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Comparison of diagnostic tests for chronic endometritis and endometrial dysbiosis in recurrent implantation failure: Impact on pregnancy outcomes

Daiki Hiratsuka[1], Mitsunori Matsuo[1], Kosuke Kashiwabara[2], Mariko Inoue[2], Chihiro Ishizawa[1], Rei Iida[1], Yamato Fukui[1], Shizu Aikawa[1], Takehiro Hiraoka[1], Miyuki Harada[1], Osamu Wada-Hiraike[1], Yutaka Osuga[1] & Yasushi Hirota[1]✉

Chronic endometritis (CE) and endometrial dysbiosis (ED) are major causes of recurrent implantation failure (RIF). CE is diagnosed via hysteroscopy or the endometrial CD138 test; ED is examined using endometrial microbiome testing with next-generation sequencing. ED is characterized by a reduction in *Lactobacillus* species. However, correlations between the results of the three tests and the efficacy of treatment against CE and ED in pregnancy outcomes remain unclear. We analyzed 73 patients with RIF who underwent all three tests (hysteroscopy, endometrial CD138 test, and endometrial microbiome test). Patients with CE received antibiotics, whereas those with ED received antibiotics and vaginal *Lactobacillus* probiotics. The incidences of CE diagnosed using hysteroscopy and the CD138 test were 56.2 and 49.3%, respectively, and the prevalence of ED was 53.4%. No correlations were observed among the test-positive individuals in these three tests. Among patients with ED, 88.9% had a post-treatment clinical pregnancy, a significantly higher rate than that in patients without ED (p = 0.021). Multivariate analysis demonstrated that ED was associated with clinical pregnancy (odds ratio (OR): 6.29, p = 0.031). In conclusion, the three tests detected different populations of patients with RIF. ED diagnosed using the endometrial microbiome test was associated with favorable pregnancy outcomes after testing.

Keywords Chronic endometritis, Endometrial dysbiosis, Endometrial microbiome test, *Lactobacillus*, Recurrent implantation failure

Approximately 17.5% of couples who wish to conceive suffer from infertility for > 1 year[1] and they are treated using timing therapy, artificial insemination, and in vitro fertilization. However, some couples are unable to conceive even after IVF. When a couple is under 40 years of age and has transferred four or more good embryos three or more times without achieving pregnancy, the condition is referred to as recurrent implantation failure (RIF)[2]. RIF accounts for approximately 20% of all infertility cases and is an intractable form of infertility[2]. Chronic endometritis (CE) is observed in 30–57% of patients with RIF and is an important uterine factor of infertility[3–5]. CE is generally diagnosed using hysteroscopic findings and the endometrial CD138 test, and treated with antibiotics such as doxycycline[3–6]. However, antibiotic therapy for CE does not improve pregnancy rates[7], and the treatment of CE in patients with RIF has not yet been unified. The endometrial microbiome has recently been recognized as another uterine factor involved in RIF. The uterus contains a small number of bacteria, forming a bacterial flora similar to that of intestinal bacteria, and the endometrial microbiome test is considered normal when *Lactobacillus* species account for > 90% of the endometrial microbiome[8]. When an endometrial microbiome is abnormal the condition is termed endometrial dysbiosis (ED); ED reduces pregnancy rates[8], and patients with RIF associated with CE reportedly have a higher rate of ED[9]. The treatment of ED is not unified, but combined antibiotic and vaginal *Lactobacillus* probiotic treatment has been reported as one of the options[10,11].

[1]Department of Obstetrics and Gynecology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan. [2]Clinical Research Promotion Center, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan. ✉email: hirotay-gyn@h.u-tokyo.ac.jp

Based on these reports, it may be inferred that CE and ED in patients with RIF are related and that diagnosis can be made using hysteroscopy, the endometrial CD138 test, and the endometrial microbiome test. However, the correlations between the results of these three tests, including the endometrial microbiome test, and the usefulness of CE and ED detection and treatment on pregnancy outcomes remain unclear. In this study, we analyzed the results of these tests and clinical pregnancy.

