BMC Pregnancy and Childbirth

RESEARCH

Open Access

Influences of gestational diabetes mellitus on the changes in the vaginal microbiota from antepartum to postpartum

Ying Liao[1], Ting Liu[1], Xinfang Zeng[1,2], Bin Xiao[3], Meiqun Xiao[1], Yuanfang Zhu[1,3*] and Qiying Song[4,5,6*]

Abstract

Background No consensus has yet been reached concerning whether there were significant differences in the vaginal microbiota according to maternal gestational diabetes mellitus (GDM) status. This study aimed to compare the vaginal microbiota of women with GDM and normal blood glucose before and after delivery and to prospectively evaluate the influence of GDM on the dynamic changes of vaginal microbiota from antepartum to postpartum.

Methods This study included 20 GDM patients and 31 average pregnant women who gave birth at the Shenzhen Baoan Women's and Children's Hospital. Vaginal secretions samples were collected one week before delivery (D0), on the first day of delivery (D1), and 42 days after delivery (D42). Vaginal microbiota was detected using 16S rRNA gene sequencing.

Results There was no significant difference in alpha and beta diversity between the GDM and non-GDM groups at each time point (all $p > 0.05$). However, the overall change patterns in Shannon and Pielou's evenness index from D0 to D1 to D42 significantly differed between the GDM and non-GDM groups ($p = 0.046$ and $p = 0.032$, respectively). The abundance of Lactobacillus decreased obviously after delivery, especially in the GDM group, showing a more severe imbalance of the vaginal microbiota.

Conclusions We found that GDM affected the succession of vaginal microbiota in the perinatal period. Our findings provided additional evidence for regulating the vaginal microbiota during pregnancy and postpartum to reduce adverse pregnancy outcomes and achieve long-term vaginal health outcomes.

Keywords Gestational diabetes, Vaginal microbiota, Bacterial vaginosis, Gene sequencing, Antenatal period, Postpartum period

*Correspondence:
Yuanfang Zhu
zhuyf1027@163.com
Qiying Song
songqiying@163.com
[1] Department of Obstetrics, Shenzhen Baoan Women's and Children's Hospital, No.56 Yulv Road, Shenzhen, Baoan 518100, China
[2] First Clinical Medical College, Jinan University, Guangzhou 510000, China
[3] Maternal-Fetal Medicine Institute, Shenzhen Baoan Women's and Children's Hospital, Shenzhen 518100, China
[4] Department of Child Healthcare, Shenzhen Baoan Women's and Children's Hospital, Shenzhen 518100, China
[5] Center for Energy Metabolism and Reproduction, Institute of Biomedicine and Biotechnology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China
[6] Health Science Center, Shenzhen University, Shenzhen 518055, China

Introduction

Gestational diabetes mellitus (GDM) is one of the common pregnancy complications in obstetrics, mainly manifested by the first discovery of impaired glucose tolerance during pregnancy, which profoundly impacts the health of mothers and babies. GDM may increase the risks of postpartum metabolic syndrome and cardiovascular diseases, including central obesity, hypertension, insulin resistance, and dyslipidemia [1–3]. A retrospective cohort study shows that more than 25% of GDM will develop into type 2 diabetes mellitus at 15 years postdiagnosis [4, 5]. Gestational diabetes will also increase the risks of adverse neonatal outcomes, especially macrosomia, overdue pregnancy, premature delivery, poor Apgar score, and the incidence of metabolic diseases in offspring [5]. Furthermore, GDM is usually accompanied by advanced maternal age and obesity, leading to a more complicated pregnancy course and worse outcomes in GDM patients [6, 7].

Compared to the non-pregnant women, the pregnancy vaginal microbiota was dominated by Lactobacillus and characterized by low alpha diversity. The composition of the vaginal microbiota was dynamically restructured in the postpartum period, with less Lactobacillus and increased alpha diversity [8]. In particular, Lactobacillus played vital protection in women, acting as a probiotic multi-microbial consortium instead of providing individual probiotic protection [9, 10]. Recent researchers found that GDM might be associated with disturbances in the vaginal microbiota during pregnancy [3]. Zhang et al. [3] found that vaginal Lactobacillus species were vastly different between the GDM group and the healthy control, with L. listeri, L. amylovorus, and L. fructivorans specific to the vagina of the GDM group, while L. salivarius was only observed in the healthy pregnant women. However, Wang and colleagues [5] collected 259 vaginal samples and performed various microbial analyses, but they did not observe obvious variation in vaginal microbiota in women with GDM, compared with those with normal glucose tolerance. In brief, no consensus has yet been reached concerning whether there were significant differences in the vaginal microbiota during pregnancy according to maternal GDM status.

In addition, most previous studies only focused on the changes in vaginal microbiota during pregnancy, and few studies described the differences in the postpartum period. The imbalance of postpartum vaginal microbiota is one of the causes of puerperal infection, postpartum hemorrhage, and endometritis. GDM may affect postpartum vaginal microbiota and thus affect maternal health after delivery. Therefore, it is important to understand postpartum vaginal microbiota changes in GDM versus non-GDM women. Moreover, to the best of our knowledge, no studies have explored whether there are differential changes in the vaginal microbiota from antepartum to postpartum in women with and without GDM.

We hypothesized that GDM would alter the vaginal microbiota not only during pregnancy but also after pregnancy, as well as the dynamic changes from antepartum to postpartum. Therefore, in this study, we aimed to detect the vaginal microbiota of pregnant women in the third trimester of pregnancy, one day and 42 days after delivery, respectively, and to compare the differences at each time point between women with GDM and with normal glucose tolerance. In addition, we would prospectively evaluate the influence of GDM on the dynamic changes of vaginal microbiota from antepartum to postpartum.

Methods

Participants

In this study, 51 women who gave birth at the Shenzhen Baoan Women's and Children's Hospital were included with the criteria: (1) with full-term, singleton pregnancy and (2) without known fetal anomalies or complications. Because the use of antibiotics will lead to an imbalance in the microbiota, those who received any antibiotic treatment during the pregnancy were excluded to control for confounding factors. In the 24–28 weeks of gestation, an oral glucose intolerance test (OGTT) was conducted; blood was collected to monitor blood sugar for pregnant women on an empty stomach after oral administration of 75 g of glucose in 1 h and 2 h. Any of the results are greater than the reference value (fasting blood glucose: 5.1 mmol/L, 1 h after taking sugar: 10.0 mmol/L, and 2 h after taking sugar: 8.5 mmol/L), can be diagnosed as GDM according to the International Association of the Diabetes and Pregnancy Study Group (IADPSG) criteria [11] while the rest is classified as a non-GDM group. The Ministry of Health of China refers to the guidelines for the detection and diagnosis of GDM formulated by IADPSG since IADPSG standards can detect more GDM and better identify women with poor pregnancy outcomes [12]. In the present study, pregnant women with GDM were included only if they received diet and/or exercise management to control blood glucose. Those treated with medications (such as insulin or oral agents) were excluded because using medication to control blood glucose may affect vaginal microbiota and bias the association between GDM and vaginal microbiota.

The study received ethical approval from the Ethical Committees of Shenzhen Baoan Women's and Children's Hospital (approval number: LLSC 2020–09-02-KS), and all participants signed written informed consent. This study was compliant with the Strengthening the

Reporting of Observational Studies in Epidemiology (STROBE). Demographic and clinical features of all participants, including the gestational week of labor, pre-pregnancy BMI, gestational weight gain, mode of delivery, feeding practices, and OGTT results, were collected through interviews and a review of medical records.

