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Is there an association between the vaginal microbiome and first trimester miscarriage? A prospective observational study

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Is there an association between the vaginal microbiome and first trimester miscarriage? A prospective observational study

Abstract

Aim. To examine whether there are differences in the vaginal microbiome of women who miscarry compared to those who have normal pregnancy outcomes.

Methods. Prospective observational study conducted at the Canberra Hospital, Australia with 24 participant women in the first trimester of pregnancy. The vaginal microbiomes of the 24 women were characterised using sequencing analysis of the V4 region of the 16S rRNA gene employing an Illumina MiSeq instrument with QIAGEN reagents. Vaginal microbiome data were correlated with pregnancy clinical metadata.

Results. Ordination plots showed differences in the composition of microbiomes of women who miscarried and controls. In nulliparous women, Lactobacillus crispatus was the dominant bacterium in 50% of women. Lactobacillus iners was the dominant bacterium in 50% of women with a history of prior miscarriage and a miscarriage in the study compared to 15% (p=0.011) in those with no history of miscarriage and no miscarriage in the study. There were significant differences in the number of OTU and the richness of the microbiomes of women who miscarried compared to those who delivered at term. Eight taxa were found in different relative abundances in both groups of women.

Conclusions. The study indicated that the composition of the vaginal microbiome varies with pregnancy history. Also, there was a significant difference in the vaginal microbiomes between women who suffered miscarriage and those who continued to term delivery both in the overall microbiome populations and in the abundances of individual taxa.

Keywords. Bacterial diversity, early pregnancy complications, miscarriage, vaginal microbiome.
Introduction

Miscarriage, or spontaneous pregnancy loss, is the unplanned and unexpected loss of an established intra-uterine pregnancy before the fetus reaches 20 weeks of gestation\textsuperscript{1,2}. It is the most frequent complication of pregnancy, occurring in up to 50\% of pregnant women\textsuperscript{3}. It is a clinically recognised event in 10\% of pregnancies\textsuperscript{3,4}. Although miscarriage is not usually associated with severe physical maternal morbidity and mortality, frequently it is coupled with psychological and social distress (Bottomley & Bourne 2009). For many couples, miscarriage causes elevated levels of anxiety and depression, and periods of grief similar to that of a loss of a close relative\textsuperscript{1}.

Miscarriage is sub-classified as early, before 12 weeks of gestation, or late, after 12 weeks of gestation\textsuperscript{1}. Recognised causes of early miscarriage include chromosomal abnormalities such as trisomies, triploidies, or monosomy X, as well as implantation abnormalities, teratogenic drugs, antiphospholipid syndrome, maternal uterine anatomical abnormalities, infections and maternal medical conditions\textsuperscript{5}. Recognised aetiological factors of late miscarriage include the presence of pathogenic microbes, cervical weakness, thrombophilia and structural uterine abnormalities. Chromosomal and genetic abnormalities can cause late miscarriage; however, they are abnormalities also seen in term pregnancy, e.g. trisomy 21, or involve gene deletions or mutations\textsuperscript{1}. Management of women who experience recurrent miscarriage is directed towards identified underlying causes when they have been determined. Nonetheless, a reliable treatment or prevention therapy for miscarriage does not exist and not all causes of miscarriage can be identified.

The community structures of the vaginal microbiomes of non-pregnant and of pregnant healthy women from various racial backgrounds have been classified in five community state types (CST)\textsuperscript{6,7}. The species with higher relative abundance in CST I is \textit{L. crispatus}, and \textit{L. iners} is associated with CST III. The species with more relative abundances in CST II and CST V are \textit{L. gasseri} and \textit{L. jensenii}, respectively. Community type IV is characterised by low abundance of lactobacilli and higher abundance of anaerobic bacteria of several genera including \textit{Atopobium}, \textit{Dialister}, \textit{Gardnerella}, \textit{Prevotella} and others\textsuperscript{8}. The healthy vaginal microbiome of Aboriginal Australian, African American, Asian, Caucasian and Hispanic American pregnant women is usually dominated by one or two of four \textit{Lactobacillus} taxa: \textit{L. crispatus}, \textit{L. iners}, \textit{L. gasseri}, \textit{L. jensenii}\textsuperscript{7,12}. Lactobacilli in the vagina make lactic acid that lowers vaginal pH, reducing the risk of colonisation by pathogenic organisms\textsuperscript{13}. Disruption of the vaginal microbiome is associated with a number of pregnancy complications including miscarriage, tubal factor infertility, spontaneous preterm labour and chorioamnionitis\textsuperscript{14-17}.

