The first glimpse of the endometrial microbiota in early pregnancy

Inmaculada Moreno, PhD[1]; Iolanda Garcia-Grau, BS[1]; Davide Bau, PhD; David Perez-Villaroya, BS; Marta Gonzalez-Monfort, BS; Felipe Vilella, PhD; Roberto Romero, MD, DMedSci; Carlos Simón, MD, PhD

Investigation of the microbial community in the female reproductive tract with the use of sequencing techniques has revealed that endometrial samples obtained through a transvaginal catheter are dominated by *Lactobacillus* species. Dysbiotic changes in the endometrial microbiota may be associated with implantation failure or early spontaneous abortion in patients who undergo assisted reproductive technology treatment. Whether or not there is an endometrial microbiota in early pregnancy is unknown. Herein we describe, the human endometrial microbiota in a patient who subsequently had an 8th week spontaneous clinical miscarriage with euploid embryos in the next cycle and, for the first time, during a successful pregnancy in which the endometrial fluid was sampled at 4 weeks of gestation. The microbial profile found on the endometrial sample before the spontaneous abortion had higher bacterial diversity and lower *Lactobacillus* abundance than the endometrial fluid from the healthy pregnancy. Functional metagenomics detected different *Lactobacillus* species between the 2 samples. *Lactobacillus crispatus* was present in the endometrium before the spontaneous abortion, as were other bacteria involved in dysbiosis, which had an unstable functional pattern characterized by transposases and insertion elements. *Lactobacillus iners* was the most prevalent microbe found in the endometrium during early pregnancy; its presence was associated with defense mechanisms and basal functions. These novel observations prompt future investigations to understand the potential implications of microbiology on healthy and pathologic human pregnancy.

Key words: 16S rRNA, endometrial microbiota, *Lactobacillus crispatus*, *Lactobacillus iners*, metagenomic, microbiome, pregnancy, reproductive tract, sequencing, spontaneous abortion

The efforts of the Human Microbiome Project have highlighted the importance of microorganisms and their genomes in several human niches and emphasized the importance in human health and disease.[1] The female reproductive tract contributes up to 9% of the human microbiota.[2] Until recently, the main research focus has been on the vaginal microbiota.[3] However, accumulating evidence suggests the existence of a different bacterial ecosystem in the endometrium,[4–8] challenging the traditional dogma of the sterility of the human uterus.[9,10]

The vaginal microbiota has been investigated for years with the use of microbial culture, microscopy, and culture-independent techniques, which show that the predominant bacteria are *Lactobacilli*.[3] The endometrial cavity has traditionally been considered sterile, and the isolation of Enterobacteriaceae, *Streptococcus*, *Staphylococcus*,

From the Igenomix Foundation-Instituto de Investigación Sanitaria Hospital Clínico (INCLIVA; Drs Moreno, Vilella, and Simón and Ms Garcia-Grau and Ms Gonzalez-Monfort); Igenomix S.L. (Drs Moreno, Bau, and Simón and Ms Gonzalez-Monfort, and Mr Perez-Villaroya); the Department of Pediatrics, Obstetrics and Gynecology, School of Medicine, University of Valencia (Dr Simón and Ms Garcia-Grau), Valencia, Spain; the Perinatology Research Branch, Division of Obstetrics and Maternal-Fetal Medicine, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD, and Detroit, MI; the Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI (Dr Romero); the Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI (Dr Romero); Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI (Dr Romero); the Detroit Medical Center, Detroit, MI (Dr Romero); the Department of Obstetrics and Gynecology, Florida International University, Miami, FL (Dr Romero); and the Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX (Dr Simón); IVI Valencia - Clínica de Fertilidad y Reproducción Asistida, Valencia, Spain (Dr Simón); and Beth Israel Deaconess Medical Center (BIDMC), Harvard University, Boston, MA (Dr Simón).

[1]These authors contributed equally to this article.

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Corresponding authors: Carlos Simón, MD, PhD. csimon@bidmc.harvard.edu and Inmaculada Moreno, PhD. inmaculada.moreno@igenomix.com

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AJOG at a Glance

Why was the study conducted?

The purpose of this study was to address the question of whether there is a human endometrial microbiota in early pregnancy. This question became tractable because endometrial fluid was collected when pregnancy had not been diagnosed. Therefore, it was possible to characterize the endometrial microbiota in the cycle before a spontaneous abortion and during a successful pregnancy.

Key findings

There were taxonomic and functional differences between the microbiota found in endometrial fluid collected during an early successful pregnancy and before a spontaneous abortion with euploid embryos in the same patient.

What does this study add to what is already known?