Sample collection and sequencing

The first vaginal secretions sample was collected within seven days of delivery, one week before delivery (henceforward referred to as the "D0 phase"). In the case of elective cesarean section, the first vaginal secretions samples were obtained the day before the cesarean section. In contrast, in the cases of vaginal delivery and emergency cesarean section, the vaginal secretions samples were obtained when pregnant women were hospitalized due to signs of labor. For the latter cases, most of the vaginal secretions samples were obtained within 48 h before delivery, except that a few pregnant women were admitted to the hospital because of false labor and did not give birth until several days later. However, all the vaginal samples were obtained within one week before delivery at baseline in the present study. Then another two vaginal secretions samples were collected for the pregnant women on the first day of delivery (henceforward referred to as "D1 phase") and 42 days after delivery (henceforward referred to as "D42 phase"), respectively.

During sampling, the vagina was fully exposed with a sterile speculum, and the secretions from the posterior vault of the vagina and the inner wall of 1/3 of the vagina were scraped with a sterile swab. Regarding the collection of vaginal samples on the first day after delivery, the blood, lochia, and necrotic decidua tissue were wiped with sterile cotton swabs, and then vaginal secretions were collected. All samples were placed in a cryogenic transfer box and then frozen in a refrigerator at −80 °C as soon as possible. Since there was a large amount of blood, lochia, and necrotic decidua tissue in the vagina on the first day after delivery, we first dried the blood with a sterile cotton swab. Then we took a sample, obtaining the actual microecological situation of the vagina after delivery and amniotic fluid washing.

After sample collection, DNA was extracted from vaginal swabs using QIAamp DNA Mini kit (Qiagen, Germany). The 16S rRNA gene encodes small ribosome subunits of prokaryotes, including 10 Conserved Regions and 9 Hypervariable Regions. The Hypervariable Regions have the specificity of genus or species and vary with different genetic relationships, which is one of the most suitable indicators for bacterial phylogeny and classification. Among them, V3, V4, and V5 regions have reasonable specificity and complete database information, which is the best choice for bacterial diversity analysis and annotation. The V3-V4 region is usually examined, and its resolution for most species in the microbial community can be up to the relative abundance at the genus level. Some can be up to the species level. By comparing the sequencing data with the 16S rRNA gene reference database, the sequence variation and relative abundance can be obtained. Then much information such as species classification, species abundance, population structure, systematic evolution, and community comparison can be obtained. The V3-V4 hypervariable region of the 16S rRNA gene was amplified with the forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Then, the generated 16S rRNA gene sequences were analyzed using the bioinformatics software package QIIME2(version 2019.4) [13]. Paired-end reads were firstly denoised by QIIME2 with the command "qiime dada2 denoise-paired". Aimed to merge paired-end reads, quality filtering, and exclude chimeric and phiX sequences. Taxonomic assignment was performed against Greengenes (13_8 revision) database using the command "qiime feature-classifier classify-sklearn." Meanwhile, an array of alpha- and beta-diversity measures was generated using the commands "qiime phylogeny align-to-tree-mafft-fasttree" and "qiime diversity core-metrics-phylogenetic". For 16S rRNA sequencing data, bioinformatic software QIIME2 and the database Greengenes have been cited thousands of times. They were widely used because of their high reliability and validity. Therefore, in the present study, we also chose QIIME2 and Greengenes.

At the 97% similarity level, sequences are divided into operational taxonomic units (OTUs) according to their classification levels. Then, alpha- and beta-diversity measures were generated. The data that support the findings of this study have been deposited into the CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0003769.

Statistical analysis

Statistical analysis was performed using R software (version 3.6.1) [14], with continuous variables expressed as mean ± standard deviation (SD), compared using Student's independent t-test, and categorical variable comparison using the chi-square test or Fisher precision test. $P < 0.05$ is statistically significant for the difference.

Alpha diversity was expressed by the Shannon diversity index, Pielou's evenness index, and observed features value. The Shannon diversity index is an index for calculating bacterial diversity, which represents the uncertainty in the community. The greater the Shannon

diversity index value, the more unknown factors in the community, and the richer the bacterial diversity. Pielou's evenness index represents the evenness of species distribution in the community. The closer the relative abundance of each species in the community is, the closer Pielou's evenness is to 1.

Beta diversity analyses were performed using the vegan R packages (https://cran.r-project.org/web/packages/vegan/vegan.pdf). Beta diversity reflects the community differences among different samples, expressed as the weighted UniFrac distances in the present study. The difference of microbial community profiles was assessed based on principal co-ordinates analysis (PCoA), supplemented by permutation multivariate variance analysis (PERMANOVA, 999 permutations). The more significant the community difference between the two samples, the further the distance in the PCoA. Thus, differences in vaginal microbiota between the GDM and non-GDM groups could be analyzed and visualized.

The LEfSe (Linear Discriminant Analysis Effect Size) program was used to identify taxa with significantly different relative abundances. LDA (Linear Discriminant Analysis) was applied to draw a straight line that completely separates two class data points, following the criteria of maximizing the distance between the means of two classes and minimizing the variance within individual courses to get the difference between the two groups. Only when the LDA score > 2.0 can the difference of a particular flora between the two groups be considered statistically significant [15]. Although LDA, as a supervised learning algorithm, was unsuitable for unlabeled data and might not effectively discriminate between classes with overlapping statistical properties, this method is still widely used.

PICRUSt2.0 software was applied to perform the functional annotation analysis based on the MetaCyc database. The differences in metabolic pathways between the GDM and non-GDM groups were explored using STAMP software [16, 17].

Results

Analysis of clinical data of the examined mother

A total of 51 pregnant women (GDM ($N=20$) and non-GDM ($N=31$)) were included in this study. A comparison of maternal and infant characteristics between the GDM and non-GDM groups was presented in Table 1. There were no statistically significant differences in gestational age, maternal pre-pregnancy BMI, gestational weight gain, group B streptococcus infection, feeding practices, and mode of delivery between the two groups ($p > 0.05$). The difference in OGTT results between the two groups was statistically significant ($p < 0.05$).

Differences in alpha diversity of vaginal microbials

Compared with the D0 phase (1.53 ± 1.96, and 0.30 ± 0.26), the Shannon diversity and Pielou's evenness index of the D1 (3.37 ± 1.95, and 0.53 ± 0.22) and D42 (2.85 ± 1.44, and 0.54 ± 0.22) phases increased

Table 1 Maternal and infant characteristics comparison between the GDM and non-GDM groups ($N=51$)

	GDM ($N=20$)	non-GDM ($N=31$)	*p*-value
Maternal characteristics			
Age at delivery, year	32.6 ± 4.3	30.5 ± 3.3	0.068
Pre-pregnancy BMI, kg/m2	23.3 ± 2.9	21.9 ± 3.7	0.125
Gestational weight gain, kg	12.5 ± 4.0	13.6 ± 5.0	0.379
Delivery mode			0.743
Vaginal	14 (70.0)	23 (74.2)	
Cesarean	6 (30.0)	8 (25.8)	
Feeding practices			0.390
Breastfeeding	11(55.0)	23(74.2)	
Mixed	7(35.0)	7(22.6)	
Formula	1(5.0)	1(3.2)	
Missing	1(5.0)	0(0)	
Group B streptococcus infection	3 (15.0)	5 (16.1)	1.000
Fasting glucose, mmol/L	5.1 ± 0.9	4.3 ± 0.3	0.004
OGTT-1 h, mmol/L	9.9 ± 2.2	7.7 ± 1.4	0.001
OGTT-2 h, mmol/L	8.0 ± 2.2	6.3 ± 1.1	0.010

Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student's independent t-test; categorical variables were presented as numbers and percentages and analyzed using the chi-square test or Fisher's exact test

Abbreviations: BMI body mass index, *OGTT* oral glucose tolerance test

significantly (Fig. 1a and b). The observed features value of the D1 phase (97.35 ± 88.58) was higher than that of the D0 phase (39.87 ± 71.83) and the D42 phase (38.96 ± 25.81) (Fig. 1c).

