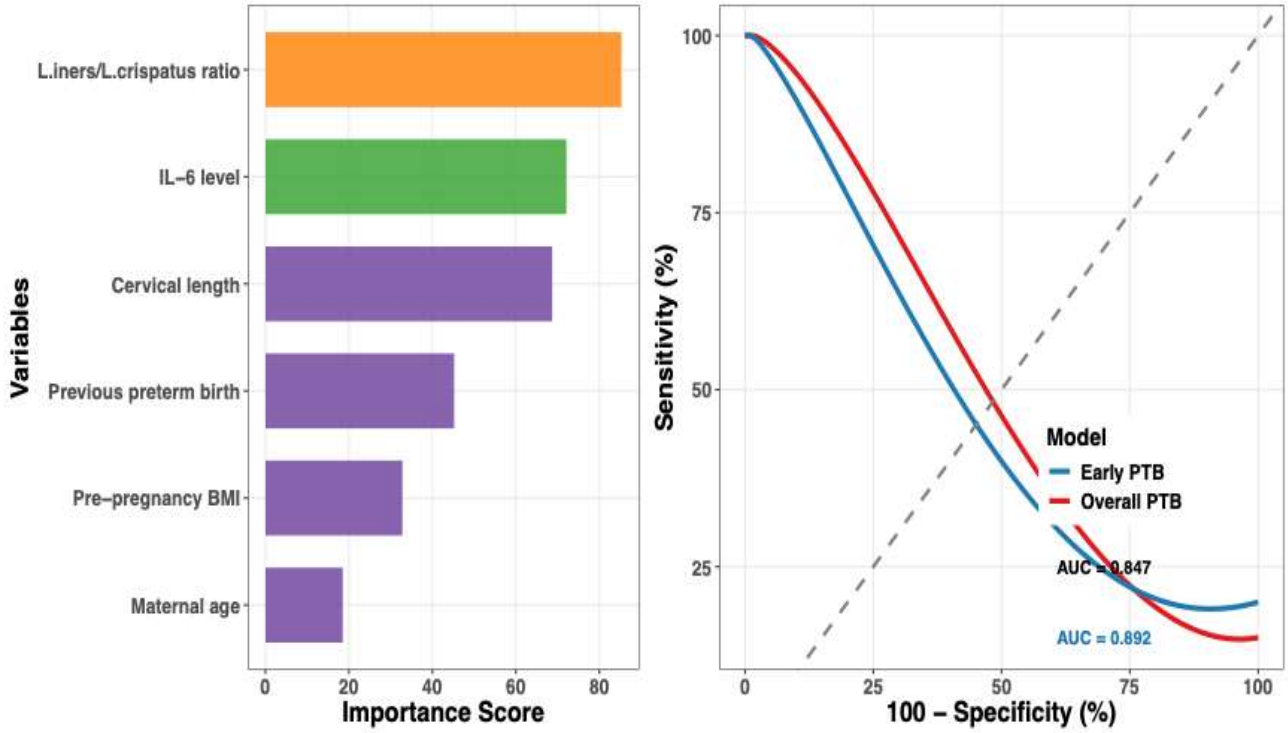

Figure 4: Predictive model performance and variable importance analysis. A: Variable importance ranking; B: ROC curves for preterm birth prediction

For early preterm birth (<34 weeks), the model performance was even better with an AUC of 0.892 (95% CI: 0.821-0.963).Random forest modeling achieved comparable performance with an AUC of 0.839 (95% CI: 0.754-0.924). Variable importance analysis revealed that the L. iners/L. crispatus ratio was the most important predictor, followed by IL-6 levels and cervical length measurements (Figure 4).

The integration of mid-trimester (18-22 weeks) microbiome data with cervical length measurements further improved prediction accuracy, with the combined model achieving an AUC of 0.901 (95% CI: 0.843-0.959). Cross-validation analysis confirmed the robustness of the predictive models, with minimal overfitting observed across different validation folds.

Discussion

This study analyzed the dynamic characteristics of cervical microbiome changes during pregnancy and successfully constructed a preterm birth risk prediction model based on microbiome features. The results demonstrate that the relative abundance ratio of *L. iners* to *L. crispatus* and associated inflammatory markers can serve as effective predictive indicators for preterm birth risk.