This study describes the differences in the microbial community of the endometrium in a successful pregnancy compared with that of a pregnancy failure. This observation suggests that an endometrial microbiota is present in normal pregnancy and that its composition can be different before a spontaneous abortion. These observations support that the endometrial microbiota may be associated with different reproductive outcomes.

and *Escherichia coli* from the tip of the embryo transfer catheter has been linked with poor reproductive outcomes in patients who undergo in vitro fertilization (IVF).[11] The development of culture-independent techniques, especially 16S ribosomal RNA (16S rRNA) gene sequencing, allows interrogation of low-biomass sites. Shotgun metagenomics sequencing/whole metagenome sequencing (WMS) allows investigation of species diversity and certain functional properties.[12,13]

With the use of 16S rRNA sequencing in specimens obtained through a transcervical catheter, the microbiota profile in the human endometrial fluid can be classified as *Lactobacillus*-dominated or non—*Lactobacillus*-dominated, which was established by a cut-off of 90% *Lactobacilli*. Dysbiotic profiles (ie, imbalanced bacterial composition for a given niche) characterized by a non—*Lactobacillus*-dominated microbiota together with specific pathogens have been associated with lower implantation, pregnancy, ongoing pregnancy, live birth rates, and an increase in clinical spontaneous abortions.[5,14]

During pregnancy, the presence of pathogenic bacteria in the reproductive tract has been associated with obstetric complications such as spontaneous preterm birth and fetal death.[15,16] The vaginal microbiota is significantly different between pregnant and nonpregnant women. These differences can be observed in terms of structure and stability; during pregnancy, it is more stable and less diverse than that in nonpregnant women given the domination by *Lactobacillus* spp and a lower frequency of bacteria associated with bacterial vaginosis.[17—20] The higher stability of the vaginal microbiota during pregnancy can be attributed to a high hormonal concentration of estrogen, the absence of menses, or changes in cervical and vaginal fluids.[18] The dominance of vaginal *Lactobacillus* spp. in pregnancy may have a protective role against pathogenic bacteria ascending to the maternal-fetal interface, where they can confer risk for the ongoing pregnancy.[21,22] Here, we report the first incidental case to characterize the endometrial microbiota taxonomically and functionally with the use of 16S rRNA sequencing and WMS before an embryo transfer that resulted in spontaneous abortion and during a 4-week gestation in the same woman who subsequently had a successful pregnancy (Figure 1).

