Recurrent pregnancy loss is associated with unfavourable composition of endometrial and vaginal microbiota.

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Research Article

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Abstract

Research Question Does endometrial or vaginal microbiota composition associate with recurrent pregnancy loss (RPL)?

Design Endometrial and vaginal samples were collected from 47 women with two or more consecutive pregnancy losses and 39 healthy control women without a history of pregnancy loss between March 2018 and December 2020 in Helsinki University Hospital, Helsinki, Finland. The endometrial and vaginal microbiota compositions, analysed using 16S rRNA gene amplicon sequencing, were compared between RPL and control women, and between individual vaginal and endometrial samples. The mycobiota composition was analysed using internal transcribed spacer 1 amplicon sequencing for a descriptive summary. The models were adjusted for BMI, age, and parity. False discovery rate -corrected P -values (q -values) were used to define nominal statistical significance at $q < 0.05$.

Results *Lactobacillus crispatus* was less abundant in the RPL women's endometrial samples compared to controls (mean relative abundance 17.2% vs. 45.6%, $q = 0.04$). *Gardnerella vaginalis* was more abundant in RPL women than in controls in both endometrial (12.4% vs. 5.8%, $q < 0.001$) and vaginal samples (8.7% vs. 5.7%, $q < 0.01$). The individual vaginal and endometrial microbial compositions correlated strongly ($R = 0.85$, $P < 0.001$). Fungi were detected in 22% of the endometrial and 36% of the vaginal samples.

Conclusions Unfavourable reproductive tract microbiota is associated with RPL and may represent a novel risk factor for pregnancy losses.

Key Message

Lactobacillus crispatus was less and *Gardnerella vaginalis* more abundant in endometrial samples of 47 women with recurrent pregnancy loss, compared to 39 healthy control women. Unfavourable endometrial microbiota may be a novel risk factor for RPL, a condition that currently often remains unexplained in standard examinations.

Introduction

Recurrent pregnancy loss (RPL) is one of the most challenging issues in reproductive medicine because its causes are often unknown, and effective treatment is rarely available. European Society of Human Reproduction and Embryology (ESHRE) defines RPL as the spontaneous loss of two or more pregnancies (Bender Atik et al., 2018), and it affects 1-3% of couples trying to have a child. Recognised causes for RPL are chromosomal abnormalities, uterine malformations, antiphospholipid syndrome, and endocrinological disorders (Rai and Regan, 2006), while over half of the RPLs still remain unexplained.

Several studies have suggested an infectious aetiology behind miscarriages (Giakoumelou et al., 2016; McQueen et al., 2015). Chronic endometritis has been associated with RPL (McQueen et al., 2021) and bacterial vaginosis (BV) has been linked to the risk of miscarriage (Haahr et al., 2019; Ralph et al., 1999). Recent studies using high-throughput DNA sequencing techniques have shown that *Lactobacillus* spp. dominate the vaginal bacterial composition in healthy early pregnancy (Freitas et al., 2017; MacIntyre et al., 2015), while the reduced prevalence of lactobacilli has been associated with pregnancy loss (Al-Memar et al., 2020). Mycobiota, consisting of various species of fungi, is another important element of the vaginal ecosystem (Bradford and Ravel, 2017). However, there are no studies examining mycobiota in relation to reproductive outcomes.

The uterine cavity has long been thought to be sterile, but recent studies have reported that endometrium may have a distinct microbiome (Chen et al., 2017; Koedooder et al., 2019). Like in the vagina, the dominant species in the endometrium are usually lactobacilli (Chen et al., 2017, Oberle et al., 2021), and alteration of this composition may affect reproductive outcomes. In patients treated with in vitro fertilization (IVF), non-*Lactobacillus* dominated endometrial microbiota has been associated with lower implantation rates, clinical pregnancy rates, and live birth rates compared to *Lactobacillus*-dominated microbiota (Moreno et al., 2016). The impact of endometrial microbiota on RPL has not been investigated before.

The goal of our study was to explore the composition of microbiota and mycobiota in endometrial and vaginal samples in women with RPL and compare the results to healthy women without a history of miscarriages. We also investigated whether the composition of vaginal microbiota reflects the composition of endometrial microbiota.

