Translocation of vaginal microbiota is involved in impairment and protection of uterine health

Jinfeng Wang1,2,7, Zhanzhan Li1,2,7, Xiuling Ma1,7, Lifeng Du1, Zhen Jia1,3, Xue Cui3, Liqun Yu3, Jing Yang4, Liwen Xiao1, Bing Zhang1, Huimin Fan1 & Fangqing Zhao1,2,5,6

The vaginal and uterine microbiota play important roles in the health of the female reproductive system. However, the interactions among the microbes in these two niches and their effects on uterine health remain unclear. Here we profile the vaginal and uterine microbial samples of 145 women, and combine with deep mining of public data and animal experiments to characterize the microbial translocation in the female reproductive tract and its role in modulating uterine health. Synchronous variation and increasing convergence of the uterine and vaginal microbiome with advancing age are shown. We also find that transplanting certain strains of vaginal bacteria into the vagina of rats induces or reduces endometritis-like symptoms, and verify the damaging or protective effects of certain vaginal bacteria on endometrium. This study clarifies the interdependent relationship of vaginal bacterial translocation with uterine microecology and endometrial health, which will undoubtedly increase our understanding of female reproductive health.
The microbiota in different niches of the human body may not be as separate from each other as expected1–3. Heterologous bacteria may be transmitted and colonize another tissue or organ universally, stimulate inflammation, and increase the risk of diseases such as cancer. A series of examples of microbial cross-colonization have been noted, such as transmission of symbiotic bacteria from other sites to the uterine cavity and fetus in the body of pregnant women4,5 and the induction of preeclampsia due to translocation of intestinal bacteria to the placenta6. Similarly, it was believed that gastric acid prevents effective microbial communication and translocation between the oral cavity and intestine. However, recently, oral bacteria have been determined to frequently migrate to the intestine via the digestive tract7,8. Enrichment of specific oral bacteria such as Fusobacterium nucleatum was observed in both pancreatic cancer and colorectal cancer9,10.

In abnormal cases, the female reproductive tract and elucidate the effect of perturbation of the vaginal microbiota11,12, there was a lack of understanding of the microbial community in the uterine cavity. Fortunately, such knowledge has been rapidly accumulating owing to the increased sensitivity of microbial detection13–15. It has been reported that living microbes may be ubiquitous in the female upper reproductive tract, including in the uterus16,17. With respect to the microbial taxa largely shared among different parts of the reproductive tract of the same individual, which demonstrate gradual changes from the vagina to the peritoneum, the microbes harbored in the female upper reproductive tract have been speculated to migrate from the lower genital tract or be transferred from the peritoneal fluid18.

Various Lactobacillus species are the dominant bacteria in the vagina of healthy reproductive-age women19. The lactic acid they produce maintains the low pH of the vaginal environment, inhibits the growth of harmful bacteria, and maintains the microecology in a relatively balanced state20. In abnormal cases, however, the abundance of vaginal Lactobacillus may decrease significantly, causing the pH to rise. Elevated pH leads to the proliferation of harmful bacteria such as Gardnerella and Prevotella, which leads to dysbiosis and an increased risk of various diseases, including bacterial vaginosis (BV) and urinary tract infections (UTIs)21,22. Likewise, alteration of the uterine microbiome is closely associated with various intrauterine diseases23, such as endometriosis, endometrial polyps, and endometrial cancer24,25, and can even affect endometrial receptivity to blastocyst26. Therefore, the ecological stability of the vaginal and uterine microbiota plays an important role in the health of the female reproductive system. The uterine cavity and the vagina are physiologically adjacent channels. Hence, theoretically, the bacteria that colonize the vagina have the opportunity to migrate upward to the uterus via the cervix. Although certain studies have speculated that intrauterine infection is caused by vaginal bacteria ascending to the uterine cavity27, the communication of microbes between these two body sites is still unclear, and the mechanisms underlying the modulation of the microbiota in uterus and induction of disease when vaginal bacteria translocate to the upper reproductive system remain obscure.

In this work, we collect both vaginal and uterine samples from healthy women and women with chronic endometritis and conduct data mining of the reproductive tract microbiomes of more than 1000 samples. By combining these results with those of animal experiments, we reveal microbial translocation in the female reproductive tract and elucidate the effect of perturbation of the vaginal microbiota on intrauterine microbiota and reproductive health. These results demonstrate the interdependent effect of the vaginal microbiota and bacterial translocation on uterine microecology and endometrial health, which will increase our understanding of microbial cross-transmission in the female reproductive system as well as their role in modulating the physical health of women.

Results
We recruited 145 women aged 19–71 years in the first cohort of our study, 106 of whom had experienced at least one abortion and 95 of whom had given birth with different modes of delivery (72 vaginal delivery vs 23 cesarean section) (Fig. 1a). Both uterine and vaginal samples were collected from each subject. The 16S rRNA gene V3–V4 regions were amplified and sequenced successfully from these samples, yielding a total of 48,659,278 PE250 reads. After merging the PE reads into long sequences and trimming out low-quality and chimera sequences, 40,348,849 V3–V4 region sequences were obtained for subsequent analysis, with an average of ~149,000 reads for uterine samples and ~158,000 reads for vaginal samples. We also retrieved the 16S rRNA gene sequencing data of 308 uterine and 653 vaginal samples of women (aged 15–83 years) from six previous projects16,18,24,28–30. Together with the new samples obtained in the present study, a total of 1223 samples were included in the meta-analysis.

Dynamic changes in the uterine and vaginal microbiome during aging. Given that aging in humans results in extensive changes in physiological functions and metabolism, it is likely that aging is one of the key factors that affect the human microbiota, including that of the reproductive system. We divided the women into six groups according to their age at 10-year intervals. The uterine and vaginal microbiome were profiled to elucidate the microbial transitions during aging and to explore the potential relationship between the aging process and female reproductive tract disorders.

We first observed that the microbial diversities of both the vaginal and uterine microbiome varied with age (Fig. 1b). The trend lines representing the average Shannon diversities were relatively stable in the age groups under 40 but began to fluctuate in the group of women above 40. In the uterine cavity, the youngest women demonstrated the highest alpha diversity, and the diversity decreased slightly with advancing age. However, in the vagina, microbial diversity and evenness increased gradually with age and demonstrated the highest value in the oldest women (Supplementary Fig. 1), which suggests that both the uterine and vaginal microbiome undergo alterations with aging. We further applied a Pearson’s correlation test on unbalanced data and found that age was significantly correlated with the microbial diversity in both the uterus (r = –0.173, P < 0.01) and vagina (r = 0.29, P < 0.001).

To characterize the uterine and vaginal microbiome at different ages, we calculated the Bray-Curtis (BC) distance of the microbial community between individuals in each age group at the OTU level (Fig. 1c). The interindividual community dissimilarities demonstrated a pattern of an initial increase with age (r = 0.17, P < 0.001 for uterus; r = 0.28, P < 0.001 for vagina, Pearson’s test), followed by a gradual decrease (r = –0.05, P = 0.006 for uterus, r = –0.07, P < 0.001 for vagina), although the alteration was less apparent in the uterine cavity than in the vagina. More specifically, young women aged 20 years and younger exhibited the highest interindividually similarity. In contrast, the greatest interindividual difference was observed in women aged 41–60 years. We further calculated the BC distance of microbiome between individuals across different age groups (Fig. 1d). The uterine microbiome gradually deviated from that of young women, and dissimilarity accumulated with increasing age.
Women with advanced age (over 60 years) exhibited the greatest dissimilarities in the uterine microbiome, compared with women belonging to other age groups. In contrast, variations appeared earlier in the vaginal microbiome, with the largest difference present in the women aged 41–60 years. Both the uterine and vaginal microbiome showed remarkable alterations in the 40+ (40–49) and 50+ (50–59) age groups relative to the neighboring age groups, which is consistent with the results of intragroup alpha and beta diversity, suggesting that the uterine and vaginal microbiota of women in this age range undergo significant perturbation.