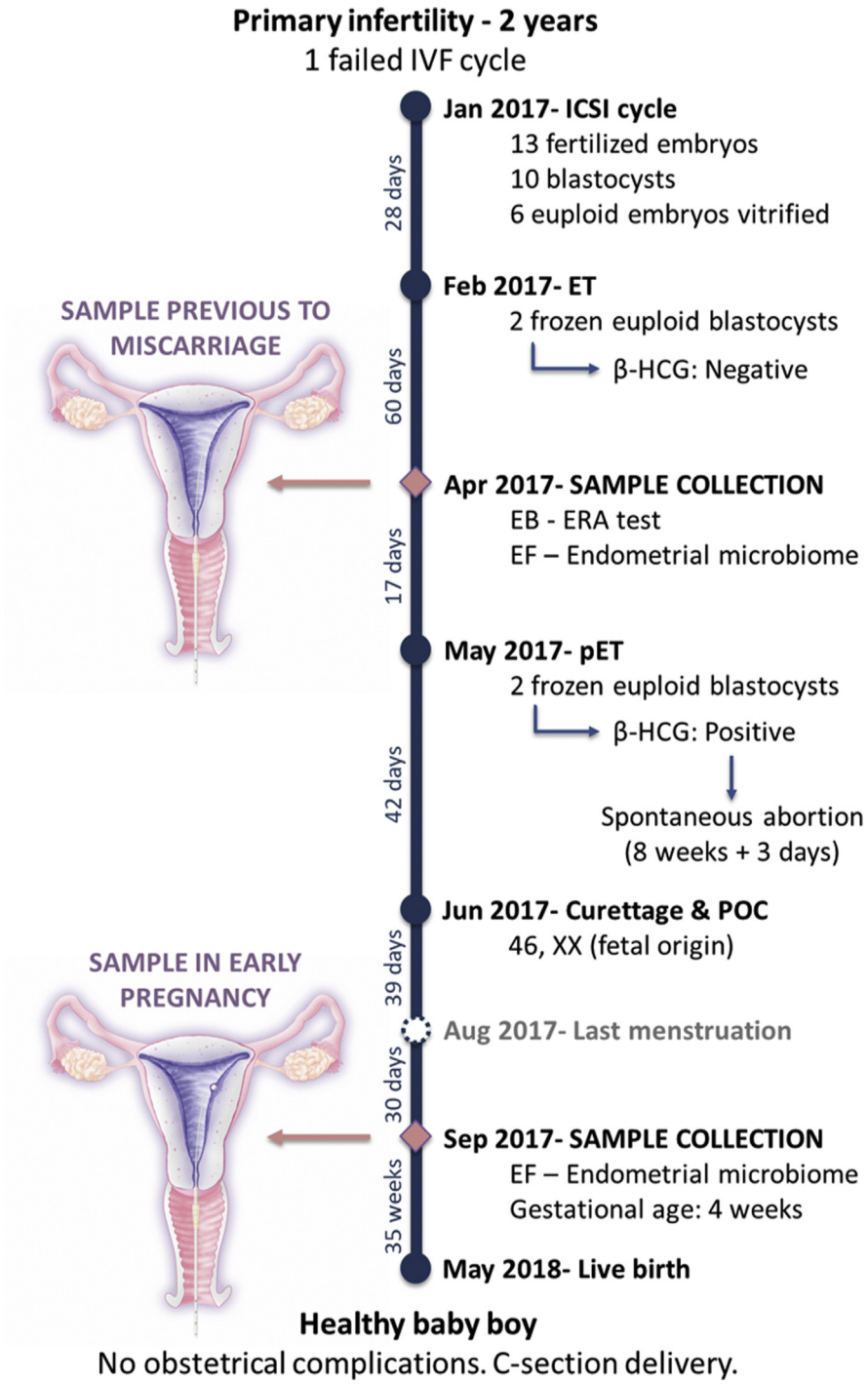
Patient and Methods

A 28-year old woman with primary infertility for 2 years had undergone 1 unsuccessful IVF cycle (Figure 1). The patient did not have medical or surgical complications, had a body mass index of 22 kg/m^2, and a negative serologic test result for human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and syphilis. Her husband had normal semen analysis results, and neither had chromosomal abnormalities.

As a result of her first intracytoplasmic sperm injection cycle, 14 metaphase-II oocytes were retrieved that resulted in 13 zygotes after the intracytoplasmic sperm injection. Of these, 10 embryos reached the blastocyst stage and resulted in 6 euploid embryos identified by preimplantation genetic testing for aneuploidies that were vitrified.

After the first embryo transfer of 2 euploid blastocysts, the pregnancy test was negative. Two months later, a sample of endometrial fluid was collected and stored for microbiota analysis prior to the endometrial biopsy and then used for the endometrial receptivity analysis to guide personalized embryo transfer. Subsequently, 2 euploid blastocysts were transferred in April 2017. Pregnancy was achieved, and the beta human chorionic gonadotropin concentration was 278.9 mIU/mL. One gestational sac, 8 mm in diameter, was visualized with the use of transvaginal ultrasound scanning during Week 5 of pregnancy. A spontaneous clinical miscarriage occurred at Week 8 of gestation, and dilation and curettage was performed. The patient received azithromycin: 500 mg per day for 3 days. The analysis of the products of conception confirmed that the embryo was chromosomally normal with a profile 46, XX of fetal origin. Two months after the dilation and curettage, the patient was seen at the time of the expected menstruation to start a new embryo transfer cycle. In this visit, endometrial fluid was collected and stored to investigate changes in the microbiota. Subsequently, it became evident that the patient had conceived spontaneously and was 4 weeks pregnant when the sample

FIGURE 1
Flow chart



Flow chart shows the clinical evolution of the patient during the spontaneous abortion and successful pregnancy investigated.

C-section, cesarean delivery; *EB*, endometrial biopsy; *EF*, endometrial fluid; *ERA*, endometrial receptivity analysis; *β-HCG*, beta human chorionic gonadotropin; *ET*, embryo transfer; *ICSI*, intracytoplasmic sperm injection; *IVF*, in vitro fertilization; *pET*, personalized embryo transfer after the recommendation of endometrial receptivity analysis test; *POC*, product of conception.

Moreno. The endometrial microbiome in early pregnancy. Am J Obstet Gynecol 2020.

of endometrial fluid was obtained. The pregnancy continued uneventfully, and the patient delivered a healthy male infant who weighed 3700 g by cesarean section at 40 weeks of gestation.

Endometrial fluid had been collected under a protocol approved by the local Ethics Committee at the Instituto Valenciano de Infertilidad (Federal Wide Assurance number: FWA00027749; protocol number 1606-IGX-044-CS). The patient provided written informed consent for the aspiration of the endometrial fluid and the subsequent publication of her case.

Sample collection

Endometrial fluid samples were obtained by transcervical aspiration with a double lumen embryo transfer catheter as previously described.[23] The specimens were collected in sterile tubes containing 50 μL of RNA*later* solution (ThermoFisher Scientific, Waltham, MA) according to the manufacturer's instructions and stored at −80°C until use.

DNA extraction

Total DNA was isolated by performing a predigestion step with lysozyme, lysostaphin and mutanolysin to degrade the cell wall of bacteria, followed by extraction with a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The genomic DNA was quantified with the use of Tape Station (Agilent Technologies, Waldbronn, Germany) and subjected to preamplification and sequencing for the identification of microbiota represented in the endometrial fluid.

16S ribosomal RNA sequencing

The 16S rRNA gene microbiota profiles were obtained with the use of the Ion 16S metagenomics kit (ThermoFisher Scientific). This kit includes 2 primer sets (V2-4-8 and V3-6, 7-9) that selectively amplify the corresponding hypervariable regions of the 16S ribosomal subunit. The amplified fragments were sequenced on the Ion S5 XL system (ThermoFisher Scientific), and the results were analyzed with the use of the QIIME 2.0 package (https://qiime2.org/) and RDP classifier

2.2 for taxonomic assignment. QIIME was used to calculate the alpha diversity and rarefaction curves before filtering. Positive controls of *E coli* DNA along with blank controls were included in the assays to detect any potential contamination from reagents.