Material And Methods

Study population

TOIVE is a prospective cohort study of the immunological and microbiological causes of RPL, conducted at the University of Helsinki and Helsinki University Hospital (HUS), Finland. Between March 2018 and June 2020, we recruited 51 women referred to the Reproductive Medicine Unit and the Department of Obstetrics and Gynaecology of Hyvinkää Hospital due to RPL (Figure 1). Women were eligible if they had a history of three or more consecutive clinical first-trimester pregnancy losses or two losses, with at least one of them in the second trimester. We defined clinical pregnancy loss as a spontaneous loss of an intrauterine pregnancy confirmed by an ultrasound, or positive urine or serum human chorionic gonadotrophin and over six weeks of amenorrhea.

As controls, we recruited 40 women investigated for male factor infertility between June 2018 and December 2020. They represented healthy Finnish women since they had no history of pregnancy losses, endometriosis, anovulation, or fallopian tube defects. Participants were excluded if they were <18 or ≥40 years of age, had hepatitis or HIV infection, or had an irregular menstrual period (<21 or >42 days).

Power calculations were not applicable to our associative and novel setting since earlier studies on microbiota composition and RPL are lacking. The ethics committee of HUS approved the study (No. HUS/3635/2017).

Collection of clinical data

All participants gave their written informed consent. Upon enrolment, we took a detailed medical and reproductive history, and participants received a questionnaire related to previous infections, the use of anti- or probiotics, sexual behaviour, and educational background. The basic examinations for RPL included transvaginal 2D-ultrasound, complete blood count, phospholipid antibodies, karyotyping (of both partners), thyroid function tests, thyroid peroxidase antibodies, fasting glucose, and glycosylated haemoglobin. Screening for thrombophilia, celiac disease, hyperprolactinemia, or uterine anomalies with 3D-ultrasound or hysteroscopy were performed when necessary. Women were not routinely tested for sexually transmitted diseases nor chronic endometritis.

Endometrial and vaginal sampling

We collected vaginal and endometrial samples in the mid-luteal phase, 6-8 days after a positive ovulation test (Clearblue Digital®, Swiss Precision Diagnostics GmbH, Switzerland). Couples were advised to use a condom or abstain from sexual intercourse during the menstrual cycle in which the samples were taken for contraception and to avoid the effects of seminal fluid on the microbiota. Vaginal samples were collected in a speculum examination from the right and left fornix with sterile flocked swabs (FLOQSwabs, Copan spa®, Italy) and severed to 1.5 mL Eppendorf tubes. We used non-sterile examination gloves and a white coat during sampling, but not a mask or hair cover. Lubricants were not used. Endometrial samples were collected to 1.5 mL Eppendorf tubes with endometrial biopsy curette (Pipelle®, Prodimed, France) without contact to vaginal walls. Eppendorf tubes were frozen at -20 °C immediately after sampling and moved to -80 °C within two weeks.

DNA extraction

Microbial DNA was extracted from the vaginal samples using a beat beating method as previously described (Virtanen et al., 2019). One-sixth of the endometrium biopsies (average weight 33 mg; SD 22) was used for DNA extraction with negative controls using the same method as for the vaginal samples. DNA was quantified using Quanti-iT Pico Green dsDNA Assay (Invitrogen®, San Diego, CA, USA). The DNA yields for both sample types were comparable; mean 78.8 (SD 60) ng/μl in vaginal and 43.8 (SD 30) ng/μl in endometrial samples. The negative controls (no input sample for DNA extraction) did not contain detectable amounts of DNA.

16S rRNA gene and Internal transcribed spacer (ITS) amplicon sequencing

Illumina MiSeq paired-end sequencing of PCR-amplicons from the hypervariable V3-V4 regions of the bacterial 16S rRNA gene (primers 341F/785R) and fungal ITS-1 region (primers ITS1F and ITS2) was prepared and performed as explained in detail elsewhere (Virtanen et al., 2021). Briefly, bacterial and fungal amplicons were prepared separately, combined for indexing in a 1:1 ratio, and an equimolar pool was sequenced using 2 × 300 bp reads and a MiSeq v3 reagent kit at the Biomedicum Functional Genomics Unit (FuGU), Helsinki, Finland.

Sequence processing and analysing

The primary step of the analysis was to split the combined 16S rRNA gene and ITS sequence fastq-files into separate datasets. This was done by first removing ambiguous (N) bases from the reads, followed by primer-based separation using cutadapt v3.5, removing the primers in the process. From this step onwards, all pre-processing was carried out individually on both datasets. The bacterial 16S rRNA gene dataset was processed using dada2 v1.20, following the pipeline tutorial v1.16, while the ITS pipeline tutorial v1.8 was used for processing the ITS sequence dataset.