Although the difference in alpha diversity metrics of the GDM group and the non-GDM group was not statistically significant at each time point (all $p > 0.05$), the results of mixed effect model analyses showed that the overall changes in Shannon and Pielou's evenness index from D0 to D1 to D42 were significantly different over time between GDM and non-GDM groups ($p = 0.046$ and $p = 0.032$, respectively).

Differences in beta diversity of vaginal microbials in GDM and non-GDM

The results of principal coordinate analysis (PCoA) showed that the vaginal microbiota of the GDM and non-GDM groups overlapped in the three phases of D0, D1, and D42 (PERMANOVA, $p = 0.403$, 0.395, and 0.619, respectively). That is, there was no significant difference in beta diversity of the vaginal microbiota between GDM and non-GDM women (Fig. 2).

Relative abundance

The trends in vaginal microbiota from D0 to D1 to D42 at the phylum and genus level are shown in Fig. 3. The main phyla included Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria. Lactobacillus, Gardnerella, Prevotella, and streptococcus were the most abundant at the genus level.

The results also showed that Firmicutes remained the predominant phylum before and after delivery. The relative abundance of Firmicutes decreased on the day of delivery and stayed at this level through 42 days postpartum. However, the relative abundance of Actinobacteria and Bacteroidetes phyla significantly increased after childbirth, especially 42 days after delivery. Interestingly, the phylum Proteobacteria had a significant increase in

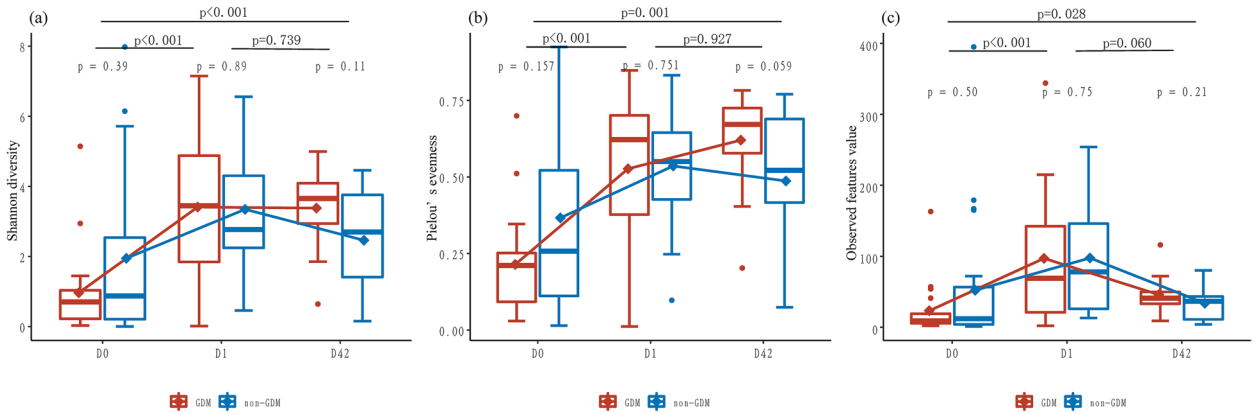

Fig. 1 Alpha diversity of vaginal microbiota in women with and without GDM at different time points. **a** Shannon diversity; **b** Pielou's evenness; **c** observed features value

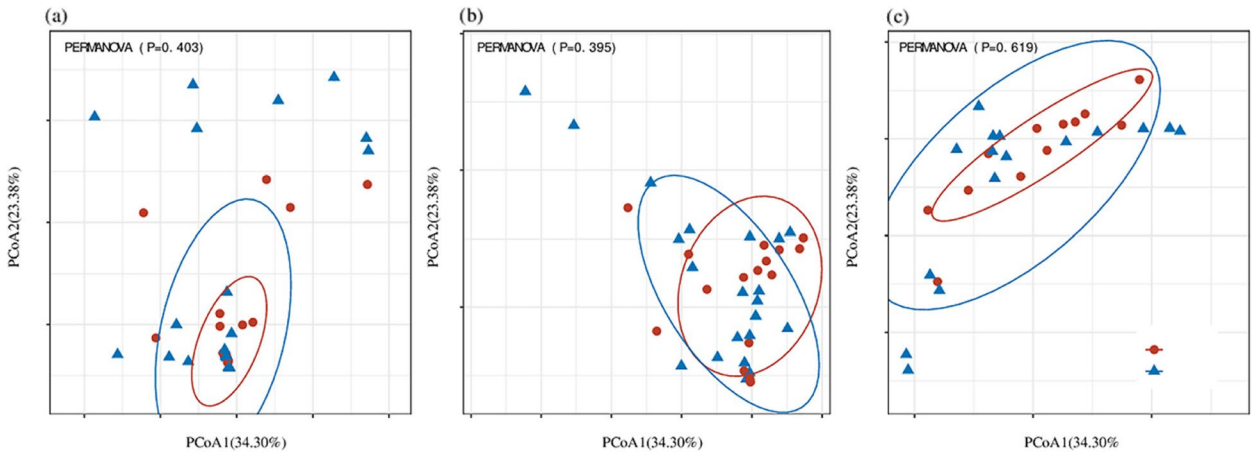

Fig. 2 Principal ordination analysis based on weighted UniFrac distances in the D0 (**a**), D1 (**b**), and D42 (**c**) phases