Whole metagenome sequencing

The endometrial microbiome functional composition was assessed by WMS with the Illumina platform and the use of the Nextera DNA Flex Library Preparation kit (Illumina) according to the manufacturer's instructions. The sample collected early in the successful pregnancy yielded sufficient DNA to analyze in 2 technical replicates, starting from the same preparation of genomic DNA and sequencing the sample twice with independent amplifications and library preparations. Because both technical replicates yielded equivalent results, the results presented herein are representative of both aliquots. The libraries were sequenced on the NextSeq 500 system (Illumina). The reads generated by the Illumina sequencing platform were quality trimmed and length filtered with the use of PRINSEQ.[24] Paired-end reads were merged with the use of the FLASH (Fast Length Adjustment of SHort reads) software tool[25]; finally, host-reads were removed by using the Burrows-Wheeler Aligner mapped against the human genome reference.[26]

Functional and taxonomical joint profiling was performed with the use of the Human Microbiome Project Unified Metabolic Analysis Network pipeline.[27] This method combines taxonomic profiling of samples using MetaPhlAn2,[28] which provides a pan-microbial annotation, and the use of a combination of clade-specific markers and functional annotation inferred by the pangenomic database resulting from MetaPhlAn2 taxonomic classification. Another annotation assessing the robustness of taxonomic classification was obtained by using KRAKEN software, ie, MiniKraken DB_8GB, formed from complete bacterial, archaeal, and viral genomes in the National Center for Biotechnology Information reference sequence.[29] The presence of biomedically interesting protein families, such as G protein-coupled receptor ligand producers, was assessed with InterProScan 5 and PFAM reference protein database.[30,31] Finally, the pipeline outputs were processed with the use of R statistical software[32] for statistical description and graphical representation of the sample's taxonomic and functional profile.

Data availability

The sequence data that support the findings of this study have been deposited as compressed fastq.gz files in the Sequence Read Archive with the primary accession codes PRJNA514966 (http://www.ncbi.nlm.nih.gov/bioproject/514966).

Results

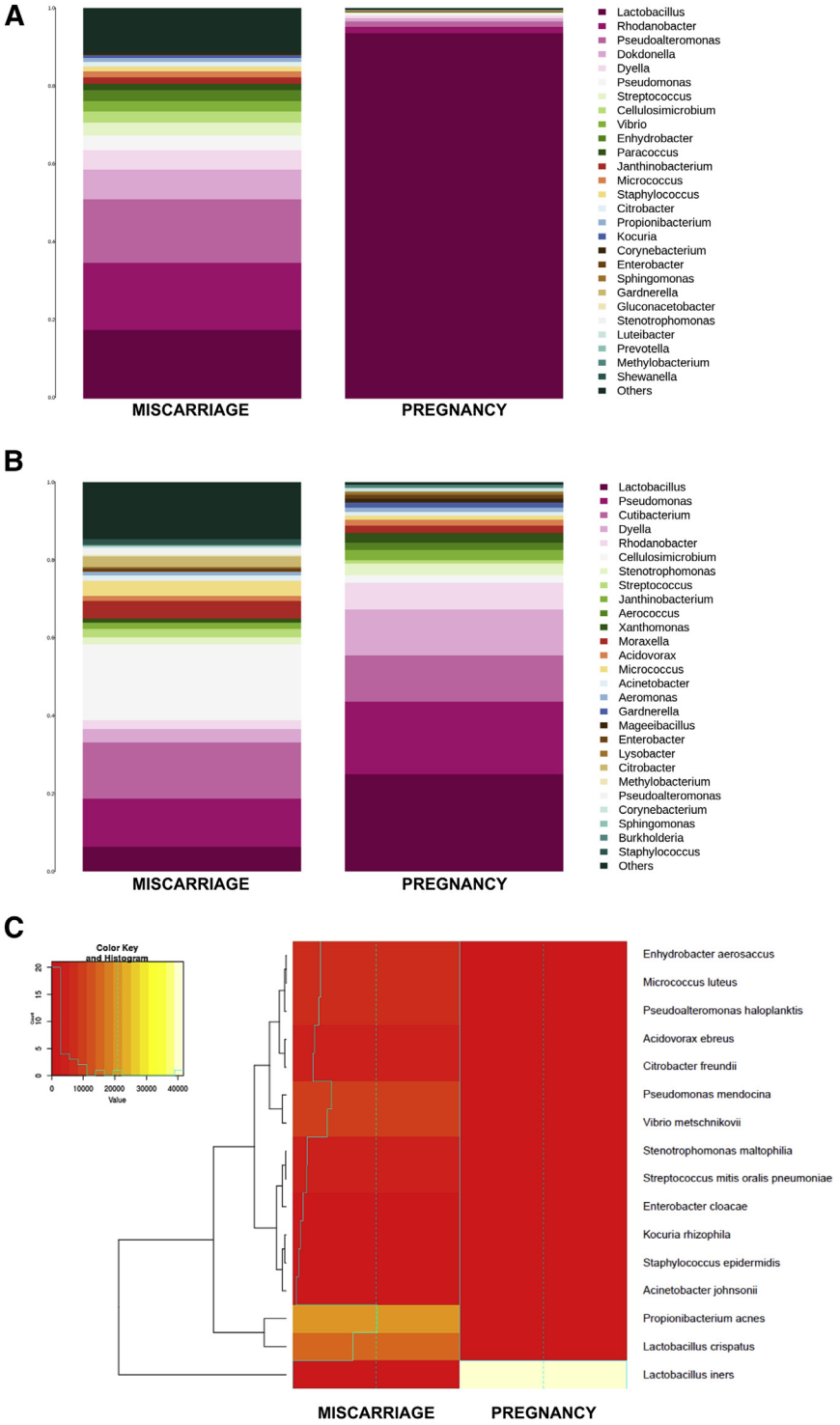
The 16S rRNA sequencing of the endometrial fluid obtained in the cycle before the spontaneous miscarriage showed a non−*Lactobacillus*-dominant profile with 5% Actinobacteria, 19% Firmicutes, and 76% Proteobacteria. From these phyla, 15% of *Lactobacilli* was encountered together with several pathogenic bacterial genera previously reported to affect the reproductive tract such as Enterobacteriaceae (3%), *Streptococcus* (2%), *Pseudomonas* (2%), and *Staphylococcus* (0.8%). The microbiota in the sample collected at the 4th week of the successful pregnancy in the same patient revealed a *Lactobacillus*-dominated profile with 91% of Firmicutes and only 9% of Proteobacteria. Interestingly enough, *Lactobacillus* was the only bacteria present under the Firmicutes phylum, accounting for 91% of the sample (Figure 2, A).

