Analysis of Vaginal Microbiome in Women with or Without Episodes of Spontaneous Abortion in Eastern Nigeria

Felix E. EMELE1, Prisca N. ONYEULOR1, Francisca O. NWAOKORIE2, Damian C. ASOGWA3
Nnewi, Nigeria

ABSTRACT

OBJECTIVES: Spontaneous abortion (miscarriage) is a common adverse pregnancy outcome worldwide, and has remained a challenge in Nigeria. This study aimed at comparing the vaginal microbiome of women who have had episodes of spontaneous abortion with those who have not experienced any incident - in order to find out any possible role of vaginal microbiota in spontaneous abortion.

STUDY DESIGN: High vaginal swab samples were collected from the vagina fornix of 6 women of reproductive age, with a history of recurrent spontaneous abortion, as well as those without such history (non-spontaneous abortion). The samples were analyzed and interpreted by standard metagenomic and bioinformatic techniques.

RESULTS: The following phyla were encountered in spontaneous abortion and non-spontaneous abortion, respectively: Firmicutes (69.4%, 94.9%), Actinobacteria (12.7%, 1.1%), Bacteroidetes (9.5%, 2.8%), Proteobacteria (7.9%, 0.3%), Chloroflexi (0.2%, 0.0%), Fusobacteria (0.2%, 0.0%), Tenericutes (0.02%, 1.0%). There was more bacterial diversity in spontaneous abortion (H=2.34856) than in non-spontaneous abortion (H=0.61384), with evenness (EH) of 0.60668 and 0.24703, respectively. On the contrary, Lactobacillus had more relative abundance in non-spontaneous abortion (83%) than spontaneous abortion (23.5%). The following genera (among others) occurred exclusively in spontaneous abortion: Enterococcus (relative abundance=26%), Peptostreptococcus (5.1%), Anaerococcus (2.4%), Dialister (2.1%), Streptococcus (1.9%), Megasphaera (1.3%), Mobiluncus (1.0%), Peptinophilus (0.9%), and Veillonella (0.7%). The efficiency of taxonomic identification, using the operational taxonomic unit clustering method, declined, downstream, from family to species levels.

CONCLUSION: Recurrent spontaneous abortion appears to be associated with low vaginal Lactobacillus abundance and high bacterial diversity. We recommend that the current operational taxonomic unit -based sequence taxonomic analysis technique be reviewed.

Keywords: Eastern nigeria, Spontaneous abortion, Vaginal microbiome

Gynecol Obstet Reprod Med 2022; (Article in Press)

Introduction

The human body is estimated to contain as many bacterial cells as human cells, which represents a magnitude of about 10^{13} bacterial cells; these organisms inhabit different parts of the body, such as the gastrointestinal tract, vagina, breast, skin, and oral cavity - with or without adverse consequences (1). The complex bacterial community that resides in the body, along with its total genomic materials, is referred to as the body microbiome. During the past decade, the microbiome has been identified as a major contributor to human health (1-4); the vaginal microbiome has a significant role in women’s reproductive health. Imbalances in the microbiota, also referred to as “dysbiosis”, may lead to several undesirable conditions, including negative reproductive outcomes (5).
tion by pathogenic organisms (6). Vaginal communities where *Lactobacilli* are either unstable or not dominant are dysbiotic and engender overgrowth of other species often implicated in many female reproductive tract disorders (5,7).

One of the commonly reported negative reproductive outcomes in Nigeria is spontaneous abortion (8), commonly referred to as miscarriage (4), and which is believed to be associated with the following identifiable risk factors: febrile illnesses such as malaria, urinary tract or lower genital tract infections, smoking, alcohol ingestion, advanced maternal age, increasing parity, increasing paternal age, previous miscarriages, smoking, obesity, among others (9-11). Several authors have included vaginal dysbiosis as a risk factor in spontaneous abortion (SA) (12,13). No work emanating from our locality has investigated the taxonomic profile of vaginal microbiome in SA, hence the need for this study.

**Material and Method**

This was a cross-sectional study involving women of different age groups attending Obstetrics and Gynaecology clinics, with complaints of recurrent spontaneous abortions with or without clinical symptoms of infection. A simple random sampling technique was used in the recruitment of subjects.

