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The first glimpse of the endometrial microbiota in early pregnancy

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AUTHORS CONTRIBUTIONS

CS obtained the samples. IM and CS designed experiments. IG-G and MG-M performed experiments. DB, DP-V analysed data. IG-G, IM, FV, RR and CS interpreted the data. IM, IG-G, DB, DP-V, MG-M, FV, RR and CS wrote and approved the manuscript. RR did not have involvement with the patient management, collection of samples, and only contributed to the interpretation of the results and writing of the manuscript.

Conflict of interest

IM, DB, DP-V, MG-M, and CS are partial or full-time employed by Igenomix S.L. IG-G, FV and RR report no conflict of interest.

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Abstract

Investigation of the microbial community in the female reproductive tract using 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 undergoing assisted reproductive technology (ART) 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 prior to 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 two samples. *Lactobacillus crispatus* was present in the endometrium prior to 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, associating its presence 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.

Condensation

The endometrial microbiota in the same woman who subsequently had a spontaneous abortion with euploid embryos had a different profile than that of an early successful pregnancy.

Keywords

16S rRNA; Assisted Reproductive Treatments; Endometrial microbiota; *Lactobacillus crispatus*; *Lactobacillus iners*; Pregnancy; Reproductive tract microbiome; Spontaneous abortion; Whole Metagenomic Sequencing

INTRODUCTION

The efforts of the Human Microbiome Project (HMP) has highlighted the importance of microorganisms and their genomes in several human niches and has 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 using microbial culture, microscopy, and culture-independent techniques, showing that the predominant bacteria are *Lactobacilli*.[3] The endometrial cavity has been traditionally considered sterile, and the isolation of Enterobacteriaceae, *Streptococcus*, *Staphylococcus*, and *Escherichia coli* from the tip of the embryo transfer catheter has been linked with poor reproductive outcomes in patients undergoing 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 (SMS/WMS) allows investigation of species diversity and certain functional properties.[12,13]

Using 16S rRNA sequencing in specimens obtained through a transcervical catheter, the microbiota profile in the human endometrial fluid can be classified as *Lactobacillus*-dominated (LD) or non-LD (NLD), established by a cut-off of 90% *Lactobacilli*. Dysbiotic profiles (i.e., imbalanced bacterial composition for a given niche) characterized by an NLD microbiota together with specific pathogens have been associated with lower implantation, pregnancy, ongoing pregnancy, and live birth rates, as well as 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 non-pregnant 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 due to 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 high hormonal concentration of estrogen, the absence of menses, or changes in cervical and vaginal fluid.[18] The dominance of vaginal *Lactobacillus* 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 characterizing the endometrial microbiota taxonomically and functionally – using 16S rRNA sequencing and WMS – prior to an embryo transfer that resulted in spontaneous abortion and during a 4th 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 two years had undergone one unsuccessful IVF cycle (Figure 1). The patient did not have medical or surgical complications, had a body mass index (BMI) of 22, and a negative serological test 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 (ICSI) cycle, 14 metaphase-II oocytes were retrieved resulting in 13 zygotes after ICSI. Of these, 10 embryos reached the blastocyst stage, resulting in 6 euploid embryos identified by pre-implantation genetic testing for aneuploidies (PGT-A), that were vitrified.

After the first embryo transfer (ET) of two 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 used for the endometrial receptivity analysis (ERA) to guide personalized embryo transfer (pET). Subsequently, two euploid blastocysts were transferred in April 2017. Pregnancy was achieved, and the β-HCG concentration was 278.9 mIU/mL. One gestational sac 8 mm in diameter was visualized using transvaginal ultrasound during the 5^{th} week of pregnancy. A spontaneous clinical miscarriage occurred at the 8^{th} week of gestation, and dilation and curettage (D&C) 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 D&C, 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 of endometrial fluid was obtained. The pregnancy continued uneventfully, and the patient delivered a healthy male infant weighing 3,700 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 (Thermo Fisher Scientific, Waltham, MA) following manufacturer's instructions and stored at −80°C until use.