The vaginal microbiome may affect the risk of miscarriage in various ways. For example, reduced vaginal \textit{Lactobacillus} spp. abundance and increased diversity and richness of bacterial community composition are associated with miscarriage in the first trimester\textsuperscript{18}. Infection of human endometrial stromal cells by \textit{Chlamydia trachomatis} induces defective decidualisation and chemokine release that
can cause miscarriage\textsuperscript{19}. Group B \textit{Streptococcus} vaginal infection that leads to exfoliation and subsequent bacterial ascension is associated with increased rates of miscarriage\textsuperscript{20}. In a group of recurrent miscarriage patients, \textit{Atopobium}, \textit{Prevotella} and \textit{Streptococcus} were significantly more abundant than in the control group, and \textit{Ureaplasma} was found at significant high abundances in women with a history of miscarriage\textsuperscript{21,22}.

The use of culture-independent methods has allowed a better identification of the vaginal microbiome to the taxum level. Techniques that extract bacterial DNA, amplify it with polymerase chain reaction (PCR) and target gene identification, commonly using the bacterial 16S rRNA gene have allowed microbiomes to be studied with greater sensitivity and accuracy than traditional culture-based techniques\textsuperscript{23}. Furthermore, vaginal swabs can be collected, stored and analysed in batches in similar manners to previously used methods\textsuperscript{24}.

The microbial richness and diversity of bacterial populations are reduced at the beginning of pregnancy. The composition of the microbial community of the vagina remains stable during normal pregnancy with an increase of the microbial diversity before birth of a healthy infant at term\textsuperscript{10,25}. The diversity of the vaginal microbiome of pregnant women in the first trimester is influenced by personal pregnancy history. A study by Nasioudis et al (2017) recruited 155 women from varied racial backgrounds, with Caucasian (57\%), mixed race (25\%), Asian (12\%), Hispanic (5\%) and Black (1\%). In 52 nulliparous participants, \textit{L. crispatus} was the dominant bacterium in 76.4\% of women, but in 77 multiparous participants this taxon was the most abundant one in only 22.1\% of women, whereas in 26 participants whose pregnancy did not reach term, it was the most abundant taxon in 50\% of women\textsuperscript{26}. \textit{L. iners} was the dominant bacterium in 9.5\% of women with no history of miscarriage, and had relative abundances of 26.9\% and 45.4\% in women who experienced one or two prior miscarriages, respectively\textsuperscript{26}. The relative abundances of \textit{L. crispatus} and \textit{L. iners} were similar between Caucasian and non-Caucasian subjects\textsuperscript{26}. This interesting observation requires further exploration, firstly to validate the observation, and secondly to understand it better.

The aim of this study was to compare the vaginal bacterial communities in women who miscarried and in women with healthy pregnancies, to validate previous observations of the roles of \textit{L. crispatus} and \textit{L. iners}, and to document any differences in the dominant bacteria in both groups of women.

\textbf{Methods}

\textbf{Participant recruitment and ethics approval}
A prospective observational study was performed with women recruited between October 2018 and April 2019 from an Early Pregnancy Clinic and a private clinic in Canberra, Australia. Women attending the clinics had higher risk of miscarriage or adverse pregnancy outcomes than average. Inclusion criteria were pregnant women in the first trimester, not experiencing an ectopic pregnancy. Exclusion criteria from participation were pregnancy of more than 14 weeks of gestation, ectopic pregnancy, or inability to give informed consent. Patients were considered to have had a miscarriage if the pregnancy spontaneously ended prior to 20 weeks; participant women delivering at term served as controls. The study was approved by the ACT Health Human Research Ethics Committee (HREC# 2018/ETH00172) and the Australian National University Ethics Committee (HREC# 2018/649). Participants provided written informed consent.

**Baseline data**

Baseline demographic and clinical data were collected using questionnaires that requested information including age, racial background, gravidity, parity, gestational age at birth, infection during pregnancy, antibiotics use (they were administered on grounds of clinical information), fever, bleeding, pelvic pain, and outcomes of previous pregnancies. Data were requested also on smoking, alcohol consumption and nutritional supplements. To gather as complete as possible details, clinical files also were consulted.

**DNA extraction, amplification and sequencing**

Two vaginal swabs were sequentially collected from each participant under sterile conditions by a qualified practitioner. Previous investigations have reported that during pregnancy bacterial communities are stable in the introitus, midpoint and posterior fornix of the vagina27,28. Samples were acquired from the vaginal midpoint (3 cm from introitus) using a sterile plastic swab with a Dacron tip. Swabs were de-identified and stored at 4 °C until further processed.