We next conducted a principal coordinate analysis (PCoA) based on unweighted UniFrac distance to measure the age-related changes in microbial communities and their clustering relationships. There was a little change in the uterine microbiome. In contrast, the vaginal microbiome showed gradual changes with advancing age (Fig 1e). It can be observed from the distribution of vaginal samples that the core regions (center of gravity) of the three younger age groups (10+, 20+, and 30+) were relatively close to each other. In particular, in the 20+ and 30+ year-old samples, the core regions almost overlapped. The vaginal samples of women over 40 years old had a large spatial displacement in the core regions and gradually moved closer to the uterine samples. This is highly consistent with the alpha diversity calculations and the intragroup distances (Fig. 1b, c), suggesting that the age range between 40 and 50 years old may be one of the key stages when the microbiota is more fragile and prone to dysbiosis. For women over the age of 50, the densely distributed area of their vaginal samples overlapped with the uterine samples in PCoA, indicating a more similar community composition.
We attempted to explain the aforementioned changes based on the taxonomic classification. The primary difference between the two body sites was that compared with the vagina, the uterine cavity demonstrated higher amounts of Bacteroides and Proteobacteria and fewer Firmicutes and Actinobacteria. However, the dominant bacterial phyla in the uterine and vaginal microbiome were identical (Fig. 1f). Regardless of body site, the ratio and rank of microbes were relatively stable among women under 40 years old but fluctuated above age 40. Particularly for women over the age of 50, the proportion of Firmicutes and Proteobacteria in the uterine microbiome increased and decreased, respectively, compared with those in the younger age groups. The temporal variation in the vaginal microbiome was accompanied by an increase in the amount of Bacteroides species and a decrease in the amount of Actinobacteria. These variations may account for the alterations in the microbial diversities in both the uterine and vaginal microbiome in this age group, also leading to similarities in the community structures of the two sites.

**Dysbiosis of the uterine and vaginal microbiota associated with chronic endometritis.** To examine the possible impact of age-related changes in the bacterial community on uterine health, we analyzed the uterine and vaginal microbiome in case of intrauterine disease. Among the volunteers recruited in this study, 71 women suffered from chronic endometritis. We compared the differences in microbiome between these patients and women without endometritis (healthy control) to evaluate the correlation between the microbial community and endometrial disease and to explore the intrinsic link between the health of the female reproductive system and age.

The results showed that for women with intrauterine inflammation, both the uterine and vaginal microbiome were significantly different from those of healthy women. First, the microbial diversity of the endometritis and healthy control groups was different (Fig. 2a), with the diversity of the uterine microbiome significantly lower in the endometritis group than in the controls \( (P = 0.019, \text{Wilcoxon test}) \). An opposite trend was observed with respect to the vaginal microbiome; that is, the diversity in the vaginal microbiome was higher in the endometritis group than in the controls \( (P = 0.005, \text{Wilcoxon test}) \), which is consistent with the alterations in alpha diversity associated with age. Second, the community composition was different between the two groups (Fig. 2b). In the PCoA at the OTU level, PC 2 divided samples into two independent clusters \( (P = 0.044, \text{Adonis test}) \), which indicates that endometritis and control women had distinct microbial communities in both the uterine cavity and vagina. While calculating the BC distance between the samples within each group, the distances in the endometritis group were significantly larger than those in the control group \( (P < 0.05, \text{Wilcoxon test}) \), indicating that the microbiome in the endometritis group was highly dissimilar among individuals (Fig. 2c). Likewise, in the vaginal microbiome, the interindividual divergence among the women with endometritis was particularly noticeable and was comparable to the dissimilarities between the endometritis and control groups.

To further illuminate these differences in microbial diversity and community structure, we subsequently grouped samples based on their health status and calculated the relative abundance of the dominant bacteria (Fig. 2d). The proportions of the dominant bacteria in the uterine cavity were similar among controls but fluctuated in women with endometritis. Increases in *Ruminococcus* and *Clostridium* abundance and decreases in *Megamonas* and *Lactobacillus* abundance significantly reduced the similarities of the microbiome among endometritis individuals. A similar phenomenon was also observed in the vaginal microbiome. *Lactobacillus*, which had an absolute predominance in the vaginal microbiome of most healthy controls, was reduced to less than 50% in more than half of the women with endometritis. With the decrease in *Lactobacillus* abundance, the amounts of other bacteria such as *Prevotella* and *Gardnerella*, increased. The diversity and dissimilarity of the microbial communities among women with endometritis increased significantly (Fig. 2a–c).

To avoid bias due to differences in age distribution, we then chose the individuals with age between 35 and 45 for comparisons, and similar trends on alpha diversities and community structure were observed (Supplementary Fig. 2a, b). Similar results were also found when we used the samples with the same delivery mode (Supplementary Fig. 2c, d). To quantify the distinctions corresponding to health and disease status, we used LEfSe to identify 43 and 51 discriminatory bacterial taxa in the uterine and vaginal microbiome, respectively (Supplementary Fig. 3). Bacteria such as Fusobacteriales, *Clostridium*, and *Sneathia* were discriminatory taxa among both the uterine and vaginal microbiome, suggesting that variations in the proportion of specific bacteria associated with health status may take place at these sites simultaneously.

**Synchronous variations in the uterine and vaginal microbiome.** Considering the dysbiosis of both the uterine and vaginal microbiota in women with chronic endometritis, we wanted to understand whether such changes were similar or synchronized across the two sites. We first explored the disease-discriminatory bacteria identified by LEfSe in each sample and observed that the composition and relative abundance of the discriminatory bacteria were quite similar among individuals with the same health status as well as between body sites (Fig. 2e), indicating that the uterine and vaginal microbiota may have undergone similar selective pressure in response to uterine disease status. The rank of highly abundant discriminatory bacteria was almost the same regardless of health status, and the relative abundance of these taxa at both sites was more variable in the endometritis group than in the healthy group (Fig. 2e and Supplementary Fig. 4a). These results indicate the synchronous alteration of the microbiome between the uterus and vagina in women in association with uterine disease. This synchronous relationship was also demonstrated by showing the direction of change with respect to the relative abundance of the discriminatory bacteria between the healthy and endometritis groups (Supplementary Fig. 4b). We investigated whether the direction of their alterations was towards enrichment or depletion in the endometritis group by calculating the difference in the mean relative abundance of each discriminatory taxon between the healthy and endometritis groups. Most of these taxa (37/51) changed in the same direction in both the endometritis and healthy control groups. Taken together, these findings strongly indicated that there is cross-consistency in the relative abundance of specific bacterial taxa and their directions of change associated with chronic endometritis.

To reconstruct the broader relationships among reproductive tract microbes, we calculated the correlation coefficient between the uterine and vaginal microbiome for each OTU (Fig. 2f). Most of the OTUs were negatively correlated in either the healthy group (257/300) or the endometritis group (203/300). Notably, 232 of 300 OTUs maintained the same direction of correlation irrespective of the health status, indicating that the correlation in the relative abundance of any given bacterial taxon was relatively constant between the uterine cavity and vagina. Based on the bacterial abundance, the correlation at the OTU level, and the differences associated with the disease, 7 genera were screened to...
analyze the co-occurrence relationship between the same and different bacterial taxa across body sites (Supplementary Fig. 4c). Only the different bacterial genera from different body sites (the pies on both sides of the blue dotted line) were involved in distinct co-occurrence relationships under different health statuses. In contrast, the positive or negative correlation of the same genus between the uterine and vaginal microbiome was consistent in the control and endometritis groups despite the differences in the strength of the correlation (the pies on the two green dotted lines), which was consistent with the analysis results at the OTU level. Accordingly, the co-occurrence of the same genus across body sites or different genera at the same site was
Dysbiosis of uterine microecology triggered by vaginal perturbation. Since the uterine and vaginal microbiome changed simultaneously or even converged under a variety of clinical contexts, two animal experiments were performed to investigate the possibility of vaginal bacteria passing through the cervical barrier and in utero transplantation, as well as to explore the potential impact of vaginal dysbiosis on the intrauterine microecology. We first exchanged the vaginal microbiota of Brown Norway (BN) and Sprague Dawley (SD) rats (n = 12 pairs) three times within 1 week and collected the fluid from lavage of the uterine cavity of each rat 3 weeks later. We subsequently performed amplicon sequencing for the V3–V4 regions of the 16S rRNA genes (Fig. 4a). On average, 155,770 sequences were obtained from each sample. The analysis of microbial diversity at the OTU level showed that the species richness of the uterine microbiome was significantly increased in each rat after exchanging the vaginal microbiota (Fig. 4b). The Shannon diversity index also showed a significant increase (P < 0.001, Wilcoxon test) in post-exchange rats (Fig. 4c).