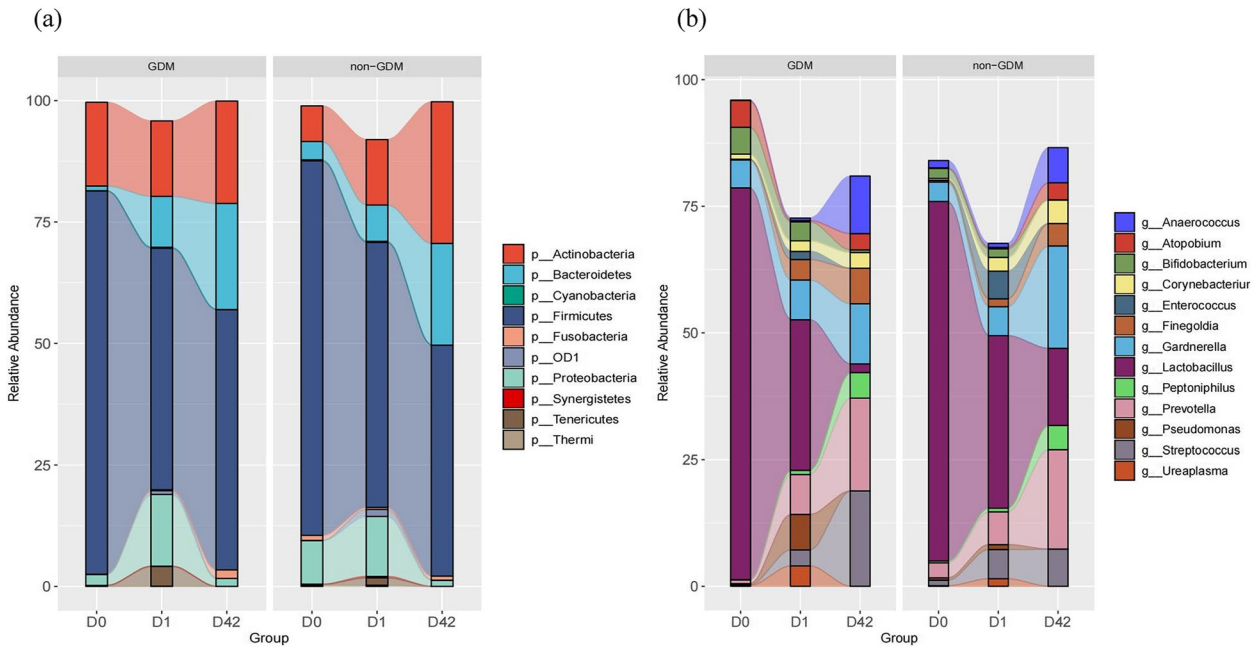

Fig. 3 Trends in vaginal microbiota at the phylum (**a**) and genus (**b**) level

the D1 phase, gradually decreasing to a minimum in the D42 phase (Fig. 3a).

As shown in Fig. 3, the change trend of vaginal microflora in GDM and non-GDM in different periods is similar. At the phylum level, whether GDM or non-GDM, from prenatal to postpartum (D0 to D42), Actinobacteria and Bacteroidetes showed an upward trend, while Firmicutes and Proteobacteria gradually decreased. At the genus level, Lactobacillus was the most dominant genus in the D0 phase, while after delivery, the relative abundance of Lactobacillus significantly reduced, with more reduction in the D42 phase, especially in the GDM group. Meanwhile, Prevotella, Streptococcus, Peptoniphilus, Gardnerella, and Anaerococcus showed an upward trend over time (Fig. 3b).

Comparison of the vaginal microbiota between the two groups at different time points

Taxa with different relative abundance were identified using the LEfSe program in the set threshold. It can be seen that in the D0 phase, the abundance of the family Staphylococcaceae, genus Staphylococcus, and genus Fusobacterium was higher in the non-GDM group (Fig. 4a). However, in the D1 phase, family Ellin6075, family Christensenellaceae, genus Phascolarctobacterium, and genus Mobiluncus were enriched in the non-GDM group. In contrast, the family Bacillaceae, genus Moryella, family Rhodospirillaceae, and genus Gemella were more abundant in the GDM group (Fig. 4b). In the D42 phase, the family Carnobacteriaceae, and genus Varibaculum, Desemzia, and Salinicoccus in the GDM group showed higher abundance (Fig. 4c).

Functional and pathway analysis

The results in the D0 phase showed that naphthalene degradation, naphthalene degradation to acetyl-CoA, norspermidine biosynthesis, and ethylmalonyl-CoA pathway were significantly enriched in the non-GDM group (Fig. 5a). However, in the D42 phase, more pathways were enriched in the GDM group, such as glycogen degradation I, superpathway of histidine, purine, and pyrimidine biosynthesis, superpathway of purine nucleotides de novo biosynthesis I and II, superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (Fig. 5b). There were no different metabolic pathways between the GDM and non-GDM groups in the D1 phase.

Discussion

In this study, we explored the dynamic changes in the vaginal microbiota of GDM and non-GDM women during the perinatal period for the first time. We haven't found any differences in the alpha diversity (Shannon diversity and Pielou's evenness) of GDM and the GDM. However, when using mixed effect model analyses, research from D0 to the change of the D42 found that different periods of the shift in Shannon diversity and Pielou's evenness are different which indicated that GDM affected the succession of vaginal microbiota in the

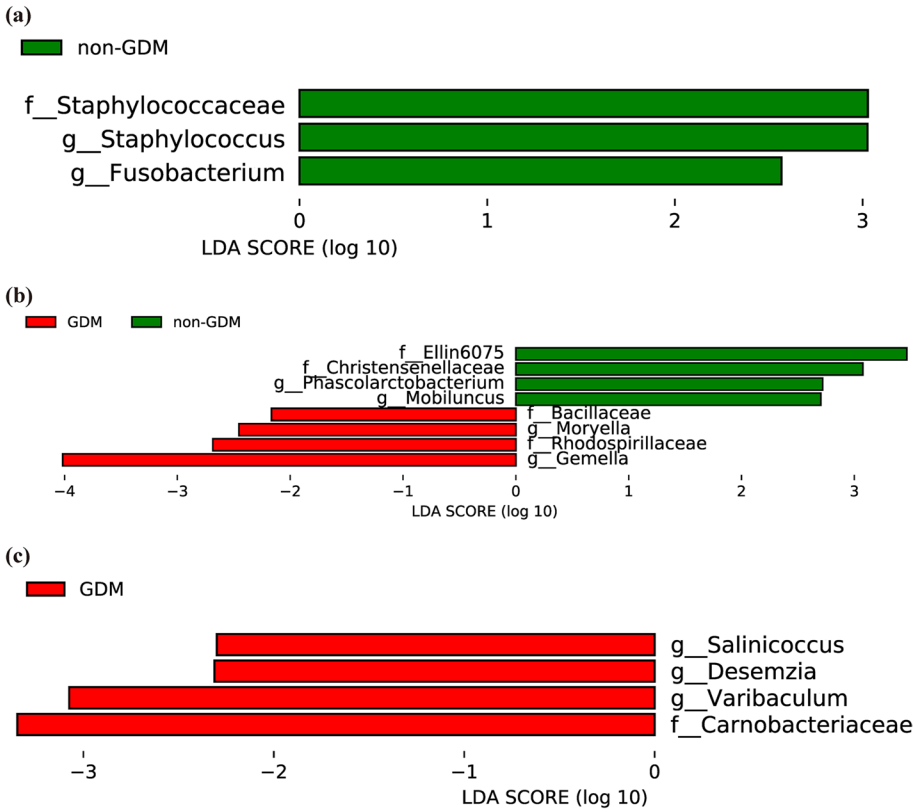

Fig. 4 Histogram of the LDA scored for differentially abundant genera between the GDM and non-GDM groups. LDA scores were calculated by LDA effect size using linear discriminant analysis. (**a**) was D0, (**b**) was D1, and (**c**) was D42

perinatal period. Figure 2 showed that GDM and non-GDM groups overlap in the PCoA coordinate system for beta diversity in different periods. We can understand that there is no noticeable difference in the species composition of vaginal microbiota between the two groups. It showed that GDM may not affect the species composition structure of vaginal microbiota. This is only one of the findings of this study, and the specific significance needs further research with larger samples. In addition, we found a notable decrease in the abundance of the genus Lactobacillus from antepartum to postpartum, especially in the GDM group. These trends are similar to a study by Elizabeth et al. [18]. The composition of vaginal microorganisms after delivery is characterized by lactic acid bacteria being replaced by various anaerobic bacteria, including Peptoniphilus, Prevotella, Anaerococcus, and so on. This shows that vaginal microorganisms after delivery are not as stable as when pregnant, and premature pregnancy after delivery may lead to premature delivery.