DNA extraction and purification were performed in a single batch using the QIAamp DNA Mini Kit (QIAGEN, Chadstone Centre, VIC, Australia); the concentration and quality of the DNA was assessed using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies; Wilmington, DE, USA). The composition of the microbial communities was determined at the Ramaciotti Centre for Genomics (University of NSW, Sydney, Australia) by high-throughput sequencing of the V4 region of the 16S rRNA gene employing the Earth Microbiome primers (515F-806R) and an Illumina MiSeq instrument (2 x 250 bp chemistry). The Ramaciotti Centre is an institution with a quality management system according to the ISO/IEC17025:2017 Standard, and it follows recommended best practice protocols29. All de-identified samples were assigned a code number and analysed individually keeping track of their origin through the coding system.
Sequence raw reads were quality checked, trimmed and aligned against the SILVA reference database using standard operating procedures implemented in Mothur v1.37.3. DNA extraction procedures and microbiome analyses have been previously employed and validated.

The two vaginal samples were analysed independently and served as an intrasubject control using hierarchical clustering. There was a 99.99% similarity across all samples with the exception of one set of samples which was excluded from subsequent analysis. Duplicates of the four reagents in the DNA extraction kit were included randomly in the sequencing as negative controls. All operational taxonomic units (OTU) with an abundance >1% in the negative controls were deleted for the analyses. Samples were subsampled, leading to a total number of 1688 OTU.

Analyses of vaginal microbiomes

De-identified demographic data were analysed using Statistical Package for the Social Sciences (SPSS), Version 22. One-way Analyses of Variance (ANOVA) were performed to determine differences in the microbiomes of miscarriage and non-miscarriage participants. Logistic regression modelling was conducted to control for potential confounding variables, such as previous preterm birth history, maternal age and drug and alcohol use during pregnancy.

Analyses of the sequence data yielded the number of reads (abundance) and identification of the bacterial taxa (diversity) for each participant. Taxon abundance was determined from sequence counts and expressed in percent. Vaginal taxa sequence data were analysed to determine species diversity and richness, and Shannon’s Diversity Index for each participant and the entire cohort. To evaluate taxa beta-diversity in these participants, multivariate analyses including Distance Linear Model (distLM) analyses following square-root transformation and Bray-Curtis similarity resemblance, non-metric multidimensional scaling (nMDS) and PERMDISP were performed on relative abundances using Primer-E v7.

Probability tests were used to combine the clinical metadata and sequencing data to generate p-values of the association of microbial taxa with vaginal infection. Multivariate Association with Linear Models analyses (MaAsLIN) were conducted to evaluate associations between clinical metadata and microbial community abundance.

Results

Of 24 women recruited in the study, one was excluded owing to late diagnosis of ectopic pregnancy, and a second one over concerns about sample reliability. This left data on 22 women, of whom 8 (36%) had a miscarriage and 14 (64%) were healthy pregnancy controls. Mean gestation of sample collection in the control group and miscarriage groups were 58 days and 62 days, respectively (p = 0.10). The
study population was similar to that of the background Australian population in terms of age and race although with a higher percentage of nulliparous women.

**Clinical outcomes**

The characteristics of women who miscarried compared to controls are summarised in Table 1. Mean maternal age and gestational age at recruitment were similar between groups. In miscarriage and control groups mean maternal age was 28 and 27 years, respectively, and mean age at recruitment was 62 and 58 days, respectively (both p>0.05). None of the women who miscarried reported smoking or drinking alcohol in pregnancy compared to 7% of controls. Prior miscarriage was common with 100% of women who miscarried in this pregnancy, and 7% controls reporting a prior miscarriage. Including the index pregnancy, the mean number of miscarriages for the cohort was 0.8 (0-2); all women with greater than 3 miscarriages were formally investigated with no cause identified. Interestingly, women who miscarried were less likely to have pelvic pain, fevers and vaginal infections during pregnancy than controls.

**Percentage dominance of L. iners in women who miscarried and controls**

In the study cohort, 12 women were nulliparous; and L. crispatus was the dominant bacterium in 50% (6/12) in these women, the remaining 6 cases were equally spread between L. gasseri (CST II), L. iners (CST III), and L. jensenii (CST V). In contrast, in multiparous women the most abundant bacterial taxa were distributed between L. crispatus (CST I; 10%), L. iners (CST III; 30%), L. gasseri (CST II; 20%), mixed taxa (CST IV; 20%) and L. jensenii (CST V: 20%). The relative abundances of the four *Lactobacillus* species in the microbiome of each participant are shown in Figure 1.

