



Figure 1: (A) Correlation analysis on the contents of vaginal microecology, including AV score, Nugent score, microbial density, microbial diversity, dominant flora, Lactobacillary grades, hypha, leukocytes. The absolute value of the correlation coefficient (cor.p) was <0.2, indicating that there was no correlation between the above detection contents at the four time points of 0, 1, 2, and 3 before and after the ozonated water lavage. (B) Changes in vaginal flora composition ratio at different time points of 0, 1, 2, and 3 before and after vaginal lavage.

V1-V2 variable region of the 16S rDNA genes was sequenced by Illumina Miseq platform according to the 300PE MiSeq protocol.

Of the 30 female volunteers, 29 completed three follow-up visits and one was lost at the third week of follow-up. Before the first vaginal ozonated water lavage, the first week, the second week, and the third week after the last lavage were marked as 0-, 1-, 2-, and 3-time points, respectively. (1) The results of Pearson correlation analysis (cor.p) on the contents of vaginal microecology [Figure 1A], including AV score, Nugent score, microbial density, microbial diversity, dominant flora, Lactobacillary grades, hypha, leukocytes, the absolute values of correlation coefficients of the above items were all <0.2, indicating that the above contents were not changed at four time points. (2) As shown in Figure 1B, the top three bacteria at the 4-time points are three *Lactobacillus* spp.,

that is, *L. iners*, *L. crispatus*, and *L. jensenii*. The type and abundance of *Lactobacillus* are no significant change before and after lavage of ozonated water.

By evaluating the proportion and type of *Lactobacillus*, the dominant flora in the vagina of healthy women, and vaginal microecology before and after ozonated water lavage, we have confirmed that ozonated water lavage has no obvious side effects on intravaginal microecology and *Lactobacillus*. As far as we know, there are no reports on the effects of ozone water on *Lactobacillus* and vaginal microecology so far. The results of this study can be seen as extremely important evidence that ozonated water to treat vaginitis has a minor side effect on vaginal microecology and *Lactobacillus*.

Ozonated water is a new treatment for vaginitis, which not only kills pathogens but also protects *Lactobacillus* and

vaginal microecology from disorders. When serious vaginal secretion increases, accompanied by odor, a large number of inflammatory secretions stimulate patients with vulva and perianal, causing discomfort. Therefore, in the treatment of vaginitis, we can consider using ozone water to rinse the vagina, reduce the concentration of local vaginal pathogens, to help the recovery of vaginal dominant bacteria. As a relatively new treatment concept, ozone water anti-infection has been widely used in many industries, but there is still a lack of a large number of clinical evidence in the treatment of vaginitis. More basic and clinical studies *in vivo* and *in vitro* are needed to provide a theoretical basis for the extensive participation of ozone water in the treatment of vaginitis in the future.

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Conflicts of interest

None.

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