Furthermore, the metagenomic analyses by WMS yielded a total of 238,778,133 reads. After quality control and filtering of human reads, only 0.1−1% of reads corresponded to bacterial DNA; the vast majority of the sequences mapped to human DNA (Table 1). As in the 16S rRNA sequencing results, the taxonomic analysis by WMS showed a dysbiotic non−*Lactobacillus*-dominant profile in the endometrial fluid obtained before the spontaneous abortion and, alternatively, higher *Lactobacillus* abundance in the endometrial fluid sample collected in the presence of an embryo with successful implantation (Figure 2, B). However, when we analyzed the complexity of the microbial communities with the WMS technology in both samples, certain bacterial genera not represented in the 16S rRNA sequencing were detected such as *Cutibacterium*, *Acidovorax*, *Xanthomonas*, and *Aerococcus* (Figure 2, B). Although the taxonomic assignment derived from WMS showed greater microbial diversity than 16S rRNA sequencing, when functional and taxonomic analyses were combined, the microbial diversity present in each sample was reduced. Because of this, the functional metagenomic analysis showed that the sample collected before the clinical spontaneous abortion contained *Lactobacillus crispatus* as the predominant *Lactobacillus* spp. (15%) and a variety of bacterial genera, such as *Propionibacterium* (21%), *Pseudomonas* (10%), and *Streptococcus* (3.5%). In contrast, in the sample collected during the successful pregnancy, *L. iners* was the only microbe found in the endometrium (Figure 2, C).

Functional metagenomics analysis also revealed different *Lactobacillus* species in the 2 samples (Figure 2, C). *L. iners* was the only microbe present in the endometrium during successful early pregnancy, thus potentially associating its presence with defense mechanisms and basal functions (particularly, translation, energy production, and cell division). In contrast, *L. crispatus*, along with other non-*Lactobacillus* spp., was dominant in the endometrium before spontaneous abortion, and this community had a heterogeneous functional pattern characterized by transposases and insertion elements (Figure 3, A).

The results of the metagenomic sequencing showed both taxonomic and functional differences in the 2 endometrial microbiomes from the same patient. The functional metagenomic analysis was performed with the use of information obtained from the UniRef database and clusters of orthologous (COG) groups, considering the proteins and functions associated with a specific

FIGURE 2
Endometrial microbiota profile



Endometrial microbiota profile assessed by 16S ribosomal RNA gene sequencing and whole-metagenome sequencing. **A,** Microbiota composition profiles shows the 20 most-abundant genera and their relative abundance in the sample preceding a spontaneous clinical miscarriage or a successful pregnancy in the same woman with the use of 16S sequencing or **B,** whole-metagenome sequencing. **C,** Heatmap shows the bacterial composition with associated functional pattern analyzed by whole-metagenome sequencing.

Moreno. The endometrial microbiome in early pregnancy. Am J Obstet Gynecol 2020.

TABLE 1
Sequencing reads obtained after sequencing, quality control, and elimination of human reads

Sample	Raw reads, n	Cleaned reads, n (%)	Joined reads, n (%)	Nonhuman reads, n (%)
Miscarriage	126,325,813	115,991,731 (91.8)	56,197,765 (44.5)	1,291,879 (1)
Pregnancy	112,452,320	102,731,745 (91.4)	41,138,063 (36.6)	76,160 (0.1)

Moreno. The endometrial microbiome in early pregnancy. Am J Obstet Gynecol 2020.