Materials and methods

Data collection

This study was performed in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Biological Research Involving Human Subjects formulated by the Japanese Government. This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine at the University of Tokyo (IRB number: 10991). Written informed consent was substituted by an informed opt-out procedure because of the retrospective nature of this study.

This study retrospectively analyzed the anonymous clinical data of 73 patients under 43 years old with infertility who failed to achieve clinical pregnancy after the transfer of good-quality embryos (a Gardner blastocyst grading system score of ≥ 4BB) in a minimum of two fresh or frozen cycles. All 73 patients underwent hysteroscopy, endometrial CD138 testing, and endometrial microbiome testing at the University of Tokyo Hospital between April 2019 and March 2024 (Fig. 1). Endometrial CD138 testing and endometrial microbiome testing were performed on day 8 of progesterone vaginal supplementation of the hormone replacement cycle. Endometrial fluid was collected by inserting a pipette into the uterus after removing cervical mucus from the portio vaginalis. The endometrial fluid was injected into a collection tube and was submitted for analysis. The collected endometrial fluids for endometrial microbiome testing were analyzed using next-generation sequencing.

Clinical data including age; gravidity; parity; history of chemical abortion; spontaneous abortion; dilation and curettage; number of embryo transfers; complications such as adenomyosis, leiomyoma, or ovarian endometrioma; findings of hysteroscopy, endometrial CD138 test results, and endometrial microbiome test results; treatment history; and clinical pregnancy checked 1 year after treatment were obtained from the medical records. Of the patients with adenomyosis or leiomyomas, those with a uterus larger than a small pelvis or those with FIGO classification II leiomyomas grade 0–3 were excluded from the study. Patients with abnormal uterine cavities resulting from endometrial polyps, intrauterine adhesion, and uterine septum were also excluded to avoid the involvement of factors other than CE and ED as confounding factors.

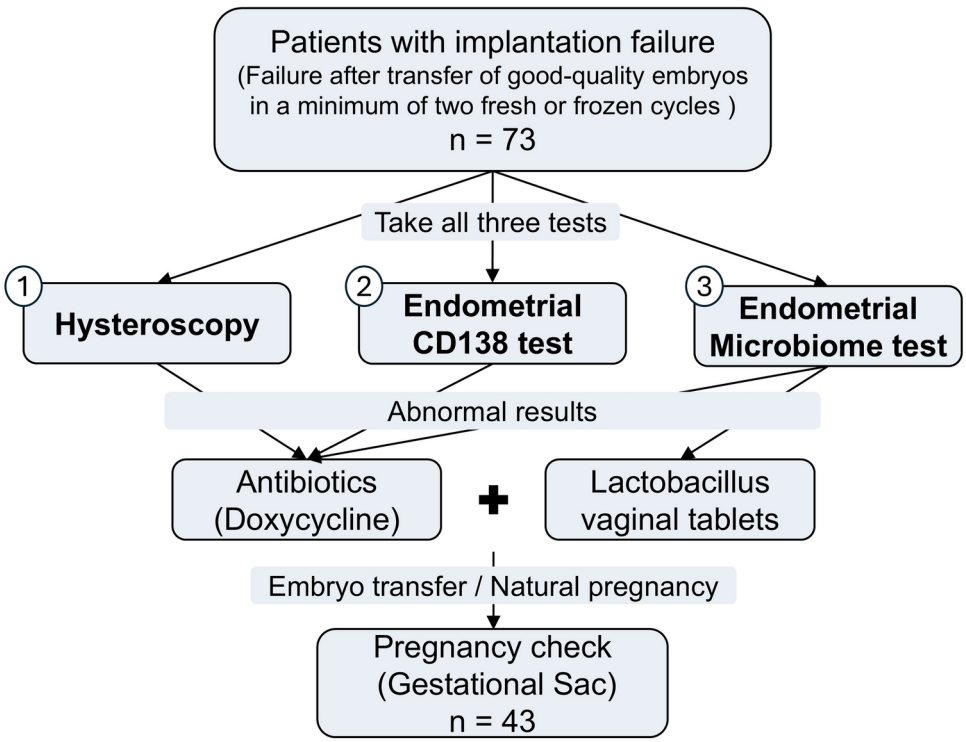

Fig. 1. Study flowchart.