The study was reviewed and approved by the ethics committee of Federal Teaching Hospital Abakaliki, Ebonyi State, Nigeria (reference number: FETHA/REC/Vol. 2/2018/084 - dated July 31, 2018) as well as Federal Medical Centre, Owerri, Imo State (reference number: number FMC/OW/HREC/223, dated September 18, 2018). The study was conducted in accordance with the Declaration of Helsinki.

A total of four sexually active women (of reproductive age), with episodes of SA, were recruited into the study; SA (or miscarriage) was defined as unplanned (spontaneous) loss of pregnancy before the fetus reaches viability; if the pregnancy losses occurred more than two consecutive times (before 20 months of gestation, in each case) it was considered as recurrent SA.

All participants signed informed written consent before being enrolled in the study and were served interviewer-administered questionnaires. Information sought through the questionnaires included: age, history of antibiotic treatment, smoking and alcohol consumption habits, obesity, symptoms of vaginal infection, organ transplant, and use of steroid hormone, among others. Women who were menstruating, or having vaginal bleeding, those with a known history of vaginal infection, organ transplant, human immunodeficiency virus (HIV) infection, and diabetes mellitus, were excluded. Also excluded were women who had received antimicrobial therapy within the previous six months, and those using any form of vaginal suppository formulations, including prebiotics or probiotic preparations. Two premenopausal women with regular menstrual periods, who did not have any of the exclusion criteria, and had carried pregnancy to term, were enrolled in comparison.

Vaginal samples were collected from each subject, using the Norgen Microbiome collection and preservation kit (Cat no 45690). On the whole, High Vaginal Swab (HVS) samples were collected from a total number of six women - 4 with episodes of (SA) and 2 healthy women, who were not experiencing SA (Non-SA).

**Metagenomic Sequencing**

The DNA from each of the HVS samples was isolated, extracted, and purified using the Norgen microbiome isolation kit (Cat No. 64100), according to manufacturers’ instructions. The purity and quantity of the DNA were checked using a Nano-Spectrophotometer (Model ND 2000, Thermo Scientific). Polymerase chain reaction (PCR) was carried out on the DNA extracts to amplify V3-V4 hypervariable regions of 16S rRNA, using paired-end universal primer 341F, 5’-CC-TACGGGNGGCWGCAG-3’ (14) and 785R, 5’-GAC-TACHVGGGTATCTAATCC-3′(15). Samples were bar-coded with a unique combination of forward and reverse indexes, allowing for the simultaneous processing of multiple samples. PCR products were pooled, column-purified, and size-selected through microfluidic DNA fractionation. Consolidated libraries were quantified by quantitative real-time PCR, using the Kapa Bio-Rad iCycler qPCR kit on a Bio-Rad MyQ machine, before loading into the MiSeq sequencer. Sequencing was carried out in a pair-end modality on the Illumina NextSeq 500 platform.

16S rRNA metagenomics sequence analysis: Raw sequence reads were demultiplexed, using Illumina’s BCL2FASTQ algorithm. The paired-end sequence FASTQ reads were imported into the Illumina Base space pipeline for quality check. Reads with an average Q-score greater than 30 (Q score >30) were filtered. Sequenced data were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (http://qiime.sourceforge.net/) (16). The sequences with the same barcode were assigned to the same sample and then the barcode and primer sequences were removed. The chimeric sequences were removed from aligned sequences, using the UCHIME algorithm (17). The valid reads obtained from Illumina MiSeq sequences were normalized to 1000 for comparison of community diversity. Reads with 97% similarity were then clustered into operational taxonomic units (OTUs) (18). The Greengenes database and ribosomal database project (RDP) classifier were used to assign the effective sequence tags to different phylogenetic bacterial taxa (19). Diversity indices and statistical analyses were by standard methods (20).

**Results**

The demographic information on the subjects recruited for this study is shown in table I.
Of a total of 112,196 high-quality sequence reads (Mean=18,699; SD=5009.405) generated, 87,541 were assigned to the phylum “Fermicutes” (giving a relative abundance of 78.0%); Actinobacteria (9,838 reads and relative abundance of 8.8%) was the next in abundance, as shown in table II.