taxonomy, respectively. After analysis of the most represented proteins in each sample, a greater functional annotation associated with several bacteria was observed in the sample preceding the spontaneous abortion, whereas in the sample obtained during the successful pregnancy, only proteins associated with *L. iners* were detected (Figure 3, B). We also observed distinct functional profiles when we compared the main COG groups present in both samples (Figure 3, C). "Information storage and processing" was the most represented functional category in both samples, with 2285 and 798 counts per million in the sample associated with spontaneous abortion and successful pregnancy, respectively (ftp://ftp.ncbi.nlm.nih.gov/pub/COG/COG/fun.txt). Moreover, of the 25 COG subcategories established in the database, the endometrium before miscarriage showed an unstable functional pattern characterized by transposases and insertion elements belonging to the subcategory "[L] Replication, recombination and repair." For instance, we found transposases and mobile elements, like Tra8, the only member of the superfamily cl28582, (COG2826), and a member of the superfamily cl27435 (COG3547; Figure 3, B). In contrast, the microbiome during early pregnancy subcategory "[J] translation, ribosomal structure and biogenesis" was the most represented. Notably, functions associated with defense mechanisms (subcategory [V]), carbohydrate metabolism and energy production (subcategories [C] [G]), and cell division (subcategory [D]) were represented only in the sample from the successful pregnancy, where the predominant bacterium was *Lactobacillus* (Figure 3, A).

Microbes produce G protein-coupled receptor ligands to communicate with the human host and to regulate its physiologic condition.[33] In both endometrial fluid samples, we sought sequences associated with the N-acyl synthase protein family PF13444, the consensus PFAM profile of the G protein-coupled receptor. In the endometrial microbiome before the spontaneous abortion, we identified 44 sequences that corresponded to molecules of the Gcn5-related N-acetyltransferases (GNAT) domain; in the microbiome of the early pregnancy, these sequences were not found.

Comments

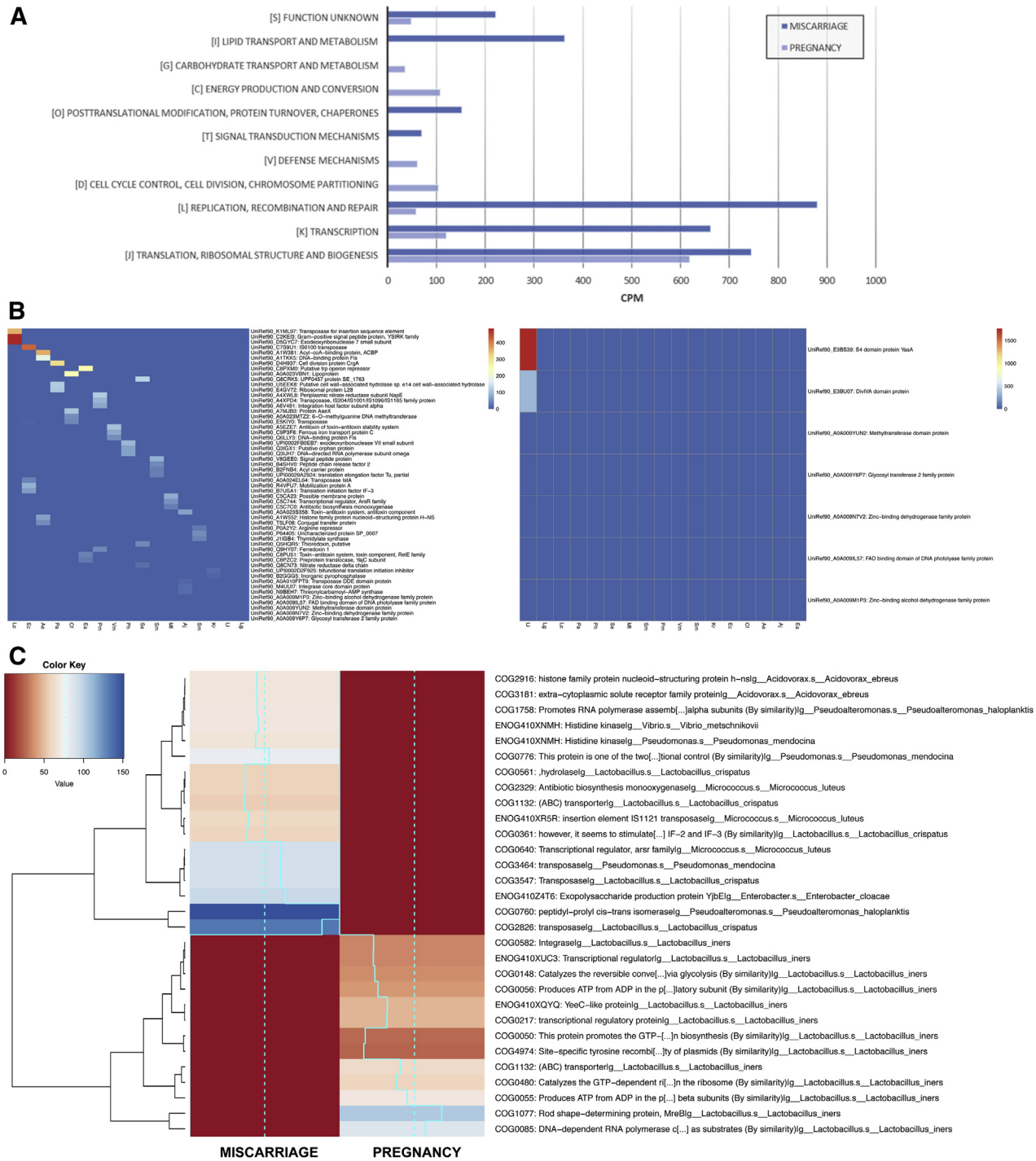
This case represents the first glimpse of the endometrial microbiome during a successful pregnancy. Moreover, we found an abnormal endometrial microbiome before spontaneous abortion in the same patient, with euploid embryos.