The dada2 tutorials were only followed until ASV table construction and removal of chimeras, after which the taxminer package was used to assign taxonomic annotations (Saqib, 2021). This is a BLAST-based annotation tool combined with text-mining-based filtration to assign the most likely annotations. A detailed description of this approach has been communicated elsewhere (Oksanen et al., 2020).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 25 (IBM Corporation, Armonk, NY, USA) software for univariate data. Two-sample t-test and Mann–Whitney U test compared the continuous background variables, Pearson chi-square, and Fisher's exact tests compared the categorical variables between RPL women and controls. Parity was categorized as nulliparous and parous (≥ 1 delivery).

The primary outcomes were mean relative abundances of bacteria and fungi in the endometrial and vaginal samples. Associations between background variables and the microbiota were analysed with permutational analysis of variance (PERMANOVA) for the overall microbiota variation using the `adonis2` function with 99 999 permutations from the `vegan` package (18). Potential confounders to adjust models for differential abundance analysis downstream were identified based on the following criteria: 1) factors previously identified to influence microbiota and RPL and 2) confounders with statistical significance, determined by PERMANOVA analysis, ordination plots, and the `GroupTest` function from the package `mare` (Korpela, 2016). `GroupTest` performs differential abundance analysis for taxa within user-defined groups, in this case each background variable. The function finds the most suitable model for individual taxa and uses read counts as an offset within the model formula to account for sequencing depth. Lastly, cases and controls were used as the grouping variable in `GroupTest` to determine the significantly different taxa in endometrial and vaginal samples between RPL and control women. Based on the criteria mentioned above, body mass index (BMI), parity, and age were selected as confounders. The P -values obtained were adjusted for multiple testing by False Discovery Rate (FDR)-correction and reported as q -values. The relationship between individual vaginal and endometrial microbiota was investigated using Pearson correlation coefficient.

Results

We sampled 47 women with RPL and 39 control women to study the composition of the endometrial and vaginal microbiota and mycobiota. In one RPL woman, only the vaginal sample was obtained. RPL women had a history of three (range 2–5) consecutive miscarriages, of which 13 (15.3%) had occurred during the second trimester of pregnancy. Nine women had a likely explanation for their miscarriages: five were diagnosed with congenital uterine malformation, three with acquired thrombophilia, and one with antiphospholipid syndrome and chromosomal translocation.

The RPL women were older (mean 33.2 [22–39] vs. 32.1 [26–38] years, $P = 0.04$), had a higher BMI (mean 25.4 [19.5–39.4] vs. 23.3 [19.4–33.1] kg/m², $P = 0.02$), and were parous more often than the controls (50.0% vs. 15.4%, $P < 0.001$) (Table S1). The RPL women had more often self-reported recurrent (≥ 3) candida vaginitis, BV, and regular oral or vaginal probiotics use than the controls.

16S rRNA gene sequencing results

After taxonomic annotations and quality filtration (> 500 reads), 74% (34/46) of the RPL and 77% (30/39) of control women's endometrial samples remained for bacterial analysis with an average read count of $\sim 4\,500$ (535–20 829), and $\sim 4\,500$ (569–11 540), respectively. Similarly, 98% of the vaginal RPL samples

(46/47) and 100% of controls (39/39) remained, with an average read count of ~23 600 (3 728–47 923) and ~24 300 (1 322–55 011).

PERMANOVA analysis showed a difference in the overall endometrial microbiota composition between the RPL and control women ($R^2 = 0.050$, $P = 0.01$, Figure S1, Table S2), but the vaginal bacterial compositions were similar between the groups (Figure S2, Table S2). BMI was a strong clinical explanatory factor for the endometrial microbiota ($R^2 = 0.057$, $P = 0.005$), especially among RPL women ($R^2 = 0.09$, $P = 0.005$). Current vaginitis symptoms, use of vitamin D and folic acid, microbiota diversity, and read count explained the variability of the vaginal microbiota in the whole study population, and among the RPL women also BMI, age, and probiotics. Parity, gravidity, or curettages had no impact on the endometrial or vaginal microbiota variation.