In all, a total of seven taxonomic phyla were identified in vaginal samples of women who experienced episodes of SA, while five were identified in those who had no history of SA (Non-SA). In both groups, Fermicutes was the dominant phylum (SA=69.4%, Non-SA=94.9%); other phyla present were, respectively, as follows: Actinobacteria (12.7%, 1.1%), Bacteroidetes (9.5%, 2.8%), Chloroflexi (0.2%, 0.0%), Fusobacteria (0.2%, 0.0%), Proteobacteria (7.9%, 0.3%), and Tenericutes (0.02%, 1.0%), as expressed in figure 1.

### Table I: Demographic information on study participants

| Demographic information | #1 | #2 | #3 | #4 | #5 | #6 |
|-------------------------|----|----|----|----|----|----|
| Age                     | 38 | 32 | 40 | 35 | 42 | 34 |
| State of origin         | Anambra | Imo | Ebonyi | Abia | Imo | Ebonyi |
| Marital Status          | Married | Married | Married | Married | Married | Married |
| Level of Education      | Tertiary | Secondary | Secondary | Secondary | Tertiary | Secondary |
| Profession              | Trader | Trader | Teacher | Tailor | Teacher | Technician |
| Employment              | Trading | Trading | Teaching | Sewing | Teaching | Civil servant |
| Alcohol/tobacco intake  | No | No | No | No | No | No |
| HIV positivity          | No | No | No | No | No | No |
| History of recurrent abortion | Yes | Yes | Yes | Yes | None | None |
| Recent antibiotics therapy | None | None | None | None | None | None |
| History of organ transplant | None | None | None | None | None | None |

### Table II: Sequence reads and relative abundances of phyla identified in samples from the vagina of women with episodes of spontaneous abortion and in those without abortion

| S/No | OTUs Identified at Phylum level | SA | Non-SA | Over-all (1-6) |
|------|---------------------------------|----|--------|---------------|
|      |                                 | #1 | #2     | #3           | #4 | Total | #5 | #6 | Total | Total |
| 1    | Actinobacteria                  | 250 | 3427   | 2078 | 3667 | 9422 | (12.7%) | 199 | 217 | 416 | (1.1%) |
| 2    | Bacteroidetes                   | 24  | 6180   | 602  | 284  | 7090 | (9.5%)  | 1033 | 18  | 1051 | (2.8%) |
| 3    | Chloroflexi                     | 0.0 | 17     | 0.0  | 160  | 177  | (0.2%)  | 0.0  | 0.0  | 0.0  | (0.0%) |
| 4    | Fermicutes                      | 11390 | 7218 | 23539 | 9408 | 51555 | (69.4%) | 18545 | 17441 | 35986 | (94.9%) |
| 5    | Fusobacteria                    | 0.0 | 35     | 0.0  | 124  | 159  | (0.2%)  | 0.0  | 0.0  | 0.0  | (0.0%) |
| 6    | Proteobacteria                  | 238 | 433    | 1121 | 4057 | 5849 | (7.9%)  | 79   | 36   | 115  | (5.3%) |
| 7    | Tenericutes                     | 0.0 | 0.0    | 0.0  | 18   | 18   | (<0.1%) | 0.0  | 362  | 362  | (1.0%) |

OTUs: Operational taxonomic units, SA: Spontaneous abortion, Non-SA: Not having spontaneous abortion

Figure 1: Chart showing relative abundances of bacterial phyla in vaginal samples from all recruited women in Eastern Nigeria
#1-4: From women with episodes of spontaneous abortion
#5-6: From women not having a spontaneous abortion
Table III shows the number of times each taxonomic category occurred; more taxonomic groups were identified in SA samples, compared with non-SA samples, as follows: Phylum (SA=7, Non-SA=5), Class (15, 8), Order (24, 10), Family (54, 20), Genus (68, 16), and species (26, 11), respectively. Five of 60 (8%) OTUs were not classifiable at the taxonomic level of “Family”; similarly, 29 of 99 (29%) could not be classified at genus levels, while 87 of 118 (74%) were unclassifiable at the species level (Table III).