The microbiota of the reproductive tract is an important determinant of health and disease.[34–37] Spontaneous abortion is a syndrome that has multiple causes, which reflect the interaction of embryonic, maternal, and microbial factors.[38] The role of the host-microbial relationship in determining pregnancy outcome is poorly understood.

Although it has been demonstrated that the reproductive tract of healthy women can be colonized by *L. iners,*[39] it has been identified often in transitional communities between bacterial vaginosis and a normal microbiota.[40] For example, *L. iners* was found to be dominant after treatment for bacterial vaginosis.[41] In our study, transition to an *L. iners*-dominated microbiota after a period of instability (clinical miscarriage, followed by dilation and curettage and antibiotic treatment) was observed in the endometrial fluid present during early pregnancy when the embryo was already implanted. The genome of *L. iners* contains an iron-sulfur cluster that limits iron availability. This system may be used as a defense mechanism by providing a competitive advantage against other bacterial pathogens, or it may play a role in providing nutrients and surviving in adverse conditions such as menstruation.[42] Correspondingly, it has been found that, during menstruation, the abundance of *L. iners* in the vaginal community increases while the number of *L. crispatus* decreases.[40,43] The potential of *L. iners* to sequester iron could confer this microorganism with an advantage in respect to other bacteria to colonize the uterine cavity after dilation and curettage, where the environmental conditions are characterized by the presence of blood, similar to menstruation.

Mendes-Soares et al[44] characterized the genomes of several *L. iners* strains and found that they lack several proteins related to the acetyltransferase GNAT family and various transcriptional regulators. Indeed, these results are in agreement with our findings. The GNAT domain is implicated in bacterial antibiotic resistance, chromatin remodeling, and anabolic and catabolic functions. Three putative ligands have been found in the ChEMBL database (The European Molecular Biology Laboratory, United Kingdom) related to the GNAT domain: Luspatercept, Ecallantide, and Rilonacept, which correspond to inhibitors of activin receptor type-2B, plasma kallikrein, and interleukin-1β, respectively (Table 2). Ecallantide (Kalbitor) and Rilonacept (Arcalyst) are Food and Drug Administration–approved drugs with important effects on human health (www.accessdata.fda.gov). Rilonacept is an interleukin-1 blocker indicated for treatment of cryopyrin-associated periodic syndrome that is associated with mutations in the cryopyrin gene, which

FIGURE 3
Functional pattern associated with taxonomy



Functional pattern associated with taxonomy assessed by whole-metagenome sequencing. **A,** Bar graph summarizes the 20 most detected functions obtained with the clusters of orthologous groups results. **B,** The functional metagenomic analysis was carried out in the sample preceding a miscarriage (*left panel*) and a successful pregnancy (*right panel*) with the use of the information obtained from UniRef database and **C,** clusters of orthologous groups associated with a specific taxonomy.

Ae, *Acidovorax ebreus*; Aj, *Acinetobacter johnsonii*; Cf, *Citrobacter freundii*; Ea, *Enhydrobacter aerosaccus*; Ec, *Enterobacter cloacae;* Kr, *Kocuria rhizophila*; Lc, *Lactobacillus crispatus*; Lg, *Lactobacillus gasseri;* Li, *Lactobacillus iners*; Ml, *Micrococcus luteus;* Pa, *Propionibacterium acnes*; Ph, *Pseudoalteromonas haloplanktis*; Pm, *Pseudomonas mendocina*; Se, *Staphylococcus epidermidis*; Sm, *Stenotrophomonas maltophilia;* Sm, *Streptococcus mitis*; Vm, *Vibrio metschnikovii.*

Moreno. The endometrial microbiome in early pregnancy. Am J Obstet Gynecol 2020.

TABLE 2
Potential ligands of the Gcn5-related N-acetyltransferases sequences found in the sample obtained before spontaneous abortion

Name	Compound identification	Drug phase	Mechanism of action	ChEMBL target
Luspatercept	3039545	3	Activin receptor type-2B antagonist	Activin receptor type-2B
Ecallantide (Kalbitor)	1201837	Approved	Plasma kallikrein inhibitor	Plasma kallikrein
Rilonacept (Arcalyst)	1201830	Approved	Interleukin-1 beta inhibitor	Interleukin-1 beta

Source: ChEMBL database (The European Molecular Biology Laboratory, United Kingdom).