This study found that the preterm birth group demonstrated significantly reduced microbial diversity throughout pregnancy, a finding consistent with recent large-scale meta-analysis results.[13,14] Serrano et al. analyzed 17 longitudinal studies and found that vaginal microbiomes with low *Lactobacillus* abundance were associated with a 1.69-fold increased risk of preterm birth.[13] Notably, this reduction in diversity was observable as early as the first trimester (10-14 weeks) and progressively intensified throughout pregnancy. This temporal pattern suggests that microbiome dysbiosis may be an early marker rather than a consequence of preterm birth occurrence, providing a window of opportunity for early clinical intervention.From an ecological perspective, healthy vaginal microbiomes typically exhibit low-diversity ecosystems dominated by single predominant bacterial species, which contrasts sharply with the high-diversity healthy pattern of gut microbiomes.[15] The observed reduction in microbial diversity in this study actually reflects decreased abundance of protective *Lactobacillus* and relative growth of opportunistic pathogens, an ecological imbalance state considered an important driver of vaginal inflammatory responses.[16,17]

This study revealed the important value of the *L. iners*/*L. crispatus* ratio in preterm birth risk prediction. The preterm birth group showed significantly higher relative abundance of *L. iners* compared to the term group, while *L. crispatus* demonstrated the opposite pattern. The protective mechanisms of these two *Lactobacillus* species differ significantly. *L. crispatus* primarily produces D-lactic acid, which not only maintains a low pH environment but also specifically inhibits the activation of extracellular matrix metalloproteinase inducer (EMMPRIN), thereby preventing MMP-9-mediated collagen degradation processes [18]. Additionally, *L. crispatus* possesses powerful immunomodulatory functions, capable of inhibiting the adhesion of pathogenic bacteria such as *G. vaginalis* to vaginal epithelial cells [19]. In contrast, the protective role of *L. iners* is relatively limited. Genomic studies have shown that *L. iners* lacks many protective genes of traditional *Lactobacillus*, including genes for the synthesis of certain bacteriocins and antimicrobial compounds [20]. More importantly, *L. iners* can survive in vaginal dysbiosis states and even coexist with bacterial vaginosis-associated bacteria, making it an indicator of microbiome instability [21]. Recent strain-level studies have further revealed that *L. iners* exists in multiple subspecies, some of which may have acquired virulence genes similar to *G. vaginalis* through horizontal gene transfer.[22]

This study found that IL-6 levels were significantly elevated in early pregnancy and positively correlated with *L. iners* abundance. IL-6, as a pleiotropic cytokine, plays a dual role in the pathogenesis of preterm birth [23]. Research has demonstrated that IL-6 produces anti-inflammatory effects through classical signaling pathways while generating pro-inflammatory effects through trans-signaling pathways, with its biological activity depending on the balance between membrane-bound IL-6 receptors and soluble IL-6 receptors.[24]

In the pathological process of preterm birth, sustained inflammatory stimulation leads to increased release of soluble IL-6 receptors, causing IL-6 trans-signaling to predominate, thereby activating downstream pro-inflammatory cascade reactions.[25] This inflammatory state not only directly promotes cervical ripening and membrane rupture but also facilitates pathogen colonization by affecting the local immune microenvironment, creating a vicious cycle of inflammation-microbiome dysbiosis.[26] The synergistic effects of TNF-α and IL-1β further amplify this inflammatory

response. Animal model studies have shown that TNF-α and IL-1β, rather than IL-6, can directly induce preterm birth.[27] These cytokines activate transcription factors NF-κB and AP-1, upregulating the expression of prostaglandin E2 synthase, collagenase, and other cervical remodeling-related genes, ultimately leading to preterm birth initiation.[28]This study revealed the important predictive value of microbiome dynamic changes through longitudinal analysis. The preterm birth group exhibited greater microbiome instability, with a progressive increasing trend in the *L. iners*/*L. crispatus* ratio. The discovery of this temporal dynamic pattern holds significant clinical importance, as it indicates that microbiome dysbiosis is a gradual process, providing multiple time windows for clinical intervention.[29]

Generalized linear mixed-effects model analysis showed that *L. iners* abundance significantly increased with advancing gestational age, while *L. crispatus* showed a declining trend. This opposing change pattern may reflect gradual alterations in the cervical microenvironment, including pH elevation, decreased antimicrobial peptide concentrations, and weakened immune surveillance functions [30]. These changes provide colonization advantages for opportunistic pathogens while undermining the competitive capacity of protective *Lactobacillus*.