In the PCoA plot based on unweighted UniFrac distance, the uterine samples taken from the rats before and after the exchange of their vaginal microbiota were grouped into two distinct clusters (P < 0.001, Adonis test) (Fig. 4d). The distribution of the post-exchange samples was more compact than that before the exchange, indicating that the exchange of the vaginal microbiota could homogenize the uterine microbiota of different individuals. The variations in the diversity and the structure of the uterine microbiome were further elucidated based on taxonomic classification and profiling (Fig. 5e). Before exchange, the uterine cavities of the rats harbored very few bacterial taxa with high abundance except for *Proteobacteria* and *Firmicutes*, and the bacterial taxa varied significantly among individuals, resulting in lower microbial richness and evenness and smaller interindividual similarities. After exchange, the values of the aforementioned parameters increased markedly, demonstrating that the vaginal perturbation could affect the uterine microbiota.

We next collected vaginal lavage fluids from 10 healthy women, 10 women with chronic endometritis, and 10 women observed with that increasing age, the trend of the fitted line and the increase/decrease relationship of the abundance of each genus were the same between the uterine cavity and vagina (Fig. 3f and Supplementary Fig. 7a). When using paired uterine and vaginal samples from the same healthy women and testing the bacterial correlations between the two body sites, we found that the correlations of four (*Lactobacillus*, *Bacteroides*, *Gardnerella*, and *Clostridium*) out of seven genera were significant (P < 0.01, Pearson’s test). Notably, *Lactobacillus*, whose relative abundance was higher in the healthy group than in the endometritis group (Fig. 2d), was depleted in the uterine and/or vaginal microbiome of women over 50 years of age (Fig. 3f). *Clostridium* and *Prevotella*, however, exhibited the opposite trend, with their abundance varying synchronously across body sites, suggesting that they might be closely related to age and the health status of the uterine cavity. Similar results were observed with respect to the variations in the abundance of these genera in women who underwent multiple abortions or vaginal deliveries (Fig. 3g, h and Supplementary Fig. 7b, c). We next measured the biomass of bacteria in vaginal (n = 40) and uterine (n = 40) flushing fluid by qPCR quantification. As shown in Supplementary Fig. 8, *Prevotella*, *Clostridium*, and *Lactobacillus* in the uterus and vagina were positively or negatively correlated with the age of the women, and each genus exhibited a significant positive correlation between the two body sites (P < 0.05, Spearman’s test).

Potential factors that may drive the convergence of uterine and vaginal microbiome. We browsed the medical records of 1612 other women with or without chronic endometritis visiting the gynecology department of one of the two hospitals and observed that age and number of deliveries or abortions were significantly higher (P < 0.01, Wilcoxon test) in women with chronic endometritis than in healthy women (Fig. 3a, b). Given that ages and health statuses were associated with the convergence of the microbiome between the two body sites, we next explored the possible impact of clinical factors, pregnancy, and delivery mode on the microbial community of their reproductive system.

The samples were categorized according to various medical factors, and for each category, the BC community distance of the paired samples obtained from the uterine cavity and vagina was calculated. In healthy women <50 years of age, the distance between the uterine and vaginal microbiome increased with age (r = 0.30, P < 0.01, Pearson’s test) and reached its maximum value in the 40–49-year-old group (Fig. 3c). Results similar to those of women 50–59 years old were observed in healthy women with a history of abortion (Fig. 3d) or vaginal delivery (Fig. 3e). The BC distance between their uterine and vaginal microbiome was smaller than that of women who had no history of abortion (P = 0.07, Wilcoxon test) or who underwent a cesarean section (P = 0.03, Wilcoxon test).

We then analyzed the changes in the abundance of the aforementioned seven dominant genera in different groups and observed that with increasing age, the trend of the fitted line and the increase/decrease relationship of the abundance of each genus were the same between the uterine cavity and vagina (Fig. 3f and Supplementary Fig. 7a). When using paired uterine and vaginal samples from the same healthy women and testing the bacterial correlations between the two body sites, we found that the correlations of four (*Lactobacillus*, *Bacteroides*, *Gardnerella*, and *Clostridium*) out of seven genera were significant (P < 0.01, Pearson’s test). Notably, *Lactobacillus*, whose relative abundance was higher in the healthy group than in the endometritis group (Fig. 2d), was depleted in the uterine and/or vaginal microbiome of women over 50 years of age (Fig. 3f). *Clostridium* and *Prevotella*, however, exhibited the opposite trend, with their abundance varying synchronously across body sites, suggesting that they might be closely related to age and the health status of the uterine cavity. Similar results were observed with respect to the variations in the abundance of these genera in women who underwent multiple abortions or vaginal deliveries (Fig. 3g, h and Supplementary Fig. 7b, c). We next measured the biomass of bacteria in vaginal (n = 40) and uterine (n = 40) flushing fluid by qPCR quantification. As shown in Supplementary Fig. 8, *Prevotella*, *Clostridium*, and *Lactobacillus* in the uterus and vagina were positively or negatively correlated with the age of the women, and each genus exhibited a significant positive correlation between the two body sites (P < 0.05, Spearman’s test).

Potential factors that may drive the convergence of uterine and vaginal microbiome. We browsed the medical records of 1612 other women with or without chronic endometritis visiting the gynecology department of one of the two hospitals and observed that age and number of deliveries or abortions were significantly higher (P < 0.01, Wilcoxon test) in women with chronic endometritis than in healthy women (Fig. 3a, b). Given that ages and health statuses were associated with the convergence of the microbiome between the two body sites, we next explored the possible impact of clinical factors, pregnancy, and delivery mode on the microbial community of their reproductive system.

The samples were categorized according to various medical factors, and for each category, the BC community distance of the paired samples obtained from the uterine cavity and vagina was calculated. In healthy women <50 years of age, the distance between the uterine and vaginal microbiome increased with age (r = 0.30, P < 0.01, Pearson’s test) and reached its maximum value in the 40–49-year-old group (Fig. 3c). Results similar to those of women 50–59 years old were observed in healthy women with a history of abortion (Fig. 3d) or vaginal delivery (Fig. 3e). The BC distance between their uterine and vaginal microbiome was smaller than that of women who had no history of abortion (P = 0.07, Wilcoxon test) or who underwent a cesarean section (P = 0.03, Wilcoxon test).

We then analyzed the changes in the abundance of the aforementioned seven dominant genera in different groups and observed that with increasing age, the trend of the fitted line and the increase/decrease relationship of the abundance of each genus were the same between the uterine cavity and vagina (Fig. 3f and Supplementary Fig. 7a). When using paired uterine and vaginal samples from the same healthy women and testing the bacterial correlations between the two body sites, we found that the correlations of four (*Lactobacillus*, *Bacteroides*, *Gardnerella*, and *Clostridium*) out of seven genera were significant (P < 0.01, Pearson’s test). Notably, *Lactobacillus*, whose relative abundance was higher in the healthy group than in the endometritis group (Fig. 2d), was depleted in the uterine and/or vaginal microbiome of women over 50 years of age (Fig. 3f). *Clostridium* and *Prevotella*, however, exhibited the opposite trend, with their abundance varying synchronously across body sites, suggesting that they might be closely related to age and the health status of the uterine cavity. Similar results were observed with respect to the variations in the abundance of these genera in women who underwent multiple abortions or vaginal deliveries (Fig. 3g, h and Supplementary Fig. 7b, c). We next measured the biomass of bacteria in vaginal (n = 40) and uterine (n = 40) flushing fluid by qPCR quantification. As shown in Supplementary Fig. 8, *Prevotella*, *Clostridium*, and *Lactobacillus* in the uterus and vagina were positively or negatively correlated with the age of the women, and each genus exhibited a significant positive correlation between the two body sites (P < 0.05, Spearman’s test).