As is known, during breastfeeding, women will secrete estrogen, progesterone, prolactin, oxytocin, growth hormone, insulin, glucocorticoid, and thyroid hormone to maintain the development and function of the breast. The association between GDM and vaginal microbiota might be biased by hormone levels. However, hormone levels in women after delivery were mainly influenced by breastfeeding. This study showed no significant difference in feeding patterns between the GDM and non-GDM groups (Table 1. $p=0.390$). Therefore, we assumed there would also be no significant difference in hormone levels between the two groups at 42 days after delivery, suggesting that hormone levels would not bias our study results.

There has been a large amount of evidence that the vaginal microbiota of pregnant women has undergone significant changes. During pregnancy, the effect of estrogen on vaginal epithelial cells leads to glycogen accumulation in epithelial cells. Glycogen is a carbon source that can be metabolized by Lactobacillus to lactic acid, thus forming a vaginal microbiota composed of Lactobacillus as the main body [19]. Lactobacillus has always been considered a beneficial vaginal bacteria, which can reduce the vaginal pH by producing lactic acid, create a variety of antibacterial and bactericidal compounds, or play a protective role by competitive rejection [20, 21]. This change during pregnancy makes the vaginal microbiota more stable than the

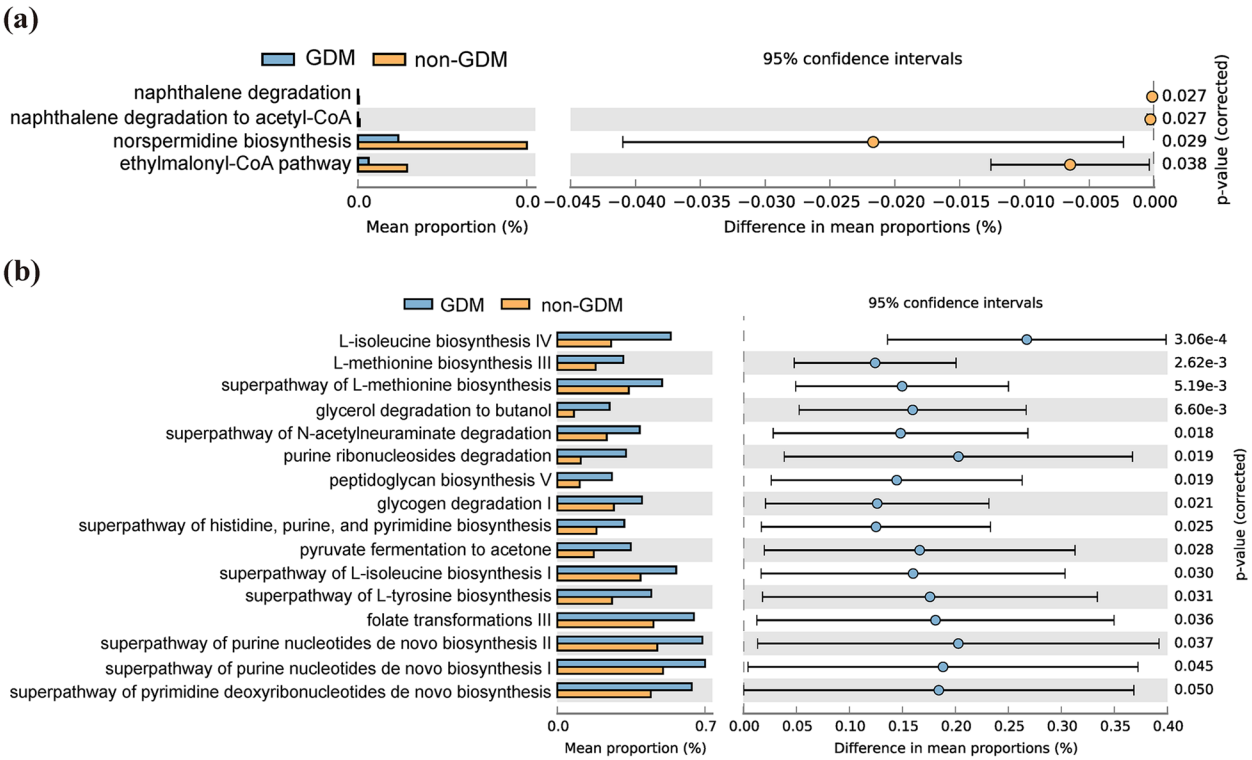

Fig. 5 Comparisons of metabolic pathways between GDM and non-GDM groups. Significantly different metabolic pathways between GDM and non-GDM groups were observed in the D0 (**a**) and D42 phases (**b**). There was no significant difference in the D1 phase

non-pregnant state, thereby significantly reducing the risk of reproductive tract infections [22, 23].

Whether the protective effect of Lactobacillus on the vagina last until postpartum? According to the treatment specifications of all hospitals and clinics in China, povidone-iodine cotton balls are used to disinfect the vulva before delivery. Disinfectants will be inevitably brought into the vagina during operations such as manually taking the placenta and lateral episiotomy and suture. And after being washed out by amniotic fluid and lochia, vaginal microorganisms undergo a redistribution. Decreased postpartum estrogen levels can also lead to an imbalance in the vaginal microbiota. This results in the microbial balance in the vagina being damaged and redistributed on the first day of delivery. We, therefore, collected vaginal microorganisms on Day 1 postpartum as a "blank" sample. This can also be a more intuitive understanding of postpartum vaginal microbiota changes.

In the present study, we found a notable change in the abundance of the genus Lactobacillus at the genus level, which decreased gradually over time. Lactobacillus mainly provides lactic acid by decomposing glycogen of vaginal epithelial cells through anaerobic metabolism. An over-acidic vaginal environment can kill white blood cells, including lymphocytes, monocytes, and macrophages, thus alleviating the development of vaginal inflammation and sexually transmitted diseases [24]. The protective mechanism of lactobacillus is related to the surface active molecules (SAMs), Peptidoglycan (PG), and Lipoteichoic acid (LTA) of lactobacillus. Lactobacillus can inhibit inflammatory cells, resist adhesion and biofilm through them, and have immunomodulatory activity. To maintain the healthy state of the vagina [25–27].

When the relative abundance of Lactobacillus is reduced, the inhibition is weakened. another flora grows wantonly, similar to the results of previous research by Srinivasan et al. [28]. The number of Lactobacillus in the vagina decreased or was absent, resulting in easy changes in the vaginal microenvironment with significant increases in Streptococcus, Staphylococcus, Enterococcus, Corynebacterium, and Peptospira to fill the space corresponding to the decreased abundance of Lactobacillus. The growth of these opportunistic pathogens also increases the chance of vaginal infection [29]. It's worth noting that Lactobacillus did not return to prenatal levels at 42 days postpartum. This change is more evident in GDM.