Strengths and limitations

The major strengths of this study include its prospective cohort design with multi-timepoint sampling throughout pregnancy, enabling comprehensive characterization of temporal microbiome dynamics. The integration of microbiome data with inflammatory markers and clinical parameters provided a multidimensional assessment of preterm birth risk. Additionally, the application of machine learning approaches enhanced predictive model performance beyond traditional statistical methods.

However, this study has several limitations that warrant consideration. First, the relatively small sample size (n=93) and single-center design may limit the generalizability of findings to broader populations. Second, the study focused exclusively on bacterial microbiomes, while potential contributions from fungi, viruses, and other microorganisms remain unexplored. Third, the study population consisted primarily of Chinese Han individuals, and microbiome characteristics may vary across different ethnic groups. Fourth, while we identified associations between microbiome patterns and preterm birth, causative relationships cannot be definitively established from observational data. Finally, the prediction model requires external validation in independent cohorts before clinical implementation.

Future research should prioritize multi-center studies with larger, ethnically diverse cohorts to validate these findings. Integration of multi-omics approaches (metabolomics, transcriptomics, proteomics) could provide deeper mechanistic insights into microbiome-host interactions. Development of point-of-care testing platforms for rapid microbiome assessment would facilitate clinical translation. Furthermore, interventional studies investigating probiotic therapy or targeted antimicrobial treatments based on individual microbiome profiles are needed to establish clinical utility.

Implications for policy and practice

These findings have important implications for clinical practice and public health policy. The identification of early pregnancy microbiome biomarkers (L. iners/L. crispatus ratio and IL-6 levels) provides opportunities for first-trimester risk stratification, enabling targeted surveillance and intervention in high-risk women. Implementation of cervical microbiome screening could complement existing preterm birth prediction methods (cervical length measurement, fetal fibronectin), potentially improving sensitivity and positive predictive value. From a policy perspective, these results support the integration of microbiome assessment into routine prenatal care protocols, particularly in resource-limited settings where traditional ultrasound-based screening may be unavailable. The strong predictive performance for early preterm birth (<34 weeks) is particularly significant, as these cases carry the highest neonatal morbidity and mortality risks. Development of evidence-based guidelines for microbiome-informed preterm birth prevention strategies could

substantially reduce the global burden of prematurity-related complications.

Conclusion

Through prospective cohort design and machine learning methods, this study successfully constructed a preterm birth risk prediction model based on dynamic changes in cervical microbiome. The *L. iners*/*L. crispatus* ratio and IL-6 levels, as key predictive factors, can identify high-risk populations in early pregnancy. This finding not only deepens our understanding of preterm birth pathogenesis but also provides new strategies for early clinical screening and individualized intervention.

Conflict of interests

The authors declare no competing interests.

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Authors' contributions

HLM and KYH made equal contributions to the conceptualisation of the study, research design, and methodology. HLM and YXW conducted the literature review. KYH and YXW were responsible for data analysis and the interpretation of results. All authors contributed to the discussion of the findings. All authors read and approved the final manuscript.

References

1. Costa A, Moller AB, Blencowe H, Johansson EW, Hussain-Alkhateeb L, Ohuma EO, Okwaraji YB, Cresswell J, Requejo JH, Bahl R, Oladapo OT, Lawn JE and Moran AC. Study protocol for WHO and UNICEF estimates of global, regional, and national preterm birth rates for 2010 to 2019. *PLoS One*. 2021 Oct20;16(10):e0258751.doi:10.1371/journal.pone.0258751.
2. Ohuma EO, Moller AB, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, Okwaraji YB, Mahanani WR, Johansson EW, Lavin T, Fernandez DE, Domínguez GG, de Costa A, Cresswell JA, Krasevec J, Lawn JE, Blencowe H, Requejo J, Moran AC. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet*. 2023 Oct 7;402(10409):1261-1271. doi: 10.1016/S0140-6736(23)00878-4.
3. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, Landoulsi S, Jampathong N, Kongwattanakul K, Laopaiboon M, Lewis C, Rattanakanokchai S, Teng DN, Thinkhamrop J, Watananirun K, Zhang J, Zhou W, Gülmezoglu AM. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health*. 2019 Jan;7(1):e37-e46. doi: 10.1016/S2214-109X(18)30451-0.
4. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012 Jun 9;379(9832):2162-72. doi: 10.1016/S0140-6736(12)60820-4.
5. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008 Jan 5;371(9606):75-84. doi: 10.1016/S0140-6736(08)60074-4.
6. Romero R, Dey SK and Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014 Aug 15;345(6198):760-5. doi: 10.1126/science.1251816.
7. Menon R. Spontaneous preterm birth, a clinical dilemma: etiologic, pathophysiologic and genetic heterogeneities and racial disparity. *Acta Obstet Gynecol Scand*. 2008;87(6):590-600. doi: 10.1080/00016340802005126.
8. Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, Girling JC, Chandirimani M, Stock SJ, Carter J, Cate R, Kurtzman J, Tribe RM and Shennan AH. Quantitative fetal fibronectin to predict preterm birth in asymptomatic women at high risk. Obstet Gynecol. 2015 May;125(5):1168-1176. doi: 10.1097/AOG.0000000000000754.
9. Honest H, Bachmann LM, Gupta JK, Kleijnen J, Khan KS. Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: systematic review. *BMJ*. 2002 Aug 10;325(7359):301. doi: 10.1136/bmj.325.7359.301.
10. Son M, Grobman WA, Ayala NK and Miller ES. A universal mid-trimester transvaginal cervical length screening program and its associated reduced preterm birth rate. *Am J Obstet Gynecol*. 2016 Mar;214(3):365.e1-5. doi: 10.1016/j.ajog.2015.12.020.

11. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1(Suppl 1):4680-7. doi: 10.1073/pnas.1002611107.
12. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DS, Wong RJ, Shaw G, Stevenson DK, Holmes SP, Relman DA. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A.* 2015 Sep 1;112(35):11060-5. doi: 10.1073/pnas.1502875112.
13. Kindinger LM, Bennett PR, Lee YS, Marchesi JR, Smith A, Cacciatore S, Holmes E, Nicholson JK, Teoh TG, MacIntyre DA. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. *Microbiome.* 2017 Jan 19;5(1):6. doi: 10.1186/s40168-016-0223-9.
14. Gudnadottir U, Debelius JW, Du J, Hugerth LW, Danielsson H, Schuppe-Koistinen I, Fransson E, Brusselaers N. The vaginal microbiome and the risk of preterm birth: a systematic review and network meta-analysis. *Sci Rep.* 2022 May 13;12(1):7926. doi: 10.1038/s41598-022-12007-9.
15. Huang C, Gin C, Fettweis J, Foxman B, Gelaye B, MacIntyre DA, Subramaniam A, Fraser W, Tabatabaei N, Callahan B. Meta-analysis reveals the vaginal microbiome is a better predictor of earlier than later preterm birth. *BMC Biol.* 2023 Sep 25;21(1):199. doi: 10.1186/s12915-023-01702-2.
16. Fettweis JM, Serrano MG, Brooks JP, Edwards DJ, Girerd PH, Parikh HI, Huang B, Arodz TJ, Edupuganti L, Glascock AL, Xu J, Jimenez NR, Vivadelli SC, Fong SS, Sheth NU, Jean S, Lee V, Bokhari YA, Lara AM, Mistry SD, Duckworth RA 3rd, Bradley SP, Koparde VN, Orenda XV, Milton SH, Rozycki SK, Matveyev AV, Wright ML, Huzurbazar SV, Jackson EM, Smirnova E, Korlach J, Tsai YC, Dickinson MR, Brooks JL, Drake JI, Chaffin DO, Sexton AL, Gravett MG, Rubens CE, Wijesooriya NR, Hendricks-Muñoz KD, Jefferson KK, Strauss JF 3rd, Buck GA. The vaginal microbiome and preterm birth. *Nat Med.* 2019 Jun;25(6):1012-1021. doi: 10.1038/s41591-019-0450-2.
17. Blostein F, Gelaye B, Sanchez SE, Williams MA, Foxman B. Vaginal microbiome diversity and preterm birth: results of a nested case-control study in Peru. Ann Epidemiol. 2020 Jan;41:28-34. doi: 10.1016/j.annepidem.2019.11.004.
18. Anton L, Sierra LJ, DeVine A, Barila G, Heiser L, Brown AG, Elovitz MA. Common Cervicovaginal Microbial Supernatants Alter Cervical Epithelial Function: Mechanisms by Which *Lactobacillus crispatus* Contributes to Cervical Health. *Front Microbiol.* 2018 Oct 8;9:2181. doi: 10.3389/fmicb.2018.02181.
19. Petrova MI, Reid G, Vaneechoutte M, Lebeer S. Lactobacillus iners: Friend or Foe? Trends Microbiol. 2017 Mar;25(3):182-191. doi: 10.1016/j.tim.2016.11.007.
20. France MT, Mendes-Soares H, Forney LJ. Genomic Comparisons of Lactobacillus crispatus and Lactobacillus iners Reveal Potential Ecological Drivers of Community Composition in the Vagina. *Appl Environ Microbiol.* 2016 Nov 21;82(24):7063-7073. doi: 10.1128/AEM.02385-16.
21. Petricevic L, Domig KJ, Nierscher FJ, Sandhofer MJ, Fidesser M, Krondorfer I, Husslein P, Kneifel W, Kiss H. Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. *Sci Rep.* 2014 May 30;4:5136. doi: 10.1038/srep05136.