DNA extraction

Total DNA was isolated performing a pre-digestion step with lysozyme, lysostaphin and mutanolysin in order to degrade the cell wall of bacteria, followed by extraction with

QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The genomic DNA was quantified using 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

16S rRNA gene microbiota profiles were obtained using the Ion 16S metagenomics kit (ThermoFisher Scientific, Waltham, MA). This kit includes two 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 using 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 *Escherichia 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, using the Nextera DNA Flex Library Preparation kit (Illumina, San Diego, CA) following the manufacturer's instructions. The sample collected during early successful pregnancy yielded sufficient DNA to analyze in two technical replicates starting from the same preparation of genomic DNA but 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, San Diego, CA). The reads generated by the Illumina sequencing platform were quality trimmed and length filtered using PRINSEQ.[24] Paired-end reads were merged using Fast Length Adjustment of Short reads (FLASh) software tool[25] and, finally, host-reads were removed using Burrows-Wheeler Aligner (BWA) mapper against human genome reference.[26]

Functional and taxonomical joint profiling was performed using the HMP Unified Metabolic Analysis Network (HUMAnN2) pipeline.[27] This method combines taxonomic profiling of samples using MetaPhlAn2,[28] which provides a panmicrobial annotation, using a combination of clade-specific markers and functional annotation inferred by the pangenomic database resulting from MetaPhlAn2 taxonomical classification. Another annotation to assess taxonomical classification robustness was obtained using the KRAKEN software with complete bacterial, archaeal and viral NCBI Reference Sequence (RefSeq) genomes database MiniKraken DB_4GB.[29] The presence of biomedical interest protein families, such as G protein-coupled receptors (GPCRs) ligands producers, was assessed with InterProScan 5 and PFAM reference protein database.[30,31] Finally, the pipeline outputs were processed using the R statistical software[32] for statistical description and graphical representation of the sample's taxonomical 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 (SRA) 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 prior to 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 during the successful 4-week 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 2A).

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 while 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 prior to the spontaneous abortion, and alternatively, higher *Lactobacillus* abundance in the endometrial fluid sample collected in the presence of an embryo with successful implantation (Figure 2B). However, when analyzing 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 2B). 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. Due to this, the functional metagenomic analysis showed that the sample collected prior to the clinical spontaneous abortion contained *Lactobacillus crispatus* as the predominant *Lactobacillus* (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, *Lactobacillus iners* was the only microbe found in the endometrium (Figure 2C).

Functional metagenomics analysis also revealed different *Lactobacillus* species in the two samples (Figure 2C). *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* species were dominant in the endometrium prior to spontaneous abortion, and this community had a heterogeneous functional pattern characterized by transposases and insertion elements (Figure 3A).

The results of the metagenomic sequencing showed both taxonomic and functional differences in the two endometrial microbiomes from the same patient. The functional metagenomic analysis was performed using the information obtained from UniRef database and Clusters of Orthologous Groups (COGs) considering the proteins and functions associated with a specific taxonomy, respectively. After analyzing 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 3B). We also observed distinct functional profiles when comparing the main COG groups present in both samples (Figure 3C). “Information storage and processing” was the most represented functional category in both samples, with 2,285 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 prior to 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 3B). 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 only represented in the sample from the successful pregnancy, where the predominant bacterium was *Lactobacillus* (Figure 3A).

Microbes produce G protein-coupled receptor (GPCR) ligands to communicate with the human host and regulate their physiology.[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 prior to the spontaneous abortion, we identified 44 sequences corresponding to molecules of the Gcn5-related N-acetyltransferases (GNAT) domain, while 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 prior to 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 caused by multiple etiologies, reflecting 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 often identified 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 D&C 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 (Fe-S) cluster that limits the iron availability. This system may be used as a defense mechanism, 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 in order to colonize the uterine cavity after D&C, where the environmental conditions are characterized by the presence of blood, similar to menstruation.