Figure 2 contains pie charts showing bacterial community distribution between SA (SAI - SAIII) and Non-SA (Non-SAI - Non-SAIII) at taxonomic levels of “Class”, “Order”, and “Family”. (In the charts, only the top 10 taxa were illustrated - the remaining taxa were condensed into one group designated as “Others”).

Table IV shows the genera represented in the vaginal samples. Lactobacillus was the most abundant of the genera in both SA (3/4; 23.5%) and non-SA (2/2; 83%), followed by Prevotella (SA:4/4, 9%; Non-SA:2/2, 2.6%), and Corynebacterium (SA:4/4, 2.2%; Non-SA:2/2, 0.4%).

Some of the genera that were exclusively present in SA included: Enterococcus (with a relative abundance of 26%), Peptostreptococcus (5.1%), Anaerococcus (2.4%), Dialister (2.1%), Arthrobacter (1.4%), Streptococcus (1.9%), Megaphaera (1.3%), Pseudomonas (1.2%), Mobiliuncus (1.0%), Bacillus (0.9%), Peptoniphilus (0.9%), Veillonella (0.7%), and Rhodomonas (0.7%), etc. On the other hand, Clostridium (9.1%) was exclusive to non-SA; Bifidobacterium occurred more abundantly in SA than non-SA (2.6% and 0.1%, respectively) as was also the case with Gardnerella (2.1% and 0.1%, respectively), as shown in Table IV. There was more evenness of distribution in SA samples (EH=0.60668) than in Non-SA (EH=0.24703); Sorenson’s similarity coefficient (CC) of 0.23333 was recorded between SA and Non-SA samples.

We noted more microbial diversity in SA (Shannon diversity index (H)=2.34856) than in non-SA (H=0.61384) – t=147.13703 (p<0.001), as shown in figure 3.

Of 70 OTUs identified at the genus level, only a total of 12 (17%) could fully be identified at the species level, as shown in table V. Species of Lactobacillus represented in the vagina of participants, and their relative abundances were as follows: L. iners (SA=14.2%; Non-SA=30.2%), L. reuteri (SA=0.1%; non-SA=0.1%), L. coelohominis (SA=0.0%; Non-SA=0.2%), Lactobacillus spp. (SA=9.2%; non-SA=52.5%) - see Table V. As can also be seen in table 5, of the 23.5% relative abundance of Lactobacilli in SA, 9.2 (40%) could not be identified up to species level; similarly, 52.5 of 83 Lactobacilli (63%), from Non-SA samples, could not be speciated (Table V). Overall,
the genus *Lactobacillus* was significantly more abundant in SA (23.5%) than in non-SA (83%) - *t*=2.4932; *p*<0.05.

In addition, 2.4 of 2.6 (85%) of OTUs corresponding to *Bifidobacterium* could not be speciated. The unclassifiable genera were distributed to their corresponding phyla, as follows: *Actinobacteria* (16.86%), *Bacteroidetes* (0.0%), *Chloroflexi* (2.68%), *Firmicutes* (27.20%), *Fusobacterium* (0.0%), *Proteobacteria* (54.79%), and *Tenericutes* (0.0%).

Figure 4 is a Pie chart showing the distribution of unclassifiable genera among the phyla represented in the vagina of women in Eastern Nigeria.
Table IV: Bacterial community distribution, at the genus level, in samples from the vagina of women with episodes of spontaneous abortion and those not having an abortion