Potential factors that may drive the convergence of uterine and vaginal microbiome. We browsed the medical records of 1612 other women with or without chronic endometritis visiting the gynecology department of one of the two hospitals and observed that age and number of deliveries or abortions were significantly higher (P < 0.01, Wilcoxon test) in women with chronic endometritis than in healthy women (Fig. 3a, b). Given that ages and health statuses were associated with the convergence of the microbiome between the two body sites, we next explored the possible impact of clinical factors, pregnancy, and delivery mode on the microbial community of their reproductive system.

The samples were categorized according to various medical factors, and for each category, the BC community distance of the paired samples obtained from the uterine cavity and vagina was calculated. In healthy women <50 years of age, the distance between the uterine and vaginal microbiome increased with age (r = 0.30, P < 0.01, Pearson’s test) and reached its maximum value in the 40–49-year-old group (Fig. 3c). Results similar to those of women 50–59 years old were observed in healthy women with a history of abortion (Fig. 3d) or vaginal delivery (Fig. 3e). The BC distance between their uterine and vaginal microbiome was smaller than that of women who had no history of abortion (P = 0.07, Wilcoxon test) or who underwent a cesarean section (P = 0.03, Wilcoxon test).
Fig. 3 Synchronous variations in the uterine and vaginal microbiome and the correlation to clinical factors. a The age distribution of 1,612 women interviewed from two hospitals. b The average (AVG) number and percentage of pregnancies, deliveries, and abortions in women with and without chronic endometritis. c Bray-Curtis (BC) community distance of the paired uterine and vaginal samples with age. The scales 10, 20, 30, 40, 50, and 60 on the x-axis represent ages <20, 20–29, 30–39, 40–49, 50–59, and ≥60, respectively. d BC distance of uterine-vaginal paired samples associated with abortion history. The distance was calculated at the OTU level in the microbiome of women with and without chronic endometritis. e BC community distance of uterine-vaginal paired samples associated with the previous delivery mode. f The relative abundance of three bacterial genera in the uterine (red) and vaginal (blue) microbiome with age. The values were normalized to 10^5 reads in each sample. g Relative abundance of three bacterial genera along with the number of abortions. h Relative abundance of three bacterial genera along with the previous delivery mode. For c-e and h, box and violin elements are defined as: center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range; points, outliers; the width of the violin represents the density distribution. P values were determined by two-tailed Wilcoxon test. For f, g the shadow around the linear regression trendline shows the 95% confidence interval (CI).
with bacterial vaginosis and transplanted their vaginal lavage fluids into the vaginas of SD rats \((n = 10)\) that had previously been submitted to 1 week of antibiotic treatment. After transplantation once a day for 3 weeks, qPCR and ELISA were performed to measure the inflammatory factors, and the changes in uterine morphology and tissue were also examined (Fig. 5f). With respect to the mRNA expression and levels of cytokine and inflammatory factors such as TNF-\(\alpha\), CD38, and IL1\(\beta\), uterine inflammation was significantly higher in rats transplanted with the vaginal microbiota of women with chronic endometritis \((P < 0.05, \text{Student's } t\text{-test})\) than in those transplanted with microbiota from the healthy group and in the control group (Fig. 5g and Supplementary Fig. 9). Interestingly, the cytokine levels in the bacterial vaginosis group were also elevated but not as high as those in the chronic endometritis group, reflecting the effectiveness of the vaginal microbiota of the latter to stimulate uterine inflammation. The uterine body of the rats in the chronic endometritis group was edematous and enlarged (Fig. 5h) and showed symptoms such as multiple punctate inflammatory lesions (5× field of view), endometrial hyperplasia (25×), and
polynucleosis (50×). The results of the immunofluorescence assay also illustrated a significant presence of TNF-α and CD38 signals in the endometrial tissues of the chronic endometritis group compared to those in the healthy group and the control group (Fig. 5i). These results demonstrate that the vaginal microbiota of women with chronic endometritis can induce inflammation in the uterine cavity, which may be caused by translocated bacteria or inflammatory factors that can cross the cervical barrier and enter the uterine cavity.

Destructive and protective effects on the endometrium mediated by vaginal bacteria. To examine the effect of the flux of certain vaginal bacteria on the uterine cavity and its stimulation of uterine inflammation, it was necessary to transplant a single bacterial species into the vagina to build an intrauterine inflammation model. Bacteria such as Prevotella and Clostridium, which were enriched in the uterine or vaginal microbiome of women with endometritis and demonstrated coaggregation across body sites were ideal candidates. We thus analyzed the metagenomic sequencing data for the selection of transplanted strains. Similar to the enriched genera identified by 16S rRNA gene analysis, Prevotella and Clostridium were identified as more abundant in the endometritis group than in the healthy group from the metagenomic data (Supplementary Fig. 10). Prevotella bivia, originally isolated from the endometrium (https://www.lgcstandards-atcc.org/products/all/29303.aspx?geo_country=gr), and Clostridium perfringens, which has been used previously to construct a model of endometritis31, were used for the following vaginal transplantation experiment.

We injected P. bivia grown to the logarithmic phase into the vaginas of SD rats (n = 17) once a day for 3 weeks and then collected samples of intrauterine tissues and uterine and vaginal lavage fluids 3 days after treatment (Fig. 5a). The same transplantation procedure was also performed for C. perfringens (n = 16). Compared with those in the control group (n = 10), the levels of inflammatory factors in the intrauterine tissue, the diameter of the uterine body, and the bacterial biomass of the uterine cavity and vagina all increased significantly in the challenge groups (P < 0.05, Student’s t-test) (Fig. 5b and Supplementary Fig. 11a, b). Significantly positive correlations were observed between these indicators (P < 0.05, Student’s t-test) (Fig. 5c and Supplementary Fig. 11c, d). The uterine body of the rats in the challenge groups was edematous and enlarged (Fig. 5d and Supplementary Fig. 11e). Tissue sections and immunohistochemical staining for TNF-α and CD38 antibodies showed multiple punctate inflammation and endometrial hyperplasia. Fluorescence in situ hybridization showed that both total bacteria and the transplanted bacterial species specifically exhibited very high biomass in the uterine cavity (Fig. 5d–f and Supplementary Fig. 11e–g). In contrast, these symptoms were absent in the control group. These results indicate that either P. bivia or C. perfringens introduced into the vagina can ascend to the uterine cavity, induce an inflammatory response in the uterine cavity, and form endometritis-like lesions.

We wondered whether any bacterial species present in the vagina could exert protective effects by reversing the inflammatory effects of P. bivia or C. perfringens. Given that the relative abundance of Lactobacillus in the uterine and vaginal microbiome of healthy women was significantly higher than that of women with endometritis, we selected Lactobacillus murinus for the evaluation of its protective effects on the endometrial health of SD rats (Fig. 5g). C. perfringens was transplanted into the vagina four times per week for 3 weeks to induce inflammation of the uterine cavity (n = 15). In the intervention group, L. murinus was transplanted into the vagina once. It was alternately supplemented after each two treatments of C. perfringens, such that C. perfringens and L. murinus were transplanted into the vagina four times and twice times per week, respectively (n = 15). To test whether the bacterial metabolites in the vagina may also cause uterine inflammation, the transplantation included a group of C. perfringens supernatants (n = 15). This treatment was similar to that in the C. perfringens group, except that the live bacteria were replaced by the supernatant of its culture solution. After 3 weeks of treatment and intervention, the results demonstrated that the inflammatory factors of the intrauterine tissue, the diameter of the uterine body, and the bacterial biomass of the uterine cavity and vagina were significantly higher (P < 0.05, Student’s t-test) in the rats transplanted with only C. perfringens than in those belonging to the other three groups (Fig. 5h and Supplementary Fig. 12a). There was no significant difference between the L. murinus transplantation group and the C. perfringens supernatant group (Supplementary Fig. 12b, c).