At the phylum level, Proteobacteria reached the highest abundance during delivery and then decreased almost

to disappear, which is a unique change. Proteobacteria include many pathogens, such as Escherichia coli (E. coli), which are facultative (aerobic and anaerobic) bacteria. Studies have shown that the growth of E. coli is inhibited by Lactobacillus since Lactobacillus causes the vaginal pH decline [30]. On the first day after delivery, with the disappearance of many Lactobacillus, this inhibitory effect is diminished, leading to an increase in the number of Proteobacteria. However, Proteobacteria gradually decreased during the extended postpartum period. We suspected that Proteobacteria were at a competitive disadvantage due to the growth of other bacteria in the vagina. More case–control experiments are needed to confirm this.

In our research, the vaginal microbiota was gradually occupied by "Bacterial Vaginosis Microorganisms" after delivery, including Gardnerella, Atopobium, and Prevotella, regardless of GDM or non-GDM groups. Bacterial Vaginosis (BV) is a vaginal dysbiosis characterized by a shift in the vaginal microbiota from the dominant Lactobacillus to a polymicrobial flora [31]. A study by Elena et al. [32]. also found that depletion of Lactobacillus species ($\leq$47% relative abundance) proved to be a highly accurate predictor of BV. The increase of Prevotella in the vagina was also related to BV [33]. Lipopolysaccharides and ammonia produced by Prevotella are part of vaginal mucus, which will destroy the normal pH of the vagina. It is also related to the production of epithelial cytokines, which promotes the growth of other "Bacterial Vaginosis Microorganisms," making the vaginal environment more susceptible to infection [34]. In normal metabolic activities, Prevotella can produce ammonia, which can increase vaginal pH and promote the growth of Gardnerella. Amino acids produced by Gardnerella can be used by Prevotella in a synergistic relationship, further stimulating the growth of Prevotella, thus making more ammonia. This cycle continued, leading to higher concentrations of both organisms [35]. Meanwhile, Gardnerella can also provide essential nutrients for the growth of other bacteria, altering the host immune response and leading to BV.

Even if our study found an increase in the proportion of "Bacterial Vaginosis Microorganisms" in the vaginal microbiota after delivery, it does not mean that postpartum women will develop BV. Since many BV cases were asymptomatic and women had no symptoms such as vaginal odor and itching, they did not seek medical treatment. Throughout a woman's life, vaginal microbes are continually influenced by external influences such as the menstrual cycle, pregnancy, hormonal changes, medications, douching, and sexual intercourse. Vaginal microbiota change their composition in response to environmental changes to maintain a healthy vaginal health environment as a whole. Although asymptomatic BV during pregnancy has been associated with adverse pregnancy outcomes such as spontaneous abortion and premature delivery, there is no clinical evidence to recommend BV screening in pregnant women [36]. On the contrary, the increasing antimicrobial rate in BV infections leads to recurrent infections, and several studies suggested the application of probiotic Lactobacilli to prevent BV infections or their recurrence in women [37–39].

The study by Wang et al. [5]. confirmed that GDM had similar effects on various parts of the mother as well as the offspring microbiota and may increase the risk of pregnancy complications or adverse pregnancy outcomes. Previous studies have found that patients with GDM in the second and third trimester of pregnancy have increased intestinal Bacteroides and decreased Bifidobacterium and Lactobacillus producing short-chain fatty acids (SCFAs) compared with non-GDM patients. This change is associated with increased blood glucose [40]. Bifidobacterium and Lactobacillus are probiotics that can alleviate insulin resistance by reducing systemic inflammation, regulating immune function, and improving intestinal mucosal permeability [41]. The effect of intestinal probiotics on the regulation of intestinal flora has been widely accepted and applied in clinical practice. A randomized controlled study found that oral probiotic preparations (L. acidophilus PBS066 and L. reuteri PBS072) and (L. plantarum PBS067, L. rhamnosus PBS070, and B. lactis PBS075) significantly increased the abundance of lactobacilli and bifidobacteria in vagina compared with the placebo control group [42]. The intravaginal administration of Lyophilized L. Crispatus IP 174178 can reduce BV's recurrence rate and prolong BV's recurrence time. And vaginal tablet consisting of L. fermentum LF15 and L. plantarum LP01 restored the acidity of imaginary pH and the threshold level of Nugent score to below 7 [43, 44]. Healthy vaginal flora is characterized by the balance of lactobacillus, and lactic acid produced by lactobacillus fermentation can inhibit bacteria related to bacterial vaginosis [45]. The recovery of vaginal microbiota is very important to prevent various vaginal infections and their recurrence. Therefore, for vaginal microbial imbalance, lactobacillus supplementation (oral or vaginal medication) can be beneficial. This study found that the decrease of lactic acid bacteria in GDM postpartum was more obvious than that in non-GDM, so it may be possible to give probiotic preparations to this group of people in advance in clinical work to prevent BV and bring about longer-term vaginal environment stability.

Although the results of this study did not find a significant difference in vaginal microbiota richness between GDM and non-GDM women, Lactobacillus was

significantly decreased in the GDM group with a postpartum vagina, which is a high-risk factor for BV, and it may be speculated that GDM women have a higher risk of BV after childbirth than non-GDM. During their subsequent pregnancy, Bifidobacterium and Lactobacilli can be appropriately supplemented to maintain the stability of the intestinal and vaginal microbiota and reduce the occurrence of adverse pregnancy outcomes.

We found many pathways enriched in the GDM group in the D42 phase through functional and pathway analysis, including multiple purine and pyrimidine nucleotide synthesis pathways. These pathways play essential roles in many cellular processes, such as DNA replication, transcription, cellular signaling, and energy metabolism [46], indicating that microbial biosynthesis in the GDM group was more active, and the microbial community metabolism in the GDM group was more abundant. This finding was consistent with our previous study and Chen et al.'s study, where pathways related to nucleotide biosynthesis were enriched in neonates born to GDM mothers [47, 48]. The possible reason was that the number of other conditional pathogens and pathogenic microorganisms increased after the abundance of Lactobacillus decreased in the GDM group. As a result, this can have significant adverse effects on vaginal health, including disrupting the vaginal microecological balance, increasing the risk of vaginitis, and causing other gynecological diseases. However, we don't know what kind of flora these abundant metabolic activities come from and whether it has a positive or negative impact on vaginal health.

The underlying mechanisms of how GDM altered the vaginal microbiota remain unclear. Some researchers suggested it might be through changes in glucose levels or immune modulation in GDM women. Hyperglycemia associated with gestational diabetes can damage the function of neutrophils, and the isolated neutrophils show apparent activation and spontaneous production of neuronal extracellular traps (Nets), which damages the immune system and makes women more susceptible to infection [49, 50]. The immune system dysfunction of diabetic patients can be mediated by immune cell migration damage, phagocytosis, intracellular killing, and chemotaxis, which is related to poor perinatal outcomes and vaginal flora disorder [51]. Howsoever, the mechanism linking GDM and the vaginal microbiota is still not clear and is worthy of more in-depth studies.

Strengths and limitations

Our study was the first one to evaluate the influence of GDM on the dynamic changes of vaginal microbiota from antepartum to postpartum, with a prospective design and multiple time-point sampling, which add robustness to the findings. Another advantage of this study was that the essential characteristics of the subjects in the GDM and control groups were similar and comparable, reducing the influence of confounding factors.

