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ORIGINAL ARTICLE

# Microbiome and its relation to gestational diabetes

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## Abstract

**Purpose** Gestational diabetes mellitus (GDM), the major endocrine pathology in pregnancy, has been associated with the development of an intense inflammatory process and increased insulin resistance. The maternal microbiota is involved in several metabolic functions; however, its role in GDM physiopathology remains unclear. The aim of this study was to assess the composition of the microbiota at different sites and evaluate its relationship with the occurrence of GDM.

**Methods** This cross-sectional study recruited women in the third trimester of gestation with and without GDM. Oral, vaginal, and stool samples were evaluated using next-generation sequencing. We included 68 participants: 26 with and 42 without GDM.

**Results** The analysis of the oral microbiome did not show significant differences in phyla and genus among the studied groups. In contrast, GDM patients presented a specific vaginal and intestinal microbiome composition, which was less diverse than those found in the control group, showing genera related to dysbiosis.

**Conclusions** Our findings suggest that changes in the composition of the vaginal and intestinal microbiome might be involved in the development of GDM. The follow-up of these patients in order to evaluate vaginal and intestinal samples after delivery may contribute to understanding the development of metabolic disease in women with previous GDM.

**Keywords** Intestinal microbiome · Vaginal microbiome · Oral microbiome · Pregnancy · Gestational diabetes

## Introduction

Gestational diabetes mellitus (GDM), the most frequent endocrine disorder of pregnancy, is defined as hyperglycemia first diagnosed during pregnancy which does not fulfill the criteria for overt diabetes [1]. In addition to immediate and long term maternal and fetal complications, GDM is associated with obesity [2].

Over the course of a normal pregnancy, women undergo diverse physiological changes, including an increase in insulin resistance (IR). In order to compensate for this physiological resistance, insulin secretion increases gradually during gestation [3]. However, some pregnant women have a limited capacity to increase insulin production and, consequently, develop GDM [3].

The composition of the microbiome also changes during pregnancy. It has recently been proposed that intestinal microflora and their metabolic activities (intestinal dysbiosis) may play a critical role in body weight control, energy homeostasis, fermentation, and absorption of non-digestible carbohydrate, and also in the development of IR; therefore, may also participate in the pathogenesis of several metabolic disorders, such as obesity, diabetes mellitus, and GDM [4, 5].

In addition to the gut microbiome, the composition of the microbial community in other body sites seems to also be involved in systemic health [6–9]. The oral microbiome seems to play an important role in obesity and diabetes, through the release of inflammatory mediators that may

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increase the IR, suggesting a link between pathogenic periodontal bacteria (such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) with glycemic control and risk of diabetes [7, 10, 11].

During pregnancy, there is a change in the structure of the vaginal bacterial community [8, 9], leading to the production of metabolites such as lactic acid, that helps to maintain low pH, which contribute to increasing the presence and stabilization of *Lactobacillus* in the vaginal microbiome [9, 12]. New data concerning the relationship between the vaginal microbiome and metabolic diseases, such as GDM, have been reported [13]. An increase of inflammatory cytokines expression been shown in GDM, as well as an increase in the abundance of potential pathogenic bacteria, characterizing a dysbiotic profile of vaginal the microbiome [13].

The complexity of the microbial community in the hosts body has been recognized, and the relationship among the microbiome at different sites of the body has been investigated [14]. Various investigators have shown that the gut/oral/vaginal microbiome influence both gestational development and metabolic disease. However, it is unclear whether there is an interaction between the microbiome of the different compartments and their role in GMD pathogenesis. Considering these observations, and the critical influence of GDM in mother and fetus health, the aim of this study was to evaluate the composition of the gut, vagina and oral microbiome of pregnant women with and without GDM.

## Materials and methods

### Subjects

This cross-sectional study recruited women in the third trimester (28–36 weeks) of pregnancy managed at the antenatal care clinics of the Obstetrics Department of the Universidade Federal de São Paulo (UNIFESP-EPM) between 2014 and 2016. The study was approved by the University's Ethic Committee and informed consent was obtained from all participants.

The inclusion criteria were: gestational age between 28–36 weeks at recruitment (based on menstrual dates confirmed through obstetric ultrasound), singleton pregnancy, and live fetus. The exclusion criteria at recruitment were: multiple pregnancy, fetal death, autoimmune diseases, pre-existing diabetes, uterine malformations, pregnancy resulting from in vitro fertilization, placental abruption, infections, cancer, or any other systemic disease, including pre-existing hypertension. All participants were followed until the end of their pregnancies.