In the endometrial samples, 37 bacterial species were identified (Figure 2A). *L. crispatus* was significantly less and *L. jensenii* more abundant in the RPL women compared to controls (mean relative abundance of *L. crispatus* 17.2% vs. 45.6%, respectively, $q = 0.04$, *L. jensenii* 5.6% vs. 3.6%, $q < 0.01$) (Figure 3, Table S3). *L. iners* was the most dominant endometrial bacterium in the RPL women (mean relative abundance 32.2% vs. 20.0%, $q > 0.05$). *Gardnerella vaginalis* was more abundant in the RPL women compared to controls (12.4% vs. 5.8%, $q < 0.001$). None of the intestinal bacteria, such as *Escherichia coli*, *Blautia spp.*, and *Faecalibacteria spp.*, nor the uncultured bacteria (bacteria that lack culture-based genomic and physiological characterization) were significantly differentially abundant in the RPL women compared to the controls.

In the vaginal samples (Figure 2B), the RPL women had more *G. vaginalis* (mean relative abundance 8.7% in RPL women vs. 5.7% in controls, $q < 0.01$) and less *G. Leopoldii* (1.0% vs. 3.2%, respectively, $q < 0.001$), whereas *L. crispatus* was the most abundant bacterium (35.1% vs. 47.5%, $q > 0.05$) (Figure 3, Table S3). In the pooled endometrial and vaginal samples, *L. crispatus* was less and *G. vaginalis* more abundant in RPL women than in controls (*L. crispatus* 27.5% vs. 46.6%, $q = 0.04$; *G. vaginalis* 10.2% vs. 5.7%, $q < 0.001$).

There were three blank samples within this pipeline that were analysed with the same pre-processing and filtration criteria. Only two out of these three samples had a considerable number of reads, which totalled to ~8500 (Figure S3). These annotated to *Streptococcus oralis* (~4000), *Fannyhessia vaginae* (~600), *Prevotella amnii* (~1700), and *Sneathia vaginalis* (~1300). *S. oralis*, a potential oral contaminant, was not detected in any other samples. *F. vaginae*, *P. amnii*, and *S. vaginae* are known vaginal microbes, and their presence in the blank sample is most likely due to contamination from the primary study samples. Due to

these reasons, we did not subtract these reads from the sample data. Furthermore, none of these bacteria were significant in our downstream analysis, and therefore not crucial for the main study comparisons.

ITS sequencing results

After taxonomic annotations and quality filtration, 24% (11/46) of RPL and 21% (8/39) of control women's endometrial samples remained for the fungal analysis, with an average read count of ~14 500 (1031–81 809) and ~12 000 (1383–23 999) respectively. Similarly, 36% (17/47) of the RPL and 36% (14/39) of control women's vaginal samples remained after filtration, with an average read count of ~6000 (742–22 500) and ~13 000 (542–30 685). Since fungi could only be detected in a small fraction of the samples, they may not sufficiently represent the entire study cohort. We therefore refrain from statistical analysis and present a descriptive overview of the taxonomic profiles.

The most prevalent genus was *Candida*, which was detected in 19/39 samples (Figure S4). *C. albicans* was not detected in RPL women's endometrium, although it was the most abundant taxa in their vaginal samples (mean relative abundance 26.9%) and in control women's endometrial and vaginal samples (mean relative abundance 49.9% and 42.9%, respectively). On the other hand, *C. parapsilosis* was only detected in RPL women, with an average relative abundance of 18.2% in endometrial and 11.8% in vaginal samples.

Comparison of microbiota in vaginal and endometrial samples

To study the relationship between microbiota colonizing endometrium and vagina, we compared samples collected from these anatomical sites of the same woman. We found a strong within-individual correlation between vaginal and endometrial microbiota composition (mean Pearson's correlation coefficient 0.85, $P < 0.001$, Figure 4, Figure S5). The overlap was strongest in *Lactobacillus*-dominated samples, while intestinal and uncultured bacteria were more abundant in the endometrium, especially in RPL women.

Data availability

The sequencing data that support the findings of this study are available in the European Nucleotide Archive (PRJEB48310). All code has been deposited to GitHub:

<https://github.com/SchahzadSaqib/TOIVE>. The clinical metadata are not publicly available due to privacy but are available from the corresponding author upon reasonable request.

Discussion

We observed an association between RPL, reduced *L. crispatus* and increased *G. vaginalis* abundance in the endometrium, and an increased *G. vaginalis* abundance in the vagina. The composition of vaginal microbiota was in concordance with the endometrial microbiota.