One limitation of this study was the small sample size. A small sample size reduces the generalizability and statistical power, while an appropriate sample size enables microbiota research to discern the differences between groups and to save resources and time. However, sample size and power calculations remain challenging [52–54]. Since the standard deviation and difference between the GDM group and the normal group in vaginal microbiota alpha or beta metrics were not available in previous studies, it was difficult to calculate a sample size. However, given the sample size, standard deviation, and difference in the present study, we would therefore calculate a statistical power of more than 85%, with a level of statistical significance of 0.05. Moreover, the essential characteristics were comparable in the two groups, strengthening the statistical power. Secondly, because we ruled out all complications except GDM, our research group couldn't represent the GDM group well. The generalizability of our results is limited, and clinicians should be more cautious in applying our findings. Thirdly, the data on their offspring was not available. It was not clear whether changes in vaginal microbiota in GDM women cause differences in growth, development, and energy metabolism in their offspring. Fourthly, unmeasured confounding factors may also affect vaginal microbiota, resulting in significant differences in microbial structure and composition. For example, high-fat eating habits and the use of medications may lead to an imbalance in the vaginal microbiota by affecting the endocrine system and metabolism. Hygiene practices, such as vaginal washing, would influence the presence and concentrations of vaginal bacteria [55]. Strategies including restriction, randomization, matching, and statistical processes could be used to control these confounding factors better in future studies. Another area for improvement was the need for the species identification of Lactobacillus and opportunistic pathogens since 16S rRNA sequencing could not identify species-level changes. Moreover, 16S rRNA sequencing is impossible to quantify species, targets only bacteria and archaea, and cannot directly interpret the functional data, thus qPCR should be added to make future studies more complete and convincing.

Conclusion

Compared with normoglycaemic women, GDM influenced the changing pattern of vaginal microbiota structure from antepartum to postpartum and was associated with a more serious disrupted vaginal microbiota composition. Women with GDM show more severe vaginal microbiota imbalance after delivery, which would

possibly result in BV. Our findings provide additional evidence for regulating the vaginal microbiota during pregnancy and postpartum to reduce adverse pregnancy outcomes and achieve long-term vaginal health outcomes.

Acknowledgements
We thank all women for their participation.

Authors' contributions
YL drafted and critically revised the manuscript. YL, TL, and XZ were involved in data collection, analysis, and interpretation. BX, QS, and MX performed the experiments and statistical analysis and interpreted the data. QS and YZ designed the study, provided overall guidance, and obtained funding. All authors were involved in the critical revision and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China under Grant 82204050; Shenzhen Science and Technology Program under Grant JCYJ20230807145901003; Shenzhen Key Medical Discipline Construction Fund under Grant SZXK028; and Research Foundation of Shenzhen Baoan Women's and Children's Hospital, Jinan University under Grant BAFY 2021001.

Data availability
The data supporting this study's findings have been deposited into the CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0003769.https://www.cngb.org/.

Declarations

Ethics approval and consent to participate
This study received ethical approval from the Ethical Committees of Shenzhen Baoan Women's and Children's Hospital (approval number: LLSC 2020–09-02-KS), and all participants signed written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 25 August 2023 Accepted: 3 March 2025
Published online: 15 March 2025

References

1. Di Cianni G, Lencioni C, Volpe L, Ghio A, Cuccuru I, Pellegrini G, et al. C-reactive protein and metabolic syndrome in women with previous gestational diabetes. Diabetes Metab Res Rev. 2007;23(2):135–40.
2. Charach R, Wolak T, Shoham-Vardi I, Sergienko R, Sheiner E. Can slight glucose intolerance during pregnancy predict future maternal atherosclerotic morbidity? Diabet Med. 2016;33(7):920–5.
3. Zhang X, Liao Q, Wang F, Li D. Association of gestational diabetes mellitus and abnormal vaginal flora with adverse pregnancy outcomes. Medicine (Baltimore). 2018;97(34):e11891.
4. Lee A, Hiscock R, Wein P, Walker S, Permezel M. Gestational diabetes mellitus: clinical predictors and long-term risk of developing type 2 diabetes: a retrospective cohort study using survival analysis. Diabetes Care. 2007;30(4):878–83.
5. Wang J, Zheng J, Shi W, Du N, Xu X, Zhang Y, et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. Gut. 2018;67(9):1614–25.
6. Santos S, Voerman E, Amiano P, Barros H, Beilin L, Bergström A, et al. Impact of maternal body mass index and gestational weight gain on pregnancy complications: an individual participant data meta-analysis of European North American and Australian cohorts. BJOG. 2019;126(8):984–95.
7. Zhang T, Tian M, Zhang P, Du L, Ma X, Zhang Y, et al. Risk of adverse pregnancy outcomes in pregnant women with gestational diabetes mellitus by age: a multicentric cohort study in Hebei. China - Sci Rep. 2024;14(1):807.
8. MacIntyre D, Chandiramani M, Lee Y, Kindinger L, Smith A, Angelopoulos N, et al. The vaginal microbiome during pregnancy and the postpartum period in a European population. Sci Rep. 2015;5:8988.
9. Pacha-Herrera D, Vasco G, Cruz-Betancourt C, Galarza J, Barragán V, Machado A. Vaginal Microbiota Evaluation and Lactobacilli Quantification by qPCR in Pregnant and Non-pregnant Women: A Pilot Study. Front Cell Infect Microbiol. 2020;10:303.
10. Pacha-Herrera D, Erazo-Garcia M, Cueva D, Orellana M, Borja-Serrano P, Arboleda C, et al. Clustering Analysis of the Multi-Microbial Consortium by Lactobacillus Species Against Vaginal Dysbiosis Among Ecuadorian Women. Front Cell Infect Microbiol. 2022;12:863208.
11. Metzger B, Gabbe S, Persson B, Buchanan T, Catalano P, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676–82.
12. Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. Diabetes Res Clin Pract. 2022;183:109050.
13. Bolyen E, Rideout J, Dillon M, Bokulich N, Abnet C, Al-Ghalith G, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7.
14. R Core Team. R: A language and environment for statistical computing: R foundation for statistical computing Vienna, Austria, 2013.
15. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
16. Douglas G, Maffei V, Zaneveld J, Yurgel S, Brown J, Taylor C, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38(6):685–8.
17. Parks, Beiko. STAMP: statistical analysis of metagenomic profiles. Encyclopedia of metagenomics 2013.
18. Elizabeth C, Daniel D, Anna R, Laura S, Ronald W, Gary S, et al. Abrupt perturbation and delayed recovery of the vaginal ecosystem following childbirth. Nat Commun. 2023;14(1):4141.
19. Miller L, Patton D, Meier A, Thwin S, Hooton T, Eschenbach D. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. Obstet Gynecol. 2000;96(3):431–9.
20. Boskey E, Cone R, Whaley K, Moench T. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Hum Reprod. 2001;16(9):1809–13.
21. Ravel J, Gajer P, Abdo Z, Schneider G, Koenig S, McCulle S, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108 Suppl 1(Suppl 1):4680–7.
22. Romero R, Hassan S, Gajer P, Tarca A, Fadrosh D, Nikita L, 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(1):4.
23. Aagaard K, Riehle K, Ma J, Segata N, Mistretta T, Coarfa C, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One. 2012;7(6):e36466.
24. Cone. Vaginal microbiota and sexually transmitted infections that may influence transmission of cell-associated HIV. J Infect Dis. 2014 ;210 Suppl 3(Suppl 3):S616–21.
25. Lebeer S, Vanderleyden J, De Keersmaecker S. Genes and molecules of lactobacilli supporting probiotic action. Microbiol Mol Biol Rev. 2008 ;72(4):728–64, Table of Contents. 2008.
26. Hajfarajollah H, Eslami P, Mokhtarani B, Akbari Noghabi K. Biosurfactants from probiotic bacteria: A review. Biotechnol Appl Biochem. 2018;65(6):768–83.
27. Chee W, Chew S, Than L. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. Microb Cell Fact. 2020;19(1):203.
28. Srinivasan S, Hoffman N, Morgan M, Matsen F, Fiedler T, Hall R, et al. Bacterial communities in women with bacterial vaginosis: high resolution