The diagnosis of GDM was made according to the criteria proposed by the World Health Organization, that is fasting glucose of 92–125 mg/dL or a 1-hour post 75 g-load ≥ 180 mg/dL or a 2-hour post 75 g-load of 153–199 mg/dL. Those with fasting glucose values ≥ 126 mg/dl or 2 h post load ≥ 200 mg were diagnosed as having overt DM and excluded from the study. The control group included pregnant women with a normal 2-hour 75 g oral glucose tolerance test and without any obstetric disorder. Pregnant women diagnosed with GDM were followed up in a specific outpatient clinic for diabetic pregnant women, where they received nutritional counseling from a dietician and insulin, if necessary, to keep their fasting glucose < 90 mg/dL and 1-hour post-meal glucose < 130 mg/dL.

GDM patients were monitored up by a multidisciplinary team that included: a psychologist, a physical educator and nutritionists. After diagnosis, they were all attended by these professionals for evaluation, dietary, and physical activity guidance. GDM patients were given weekly medical consultations and evaluation by nutritionists and a physical educator to ensure adherence to the recommendations. After adequate glycemic control, the appointments were made every 2 or 3 weeks. All patients did at least 4 daily measurements of glucometry.

### Blood Samples

Upon enrollment, peripheral blood was collected from each participant after 12 h of fasting, in tubes containing ethylenediaminetetraacetic acid (EDTA) (BD Diagnostics, USA) for glycated hemoglobin analysis. The fasting glycemia were obtained from the patient charts and corresponded to the last fasting blood glucose analysis made before inclusion in the study (between 24 and 28 weeks of pregnancy).

### Collection of stool, vaginal, and oral samples

Each study participant collected one stool sample at home using a dry and sterile stool collector and a Styrofoam container (both previously supplied to the patient). After the collection, the participants were instructed to store the stool sample in the freezer. When delivered, the sample was sent to the laboratory and immediately stored at −80 °C until DNA extraction. The vaginal sample was collected from the vaginal cervix by the doctor who attended the patient on the same day that the stool sample was delivered. After collection, the secretion was placed in buffered medium (phosphate-buffered saline with 10% glycerol). The oral secretion sample was collected the same day by sterile swab. After collection, samples were stored immediately in the freezer at −80 °C.

## Microbiome sequencing

Bacterial DNA was obtained from fecal samples according to the manufacturer's instructions (QiaAmp DNA Stool Mini Kit - Qiagen). The vaginal and oral secretion samples were submitted to the DNA extraction technique using the QiAamp DNA Blood Mini Kit (Qiagen) for extraction of DNA from body fluids and blood. For all samples, the V4 region of 16S rRNA genes was amplified (25 cycles) as previously described [15]. The amplicons were pooled and loaded onto the Illumina MiSeq clamshell style cartridge kit v2 at 500 cycles, for paired-end 250 sequencing at the final concentration of 12 pM. The library was clustered to a density of ~80.000 k/mm2. The MiSeq platform provided the image analysis, base calling, and data quality assessment. The raw read files were demultiplexed and then analyzed using QIIME software [16]. This software was used to remove barcodes and primer sequences, extract chimeric artifacts, sequence alignment, distance matrix construction, picking Operational Taxonomic Units (OTUs), and phylogenetic tree construction, as well as calculating the diversity indices and hypothesis tests. We first removed the barcodes and primer and then filtered the sequences, discarding readings smaller than about 400 base pairs. Afterwards, chimeras were checked using usearch61 [17], and sequences identified as chimeric were excluded. The sequences of the remaining libraries were grouped into OTUs, based on 97% similarity, against Silva's database version 128 [18]. The relative abundance rate of the bacteria was performed in relation to the main phyla and genera found.

The alpha and beta diversity indices were calculated for each library, and their analysis refers to the variety and complexity of species in a community. To calculate the alpha diversity, we used the Chao1 [19] richness estimation and the Shannon and Simpson diversity indices [20, 21]. To observe the differences related to beta diversity between the groups, the principal coordinate analysis (PCoA) plot based on the weighted and unweighted Unifrac phylogenetic [22] distance matrices was constructed [23]. Nucleic acid sequences are available at the Sequence Read Archive (SRA) under accession number SRP126329.

## Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) and R Studio version 1.1.453 (R Core Team, 2008). The differences in clinical and baseline data between groups were calculated using the Chi-square test and Student's *t*-test; when the data did not present values within normality, they were converted into z-scores.

For differences in the microbiota composition, PERMANOVA was performed for each site using Adonis function in vegan [24] with weighted and unweighted UniFrac distances. For each variable, 999 permutations were made. Fisher's exact test was used to compare the presence/absence of each OTU between the groups, for the sites which presented statistical significance after PERMANOVA with unweighted UniFrac values.