22. Nori SRC, Walsh CJ, McAuliffe FM, Moore RL, Van Sinderen D, Feehily C, Cotter PD. Strain-level variation among vaginal Lactobacillus crispatus and Lactobacillus iners as identified by comparative metagenomics. *NPJ Biofilms Microbiomes.* 2025 Mar 23;11(1):49. doi: 10.1038/s41522-025-00682-1.
23. Prairie E, Côté F, Tsakpinoglou M, Mina M, Quiniou C, Leimert K, Olson D, Chemtob S. The determinant role of IL-6 in the establishment of inflammation leading to spontaneous preterm birth. *Cytokine Growth Factor Rev.* 2021 Jun;59:118-130. doi: 10.1016/j.cytogfr.2020.12.004.
24. Omere C, Richardson L, Saade GR, Bonney EA, Kechichian T, Menon R. Interleukin (IL)-6: A Friend or Foe of Pregnancy and Parturition? Evidence From Functional Studies in Fetal Membrane Cells. *Front Physiol.* 2020 Jul 24;11:891. doi: 10.3389/fphys.2020.00891.
25. Brou L, Almli LM, Pearce BD, Bhat G, Drobek CO, Fortunato S, Menon R. Dysregulated biomarkers induce distinct pathways in preterm birth. *BJOG.* 2012 Mar;119(4):458-73. doi: 10.1111/j.1471-0528.2011.03266.x.
26. Robertson SA, Hutchinson MR, Rice KC, Chin PY, Moldenhauer LM, Stark MJ, Olson DM, Keelan JA. Targeting Toll-like receptor-4 to tackle preterm birth and fetal inflammatory injury. *Clin Transl Immunology.* 2020 Apr 14;9(4):e1121. doi: 10.1002/cti2.1121.
27.Sadowsky DW, Adams KM, Gravett MG, Witkin SS, Novy MJ. Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. *Am J Obstet Gynecol.* 2006 Dec;195(6):1578-89. doi: 10.1016/j.ajog.2006.06.072.
28. Menon R, Fortunato SJ. Fetal membrane inflammatory cytokines: a switching mechanism between the preterm premature rupture of the membranes and preterm labor pathways. *J Perinat Med.* 2004;32(5):391-9. doi: 10.1515/JPM.2004.134.
29. Golob JL, Oskotsky TT, Tang AS, Roldan A, Chung V, Ha CWY, Wong RJ, Flynn KJ, Parraga-Leo A, Wibrand C, Minot SS, Oskotsky B, Andreoletti G, Kosti I, Bletz J, Nelson A, Gao J, Wei Z, Chen G, Tang ZZ, Novielli P, Romano D, Pantaleo E, Amoroso N, Monaco A, Vacca M, De Angelis M, Bellotti R,

Tangaro S, Kuntzleman A, Bigcraft I, Techtmann S, Bae D, Kim E, Jeon J, Joe S; Preterm Birth DREAM Community; Theis KR, Ng S, Lee YS, Diaz-Gimeno P, Bennett PR, MacIntyre DA, Stolovitzky G, Lynch SV, Albrecht J, Gomez-Lopez N, Romero R, Stevenson DK, Aghaeepour N, Tarca AL, Costello JC, Sirota M. Microbiome preterm birth DREAM challenge: Crowdsourcing machine learning approaches to advance preterm birth research. *Cell Rep Med.* 2024 Jan 16;5(1):101350. doi: 10.1016/j.xcrm.2023.101350.

30. Oliver A, LaMere B, Weihe C, Wandro S, Lindsay KL, Wadhwa PD, Mills DA, Pride DT, Fiehn O, Northen T, de Raad M, Li H, Martiny JBH, Lynch S, Whiteson K. Cervicovaginal Microbiome Composition Is Associated with Metabolic Profiles in Healthy Pregnancy. *mBio.* 2020 Aug 25;11(4):e01851-20. doi: 10.1128/mBio.01851-20.