We finally performed 16S rRNA gene sequencing on the uterine microbiome of the rats in each transplantation group. PCoA showed that the samples transplanted with C. perfringens were significantly different from those of the other groups (Fig. 5i), with more Clostridium in the uterine cavity of the former (Fig. 5j). In addition, similar to that in older women and women who experienced multiple abortions, Bacteroides and Prevotella...
were enriched in the uterine microbiome of rats with vaginal transplantation of *C. perfringens*, whereas *Bifidobacterium* showed the opposite trend (Supplementary Fig. 12d). These findings indicate that bacteria in the vagina can ascend into the uterine cavity to colonize, grow, and cause inflammation, but they do not achieve the same level of stimulation by releasing their metabolites into the vagina only. *L. murinus* can inhibit the growth of vaginal *C. perfringens* and reduce the biomass of uterine *C. perfringens* and some other harmful bacteria and hence may play a protective role in reducing uterine inflammation.

**Discussion**

By profiling the uterine and vaginal microbiomes of females of different ages and health statuses, this study adds new evidence indicating that microbes in the uterine cavity inhabit this relatively confined space to form a unique community. It should be noted that contamination from the vagina/cervix cannot be absolutely avoided when collecting uterine samples. In this study, we have used blank and negative controls to exclude potential bacterial contaminations from DNA processing and library preparation. However, more comprehensive external controls from...
gloves and air are still needed to exclude all sources of contaminations from sample collection. In terms of community structure, composition, and variation tendency with age, the uterine microbiome is significantly different from that of the vagina, indicating that these observations are unlikely caused by sample contaminations. In addition, the results of our study show that the vaginal and uterine microbiome converge with age. This is a bit of an internal control for the argument that the synchronic variation of the uterine and vaginal microbiome is not an artifact of sample contaminations from vaginal bacteria.

For the first time, we unveiled the synchronous variations of the uterine and vaginal microbiome and observed that age, abortion history, and delivery mode can affect the community structure and similarity between these two body sites in women and uncovered the dysbiosis of the uterine microenvironment caused by vaginal perturbation through animal experiments. Exchange of the vaginal microbiota between rats shifted the structure of their uterine microbiota, and vaginal microbiota transplantation (VMT) from women with chronic endometritis induced inflammation-like lesions in the endometrial tissue of rats. This study also identified candidate bacteria that tend to spread across body sites, which may be responsible for the maintenance or disruption of microbiota homeostasis in the female reproductive system. Their protective or destructive effects on uterine health were confirmed by transplanting bacterial cultures to the vagina and observing cross-site microbial communication, translocation, and colonization from the vagina to the uterine cavity as well as the factors influencing these processes.

Our study indicated that the abundance of variations among certain bacteria was observed in the vagina of women with chronic endometritis. The vaginal microbiota was previously regarded as an indicator of either vaginal or cervical health\(^*\)\(^*\).\(^*\). Similar to the uterine cavity, which is situated deeper into the body, it is difficult to directly monitor the uterine microbial community and in utero health. The findings herein encourage us to explore novel ways for the convenient collection of samples and detection of the vaginal microbiota, which can be used as biomarkers to screen and diagnose asymptomatic uterine diseases or overlooked complications in vitro. Moreover, compared with oral and intestinal bacteria, which may spread into the uterine cavity via the bloodstream under special conditions,\(^\text{5,33}\)\(^\text{,}\) uterine bacteria seem to be more likely to ascend from the adjacent vagina, colonize and lead to adverse pregnancy outcomes or intrauterine diseases. Uterine colonization by BV-related bacteria is not only frequently associated with preterm birth\(^\text{12,34–36}\) but also believed to promote carcinogenesis via microbial-mediated pathophysiological changes\(^\text{37}\). We observed inflammatory lesions in the endometrial tissue of rats transplanted with the vaginal microbiota of BV patients. Combining the results of vaginal transplantation of the microbiota from patients or certain bacterial cultures that caused inflammation in the uterine cavity, our study demonstrated that the translocation of harmful vaginal bacteria can induce inflammation in the uterine cavity. We speculated that the infusion of \textit{Lactobacillus} into the vagina may exhibit a protective function via ecological effects, including the local suppression of the growth of harmful bacteria, decreasing its upward flux, and reducing the stimulation of the endometrium. This inspired us to consider bacterial manipulation as a choice of clinical intervention for the maintenance of uterine microenvironment homeostasis and prevention of disease, similar to the use of vaginal microbiota transplantation for the treatment of intractable BV\(^\text{38,39}\).

In this study, less dysbiosis was associated with aging and endometritis in the uterine microbiome than in the vaginal microbiome. One possible reason is that the vagina is a semi-open environment, and its microbiota can be easily influenced. However, the uterine cavity is nearly closed, and hence, its microbiota is more stable and under unique selective pressure. This relative isolation and independence can be disrupted by several factors. For example, we observed that aging, and increase in the number of abortions and vaginal delivery reduced the dissimilarity between the uterine and vaginal microbiome. We speculate that this increase in community similarity may be attributed to the perturbation of the originally confined uterine environment, which promotes bacterial transmission between the upper and lower reproductive tracts. These factors may also lead to increased laxity of the cervix or even cervical incompetence,\(^\text{40}\) thereby increasing the permeability of the cervix and the likelihood of translocation of vaginal bacteria. In addition, factors such as menopause, hormones or sexual life are also likely to affect the microbiota of the female reproductive system. In this study, we did not gather the information about these factors. However, we discovered that the BC distance of microbiome between age group 60+ and other groups is significantly higher than any other comparisons, which may be contributed to their menopausal or hormonal status.

In women with endometritis, we did not observe a significant increase in the similarity between the uterine and vaginal microbiome in response to abortion history or delivery mode, perhaps since the dysbiosis caused by the disease may mask the
effects of the other factors. Despite epidemiological investigations implying a correlation between reproductive history and endometrial disease\(^1\text{4,42}\), our study explained the potential connection between the parameters with respect to the reproductive tract microbiota. The results of our study suggested that the uterine niche of the elderly female population gradually deteriorates in women who are older or undergo multiple abortions or vaginal deliveries, and their uterine or vaginal microbiome demonstrate a higher abundance of Prevotella and Clostridium and a lower abundance of Lactobacillus. We also proved in animal experiments that these bacteria inhabiting the vagina can stimulate or inhibit endometrial inflammation. The enrichment and absence of certain bacterial taxa causing endometritis-like changes are characteristics of BV and postpartum vaginal microbiota\(^2,4,43\). This might be partially responsible for the clinical linkage of these two diseases with parturition\(^2,4,43\). The results of data mining and the experiments in our study may explain the high incidence of endometrial diseases in elderly women, which encourages us to pay attention to the potential threats to the upper reproductive system caused by microbial translocation.