A Mann–Whitney *U*-test was performed to compare the alpha diversity/richness and the differences in the relative abundance of the main (higher than 1% per group and per patient) bacterial phyla and genus between the groups.

*P*-values $< 0.01$ were considered statistically significant to control multiple comparisons in the relative abundance. A standard *P*-value $< 0.05$ was considered as significant for all other analyses.

# Results

## Clinical parameters

We included two groups of pregnant women in the third trimester of pregnancy: (a) 42 glucose tolerant ("Control" group) and (b) 26 with GDM ("GDM" group). There were no significant differences between groups in ethnicity and gestational age at sample collection. As expected, pregnant women with GDM were older and had a significantly higher pre-pregnancy body mass index (BMI) than women without GDM (Table 1). The prevalence of pre-pregnancy obesity (defined as a BMI ≥ 30) in the cases and controls was 47.83% ($n = 11$) and 21.43% ($n = 9$), respectively ($p =$

**Table 1** Main characteristics of study participants

| Variable | | Controls | GDM | *P* |
|---|---|---|---|---|
| Race | White | 16 (38.1%) | 12 (46.1%) | 0.79$^{b}$ |
| | Mixed$^{a}$ | 21 (50.0%) | 11 (42.3%) | |
| | Black | 5 (11.9%) | 3 (11.6%) | |
| Age (years) | Mean (SD) | 28.23 (5.68) | 35.07 (3.75) | 0.05$^{c}$ |
| Pre-pregnancy BMI (Kg/m$^2$) | Eutrophic | 19 (45.24%) | 4 (17.39%) | *0.04$^{b}$ |
| | Overweight | 14 (33.33%) | 8 (34.78%) | |
| | Obese | 9 (21.43%) | 11 (47.83%) | |
| Weight (Kg) gain during pregnancy | Mean (SD) | 8.79 (4.79) | 6.19 (6.30) | 0.10 $^{c}$ |
| Gestational age | Mean (SD) | 33.89 (1.97) | 32.45 (7.04) | 0.12$^{c,d}$ |
| Glycated hemoglobin (%) | Mean (SD) | 4.35 (2.16) | 5.65(0.35) | * < 0.001$^{c,d}$ |

*BMI* body mass index, *SD* standard deviation

**P*-value $< 0.05$

[a] Mixed race: Black and White ancestry

[b] $\chi^2$ test

[c] Student's *t*-test

[d] Values converted in z-score

0.04). However, according to binary logistic regression analysis, these differences did not affect the results. In addition, there was no difference in the weight gain during pregnancy between the groups (Table 1). As expected, glycated hemoglobin levels were significantly higher in the GDM group (Table 1). The blood glucose control was assessed by the analyses of glucometry data at every appointment. All women in the GDM group were being treated exclusively by diet and physical activity. Adherence to treatment was confirmed by the nutritionist and physical educator monitoring.

## Relative abundance of the fecal, vaginal, and oral microbiome

We evaluated the relative abundance rate of bacteria phyla and genera in both groups. We only assessed phyla and genera with an amount greater than or equal to 1% per patient and per group for all sample sites.

Considering the fecal samples, the two most abundant phyla found in both groups were Firmicutes and Bacteroidetes, followed by Proteobacteria. The Firmicutes phylum was more abundant in the GDM group (52.50% versus 43.00%), whereas the Bacteroidetes phylum was more abundant in the control group (43.20% versus 33.30%). However, these differences did not reach statistical significance. The main genera found in both groups were *Bacteroides*, *Ruminococcus*, *Prevotella*, and *Eubacterium*. The genus *Bacteroides* was higher in the control group in comparison to GDM patients (28.30% versus 20.20%, respectively), whereas *Ruminococcus, Eubacterium*, and *Prevotella* were higher in the GDM group (13.00% versus 11.90%, 5.90% versus 4.00% and 5.20% versus 5.00%, respectively). However, none of these differences were statistically significant ($p > 0.01$). Figure 1a shows a heatmap of the genera abundance in both groups.

