

## Washed microbiota transplantation improves the fertility of patients with inflammatory bowel disease

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*To the Editor:* Female patients with inflammatory bowel disease (IBD) have lower fertility rates and take longer time to conceive than the healthy population. How to improve the fertility in patients with IBD is a critical challenge. A recent study implied that microbiota play an important role in the reproductive endocrine system throughout a woman's lifetime by interacting with estrogens, androgens, insulin, and other hormones.<sup>[1]</sup> Host-bacterial interaction is sex-dependent, as studies have shown the ability of microbiota to modulate gonadal sex hormones. Putting forward the concept that gut microbiota dysbiosis may negatively affect reproductive fitness, fecal microbiota transplantation (FMT) is an effective way of remodeling gut microbiota. The improved methodology of FMT based on the automatic washing process and the related delivering consideration was named as washed microbiota transplantation (WMT),<sup>[2]</sup> which was released as consensus statement by the FMT-standardization Study Group in 2019.<sup>[3]</sup> Washed preparation of fecal microbiota changes the transplantation related safety, quantitative method, and delivery of microbiota suspension. The present study aimed to retrospectively assess whether the fertility of patient with IBD could benefit from WMT.

This study was approved by the Ethics Committees of the Second Affiliated Hospital of Nanjing Medical University. Written informed consents were provided by all the participants. We recommend the patients to prepare the pregnancy when they have good clinical response (such as remission), at least 1 month after the last WMT. Female patients who were in remission after treatment (medicine or WMT) were included in the analysis. The WMT and delivering methods using colonic/mid-gut transendoscopic enteral tubing or endoscopic delivering were described in WMT methodology consensus report.<sup>[3]</sup> Fresh microbiota was enriched from feces donated by public donors who were 18–24-year-old college students. A course of

treatment included three WMTs, with three to five units (one unit with  $1.0 \times 10^{13}$  bacteria) per WMT.<sup>[3]</sup> Patients were excluded if themselves or their spouses had known diseases (eg, polycystic ovarian syndrome, azoospermia) affecting their fertility. Included patients were interviewed via telephone. First, all patients were categorized according to whether they had planned pregnancy or not (yes/no). Second, if the answer was “yes” one question on the time to pregnancy with two choices (“0–12 months” or “>12 months”) was provided. If the answer was “0 to 12 months” information on the detailed time to pregnancy, pregnancy complications, pregnancy outcomes, and data of fetus and offspring was collected. If the answer was “>12 months” these patients were defined as failure to conceive within 1 year. To better evaluate the long-term effects of WMT on the fetus and offspring, patients with unintended pregnancies were also included.

The data were assessed by SPSS (SPSS Inc., Chicago, IL, USA). The differences between groups were tested using Student's *t* test, chi-squared test or Fisher's exact test. Time to pregnancy was compared using Kaplan–Meier method and compared using the Log rank test. *P* value <0.05 was considered statistically significant.

Finally, 21 patients were included for analysis. They were divided into two groups according to the treatment with WMT (WMT group, *n* = 11) or without WMT (Without-WMT group, *n* = 10). The detailed characteristics of the two groups are presented in [Table 1]. The pregnancy rate in WMT group was significantly higher than that in Without-WMT group (*P* = 0.047). In WMT group, most of the time to pregnancy were <6 months, which was shorter than that in Without-WMT group (*P* = 0.017). Patients who failed to conceive in both groups were diagnosed as Crohn's disease. No pregnancy complications were observed in both groups. There were 2/10 and 9/11 of spontaneous vaginal deliveries in the Without-

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**Table 1: Characteristics of IBD patients with WMT and without WMT.**