Our finding of a reduced abundance of *L. crispatus* in RPL endometrial samples is in line with prior studies reporting that in vagina, *L. crispatus* dominance is considered a healthy microbial environment (Kindinger et al., 2017; Petrova et al., 2017). Furthermore, *L. crispatus* has been shown to be less abundant in the endometrium of patients with chronic endometritis (Liu et al., 2019), a condition associated with RPL (McQueen et al., 2021). In contrast, *L. iners*, which was the most dominant microbe in the RPL women's endometrium, has been associated with dysbiosis (Petrova et al., 2017) and adverse reproductive outcomes, including subfertility (Campisciano et al., 2021), spontaneous miscarriage (Nasioudis et al., 2017), and preterm birth (Kindinger et al., 2017). Our observations support the beneficial effect of *L. crispatus* and an unfavourable effect of *L. iners* on reproductive outcomes.

We also showed an association between endometrial and vaginal *G. vaginalis* colonization and RPL. *G. vaginalis* is typically dominant in BV, which has been associated with early (Haahr et al., 2019; Ralph et al., 1999) and especially with late miscarriages (Leitich and Kiss, 2007). Miscarriage has also been linked with *Lactobacillus* depletion and high bacterial diversity in vaginal samples collected during early pregnancy (Al-Memar et al., 2020). Kuon et al. (2017) reported that RPL women, who had vaginal *G. vaginalis* colonization, showed higher peripheral blood natural killer (NK) cell levels suggesting a link between dysbiotic reproductive tract microbiota, inflammation, and miscarriage. Furthermore, *Lactobacillus* depletion and the presence of pathogenic bacteria such as *Bifidobacterium*, *Gardnerella*, *Klebsiella*, and *Neisseria* in endometrial biopsies has been associated with unsuccessful reproductive outcomes in IVF treatment (Moreno et al., 2021).

Beneficial endometrial microbiota may support early pregnancy, while abnormal microbiota can be detrimental, although the underlying mechanisms are still poorly understood (Al-Nasiry et al., 2020; Bardos et al., 2020; Benner et al., 2018). In vitro, *L. crispatus* has been shown to attach to the decidualized endometrial cells and occupy the attachment sites from pathogenetic microbes (Shiroda and Manning, 2020). Conversely, unfavourable endometrial microbiota may weaken the epithelial tight junctions, allowing pathogens to enter the endometrial stroma and induce a harmful immune reaction (Al-Nasiry et al., 2020). The activation of Toll-like receptors on the surface of endometrial cells by microbial molecules may elicit the secretion of cytokines that alter the local immune environment (Benner et al., 2018), leading to poor NK cell maturation. This may provoke disturbances in placentation (Al-Nasiry et al.,

2020) since NK cells are essential in trophoblast invasion (Moffett and Shreeve, 2015) and remodelling of spiral arteries (Smith et al., 2009). Abnormal endometrial microbiota may also favour endometrial T-helper 1 cell types (Bardos et al., 2020), which is thought to predispose to RPL (Wang et al., 2020).

The origin of endometrial microbes is still unclear, but vagina and gastrointestinal tract have been suggested (Bardos et al., 2020). Our and others' (Walther-António et al., 2016; Wang et al., 2021) findings of the concordance between vaginal and endometrial microbiota speak in favour of the vaginal route. Other possible mechanisms include hematogenous spreading from the gastrointestinal tract or retrograde ascension from the peritoneal cavity through fallopian tubes. Interestingly, we detected oxygen-sensitive intestinal bacteria, including *Blautia* and *Faecalibacterium spp.*, in endometrial samples. Also, Verstraelen et al. (2016) detected *Bacteroides spp.* in the endometrial samples of women with RPL or recurrent implantation failure which support the theory of peritoneal route. However, although we detected intestinal bacteria solely in the endometrial samples, it cannot be excluded that these bacteria have ascended from the perineum through vagina and remained under detection level due to unfavourable conditions, such as low pH. Finally, the role of intestinal vs. vaginal microbes in the uterus and their potential effects on reproductive health remain to be elucidated.