Regarding the vaginal microbiome, the most abundant phyla found in both groups were Firmicutes, followed by Actinobacteria and Proteobacteria. The Firmicutes phylum was more abundant in the GDM group (91.14% versus 86.50%, respectively). The phylum Actinobacteria were more abundant in the control group (10.41% versus 2.99%, respectively), whereas the phylum Proteobacteria was more abundant in the GDM group (3.96% versus 0.75%). However, these differences did not reach statistical significance. Figure 1b shows a heatmap of the genera abundance of the vaginal microbiome in both groups. The main genus found in the vaginal microbiome of both groups was *Lactobacillus*, which was slightly increased in the control group (80.68% versus 78.66%). Some genera were more abundant only in control group, such as other *Bifidobacteriaceae* and *Atopobium*; whereas others were more abundant only in the GDM group, such as *Staphylococcus*, *Megasphaera*, *Shuttleworthia*, *Enterobacter*, and *Enterococcus*. However, these differences were not statically significant. On the other hand, we found that the control group presented a significantly higher abundance of the genera *Varibaculum*, *Prevotella*, *Porphyromonas*, and *Ezakiella* ($p < 0.01$), whereas the GDM presented a significantly higher abundance of the genera *Bacteroides*, *Veillonella*, *Klebsiella*, *Escherichia-Shigella*, *Enterococcus*, and *Enterobacter* ($p < 0.01$) (Fig. 2).

The analyses of the oral microbiome showed that the most abundant phyla found were Firmicutes and Proteobacteria, followed by Bacteroidetes, and the percentage of all bacterial phyla found in both groups was similar. In addition, the most abundant genera found in the groups were *Streptococcus*, *Veillonella*, and *Haemophilus*. There were no significant differences concerning phyla and genera between the groups. Figure 1c shows a heatmap of the genera abundance in both groups.

## Alpha and beta diversity between groups

In order to analyze the alpha diversity between groups, we used the Shannon diversity index, the Simpson dominance index and the Chao1 richness estimator. Table 2 shows the results of the comparison between groups in relation to the alpha diversity indices. Regarding the fecal samples, the GDM group presented higher values in all indices analyzed, but the result of the Chao1 richness index was the only one that presented a statistically significant result ($p < 0.01$). On the other hand, all results from the vaginal microbiota were statistically significant, with the vaginal microbiome from GDM showing lower diversity indices (Shannon and Simpson) and also lower richness than that detected in the controls ($p < 0.01$). The analysis of the alpha diversity of the oral microbiota showed that the GDM group presented higher means than the control group in all indices, but these differences did not reach statistical significance.

In order to detect differences in beta diversity, we constructed the PCoA based on the weighted (considering the number of species observed in the spatial distribution) and unweighted (considering the presence or absence of species in the spatial distribution) Unifrac distance matrices (Fig. 3). The main analysis coordinates are represented by axes 1 and 2. Each point between the axes represents a single participant. The PERMANOVA test was performed to compare the two study groups. We did not find a significant difference in the weighted analysis at all sites (fecal: $p = 0.82$, vaginal: $p = 0.20$, and oral: $p = 0.99$); however, in the unweighted analysis, we found a significant difference in relation to vaginal samples and a trend in relation to fecal samples (fecal: $p = 0.09$, vaginal: $p = 0.001$, and oral: $p = 0.53$). From the results found in the vaginal and fecal