Thirteen participants had no prior miscarriage history and no miscarriage in the study. One woman had a prior miscarriage but no miscarriage in the study. The remaining 8 women had both a history of prior miscarriage, varying from one to 4 miscarriages, and experienced miscarriage in the study. In women with a miscarriage in the study, L. iners was the dominant bacterium in 50% (4/8) of cases. In contrast, it was the dominant bacterium in only 15% (2/13) of women with no history of miscarriage and no miscarriage in the study. The mean relative vaginal abundance of L. crispatus and L. iners in women who delivered at term were 22.1 and 18.6, respectively; in women who suffered a miscarriage the mean relative abundances were 13.7 and 18.6, respectively.

The most abundant taxa in women who delivered at term or experienced a miscarriage are shown in Table 2.

**Alpha and Beta diversity**

Alpha diversity of a sample refers to the number of different taxa (richness, d), or the relative abundances of different taxa (evenness, J’), or the number of taxa and the inequality between their
abundances [Shannon index, $H'(\log_e)$] (Scholtz, 2020). The alpha diversity of the vaginal microbiomes of women who miscarried and controls who did not, as well as their comparisons are shown in (Table 3).

Beta diversity measures the difference in microbial composition between samples in various environments (Scholtz, 2020). To evaluate beta-diversity, non-metric multidimensional scaling (nMDS) analyses were performed using a Bray-Curtis similarity resemblance matrix of square-root transformed relative abundances using Primer-E v7. Global analyses show that the microbiomes were dominated by Lactobacillus spp. (Table 2) and with no overall differences in beta diversity associated with miscarriage due to the prominence of lactobacillus. The total number of OTU and sample richness were significantly different between both groups of women but not the other measures of diversity (Table 3).

The composition of microbiomes of women who miscarried and controls were different in 2D nMDS plots (Figure 2), and this was confirmed in 3D nMDS plots (data not shown). The quality of these ordination plots was confirmed by the low stress values calculated: 2D stress = 0.18, 3D stress = 0.12. Although PERMDISP did not yield a significant difference ($p = 0.06$), the result suggested a trend in the dispersion of the microbiomes of both groups of women.

**Box plot analyses**

Box plot analyses were performed to delineate these differences further. A comparison was performed of the abundances of the 10 most abundant OTU in women who miscarried with those of controls. There were no significant differences in the four most common OTU dominated by Lactobacillus spp., but there were significant differences in the next five to ten most common OTU, namely, Gardnerella, Escherichia/Shigella, Bifidobacterium, Prevotella, and Staphylococcus (Table 2). PERMANOVA analyses, following square-root transformation and Bray-Curtis similarity resemblance, confirmed that the observed differences in the composition of the microbiome of women who miscarried compared to controls were significant (analysis at level of OTU, $t = 1.5787$, $p = 0.0110$, unique perm = 997).

**Linear discriminant analysis effect size**

The liner discriminant analysis (LDA) scores ($\log_{10}$) of LEfSe evaluations performed on the 50 most abundant OTU compared their relative abundances in women who miscarried and controls. The OTU with significant differences are summarised in Table 4. They were Corynebacterium, Fusobacterium, Peptoniphilus, Prevotella, Campylobacter, Finegoldia, Dialister and Staphylococcus (Table 4).

**Discussion**
The vaginal microbiome of women who experienced a miscarriage was compared to that women who delivered at term to investigate their differences. *L. iners* was the dominant vaginal bacterium in 50% of women who experienced recurrent miscarriage, including miscarriage in the study period. In contrast, it was the dominant bacterium in only 15% of women with no history of miscarriage, and who delivered at term in the study period. This finding is similar to that of a previous study where *L. iners* was also observed to be the dominant bacterium in 45% of women who experienced two prior miscarriages and was the dominant bacterium in only 9.5% of women with no history of miscarriage. Albeit, it needs to be noted that the racial compositions in both studies and are slightly different; namely, Caucasian 68% vs 57%, and other 32% vs 42%.

In this work, there was an association between CST and parity. *L. crispatus* (CST I) was the most abundant taxon in 50% of women who were nulliparous. On the other hand, in multiparous women, the most abundant taxon varied between those predominant in the five CST. This observation was similar to a prior report where *L. crispatus* was the dominant CST in 76.4% of nulliparous women, and the most abundant bacterial taxa varied between multiparous women.