Strengths and limitations

To our knowledge, this is the first study comparing RPL women's endometrial microbiota with healthy controls. Our study population was ethnically and even genetically homogenous. Sampling was timed to the mid-secretory phase to analyse the microbiota during the receptive state of the endometrium, since the composition of vaginal (Lopes dos Santos Santiago et al., 2012) and endometrial microbiota (Kadogami et al., 2020; Khan et al., 2016) may vary throughout the menstrual cycle. We used a recommended technique, transcervical biopsy, for collecting endometrial tissue (Molina et al., 2021) because biopsies represent mucosal microbiota better than superficial samples, such as swabs or endometrial fluid (Liu et al., 2018). In addition to the microbiota, we also analysed the endometrial mycobiota, which has not been previously explored. Our control women represent the general population as closely as possible since they were healthy and did not have previous miscarriages or conditions known to associate with alterations in reproductive tract microbiota, such as endometriosis (Khan et al., 2016; Wei et al., 2020), endometrial polyps (Fang et al., 2016), PCOS (Tu et al., 2020), or fallopian tube occlusion (Haahr et al., 2019). We also studied the influence of various lifestyle factors on our findings. However, the applicability of our results to the general population remains to be explored.

As a limitation, low microbial abundance specimens, such as the endometrium, are susceptible to contamination (O'Callaghan et al., 2020). Although we avoided contact with the vagina during sampling,

cervicovaginal contamination cannot be ruled out since similarities existed between individual vaginal and endometrial samples. However, growing evidence supports the theory that vaginal and endometrial ecosystems are not separate, but can share microbes (Chen et al., 2017; Walther-António et al., 2016; Wang et al., 2021), and only a minority of studies have questioned the existence of a uterine microbiota (Winters et al., 2019). Although cervical mucus protects the uterine environment, sperm pass from the vagina to the uterus and vaginally administered radioactively labelled albumin macrospheres spread in the uterine cavity within minutes (Kunz et al., 1997). Therefore, it is likely that microbes ascending from the vagina may colonize the endometrium. Also, the composition of endometrial microbiota has been reported to be highly similar in laparoscopically and trans-cervically taken samples (Chen et al., 2017), and common vaginal microbes, including lactobacilli, have been found in the endometrium even after hysterectomy (Chen et al., 2017; Miles et al., 2017; Mitchell et al., 2015). Overall, even if there were cervicovaginal contamination of our endometrial samples, it does not explain the observed differences between RPL women and controls.

Environmental contamination was not likely since bacteria found in the negative controls, such as *S. oralis*, were not found in women's samples, while reads for *Lactobacillus* and *Gardnerella* species were not substantial. The presence of vaginal microbes such as *F. vaginae*, *P. amnii*, and *S. vaginae* could be explained as contamination, however, considering their low abundance and prevalence in the study samples, as well as lack of significance within the group comparisons indicates that they had not significant effect on the overall results.

Conclusions

RPL was associated with an unfavourable female reproductive tract microbiota, especially in the uterus. An unhealthy endometrial microbial environment may be a new background cause for RPL, possibly contributing to an adverse immunological response during implantation and placentation. Further research should examine the mechanisms of how altered microbiota may contribute to RPL, evaluate if the prognosis of following pregnancies can be assessed according to the microbiota profile, and whether endometrial microbiota could be modified to increase the success of future pregnancies in some couples having RPL.

Declarations

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Figures

Figure 1

Flow chart describing the study design. RPL means recurrent pregnancy loss.

Figure 2

Stacked bar plots showing bacterial relative abundances in women with recurrent pregnancy loss (RPL) and control women, ordered based on top three most prevalent and abundant taxa (*Lactobacillus crispatus*, *L. iners*, and *Gardnerella vaginalis*). (A) Endometrial samples and (B) Vaginal samples.

Figure 3

Illustration and summary of the main results and statistical analysis. (A-B) Bacterial mean relative abundances within the endometrial/vaginal and recurrent pregnancy loss (RPL)/control subgroups. (C - E) Violin-Boxplots showing the distribution of data in each subgroup for taxa that were significantly differentially abundant after adjusting with age, parity, and BMI in the endometrial (C), vaginal (D) and pooled samples (E). Asterisks indicate whether there are any statistically significant differences between the RPL and control (reference) subgroups; *** $q < 0.001$, ** $q < 0.01$, * $q < 0.05$. Pooled means the combined results of endometrial and vaginal samples.

Figure 4

(A) Polar stacked bar plot illustrating the similarity of taxonomic profiles between endometrial (right) and vagina samples (left). The samples are arranged in the same order from top to bottom, creating a mirror-image between the two semi-circles and allowing direct comparison between the sample types. (B) Pearson's correlation between the paired endometrial and vaginal samples, arranged in the same order.

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