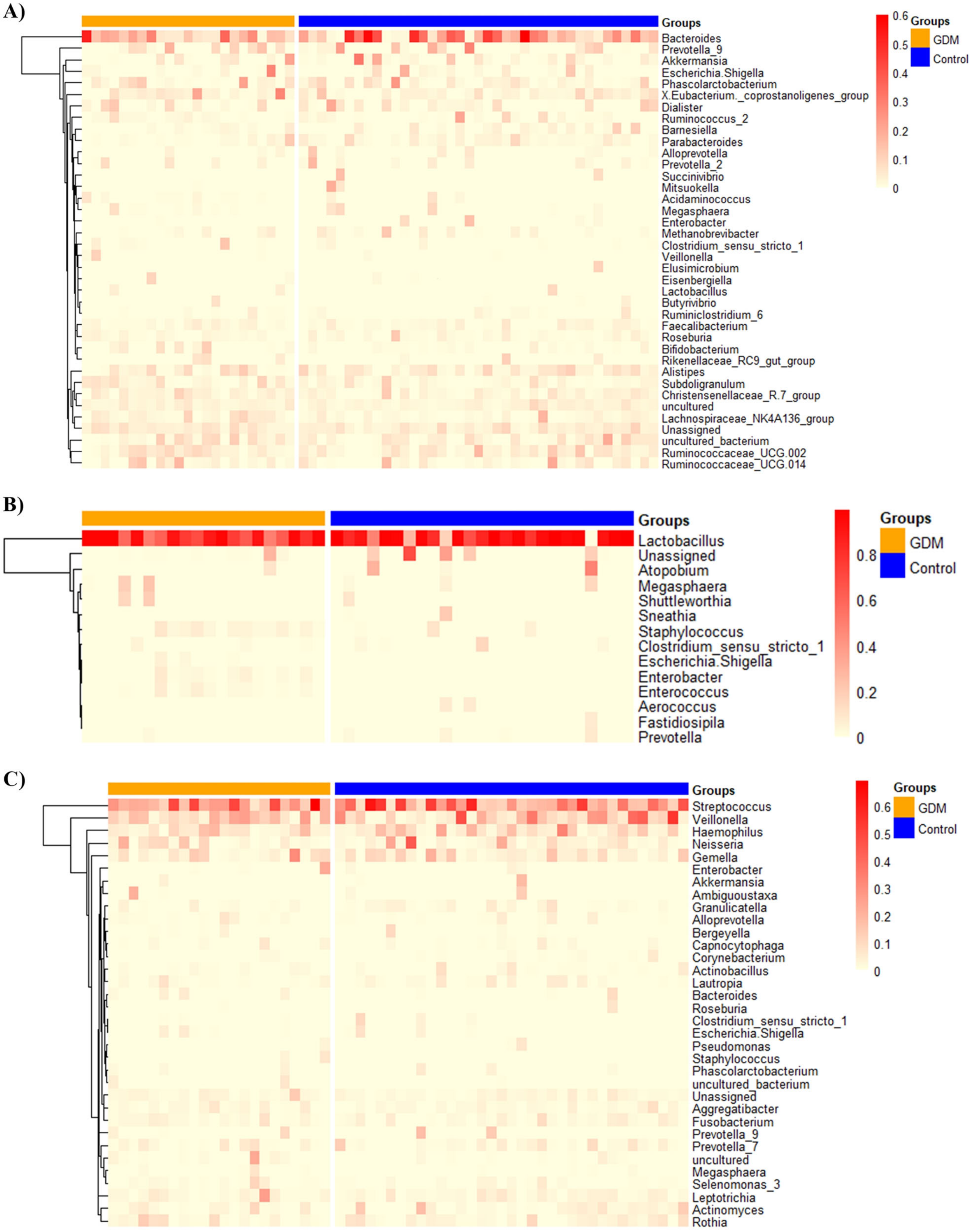

**Fig. 1** Heatmap of genera abundance of intestinal microbiome **a** vaginal microbiome **b**, and oral microbiome **c** in Control and GDM groups

microbiota in the unweighted Unifrac, we performed the statistical analysis using Fisher´s exact test to compare the presence or absence of OTUs in each group ($p < 0.01$).

Sixty-eight OTUs were found to have an increased intestinal microbiome in the GDM group, which was statistically significant when compared to that observed in controls and they were related to *Lachnospiraceae*, *Collinsella*, *Christensenellaceae*, *Dorea*, *Subdoligranulum*, and *Ruminococcus* genera, and only one *Eubacterium* OTU was found increased in the control group (Supplementary Table S1). Of the 29 vaginal OTUs that presented a statistically significant result, 23 were from the genus *Lactobacillus*, and the others were related to the *Varibaculum*, *Anaerococcus*, *Finegoldia*, *Peptoniphilus*, *Ezakiella*, and *Staphylococcus* genera, all of which had greater abundance in the control group (Supplementary Table S2).

## Discussion

The microbiome's role in various physiological processes involved in health and in the development of several diseases is not yet fully understood. Considering these observations, it has been suggested that the physiological adaptation of the microbial pattern, present in pregnancy, is altered in women with metabolic diseases, such as GDM, due to increased inflammation, IR, and weight gain in this population [13, 25].

Some studies have shown that the intestinal microbiome changes during pregnancy; however, their findings were not confirmed by other investigators [13, 26], possibly due to differences in sample size, gestational age at sample collection and in clinical parameters, making comparisons difficult. Fugmann et al. [27] evaluated the intestinal microbiome of non-pregnant women and found that those with a history of GDM had a smaller proportion of the Firmicutes phylum than the others that did not present the disease. Jost et al. [28] followed seven healthy pregnant women from the end of the third trimester until the postpartum period and reported that the phyla Firmicutes and Actinobacteria dominated the maternal microbiome. Wang et al. [14] showed lower Firmicutes phylum in oral microbiome of GDM women but did not find similar differences in the intestinal microbiome. In contrast, our results showed that GDM patients have a higher ratio of Firmicutes/Bacteroidetes when compared with healthy pregnancy patients. An imbalance in the Firmicutes/Bacteroidetes ratio has already been related to dysbiosis conditions [29, 30]. Crusell et al. [31] reported that bacteria from the phyla Firmicutes and Bacteroidetes were predominant both in GDM and in healthy pregnant women but did not evaluate and compare the ratio between these phyla among the participants.