Like all studies that use rodent models to expand physiological and medical understanding, this study also faces the problem of the significant differences in anatomy and morphology between the human and rodent reproductive systems. However, the mouse is still one of the most commonly used models to evaluate the causes and treatments of endometrial or cervical disease\(^3,14,40\), as the tissue compositions of the human and mouse cervix are very similar and are primarily composed of connective tissue and smooth muscle. Under the action of sex hormones, the variations in the volume and viscosity of cervical mucus were similar between the menstrual cycle in humans and estrus cycle in mice\(^47\). Rats exhibit a higher degree of cervical compaction than humans and have only a covert estrous cycle rather than overt menstruation, making it more difficult for microbes inhabiting the vagina to pass through the cervix and ascend to the uterus. Hence, in this study, vaginal microbiota transplantation in rats reproducing the uterine inflammation phenotype indicated that similar conditions may also occur in humans. Surprisingly, certain bacterial taxa that were undetected before the exchange of vaginal microbiota were observed in the uterine cavity with high abundance after the exchange in rats. We speculated that the original balance of the uterine microbiota was disrupted, providing opportunities for the colonization and proliferation of less abundant bacteria. In addition, the possibility of the introduction of some exogenous bacteria during transplantation cannot be ruled out. However, in either case, vaginal perturbation will affect the microecological balance of the uterine cavity. In actual situations, the translocation of pathogenic bacteria may not produce as large a biomass in as short a time as in the experimental injection. Nevertheless, it is proposed that there is a way for vaginal bacteria to enter the uterine cavity as well as a perennal and cumulative translocation effect as women age.

16S rRNA gene amplification and sequencing. Approximately 2–5 µL of extracted DNA and a pair of modified region-specific primers (341F–805R) attached to Illumina paired-end adapters, sequencing primers, and barcodes were used to amplify variable regions 3 and 4 (V3–V4) of the 16S rRNA gene using Q5 Hot Start Polymerase (NEB, Ipswich, MA). In each batch subject to amplification, negative extraction controls and blank controls were included with distilled water as the PCR template. PCR conditions consisted of an initial denaturation step for 3 min at 95 °C, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C and 30 s, and extension at 72 °C; a final extension for 5 min at 72 °C. The end products were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA), and the quality of purified amplicons was evaluated by electrophoresis on a 2% agarose gel. Positive samples with a bright primary band approximately 450 bp were pooled in equimolar ratios prior to the generation of 2 × 350 bp paired-end (PE) reads on a HiSeq2500 platform (Illumina, San Diego, CA) with v2 sequencing reagents.

16S rRNA amplicon sequence analysis. In addition to the sequencing data generated in this study, the 16S rRNA sequences of 308 uterine and 653 vaginal samples from six previous studies\(^13,14,28–39\) were retrieved from the NCBI SRA database (SRP064295, PRJEB14941, PRJEB16013, PRJEB24177, PRJNA481576, PRJNA47395) and used to explore the relationships between uterine and vaginal microbiome and the microbial variation with age in a large population. The samples for these six projects were collected from women aged 15–83 years without chronic endometritis or hyperplasia (Supplementary Table 3). Since the targeted regions of our data and the data retrieved from the databases were V3–V4, V4, or V4–V5, the sequences of the V4 region after alignment were used for integrated analysis. Briefly, the reference sequences of the 16S rRNA genes were downloaded from the SILVA rRNA database\(^31\), and the V4 regions were marked by mapping the 515F and 806R primers against the reference sequences using Bowtie 2 v2.4.1.\(^32\) Subsequently, we merged the PE reads to form long sequences and mapped them to the reference sequences to capture the sequences falling into the marked V4 regions.

Sequencing data processing and analysis were performed as described previously\(^33\), with modifications. Specifically, a quality filtering step was applied according to the Phred scores via a script split_library_fastq.py (-t 3 - p 0.75 - q 0.05) in Quantitative Insights into Microbial Ecosystems (QIME) v1.9.1.\(^34\) Chimeric sequences were identified and removed using two QHIME commands, identify_chimeric_seq.py and filter_fasta.py (usearch61 option that runs the UCHIME algorithm)\(^35\). High-quality reads were
then merged into long sequences by the overlaps of each pair of PE reads using FLASH v1.2.11 with the implementation of default options35. The merged long sequences contained 676,656, and 88,643 reads identified from the trimmed sequences with a 97% sequence identity threshold using UCLUST in QIIME. A representative sequence was selected for each OTU, and the Greengenes database was used to generate an OTU table with taxonomy36. Samples with observed species less than 50 or Good’s coverage lower than 0.8 were discarded for the following analysis.

The relative abundance of each OTU was normalized to the total abundance of all OTUs from each sample. All validated samples were used when analyzing the influence of age on microbial composition. Only the data generated in this study were used to discriminate differences of microbial (AB) containing 100 µg·mL−1 neomycin, 100 µg·mL−1 penicillin, 50 µg·mL−1 vancomycin, and 100 µg·mL−1 metronidazole (Sigma, St. Louis, MO) to reduce the amount of endogenous bacteria before they were colonized by clinical transfers. Following antibiotic treatment, the recipient rats received clinical vaginal bacteria via VMT daily for 21 consecutive days. Their vaginal and uterine tissues were collected after an adaptation period of 3 days.

Vaginal washing solution was obtained from clinical patients with endometritis (n = 10), patients with vaginosis (n = 10), and healthy women (n = 10) was transplanted to SD rats. The clinical specimens were centrifuged at 1000 rpm for 5 min to remove the visible residue, and the supernatant was harvested and mixed with 100 µL PBS. The mixture was then cultured to detect the viable bacteria. The precipitate was resuspended in 120 µL sterile and anaerobic PBS (pH = 7.0) before vaginal microbial transplantation (VMT), with PBS as the blank control. After adaptation for 7 days, SD rats (n = 10) were treated with a combination of Abdominal Bacteria (AB) containing 100 µg·mL−1 neomycin, 100 µg·mL−1 penicillin, 50 µg·mL−1 vancomycin, and 100 µg·mL−1 metronidazole (Sigma, St. Louis, MO) to reduce the amount of endogenous bacteria before they were colonized by clinical transfers. Following antibiotic treatment, the recipient rats received clinical vaginal bacteria via VMT daily for 21 consecutive days. Their vaginal and uterine tissues were collected after an adaptation period of 3 days.

Two independent experiments were conducted. In the first experiment, approximately 1 × 106 CFU of bacteria cultured in the logarithmic phase was collected and 120 µL GAM were administered to three rat groups: P. bivia (n = 17), C. perfringens (n = 16), and blank control (n = 10). The recipient rats received the cultured bacteria via VMT daily for 21 consecutive days. The vaginal and uterine washing solution and intravaginal tissues were collected after adaptation. In the second experiment, the culture medium supernatant after the elimination of C. perfringens by centrifugation at 13,000 × g for 20 min was used as a negative control, and its sterility was checked by culturing the suspension to examine the absence of the growth of bacterial clones. The rats were divided into four groups: C. perfringens (n = 15), L. murinus (n = 15), C. perfringens supernatant (n = 15), and the blank control (n = 10). In each week, the rats in the first group received 1 × 106 freshly cultured C. perfringens on the second, third, fifth, and sixth days via VMT and PBS (pH = 7.0) on the first and fourth days. The experiment was repeated for 3 weeks. The treatment course of the L. murinus group and the supernatant of the C. perfringens group was the same as that of the first group, except that PBS was replaced with L. murinus and C. perfringens was replaced with its supernatant. PBS was used in the blank control group at all the same time points as above.

The washing solutions from the vagina and the uterine cavity and intravaginal tissues were sampled on the first, eighth, fifteenth, and twenty-ninth days.