*L. crispatus* is considered to be protective against pathogenic invasion of the vagina as it does not induce a mucosal inflammatory response. In contrast, *L. iners* is observed in vaginal dysbiosis of some racial backgrounds, and can be displaced from the vaginal flora increasing the possibility of ascending vaginal infections. A study with 159 pregnant women the rate of miscarriage in women was 13%. The independent predictors of early miscarriage were chronic endometritis, the dominance of *L. iners* in the vaginal microflora, and the prevalence of non-*Lactobacillus* spp. in microscopy of vaginal preparations. The dominance of *L. crispatus* was a significant protective factor of late miscarriage. In the present study the dominance of *L. iners* in women with recurrent miscarriage may account also for the differences in species diversity and richness (Table 3). Both groups of women had microbiomes dominated by *Lactobacillus* spp., but there were significant differences in some of the most common species within the microbiome (Table 4).

Previous studies showed associations between the bacteria identified in this study as more abundant in women who miscarriaged or had pregnancy complications. *Finegoldia magna* is the type species of the genus *Finegoldia*; it is one of the species associated with the bacterial populations of CST IV most frequently observed in Black and Hispanic women. Also, it has been associated with Bartholin gland abscesses. The *Prevotella* genus has been found to be present at an increased incidence in the vaginal microbiota of women who experience recurrent miscarriages compared to healthy individuals. Another study found that *Campylobacter fetus*, a zoonotic species that is mainly present in cattle and sheep, is transmitted via the oral route and can result in disseminated infection which, if it occurs in a pregnant woman, can cause perinatal infection, placentitis or abortion. *Corynebacterium aurimucosum* taxa can colonise the female genital tract and have been related to complications during pregnancy such as late miscarriage and preterm delivery. *Fusobacterium* has been linked with preterm
birth, chorioamnionitis, still birth and neonatal sepsis via vertical transmission through haematogenous spread originating from periodontal infection. *Lactobacillus* spp. were found to play a protective role in the vaginal microbiota in the early pregnancy, and those whose microbiomes were depleted of *Lactobacillus* spp. displayed increased colonisation by potential pathogens such as *Peptoniphilus*, *Prevotella*, and *Dialister* species. These bacterial species have been shown to cause upregulation of metalloproteinase and proinflammatory cytokine expression, resulting in an increased risk of miscarriage, stillbirth and preterm birth. *Staphylococcus aureus* was identified by standard microbiological techniques in 40% of Iraqi women with UTI infection who miscarried. A study in Russia’s Northwestern district of the vaginal composition of infection-related miscarriage employing high-performing molecular techniques detected the presence in 54% of samples the presence of one or several *Enterobacteriaceae*, *Streptococcus* spp., or *Staphylococcus* spp.

A strength of this study was the stringent measures employed to ensure its quality that included double swabbing, removal of DNA extraction kit contaminants and hierarchical clustering of dual swabs. Additionally, the use of 16S rRNA analyses at the Ramaciotti Centre allowed the detection of a wider variety of microbial oligotypes. A limitation of the work was the small sample size. It is difficult to secure prospective consent for research swabs from very stressed pregnant women with a history of miscarriage when there is the possibility of experiencing a further miscarriage. Their participation in a trial demonstrated their willingness to help advance scientific knowledge that may help to prevent future miscarriages.

Nonetheless, a potential relationship between the composition of the vaginal microbiome and miscarriage was identified, albeit larger studies will be required to establish a clearer causal effect. Also, in agreement with previous work, the effect of parity on miscarriage was recognised. Finally, specific taxa were related to miscarriage. All these findings contribute to provide a foundation for diagnoses in which determining the composition of the vaginal microbial flora would serve as the basis for interventions in pregnant women at risk of miscarriage. When a firm link between vaginal microbiomes and miscarriage could be established in some women, then interventions to change the microbiome might be used to improve clinical outcomes.

**Disclosure**

The research was supported with grants the University of Notre Dame Australia. There are no financial or conflicts of interests.

**References**

Association between vaginal microbiome and miscarriage

1. https://doi.org/10.1016/j.bjog.2009.02.004


27. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 486, 207–214. [https://doi.org/10.1038/nature11234](https://doi.org/10.1038/nature11234)


Figure 1

Relative abundances (%) of the four more abundant Lactobacillus species in the vaginal microbiomes of the 22 women who participated in the study. Women 1, 7, 8, 10, 15, 16, 17 and 21 had a miscarriage and the rest delivered at term.