Mendes-Soares et al. characterized the genomes of several *L. iners* strains and found they lack several proteins related to the acetyltransferase GNAT family and various transcriptional regulators.[44] Indeed, these results are in agreement with our findings. The GNAT domain is implicated in bacterial antibiotic resistance, chromatin remodeling, as well as anabolic and catabolic functions. Three putative ligands have been found in the ChEMBL database related to the GNAT domain: Luspatercept, Ecallantide, and Rilonacept, which correspond to inhibitors of activin receptor type-2B, plasma kallikrein, and interleukin-1β (IL-1β), respectively (Table 2). Ecallantide (KALBITOR) and Rilonacept (ARCALYST) are FDA-approved drugs with important effects on human health (www.accessdata.fda.gov). Rilonacept is an IL-1 blocker indicated for treatment of cryopyrin-associated periodic syndrome, associated with mutations in the cryopyrin gene, which produces an overactive inflammasome and excessive release of IL-1β that drives inflammation. Rilonacept blocks IL-1β signaling by acting as a soluble decoy receptor that binds IL-1β, preventing activation of IL-1 receptors. In both mice and humans, IL-1ra binds to IL-1R type 1 receptor, preventing signal transduction blocking its physiological responses in vivo such as hypoglycemia, induction of IL-6, and corticosterone production.[45,46] Embryonic implantation in mice is blocked by IL-1 receptor antagonist.[47] Our group demonstrated that blockade of maternal endometrial IL-1R t1 with IL-1ra prevents implantation in the mouse by interfering with embryonic attachment, 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 two species are closely related and are thought to perform similar ecological functions. Nevertheless, there is a wide range of activity within strains of all bacteria, including *Lactobacillus spp.*, and differences in their genomes can explain their specificity for a given niche. Unlike other species studied, *L. crispatus* has the largest genome with a unique DNA polymerase, bacteriocin, and toxin-antitoxin genes that encode mobile genetic elements, especially transposases,[48,49] consistent with the large number of functions related to mobile elements observed in the sample collected prior to 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 fact that the chromosomal status of the embryos transferred was assessed prior to embryo transfer, 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, one of the main contributions of this study is to describe the distinct endometrial community in pregnancy and previous to miscarriage using 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[51,52,53,54] or the endometrium of reproductive age women, although several studies analyzing endometrial samples from abdominal hysterectomies have pointed to it.[4,6–8,55] The HMP has revealed that samples collected from the vagina contain a large amount of human DNA (~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 in the second 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, as it has been shown that DNA from bacterial genera such as *Methylobacterium*, *Stenotrophomonas*, *Janthinobacterium*, etc. 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 using a transcervical catheter. We cannot exclude that some level of contamination with cervical and vaginal microorganisms may have occurred. However, there are no alternative non-invasive means to obtain endometrial samples, particularly in early gestation. The merit of studying the endometrial microbiota using 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 physiological conditions in the uterine cavity and reproductive fitness.

Conclusions

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 prior to 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 early successful pregnancy compared to miscarriage. Ultimately, using WMS, we describe distinct functional profiles in which basal metabolism and transcription regulation are 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 to understand the causes of first trimester spontaneous abortion and facilitate development of diagnostic tools, which could be the basis for alternative and personalized therapeutic procedures with interventions to change the endometrial microbiota.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a glance:

Why was the study conducted?

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 prior to a spontaneous abortion and during a successful pregnancy.

What are the key findings?

There were taxonomic and functional differences between the microbiota found in endometrial fluid collected during an early successful pregnancy and prior to 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 to that of a pregnancy failure. This observation suggests that an endometrial microbiota is present in normal pregnancy and that its composition can be different prior to a spontaneous abortion. These observations support that the endometrial microbiota may be associated with different reproductive outcomes.