phylogenetic analyses reveal relationships of microbiota to clinical criteria. PLoS One. 2012;7(6):e37818.
29. Rasmussen M, Thorsen J, Dominguez-Bello M, Blaser M, Mortensen M, Brejnrod A, et al. Ecological succession in the vaginal microbiota during pregnancy and birth. ISME J. 2020;14(9):2325–35.
30. PL, Hung K, Bergerat A, Mitchell C. Effect of Vaginal Lactobacillus Species on Escherichia coli Growth. Female Pelvic Med Reconstr Surg. 2020;26(2):146–151.
31. Onderdonk A, Delaney M, Fichorova R. The Human Microbiome during Bacterial Vaginosis. Clin Microbiol Rev. 2016;29(2):223–38.
32. Shipitsyna E, Roos A, Datcu R, Hallén A, Fredlund H, Jensen JS, et al. Composition of the vaginal microbiota in women of reproductive age–sensitive and specific molecular diagnosis of bacterial vaginosis is possible? PLoS One. 2013;8(4):e60670.
33. Zozaya-Hinchliffe M, Lillis R, Martin D, Ferris M. Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. J Clin Microbiol. 2010;48(5):1812–9.
34. Randis T, Ratner A. Gardnerella and Prevotella: Co-conspirators in the Pathogenesis of Bacterial Vaginosis. J Infect Dis. 2019;220(7):1085–8.
35. Pybus V, Onderdonk A. Evidence for a commensal, symbiotic relationship between Gardnerella vaginalis and Prevotella bivia involving ammonia: potential significance for bacterial vaginosis. J Infect Dis. 1997;175(2):406–13.
36. Nygren P, Fu R, Freeman M, Bougatsos C, Klebanoff M, Guise J. Evidence on the benefits and harms of screening and treating pregnant women who are asymptomatic for bacterial vaginosis: an update review for the U.S. Preventive Services Task Force. Ann Intern Med. 2008;148(3):220–33.
37. Muñoz-Barreno A, Cabezas-Mera F, Tejera E, Machado A. Comparative Effectiveness of Treatments for Bacterial Vaginosis: A Network Meta-Analysis. Antibiotics (Basel). 2021;10(8):978.
38. Mastromarino P, Macchia S, Meggiorini L, Trinchieri V, Mosca L, Perluigi M, et al. Effectiveness of Lactobacillus-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis. Clin Microbiol Infect. 2009;15(1):67–74.
39. Mastromarino P, Vitali B, Mosca L. - Bacterial vaginosis: a review on clinical trials with probiotics. - New Microbiol. 2013;36(3):229–38. Epub 2013 Jun 30.
40. Kuang Y, Lu J, Li S, Li J, Yuan M, He J, et al. Connections between the human gut microbiome and gestational diabetes mellitus. Gigascience. 2017;6(8):1–12.
41. Huang L, Thonusin C, Chattipakorn N, Chattipakorn S. Impacts of gut microbiota on gestational diabetes mellitus: a comprehensive review. Eur J Nutr. 2021;60(5):2343–60.
42. Mezzasalma V, Manfrini E, Ferri E, Boccarusso M, Di Gennaro P, Schiano I, et al. Orally administered multispecies probiotic formulations to prevent uro-genital infections: a randomized placebo-controlled pilot study. Arch Gynecol Obstet. 2017;295(1):163–72.
43. Bohbot J, Daraï E, Bretelle F, Brami G, Daniel C, Cardot J. Efficacy and safety of vaginally administered lyophilized Lactobacillus crispatus IP 174178 in the prevention of bacterial vaginosis recurrence. J Gynecol Obstet Hum Reprod. 2018;47:81–6.
44. Vicariotto F, Mogna L, Del Piano M. Effectiveness of the two microorganisms Lactobacillus fermentum LF15 and Lactobacillus plantarum LP01, formulated in slow-release vaginal tablets, in women affected by bacterial vaginosis: a pilot study. J Clin Gastroenterol. 2014;48 Suppl 1:S106–12.
45. Huang B, Fettweis J, Brooks J, Jefferson K, Buck G. The changing landscape of the vaginal microbiome. Clin Lab Med. 2014;34(4):747–61.
46. Patterson D, Bleskan J, Gardiner K, Bowersox J. Human phosphoribosylformylglycineamide amidotransferase (FGARAT): regional mapping, complete coding sequence, isolation of a functional genomic clone, and DNA sequence analysis. Gene. 1999;239(2):381–91.
47. Song Q, Xiao B, Huang H, Ma L, Zhang J, Zhu Y. Influences of gestational diabetes mellitus on the oral microbiota in offspring from birth to 1 month old. BMC Pregnancy Childbirth. 2022;22(1):289.
48. Chen T, Qin Y, Chen M, Zhang Y, Wang X, Dong T, et al. Gestational diabetes mellitus is associated with the neonatal gut microbiota and metabolome. BMC Med. 2021;19(1):120.
49. Stoikou M, Grimolizzi F, Giaglis S, Schäfer G, van Breda S, Hoesli I, et al. Gestational Diabetes Mellitus Is Associated with Altered Neutrophil Activity. Front Immunol. 2017;8:702.
50. Marschalek J, Farr A, Kiss H, Hagmann M, Göbl C, Trofaier M, et al. Risk of Vaginal Infections at Early Gestation in Patients with Diabetic Conditions during Pregnancy: A Retrospective Cohort Study. PLoS One. 2016;11(5):e0155182.
51. Yefet E, Bejerano A, Iskander R, Zilberman Kimhi T, Nachum Z. The Association between Gestational Diabetes Mellitus and Infections in Pregnancy-Systematic Review and Meta-Analysis. Microorganisms. 2023;11(8):1956.
52. Qian X, Chen T, Xu Y, Chen L, Sun F, Lu M, et al. A guide to human microbiome research: study design, sample collection, and bioinformatics analysis. Chin Med J (Engl). 2020;133(15):1844–55.
53. Debelius J, Song S, Vazquez-Baeza Y, Xu Z, Gonzalez A, Knight R. Tiny microbes, enormous impacts: what matters in gut microbiome studies? Genome Biol. 2016;17(1):217.
54. Kers J, Saccenti E. The Power of Microbiome Studies: Some Considerations on Which Alpha and Beta Metrics to Use and How to Report Results. Front Microbiol. 2022;12:796025.
55. Sabo M, Balkus J, Richardson B, Srinivasan S, Kimani J, Anzala O, et al. Association between vaginal washing and vaginal bacterial concentrations. PLoS One. 2019;14(1):e0210825.

Publisher's Note