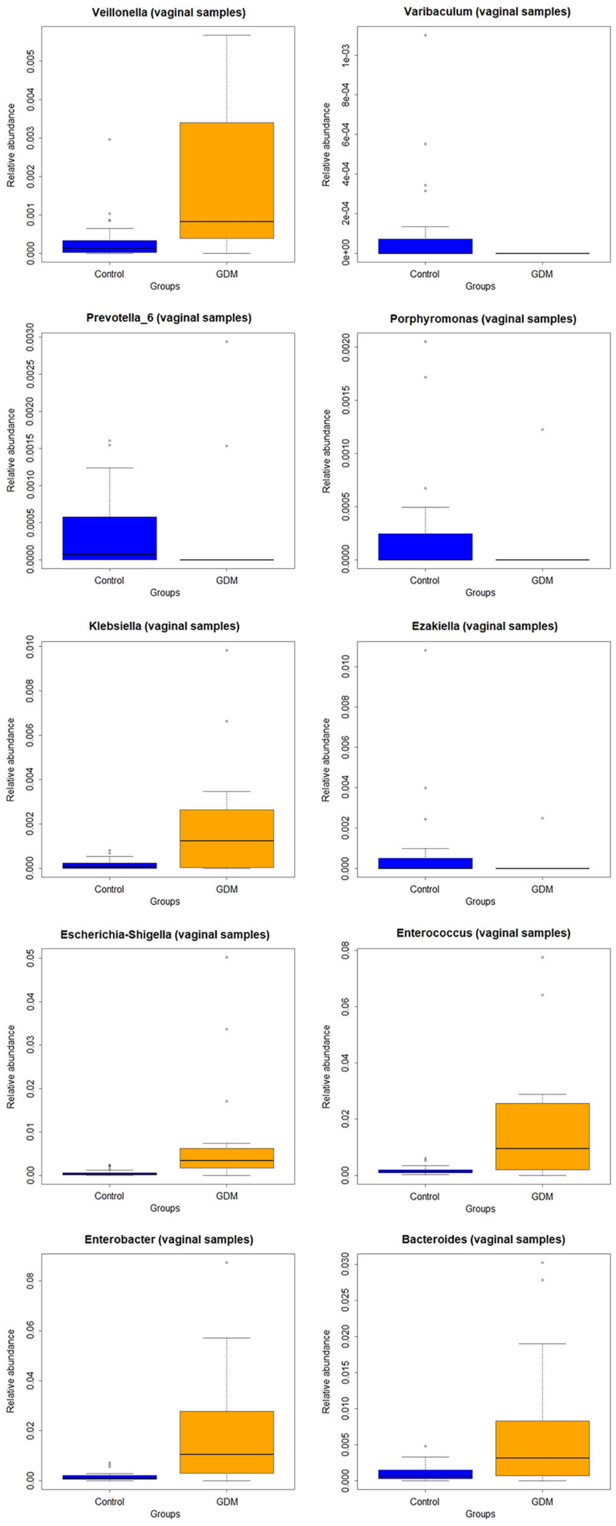

**Fig. 2** Genera in vaginal samples with significant difference between groups

Our analyses showed that healthy pregnant women presented a higher abundance of *Bacteroides, Parabacteroides, Roseburia*, *Dialister*, and *Akkermansia* genera in

**Table 2** Alpha diversity indexes between groups

| Alpha diversity | | Control<br>Range<br>mean (SD) | GDM<br>Range<br>mean (SD) | *P*-value |
|---|---|---|---|---|
| Fecal samples | Chao1 | 1228–5244.07<br>2916.38 (789.62) | 1045.84–4895.87<br>3458.51 (902.69) | * < 0.01[a] |
| | Simpson | 0.92–0.99<br>0.96 (0.02) | 0.92–0.99<br>0.97 (0.02) | 0.27[a] |
| | Shannon | 4.03–5.34<br>4.74 (0.36) | 4.11–5.62<br>4.87 (0.36) | 0.13[a] |
| Vaginal samples | Chao1 | 364.12–5547.02<br>1827.10 (1247.00) | 145.00–3386.95<br>899.54 (721.02) | * < 0.01[a] |
| | Simpson | 0.34–0.82<br>0.59 (0.12) | 0.31–0.86<br>0.70 (0.16) | * < 0.01[a] |
| | Shannon | 1.36–3.01<br>2.14 (0.47) | 1.01–3.80<br>2.59 (0.67) | * < 0.01[a] |
| Oral samples | Chao1 | 68.33–3308.00<br>1538.43 (697.10) | 32.20–3497.62<br>1694.34 (744.43) | 0.30[a] |
| | Simpson | 0.77–0.98<br>0.92 (0.05) | 0.80–0.98<br>0.93 (0.05) | 0.24[a] |
| | Shannon | 2.71–4.99<br>4.00 (0.59) | 2.81–5.01<br>4.13 (0.53) | 0.39[a] |

*SD* standard deviation

[a] Mann–Whitney *U*-Test

[*] *P*-value < .01

comparison to GDM patients. Collado et al. [32] also found a significant increase of the genus *Bacteroides* among healthy women in the third trimester of pregnancy. Santacruz et al. [33] reported similar findings in healthy women during the second trimester of pregnancy. Another genus that we found in abundance in fecal samples of the controls was *Akkermansia*. Recent studies described this as an important eubiotic genera, with systemic beneficial effects to the host [34, 35], including the control of metabolic syndromes [36, 37].