Figure 2

Non-metric multidimensional scaling plot (2D nMDS) using a Bray-Curtis similarity resemblance matrix comparing the composition of microbiomes of women who miscarried (inverted triangles) and control (upright triangles) in 2 dimensions. The outlines refer to the miscarriage (continue line oval) and control (dashed line oval) microbiomes. The analysis was valid with 2D stress of 0.18.
**Table 1**
Baseline characteristics of women who miscarried compared to controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total population</th>
<th>Miscarriage (n=8)</th>
<th>Control (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (years)</td>
<td></td>
<td>28 (23-33)</td>
<td>27 (23-32)</td>
</tr>
<tr>
<td>Racial Background</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td>5 (62%)</td>
<td>10 (72%)</td>
</tr>
<tr>
<td>Negroid</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td></td>
<td>2 (25%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>Indigenous</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>1 (13%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Mean gestational age at recruitment (days)</td>
<td>62</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>6 (75%)</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>&gt;1</td>
<td></td>
<td>2 (25%)</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td></td>
<td>6 (75%)</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>≥1</td>
<td></td>
<td>2 (25%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Smoking history (in pregnancy)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>0 (0%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Alcohol use (in pregnancy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>0 (0%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Risk factors (during pregnancy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td>1 (12%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Vaginal infection</td>
<td></td>
<td>1 (12%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td>0 (0%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td></td>
<td>5 (64%)</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>Pelvic pain</td>
<td></td>
<td>4 (50%)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>Previous miscarriages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>8 (100%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Previous preterm births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>0 (0%)</td>
<td>2 (14%)</td>
</tr>
</tbody>
</table>
Table 2

Most abundant taxa in women who delivered at term or experience miscarriage.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Relative Abundance (%)</th>
<th>Term Delivery</th>
<th>Miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. crispatus</td>
<td>22.1</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>L. iners</td>
<td>14.4</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td>L. gasseri</td>
<td>9.3</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>L. jensenii</td>
<td>9.3</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>6.1</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
<tr>
<td>Gardnerella</td>
<td>2.9</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Prevotella</td>
<td>2.7</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>2.5</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
<tr>
<td>Alloscardovia</td>
<td>2.0</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
<tr>
<td>Escherichia/Shigella</td>
<td>&lt; 0.1</td>
<td>12.6</td>
<td></td>
</tr>
</tbody>
</table>
**Table 3**

Alpha diversity of the vaginal microbiome in women who miscarried compared to controls

<table>
<thead>
<tr>
<th></th>
<th>Miscarriage Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of OTU (S)</td>
<td>159 (82)</td>
<td>270 (110)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Richness (d)</td>
<td>34 (18)</td>
<td>58 (24)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Evenness (J’)</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.1)</td>
<td>0.2903</td>
</tr>
<tr>
<td>Shannon index H’(loge)</td>
<td>1.3 (0.9)</td>
<td>1.8 (0.7)</td>
<td>0.0968</td>
</tr>
</tbody>
</table>
Table 4
Linear discriminant analysis (LDA) scores (log_{10}) of taxa corresponding to OTU found in different abundances in women who miscarried and controls using LEfSe analyses. The OTU number refers to their ordering by relative abundance.

<table>
<thead>
<tr>
<th>Microbial Taxon</th>
<th>LDA Score</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finegoldia (OTU 019)</td>
<td>3.6754</td>
<td>0.0000</td>
</tr>
<tr>
<td>Peptoniphilus (OTU 020)</td>
<td>3.6184</td>
<td>0.0004</td>
</tr>
<tr>
<td>Prevotella (OTU 030)</td>
<td>3.4033</td>
<td>0.0077</td>
</tr>
<tr>
<td>Dialister (OTU 029)</td>
<td>3.2439</td>
<td>0.0000</td>
</tr>
<tr>
<td>Campylobacter (OTU 043)</td>
<td>3.1567</td>
<td>0.0258</td>
</tr>
<tr>
<td>Corynebacterium (OTU 040)</td>
<td>3.1485</td>
<td>0.0034</td>
</tr>
<tr>
<td>Fusobacterium (OTU 047)</td>
<td>3.1445</td>
<td>0.0004</td>
</tr>
<tr>
<td>Staphylococcus (OTU 010)</td>
<td>2.8773</td>
<td>0.0000</td>
</tr>
</tbody>
</table>