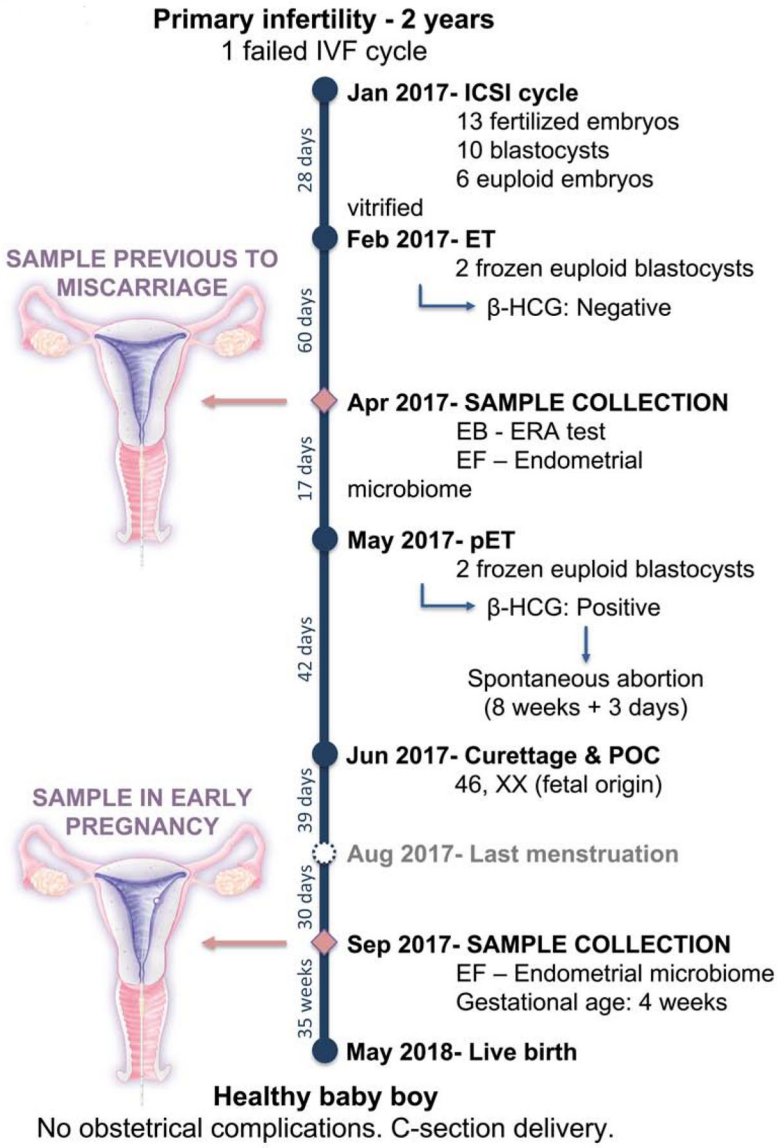

Figure 1.

Flow chart of the clinical evolution of the patient during the spontaneous abortion and successful pregnancy. 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; LD: Lactobacillus dominated; NLD: Non-Lactobacillus dominated; P: Progesterone; pET: personalized embryo transfer following the recommendation of ERA test; POC: Product of Conception.

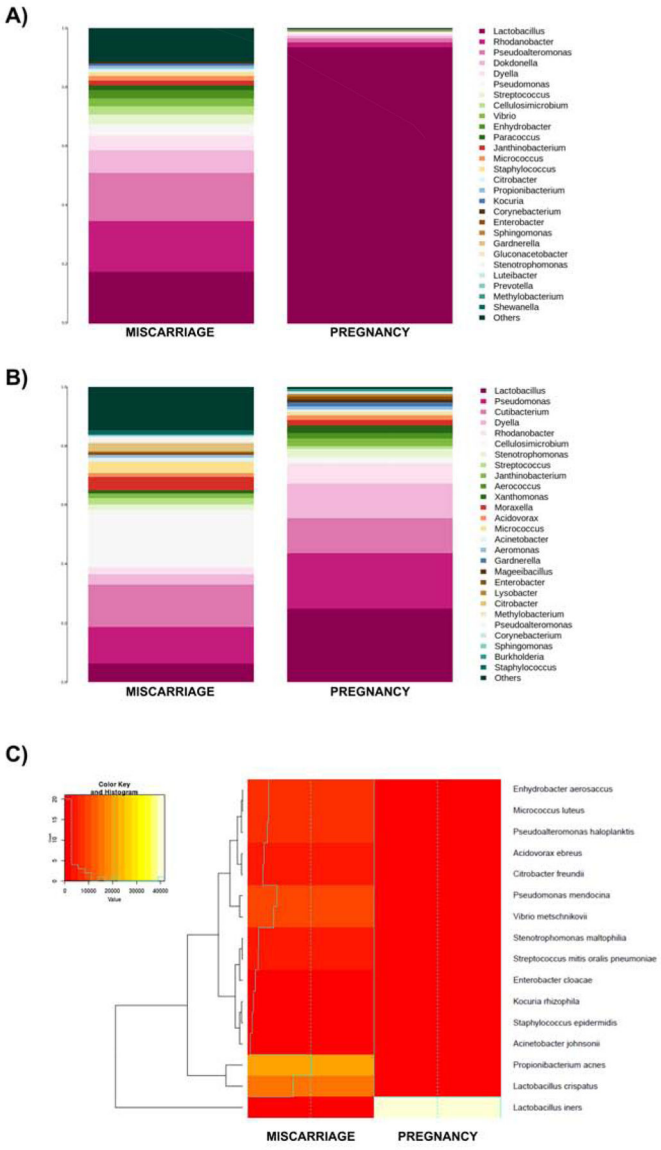

Figure 2.

Endometrial microbiota profile assessed by 16S ribosomal RNA gene sequencing and whole-metagenome sequencing (WMS). (A) Microbiota composition profiles showing the most-abundant genera and their relative abundance in the sample preceding a spontaneous clinical miscarriage (MISCARRIAGE) or a successful pregnancy (PREGNANCY) in the same woman using 16S sequencing or (B) WMS. (C) Heatmap showing the bacterial composition with associated functional pattern analyzed by WMS.