**Metagenomic sequencing and analysis.** Forty paired uterine-vaginal samples collected from 20 women (chronic endometritis vs control = 1:1) were chosen for whole-genome shotgun sequencing. Metagenomic DNA libraries were constructed using the TruSeq Sample Preparation Kit v2 (Illumina, San Diego, CA, USA) after shearing ~0.1 μm using the shearing feature in the HighSeq5000 platform with a paired end PE (150 bp) sequencing strategy. Raw reads were filtered using the FASTQ quality filter in the FASTX toolkit v0.19.146, and sequenced and mapped to one strain genome with a mapping quality of ≥30, with observed species less than 50 or Good’s coverage lower than 0.8. All validated samples were used when analyzing the influence of age on microbial composition. Only the data generated in this study were used to discriminate differences of microbial (AB) containing 100 µg·mL−1 neomycin, 100 µg·mL−1 penicillin, 50 µg·mL−1 vancomycin, and 100 µg·mL−1 metronidazole (Sigma, St. Louis, MO) to reduce the amount of endogenous bacteria before they were colonized by clinical transfers. Following antibiotic treatment, the recipient rats received clinical vaginal bacteria via VMT daily for 21 consecutive days. Their vaginal and uterine tissues were collected after an adaptation period of 3 days. The phylogenetic tree used to calculate UniFrac distance was created using QIIME2 v2.1.0.147. Linear discriminant analysis (LDA) effect size (LEfSe) was conducted to identify discriminatory bacteria, with an absolute value of log LDA score >4.0 and a false discovery rate (FDR) <0.05. Benjamini–Hochberg (BH) adjustment was used to control the false discovery rate (FDR) in multiple hypothesis tests.

**Quantitative PCR and gene expression analysis.** To determine the absolute amount of bacteria at the genus level in the uterine cavity and vagina of women, a universal 16S rRNA primer pair (1369F; 1492R) for total bacteria and three primer pairs specific for Prevotella (F: 5′-CCAGCAATGTGGTGCCGCAACG-3′; B: TGGACC TCCGATATTACGCC-3′), Cladosiurn (SF: 5′-CGGTTAAGTCTGGTATTCA-3′; SR: 5′-GGTAATAACAGAACCTGCGTCG-3′) and Lactobacillus (FLwLMA1-recB: 5′-CTCAATCAAATGACTCGTA-3′; FlwLMA1-recB: 5′-CTCAATCAAATGACTCGTA-3′) were used (Supplementary Table 4). Real-time qPCR analysis was performed using SYBR Green PCR master mix (Yeasen, Shanghai, China) on an ABI 7500 real-time PCR system (Applied Biosystems, Darmstadt, Germany).

Total RNA was extracted from the uterine tissue of rats using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions to measure the expression of inflammatory factors in the endometrial tissues. Approximately 1 µg RNA was used as a template for the reverse transcription reaction to synthesize complementary DNA (cDNA) with reverse transcription enzyme (Yeasen, Shanghai, China). The primers used are listed in Supplementary Table 5. The results of gene expression were presented as the mean expression of each gene normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a reference.

**Enzyme-linked immunosorbent assay.** Total protein was extracted from the uterine tissue of rats using RIPA lysis buffer (Beyotime, Shanghai, China). The resulting suspension was centrifuged at 2000 × g for 30 min, and the supernatants were harvested. The levels of inflammatory mediators, including TNF-α, IL-1β, IL-6, CXCL5, and CXCL10, were determined using a MILLIPLEX MAP Rat Cytokine/Chemokine Panel (Millipore, Billerica, MA) on a Luminex 100 system (Luminex, Austin, TX). All assays were conducted according to the manufacturers’ guidelines.
Fluorescence in situ hybridization (FISH) was performed as described by Choi et al.68. Briefly, formalin-fixed or paraffin-embedded sections were subjected to deparaffinization and rehydration. Specific probes for FISH, including the Eubacterial probe (EUB338 I-III) mixture, were labeled with digoxigenin, and anti-digoxigenin/horseradish peroxidase antibodies were used as secondary antibodies.

Microbial sequencing and quantification in ratsSO. DNA was extracted from the uterine and vaginal washing fluid of rats using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. 16S rRNA gene amplification, sequencing, and data analysis were performed as described above. A 10-fold serial dilution of the DNA templates (100 ng) was used to examine total bacteria. The NUN338 probe served as nonspecific control. The Clostridium-specific probe served as a genus-specific probe complementary to the partial 16S rRNA region of C. perfringens BNC185933. The Prevotella-specific probe served as a genus-specific probe complementary to the partial 16S rRNA region of P. bivia strain ATCC29303 (Supplementary Table 5). All probes were 5'-labeled with digoxigenin, and anti-digoxigenin/ horseradish peroxidase antibodies were used as secondary antibodies.

Statistical analysis of the animal experiments. The statistical analysis of the animal experiments was performed using GraphPad Prism v8.0 software (GraphPad, San Diego, CA). P values less than 0.05 as determined by unpaired two-tailed Student’s t-test or ANOVA were considered to indicate statistically significant differences. Correlation analyses were performed based on Spearman’s r statistic. Rate comparisons were performed with Pearson’s χ² test or Fisher’s exact test.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The sequencing data generated in this study have been deposited in the NCBI SRA database under accession number PRJNA573502. The public 16S RNA sequences used in this study are available in the NCBI SRA database under accession number SRP064295, PRJEB14941, PRJEB16013, PRJEB24147, PRJNA481576, and PRJNA547595. The reference sequences of the 16S RNA genes are available in the SILVA rRNA database (https://www.arb-silva.de) and the Greengenes database (http://greengenes.ucslo.edu). The reference genomes of Clostridium and Prevotella strains are available in the Human Microbiome Project (https://www.hmpdacc.org). Source data are provided with this paper.

Code availability

The code scripts used for data processing, analysis, and visualization have been deposited to Zenodo under https://doi.org/10.5281/zenodo.4925167.

Received: 21 February 2021; Accepted: 18 June 2021; Published online: 07 July 2021