| Relative Abundance (%) of OTUs in: | SA | Non-Who |
|-----------------------------------|----|--------|
| **A. Shared Genera**              |    |        |
| 1. Lactobacillus                  | 23.5 | 83.0 |
| 2. Prevotella                     | 9.0  | 2.6  |
| 3. Staphylococcus                 | 1.6  | 0.7  |
| 4. Bifidobacterium                | 2.6  | 0.1  |
| 5. Corynebacterium                | 2.2  | 0.4  |
| 6. Gardnerella                    | 2.1  | 0.1  |
| 7. Haemophilus                    | 0.1  | 0.1  |
| **B. Genera Exclusive to women with SA** |    |        |
| 1. Enterococcus                   | 26.0 | 0.0  |
| 2. Peptostreptococcus             | 5.1  | 0.0  |
| 3. Anaerococcus                   | 2.4  | 0.0  |
| 4. Dialister                      | 2.1  | 0.0  |
| 5. Streptococcus                  | 1.9  | 0.0  |
| 6. Arthrobacter                   | 1.4  | 0.0  |
| 7. Megasphaera                    | 1.3  | 0.0  |
| 8. Pseudomonas                    | 1.2  | 0.0  |
| 9. Mobiluncus                     | 1.0  | 0.0  |
| 10. Peptonophilus                 | 0.9  | 0.0  |
| 11. Bacillus                      | 0.9  | 0.0  |
| 12. Rhodococcus                   | 0.7  | 0.0  |
| 13. Veillonella                   | 0.7  | 0.0  |
| 14. Pophyromonas                  | 0.5  | 0.0  |
| 15. Parococcus                    | 0.5  | 0.0  |
| 16. Acinetobacter                 | 0.5  | 0.0  |
| 17. Campylobacter                 | 0.5  | 0.0  |
| 18. Aerococcus                    | 0.3  | 0.0  |
| 19. Sutterella                    | 0.3  | 0.0  |
| 20. Finegoldia                    | 0.3  | 0.0  |
| 21. Micrococcus                   | 0.3  | 0.0  |
| 22. Dietzia                       | 0.3  | 0.0  |
| 23. Sneathia                      | 0.2  | 0.0  |
| 24. Atopobium                     | 0.2  | 0.0  |
| 25. Bulleidia                     | 0.2  | 0.0  |
| 26. Others (n=33)                 | 3.5  | 0.0  |
| **C. Genera Exclusive to women not having SA (Non-SA)** |    |        |
| 1. Clostridium                    | 0.0  | 9.1   |
| 2. Ureaplasma                     | 0.0  | 0.9   |
| 3. Others (n=3)                   | 0.0  | 0.6   |

Discussion

Earlier reports (21,22) suggested that significant differences exist in average microbial diversity between women who experienced SA and those who successfully carried the pregnancy to term. In our study, we noted that the Shannon diversity index was much higher among SA samples, compared with non-SA (t=147.13703; p<0.001), showing that the vagina of SA patients had significantly more diversity of bacterial genera and species. Also, as can be seen from the Shannon...
Equitability Index (EH) values (SA=0.60668; non-SA=0.24703), women with SA had more species evenness in their vagina than those of non-SA (it should be pointed out that a community with low evenness contains few dominant species, whereas one with high evenness contains diverse species that are uniformly distributed in abundance). This shows that the vagina of women with SA had significantly more diverse species and that those species were more uniformly distributed in abundance than was the case with non-SA vaginal communities. This also shows that the vagina of non-SA women, unlike those of SA, was dominated by a few bacterial species. The dominant bacterium in non-SA was Lactobacillus, and this organism (Lactobacillus) was significantly more abundant in non-SA communities than in SA ($r=2.4932; p<0.05$). Lactobacilli are known to produce substances that limit the growth of other bacteria in the vagina, thereby promoting good vaginal health (23). Therefore, the relatively low abundance of Lactobacillus in the vagina of SA patients (compared with non-SA) must have resulted in the production of comparatively fewer amounts of antibacterial chemicals, thereby providing a more conducive environment for diverse bacterial species to thrive. A vaginal microbiota is considered unbalanced (dysbiotic) when the diversity is high (as seen in SA cases); such disturbed vaginal composition (dysbiosis) can lead to negative reproductive outcomes (5,7).

Based on this study, it can be concluded that SA is associated with reduced Lactobacillus levels and increased diversity of vaginal microbiota. This is possibly due to the fact that a reduced level of vaginal Lactobacilli may predispose the vagina to colonization by diverse groups of bacterial genera and species, which may, in turn, induce immunological pressures that could result in the premature ejection of the developing fetus - before the fetus attains viability; this could be attributed to the fact that presence of infecting organisms can lead to consequent activation of pattern-recognition receptors (PPRs) and release of inflammatory mediators that could ultimately stimulate uterine contractions, leading to the eventual ejection of the unviable fetus (22,24).