Characteristics	Without-WMT N = 10	WMT N = 11	P value
Age (pregnancy plan) (years), median (IQR)	29.0 (27.5–30.5)	30.0 (27.0–32.0)	0.393 <sup>†</sup>
Age (onset) (years), median (IQR)	26.0 (23.0–27.8)	24.0 (24.0–28.0)	0.594 <sup>†</sup>
Disease duration (years), median (IQR)	3.0 (1.5–7.5)	4.0 (3.0–7.0)	0.547 <sup>†</sup>
Type of IBD, <i>n</i>			
Crohn’s disease	7	7	1.000 <sup>*</sup>
Ulcerative colitis	3	4	–
Intended pregnancy, <i>n</i>	7	11	
Success	3	10	0.047 <sup>*</sup>
TTP (months), median (IQR)	6.0 (6–6)	4.5 (1–6)	0.017 <sup>‡</sup>
Mode of delivery, <i>n</i>			0.036 <sup>*</sup>
Vaginal delivery	2	9	
Cesarean section	4	1	
Live birth, <i>n</i> (%)	6	10	–
Neonatal sex, <i>n</i>			1.000 <sup>*</sup>
Male	3	4	
Female	3	6	
Neonatal jaundice, <i>n</i>			0.588 <sup>*</sup>
Yes	1	4	
No	5	6	
Infant disease, <i>n</i>			–
Eczema	1	2	
Infection	2	1	
Allergy	0	1	
Failure	4	1	
Unintended pregnancy	3	0	–
Total number of pregnancies	6	10	–

IBD: Inflammatory bowel disease; IQR: Inter quartile range; WMT: Washed microbiota transplantation; TTP: Time to pregnancy. –: Not applicable. <sup>\*</sup>Fisher’s exact test. <sup>†</sup>Student’s *t* test or Mann-Whitney *U* test. <sup>‡</sup>Kaplan–Meier method and compared using the Log rank test.

WMT group and WMT group, respectively. No significant difference was shown in the incidence of neonatal complications between two groups. All offspring were healthy in intelligence, weight, and height. The detailed information is shown in [Supplementary Table 1, <http://links.lww.com/CM9/B119>]. A total of two patients in WMT group had menstrual disorders, both of them had normal menstrual cycle for at least 6 months after WMT.

Patients with quiescent IBD have similar fertility rates compared with the general population. Compared to non-IBD pregnancies in a recent study, the adjusted relative risk ratios for time to pregnancy of more than 12 months in women with IBD, ulcerative colitis, and CD were 1.28 (95%CI, 0.99–1.65), 1.10 (95%CI, 0.80–1.51), and 1.54 (95%CI, 1.03–2.30), respectively.<sup>[4]</sup> In this study, we compared the time to pregnancy between patients who were in remission after WMT or medicine. In WMT group, we found that most of the time to pregnancy were <6 months, which was shorter than that in Without-WMT group. The results showed that the WMT shortened the time to pregnancy. The pregnancy rate in WMT group was higher than that in Without-WMT group. It should be noted that WMT appears to help improve menstrual disorders. Our pilot findings mean that patients with IBD could benefit from WMT on fertility. The potential underlying mechanism might be associated with the altered gut microbiota after WMT. Interestingly, the rate

of spontaneous vaginal delivery in WMT group was higher, however, this phenomenon needs further studies to verify and demonstrate the mechanism. The maternal gut microbiota may influence the growth of bacteria in the newborn’s gut, affecting its function and the development of the immune system.<sup>[5]</sup> No significant difference in the incidence of neonatal complications between the two groups was shown in this study, and all offspring were healthy in intelligence, weight, and height, which preliminarily indicated that WMT may be safe for fetuses and offspring.

There were several limitations in the current study. The sample size was small and the sequencing was not available to show the dynamic changes of gut microbiota. A larger sample size based on randomized and multi-center studies would provide solid evidence in the future.

In conclusion, the current study indicated that the patients with IBD might benefit from WMT on fertility. WMT may be safe for fetuses and offspring.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal.

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### Conflicts of interest

Zhang F conceived the concept of GenFMter, trans-endoscopic enteral tubing, and related devices. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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