References

1. Ding, T. & Schloss, P. D. Dynamics and associations of microbial community types across the human body. Nature 509, 357–360 (2014).
2. Stanley, D. et al. Translocation and dissemination of commensal bacteria in post-stroke infection. Nat. Med. 22, 1277–1284 (2016).
3. Kitamoto S. et al. The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis. Cell 182, 447–462.e14 (2020).
4. Rackaiyte, E. et al. Viable bacterial colonization is highly limited in the human intestine in utero. Nat. Med. 26, 599–607 (2020).
5. Aagaard, K. et al. The placenta harbors a unique microbiome. Sci. Transl. Med. 6, 237ra265 (2014).
6. Chen, X. et al. Gut dysbiosis induces the development of pre-eclampsia through bacterial translocation. Gut 69, 513–522 (2020).
7. Schmidt, T. S. B. et al. Extensive transmission of microbes along the gastrointestinal tract. Elitef 8, e42693 (2019).
8. Wang, J., Jia, Z., Zhang, B., Peng, L. & Zhao, F. Tracing the accumulation of in vivo human oral microbiota elucidates microbial community dynamics at the gateway to the GI tract. Gut 69, 1355–1356 (2020).
9. Gaiser, R. A. et al. Endocervical microbiota in early cystic precursors to invasive pancreatic cancer. Gut 68, 2186–2194 (2019).
10. Komiyama, Y. et al. Patients with colorectal cancer have identical strains of Fusobacterium nucleatum in their colorectal cancer and oral cavity. Gut 68, 1335–1337 (2019).
11. Serrano, M. G. et al. Racial/ethnic diversity in the dynamics of the vaginal microbiome during pregnancy. Nat. Med. 25, 1001–1011 (2019).
12. Fettweis, I. M. et al. The vaginal microbiome and preterm birth. Nat. Med. 25, 1012–1019 (2019).
13. Koedooder, R. et al. Identification and evaluation of the microbiome in the female and male reproductive tracts. Hum. Reprod. Update 25, 298–325 (2019).
14. Baker, J. M., Chase, D. M. & Herbst-Kralovetz, M. M. Uterine microbiota: residents, tourists, or invaders? Front. Immunol. 9, 208 (2018).
15. Molina, N. M. et al. Analysing endometrial microbiome: methodological considerations and recommendations for good practice. Hum. Reprod. 36, 859–879 (2021).
16. Chen, C. et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nat. Commun. 8, 875 (2017).
17. Winters, A. D. et al. Does the endometrial cavity have a microbial molecular signature? Sci. Rep. 9, 9905 (2019).
18. Li, F. et al. The metagenome of the female upper reproductive tract. Gigascience 7, ggy107 (2018).
19. Mitra, J. et al. Vaginal microbiome of reproductive-age women. Proc. Natl Acad. Sci. USA 108, 4680–4681 (2011).
20. Gajer, P. et al. Temporal dynamics of the human vaginal microbiota. Sci. Transl. Med. 4, 132ra152 (2012).
21. Ma, B., Forney, L. J. & Ravel, J. Vaginal microbiota: rethinking health and disease. Annu. Rev. Microbiol. 66, 371–389 (2012).
22. Ravel, J. et al. Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. Microbiome 1, 29 (2013).
23. Molina N. M. et al. New opportunities for endometrial health by modifying uterine microbial composition: present or future? Biomolecules 10, 593 (2020).
24. Walker-Antonio, M. R. S. et al. Potential contribution of the uterine microbiome in the development of endometrial cancer. Genome Med. 8, 122 (2016).
25. Fang, R. L. et al. Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. Am. J. Transl. Res. 14, 1581–1592 (2016).
26. Amsel, M., Fennewald, G., Joosten, I. & Van der Molen, R. G. How uterine microbiota might be responsible for a receptive, fertile endometrium. Hum. Reprod. Update 24, 393–415 (2018).
27. Goldenberg, R. L., Hauth, J. C. & Andrews, W. W. Intrauterine infection and preterm delivery. N. Engl. J. Med. 342, 1500–1507 (2000).
28. Bisanz, J. et al. Microbiota at multiple body sites during pregnancy in a rural Tanzanian population and effects of moringa-supplemented probiotic yogurt. Appl Environ. Micro. 81, 4965–4975 (2015).
29. Walsh D. M. et al. Postmenopause as a key factor in the composition of the uterine microbiome (ECBiome). Sci Rep. 9, 19213 (2019).
30. Bassio, C. M. et al. Vaginal microbiota of adolescents and their mothers: a preliminary study of vertical transmission and persistence. BioRxiv, 768598 Preprint at https://doi.org/10.1101/768598 (2020).
31. Lichtenberg, E. S. & Henning, C. Conservation management of dostridial endometritis. Am. J. Obstet. Gynecol. 191, 266–270 (2004).
32. Mitra, A. et al. The vaginal microbiota associates with the regression of untreated cervical intraepithelial neoplasia 2 lesions. Nat. Commun. 11, 1999 (2020).
33. Jeon, S. J. et al. Blood as a route of transmission of uterine pathogens from the gut to the uterus in cows. Microbiome 5, 109 (2017).
34. DiGiulio, D. B. et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc. Natl Acad. Sci. USA 112, 11060–11065 (2015).
35. Kindinger, L. M. et al. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. Microbiome 5, 6 (2017).
36. Callahan, B. J. et al. Replication and refinement of a vaginal microbial signature of preterm birth in two racially distinct cohorts of US women. Proc. Natl Acad. Sci. USA 114, 9966–9971 (2017).
37. Gavri, A. et al. The endocervical microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome 4, 58 (2016).
38. Lev-Sagie, A. et al. Vaginal microbiome transplantation in women with intractable bacterial vaginosis. Nat. Med. 25, 1500–1504 (2019).
39. Cohen, C. R. et al. Randomized trial of Lactin-V to prevent recurrence of bacterial vaginosis. N. Engl. J. Med. 382, 1906–1915 (2020).
40. To, M. S. et al. Cervical cerclage for prevention of preterm delivery in women with short cervix: randomised controlled trial. Lancet 363, 1849–1853 (2004).
41. Savelli, L. et al. Histopathologic features and risk factors for benign, hyperplasia, and cancer in endometrial polyps. Am. J. Obstet. Gynecol. 188, 927–931 (2003).
42. Husby, A., Wohlfahrt, J. & Melbye, M. Pregnancy duration and endometrial cancer risk: nationwide cohort study. BMJ 366, 14693 (2019).
43. Romero, R. et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome 2, 4 (2014).
44. Andrews, W. W. et al. Association of asymptomatic bacterial vaginosis with endometrial microbiol colonisation and plasma cell endometritis in nonpregnant women. Am. J. Obstet. Gynecol. 195, 1611–1616 (2006).
45. Fischer, M. et al. Fatal toxic shock syndrome associated with Clostridium sordellii after medical abortion. N. Engl. J. Med. 333, 2352–2360 (2005).
46. Monisavias, D., Peng, J., Kang, Y. R. & Matzuk, M. M. Activin-like kinase 5 (ALK5) inactivation in the mouse uterus results in metastatic endometrial carcinoma. Proc. Natl Acad. Sci. USA 116, 3883–3892 (2019).
47. Thompson, K. E., Rayhon, S. L., Bailey, G., Delille, P. & McNerney, M. E. Assessment of cervical passage of vital dyes in pregnant, nonpregnant, and mated rats and mice. Reprod. Toxicol. 59, 1–7 (2016).
48. Moreno, L. et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. Am. J. Obstet. Gynecol. 218, 602.e601–e616 (2018).
49. Cicinelli, E. et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum. Reprod. 30, 323–330 (2015).
50. Cicinelli, E. et al. Unified diagnostic criteria for chronic endometritis at fluid hysteroscopy: proposal and reliability evaluation through an international randomized-controlled observer study. Fertil. Steril. 112, 162–173.e162 (2019).
51. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596 (2013).
52. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359 (2012).
53. Wang, J. F. et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. Gut 67, 1614–1625 (2018).
54. Caporaso, J. G. et al. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336 (2010).
55. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461 (2010).
56. Magoc, T. & Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963 (2011).
57. McDonald, D. et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618 (2012).
58. Dixon, P. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930 (2003).
59. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol. Biol. Evol. 26, 1641–1650 (2009).
60. Freedman, J. et al.Inferring correlation networks from genomic survey data. PLoS Comput. Biol. 8, e1002687 (2012).
61. Knights, D. et al. Bayesian community-wide culture-independent microbial source tracking. Nat. Methods 8, 761–U107 (2011).
62. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760 (2009).
63. Truong, D. T. et al. MetaFlaAn2 for enhanced metagenomic taxonomic profiling. Nat. Methods 12, 902–903 (2015).
64. Li, H. et al. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).
65. Martin, F. E., Nielharni, M. A., Jacques, N. A. & Hunter, N. Quantitative microbiological study of human carious dentine by culture and real-time PCR-association of anaerobes with histopathological changes in chronic pulpitis. J. Clin. Microbiol. 40, 1698–1704 (2002).
66. Hsu, X. L., Wang, H. Y., Wu, Q. & Xu, Y. Development, validation and application of specific primers for analyzing the clostridial diversity in dark fermentation pit mud by PCR-DGGE. Bioresour. Technol. 163, 40–47 (2014).
67. Dubernet, S., Desmasures, N. & Gueguen, M. A PCR-based method for identification of lactobacilli at the genus level. FEMS Microbiol. Lett. 214, 271–275 (2002).
68. Choi Y. S., Kim Y. C., Baek K. J. & Choi Y. In situ detection of bacteria within paraffin-embedded tissues using a digoxin-labeled DNA probe targeting 16S rRNA. J. Vis. Exp. e52836 (2015).

Acknowledgements
This work was supported by grants from the Beijing Natural Science Foundation (JQ18020) and the National Natural Science Foundation of China (32025009, 31722031, 31670119, 31870107, 32070120). We thank Lina Hou, Xuehan Li, Ming He, Shuai Chen, Ning Wang, and Zhenqiang Zuo for their assistance in sample preprocessing.

Author contributions
F.Z. conceived and supervised the study. F.Z. and J.W. designed the study, interpreted the results, and wrote the manuscript. Z.J., X.C., I.Y., and J.Y. collected the samples and clinical information. J.W. and Z.L. processed the samples, prepared the DNA for sequencing, and conducted the experiments. F.Z., J.W., Z.L., X.M., L.D., L.X., B.Z., and H.F. analyzed the data and prepared figures and tables.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-24516-8.

Correspondence and requests for materials should be addressed to F.Z.

Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.