We also noted a low similarity coefficient between the vagina of SA and non-SA women (CC=0.23333). Sorensen’s similarity coefficient (CC) ranges in value between 0 and 1; the lower the value between two communities, the less the communities have in common. This shows that the bacterial community in SA was quite dissimilar to those in non-SA, and further suggests that the vaginal microbiome profile may have a role in negative reproductive outcomes, such as SA. The apparent involvement of vaginal dysbiosis as a risk factor in SA (as noted in this study) agrees with the report of earlier authors (25), who attributed miscarriages to reduced concentration of hydrogen peroxide-producing Lactobacillus and the presence of bacterial vaginosis. The use of probiotics or prebiotic preparations may be an effective way to address such a dysbiotic state and restore the integrity of the vagina.

Ravel et al. (6) placed black women in vaginal community type IV - a type lacking Lactobacillus-dominated microbiota communities. However, the result of this study showed that the relative abundance of vaginal Lactobacilli was higher than the level that defines the community state type IV that is ascribed to African women. This difference is not surprising, because the black population investigated by Ravel et al. (6) were not African residents but black women in the diaspora; the vaginal flora of such “westernized” African women might not be truly reflective of those of non-diaspora African women, especially as cultural and personal (westernized) practices can influence the body microbiota.

Most of the genera we found to be exclusive to SA (i.e. Sneathia, Megaphaera, Dialister, Peptostreptococcus, Gardnerella, and other facultative anaerobes encountered) have previously been associated with vaginal dysbiosis by other authors (22,26). Also, the facultative anaerobes identified (exclusively or more abundantly in SA) in this study included: Anaerococcus, Gardnerella, Peptostreptococcus Megaphaera, Dialister, Prevotella, and Peptoniphilus, among others. These bacteria have been found to be among the causative agents of pelvic inflammatory diseases (PID), fallopian tube blockade, and miscarriage (27). It has been shown that SA is one of the most common adverse outcomes of pregnancy, affecting an estimated 12-24% of known pregnancies (4). Deaths caused by this condition can be avoided, or drastically reduced, if pregnancies at risk of SA are identified early and necessary interventions promptly administered. This can be achieved by monitoring the vagina of pregnant women for the dysbiotic state.

In the course of this study, we found out that some OTUs lacked classification at taxonomic levels of “Family” (5/60 or 8%), Genus (29/99 or 29%), and species (84/114 or 74%). Consequently, 39% of Lactobacilli OTUs in SA and 63% of those in Non-SA could not be speciated; this situation made it difficult to precisely determine all the species of vaginal Lactobacilli involved in health and disease among the subjects investigated. This was also the case with the genus Bifidobacterium, 85% of which could not be speciated. The high prevalence of unclassified OTUs (at genera and species levels) underscores the need to review the currently used 16S amplicon analysis method, which involves clustering sequences within arbitrarily chosen sequence similarity threshold (usually 97%) into the operational taxonomic units (OTUs), to delineate species; poorly clustered OTUs can have significant impacts on downstream analyses and can lead to overestimation of evolutionary similarity between pairs being aligned (28). Amplicon sequence variant (ASV), also referred to as exact sequence variants (ESVs), has been suggested as a better option for the current use of the operational taxonomic unit (29), and is hereby recommended. However, Werner et al. (30) are of the view that OTUs without genus/species information is frequently both more abundant and more represen-
tative of total diversity than are OTUs with genus /species names.

The fact that the efficiency of taxonomic identification of the OTU clustering method steadily declined, downstream, from family level (92%) to genus level (71%), and species level (26%) makes us believe that more family, genera, and species would have been revealed, if the sequence similarity threshold was set higher than 97%, or possibly if an alternative technique was adopted.

Conclusion/Recommendations

The results of this study tend to affirm that vaginal dysbiosis is a risk factor in SA. It is, therefore, recommended that vaginal microbiome examination be listed among routine investigations in pregnancy, especially for those with a history of miscarriage(s); the use of prebiotics or probiotic preparations is suggested for those whose vaginal analyses indicate dysbiosis. Based on our results, it is also recommended that the current sequence taxonomic analysis technique, involving a 97% similarity threshold, be reviewed. The inability to employ a larger sample size (due to technical limitations and the small size of funds) was a limitation of this study.