We observed that pregnant women with GDM have increased *Lachnospiraceae*, *Phascolarctobacterium*, and *Christensenellaceae*, which have been previously described in dysbiotic conditions such as GDM [31] and obesity [38–40]. However, a higher abundance of *Eubacterium* and *Ruminococcus* was observed in the GDM group, which has been considered a sign of homeostasis in the intestinal microbiome.

There were no significant differences in genera detected in the intestinal microbiome between our groups, which may be due to the small sample size enrolled in this study. However, despite the presence of certain beneficial genera being shown, we observed a tendency toward a higher abundance of certain pathogenic genera in GDM patients, suggesting that this is a dysbiotic condition.

The oral microbiome has been associated with health and diseases in the host. A number of studies have shown that an altered oral microbiome may lead to periodontal complications [41, 42]. We didn't find any difference in the oral microbiome between groups, suggesting that there is no relationship between the development of GDM and the oral microbiome, nor a relationship between the bacteria profile and the glucose level. In contrast, Wang et al. [14] evaluated the oral, gut, and vaginal microbiome of GDM patients and found that when comparing the three sites, the largest changes on the phyla level was observed in oral samples. In addition, these investigators showed a positive correlation between the abundance of *Neisseria/Leptotrichia* in the oral microbiome of pregnant women and glucose levels, fasting or OGTT. Acuna et al. [43] found differences in the microbiome composition of the three compartments (oral, vaginal, and intestinal) in a small group of GDM patients in comparison to healthy pregnant women. However, the authors did not describe the genera profile observed in each compartment, but only the numeric differences that were observed.

We observed interesting results concerning the composition of the vaginal microbiome. There was an increased in the abundance of *Lactobacillus* and *Atopobium* in the healthy pregnant women and, on the other hand, an increase of certain dysbiotic genera such as *Shuttleworthia*, *Enterobacter*, and *Enterococcus* in the GDM group. However, the significant differences in the vaginal microbiome composition between groups were defined by the presence of rare genera in the GDM group, as seen in the unweighted PCoA results. Notably, the GDM group had a significant