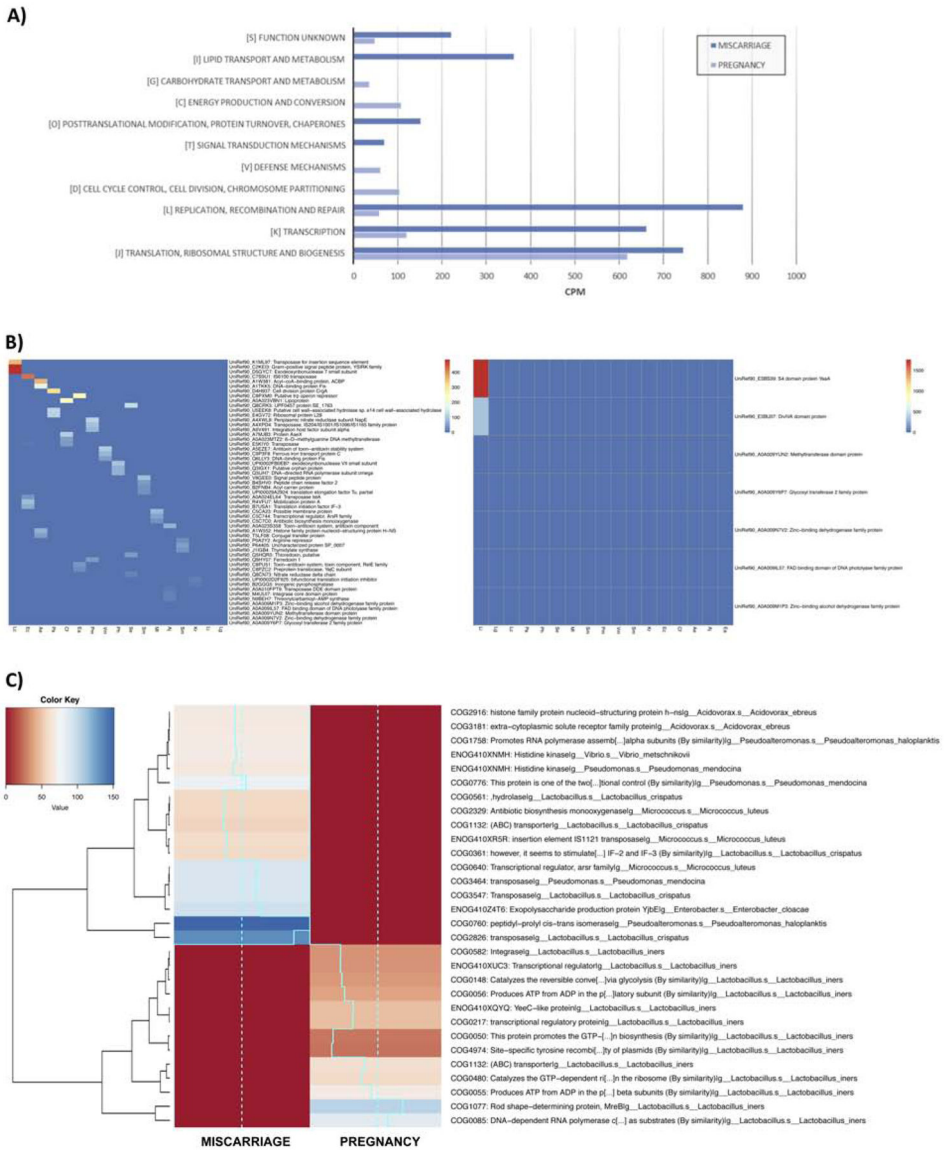

Figure 3.

Functional pattern associated with taxonomy assessed by whole-metagenome sequencing. (A) Bar graph summarizing the 20 most detected functions obtained with the COGs results. (B) The functional metagenomic analysis was carried out in the sample preceding a miscarriage (left panel) and a successful pregnancy (right panel) using the information obtained from UniRef database and (C) Clusters of Orthologous Groups (COGs) 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*.

Table 1.

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

Sample	Raw reads	Cleaned reads (%)	Joined reads (%)	Non-human reads (%)
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%)

Table 2.

Potential ligands of the GNAT sequences found in the sample obtained prior to spontaneous abortion. Source: ChEMBL database.

Name	Compound ID	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	lnterleukin-1 beta inhibitor	lnterleukin-1 beta