Declarations

Ethics approval and consent to participate: Availability of data and materials. The data supporting this study is available through the corresponding author upon reasonable request.

Conflict of interest: The authors declare no conflict of interest.

Funding: The funding for this work was provided, in part, through a research grant award from Nigerian Tertiary Education Trust Fund (Ref No: TETFUND/ES/AST&D/POLY/IMO/2016/ VOL 1).

Authors’ contributions: FEE: and PNO: Raised the presented idea. FEE., PNO: and FON: Designed the study. DCA: PNO: and FON: Conducted the analyses, writing of the manuscript was carried out by. FEE: All authors approved the final manuscript.

Acknowledgments: We would like to thank “Nigerian Tertiary Education Trust Fund” for the funding support.

References

1. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? revisiting the ratio of bacterial to host cells in humans. Cell. 2016;164(3):337-40. doi: 10.1016/j.cell.2016.01.013. PMID: 26824647.
2. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet. 2012;13(4):260-70. doi: 10.1038/nrg3182. PMID: 22411464, PMCID: PMC3418802.
3. Li K, Bihan M, Yousef S, Methé BA. Analyses of the microbial diversity across the human microbiome. PLoS One. 2012;7(6):e32118. doi: 10.1371/journal.pone.0032118. PMID: 22719823, PMCID: PMC3374608.
4. Wang YX, Mínguez-Alarcón L, Gaskins AJ, Misser SA, Rich-Edwards JW, Manson JE, et al. Association of spontaneous abortion with all cause and cause specific premature mortality: prospective cohort study. BMJ. 2021;372:n530. doi: 10.1136/bmj.n530. PMID: 33762255, PMCID: PMC7988453.
5. Ness RB, Hillier SL, Kip KE, Soper DE, Stamm CA, McGregor JA, et al. Bacterial vaginosis and risk of pelvic inflammatory disease. Obstet Gynecol. 2004;104(4):761-9. doi: 10.1097/01.AOG.0000139512.37582.17. PMID: 15458899.
6. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108 Suppl 1(Suppl 1):4680-7. doi: 10.1073/pnas.1002611107. PMID: 20534435, PMCID: PMC3063603.
7. Al-Memar M, Bobdiwala S, Fourie H, Mannino R, Lee YS, Smith A, et al. The association between vaginal bacterial composition and miscarriage: a nested case-control study. BJOG. 2020;127(2):264-74. doi: 10.1111/1471-0528.15972. PMID: 31573753, PMCID: PMC6972675.
8. Adeniran AS, Fawole AA, Abdul IF, Adesina KT. Spontaneous abortions (miscarriages): Analysis of Cases at a tertiary centre in North Central Nigeria. J Med Trop. 2015;17(1):22-6. doi: 10.4103/2276-7096.148571.
9. Pam IC, Otubu JA. Miscarriages. In: Agboola A, editor. Textbook of Obstetrics and Gynaecology for Medical Students. 2nd ed. Lagos: Heinemann Educational Publishers; 2006. p. 95-100.
10. Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Häberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. BMJ. 2019;364:l869. doi: 10.1136/bmj.l869. PMID: 30894356, PMCID: PMC6425455.
11. Sharma B, Deep J, Pandit C, Basnyat B, Khanal B, Raut B, et al. Overview on current approach on recurrent miscarriage and threatened miscarriage. Clin J Obstet Gynecol. 2020; 3:151-7. doi: 10.29328/journal.cjog.1001070.
12. Feehily C, Crosby D, Walsh CJ, Lawton EM, Higgins S, McAuliffe FM, et al. Shotgun sequencing of the vaginal microbiome reveals both a species and functional potential signature of preterm birth. NPJ Biofilms Microbiomes. 2020;6(1). doi: 10.1038/s41522-020-00162-8. PMID: 33184260, PMCID: PMC7656020.
13. Zhang F, Zhang T, Ma Y, Huang Z, He Y, Pan H, Fang M, Ding H. Alteration of vaginal microbiota in patients with unexplained recurrent miscarriage. Exp Ther Med. 2019;17(5):3307-3316. doi: 10.3892/etm.2019.7337. PMID: 30988706, PMCID: PMC6447762.
14. Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF. Transitions in bacterial communities along the 2000km salinity gradient of the Baltic Sea. ISME J. 2011;5(10):1571-9. doi: 10.1038/ismej.2011.41. PMID: 21472016, PMCID: PMC3176514.
15. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41:e1. doi: 10.1093/nar/gks808. PMID: 22933715, PMCID: PMC3592464.

16. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6. doi: 10.1038/nmeth.f.303. PMID: 20383131, PMCID: PMC3156573.

17. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-200. doi: 10.1093/bioinformatics/btr381. PMID: 21700674, PMCID: PMC3150044.

18. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460-1. doi: 10.1093/bioinformatics/btq461. PMID: 20709691.

19. Qin Y, Hou J, Deng M, Liu Q, Wu C, Ji Y, He X. Bacterial abundance and diversity in pond water supplied with different feeds. Sci Rep. 2016;6:35232. doi: 10.1038/srep35232. PMID: 27759010, PMCID: PMC5069485.

20. Chao A, Bunge J. Estimating the number of species in a stochastic abundance model. Biometrics. 2002;58(3):531-9. doi: 10.1111/j.0006-341x.2002.00531.x. PMID: 12229987.

21. Shahid M, Quinlivan JA, Peek M, Castaño-Rodríguez N, Mendz GL. Is there an association between the vaginal microbiome and first trimester miscarriage? A prospective observational study. J Obstet Gynaecol Res. 2022;48(1):119-28. doi: 10.1111/jog.15086. PMID: 34761471.

22. Grewal K, Lee YS, Smith A, Brosens JJ, Bourne T, Al-Memar M, et al. Chromosomally normal miscarriage is associated with vaginal dysbiosis and local inflammation. BMC Med. 2022;20(1):38. doi: 10.1186/s12916-021-02227-7. PMID: 35090453, PMCID: PMC8796436.

23. Witkin SS, Linhares IM. Why do lactobacilli dominate the human vaginal microbiota? BJOG. 2017;124(4):606-11. doi: 10.1111/1471-0528.14390. PMID: 28224747.

24. Ekman-Ordeberg G, Dubicke A. Preterm Cervical Ripening in humans. Facts Views Vis Obgyn. 2012;4(4):245-53. PMID: 24753916, PMCID: PMC3987477.

25. Eckert LO, Moore DE, Patton DL, Agnew KJ, Eschenbach DA. Relationship of vaginal bacteria and inflammation with conception and early pregnancy loss following in-vitro fertilization. Infect Dis Obstet Gynecol. 2003;11(1):11-7. doi: 10.1155/S1064744903000024. PMID: 12839628, PMCID: PMC1852261.

26. Kumar S, Kumari N, Talukdar D, Kothidar A, Sarkar M, Mehta O, et al. The vaginal microbial signatures of preterm birth delivery in Indian women. Front Cell Infect Microbiol. 2021;11:622474. doi: 10.3389/fcimb.2021.622474. PMID: 34094994, PMCID: PMC8169982.

27. Walker CK, Workowski KA, Washington AE, Soper D, Sweet RL. Anaerobes in pelvic inflammatory disease: implications for the Centers for Disease Control and Prevention's guidelines for treatment of sexually transmitted diseases. Clin Infect Dis. 1999;28 Suppl 1:S29-36. doi: 10.1086/514720. PMID: 10028108.

28. Goodrich JK, Di Rienzi SC, Poole AC, Koren O, Walters WA, Caporaso JG, et al. Conducting a microbiome study. Cell. 2014;158(2):250-262. doi: 10.1016/j.cell.2014.06.037. PMID: 25036628, PMCID: PMC5074386.

29. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 2017;11(12):2639-43. doi: 10.1038/ismej.2017.119. PMID: 28731476, PMCID: PMC5702726.

30. Werner JJ, Koren O, Hugenholtz P, DeSantis TZ, Walters WA, Caporaso JG, et al. Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. ISME J. 2012 Jan;6(1):94-103. doi: 10.1038/ismej.2011.82. PMID: 21716311, PMCID: PMC3217155.