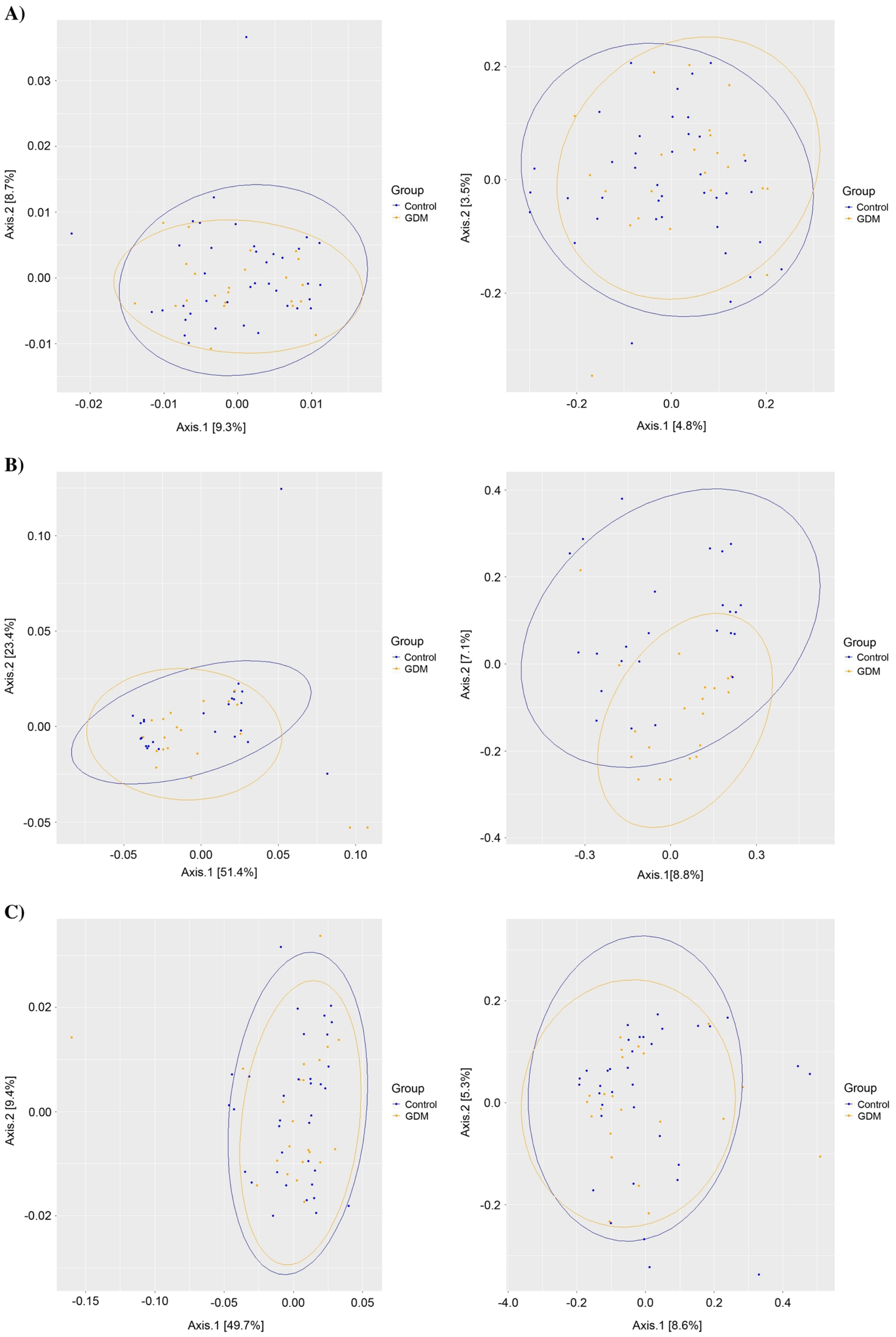

**Fig. 3** PCoA weighted (left) and unweighted (right) of the intestinal **a** vaginal **b**, and oral **c** samples based on Unifrac distance matrices

increase of *Veillonella*, *Klebsiella*, *Enterobacter*, *Enterococcus,* and *Escherichia* in vaginal samples, all of them associated with a dysbiotic microbiome [44]. Wang et al. [14] also found differences in the vaginal microbiome composition; however, the prevalent genera were not the same ones as we observed.

In this study, all GDM patients were controlled with diet alone, whereas other recent studies on this topic did not report if the patients were submitted to insulin treatment or not [14, 31]. These differences may explain some of the observed controversies. As it was previously proposed, energy intake may influence the microbial richness [45], so the diet may account for significant differences in microbiome composition, and we might find different results if insulin treated patients or those with a different diet were included. Another issue that may explain the divergent results are the selection criteria. Crussell et al. [31] selected overweight participants, and only recruited patients that presented high risk factors for GDM, including women with polycystic ovary syndrome and with higher blood pressure than the controls. In the present study, although the percentage of obese participants, defined by using the pre-pregnancy BMI, was higher in the GDM group than in the controls, this difference did not affect the results. Moreover, there was no difference in pregnancy weight gain between the groups. Wang et al. [14] analyzed samples collected in other gestational ages, one or two days before delivery, whereas in this study we collected samples at 28–36 weeks of pregnancy.

The results presented here are a semi-quantitative analysis of women's microbiomes at different sites during pregnancy, showing a tendency toward dysbiosis in the GDM condition, characterized by the presence of certain pathogenic genera and decreased diversity. The interaction between the microbiomes of different sites of the organism is still under investigation [14, 26]. There are several lines of evidence supporting that almost 50% of the women that have had GDM develop type 2 DM later in life [2]. Crussell et al. [31] reported that the intestinal microbiome is dysbiotic 8 months after delivery in patients that presented GDM. On the other hand, Hasan et al. [46] evaluated the intestinal microbiome of a group of patients who had GDM and another group who did not have GDM, five years after the delivery. There were no differences in the microbiome composition between groups. Therefore, the authors suggested that the microbiome does not allow the identification of the patient at high risk of developing GDM. We may not discard this, but in order to provide confirmation it will be necessary to develop a cohort study including a significant and well characterized number of patients.

Besides the sample size, a limitation of our study was that we only included patients that seem to present a mild form of the disease. However, we assessed the microbiome of different sites of healthy and GDM pregnant women and found significant differences in the vaginal microbiome composition, and a tendency toward dysbiosis in the intestinal microbiome of the GDM group. There was a large variation of the microbial populations within each group, which reinforces the concept of inter-individuality of the microbial pattern. These results are important for an overall understanding of the human microbiome in health and disease, especially in pregnancy and GDM.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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