Moreno. The endometrial microbiome in early pregnancy. Am J Obstet Gynecol 2020.

produces an overactive inflammasome and excessive release of interleukin-1β that drives inflammation. Rilonacept blocks interleukin-1β signaling by acting as a soluble decoy receptor that binds interleukin-1β, thereby preventing activation of IL-1 receptors. In both mice and humans, interleukin-1ra binds to the interleukin-1R type 1 receptor to prevent signal transduction blocking its physiologic responses in vivo (such as hypoglycemia, induction of interleukin-6, and corticosterone production).[45,46] Embryonic implantation in mice is blocked by the interleukin-1 receptor antagonist.[47] Our group demonstrated that blockade of maternal endometrial interleukin-1R t1 with interleukin-1ra prevents implantation in the mouse by interfering with embryonic attachment, but without adverse effects on blastocyst formation, hatching, fibronectin attachment, outgrowth, and migration in vitro.[47]

L. crispatus and *L. iners* are common inhabitants of the healthy reproductive tract. These 2 species are closely related and are thought to perform similar ecologic functions. Nevertheless, there is a wide range of activity within strains of all bacteria, including *Lactobacillus* spp; differences in their genomes can explain their specificity for a given niche. Unlike other species studied, *L. crispatus* has the largest genome with unique DNA polymerase, bacteriocin, and toxin-antitoxin genes that encode mobile genetic elements, especially transposases,[48,49] which are consistent with the large number of functions related to mobile elements observed in the sample collected before spontaneous abortion. Also, other factors may influence the reproductive tract microbiota. Further studies are needed to determine the precise role of these interesting species in endometrial health and disease and whether these strains can serve as biomarkers of reproductive success or failure.

The main cause of clinical miscarriage in humans is embryo aneuploidy.[50] The strength of the investigation of the endometrial microbiota is based on the chromosomal status of the transferred embryos—assessed before embryo transfer—that was confirmed in the products of conception after spontaneous abortion and in the baby born after a successful pregnancy, ruling out embryo aneuploidy as a possible cause of miscarriage.

Predominantly, most of the high-throughput studies that characterize the endometrial microbiota have identified bacterial taxa to the genus, family, or order level but have not been able to distinguish between bacterial species. For this reason, 1 of the main contributions of this study is to describe the distinct endometrial community in pregnancy and previous to miscarriage with the use of WMS and bioinformatics tools that provide resolution at the species level.

However, some limitations must be acknowledged. First, there is some controversy about the existence of an indigenous intrauterine microbiome in the placenta or amniotic fluid in uncomplicated pregnancies[51–54] or in the endometrium of women of reproductive age, although several studies that have analyzed endometrial samples from abdominal hysterectomies have pointed to it.[4,6–8,55] The Human Microbiome Project has revealed that samples collected from the vagina contain a large amount of human DNA (approximately 96%).[1] Considering that the endometrial microbiota is a low-biomass ecosystem and its bacterial load is estimated to be between 100 and 10,000 times lower than the vaginal microbiota,[4,8] the percentage of reads corresponding to bacteria found in our study was not unexpected. Despite the limited coverage, there were enough reads to perform the analysis with 1,291,879 and 76,160 reads in the first and second samples of endometrial fluid, respectively.

Also, we have observed differences between the microbial profiles obtained by taxonomic-only or taxonomic coupled to functional analysis. A possible explanation for such differences could be the potential noise introduced in the sample by the DNA extraction kit, because it has been shown that DNA from bacterial genera (such as *Methylobacterium*, *Stenotrophomonas*, *Janthinobacterium*) could be contained in laboratory reagents, hence affecting microbiota analysis in low-biomass samples at the taxonomic-only level.[56]

Finally, the samples of endometrial fluid were collected with a transcervical catheter. We cannot exclude that some level of contamination with cervical and vaginal microorganisms may have occurred. However, there are no alternative noninvasive means to obtain endometrial samples, particularly in early gestation. The merit of studying the endometrial microbiota with the use of endometrial fluid collected in this manner needs to be ascertained by clinical studies that examine

reproductive success, given a particular microbial profile. Our findings are consistent with reports by other investigators that isolation of bacterial pathogens from the embryo transfer catheter tip is associated with poor IVF outcomes.[57–62] This raises the question of whether the microbial communities present in the reproductive tract exert their effects either inside or in close proximity to the uterine cavity, modifying physiologic conditions in the uterine cavity and reproductive fitness.

Bacteria may facilitate or hamper human conception. Our results are the first observation of taxonomic and functional differences in the endometrial fluid microbiota between an early successful pregnancy and before spontaneous miscarriage with euploid embryos in the same patient. Functional metagenomic and 16S rRNA sequencing showed a bacterial community with lower richness and diversity and higher *Lactobacillus* abundance in the early successful pregnancy compared to the miscarriage. Ultimately, using WMS, we describe distinct functional profiles in which basal metabolism and transcription regulation are the main functions in successful pregnancy. If confirmed, these findings would highlight the emerging relevance of commensal microbes in the endometrium. Our observations may also have implications in the understanding of the causes of first-trimester spontaneous abortion and to facilitate the development of diagnostic tools, which could be the basis for alternative and personalized therapeutic procedures with interventions to change the endometrial microbiota. ■

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