Diagnostic criteria and treatment of chronic endometritis detected using hysteroscopy and the endometrial CD138 test, and endometrial dysbiosis detected using the endometrial microbiome test

CE was diagnosed on hysteroscopy when hyperemia, stromal edema, the presence of micropolyps, or the typical strawberry aspect was observed[6]. An endometrial CD138 test was considered positive when one or more plasma cells visualized using CD138 were detected in 10 high-power fields[3,6]. ED was diagnosed when the proportion of *Lactobacillus* species was < 90% according to the endometrial microbiome test.

For the treatment of CE diagnosed using hysteroscopy or an endometrial CD138 test, antibiotic therapy was administered performed with two weeks of doxycycline (200 mg on day 1, 100 mg on subsequent days). For the treatment of ED diagnosed using an endometrial microbiome test, in addition to the two weeks of doxycycline, supplementation with *Lactobacillus* vaginal tablets was administered. Tablets containing at least 2 billion *Lactobacilli* were used once a day from 4 weeks prior to embryo transfer to confirmation of the gestational sac.

Evaluations of correlations between the three tests and assessment of the factors influencing clinical pregnancy

First, to detect correlations among the three tests, the groups of patients who tested positive for each test were compared. Second, 43/73 patients who were tracked for clinical pregnancy outcomes were reviewed. Pregnancy rates were compared between the groups of patients who tested positive for each test. Third, to detect factors related to the achievement of clinical pregnancy, the patients were divided into two groups according to the presence or absence of each factor, and multivariate logistic regression analysis was performed. Among the 43 patients, we assessed the influence of the following eight factors: 1) Age ≥ 40 years; 2) Nulliparity, defined as no previous delivery; 3) History of ≥ 4 cycles of embryo transfer; 4) Presence of leiomyoma; 5) Presence of adenomyosis; 6) Positivity on hysteroscopy; 7) Positive endometrial CD138 test results; 8) Positive endometrial microbiome test results.

Statistical analysis

All statistical data were analyzed using R version 4.3.2. (R Foundation for Statistical Computing, Indianapolis, IN, USA) and Excel (Microsoft Corporation, Albuquerque, NM, USA). Correlations between the three tests were evaluated using McNemar's test, and those between the tests and pregnancy outcomes was evaluated using Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated to determine the strengths of the correlations. Statistical significance was set at $p < 0.05$.

Results

No correlations were observed between patients with positive results in the three tests

The data of 73 patients with RIF were reviewed (Table 1). For all three tests, the percentage of positive results was approximately 50% (hysteroscopy, 56.2%; the endometrial CD138 test, 49.3%; and the endometrial microbiome test, 53.4%). Of the 73 patients, 9 (12.3%) had all negative tests, 24 (32.9%) had one positive test, 28 (38.4%) had two positive tests, and 12 (16.4%) had all positive tests (Fig. 2). As shown in Table 2, no clear correlations were observed among the test-positive individuals in these tests (hysteroscopy and endometrial CD138 test, $p = 0.384$;

Characteristics	Average ± SD (Minimum—Maximum)
Age	35.7 ± 3.7 (28–42)
Age < 40 (years old)	84.9% (n = 62/73)
40 ≦ Age < 43 (years old)	15.1% (n = 11/73)
Gravidity	0.7 ± 1.0 (0–5)
Parity	0.2 ± 0.5 (0–2)
Chemical aborsion	0.1 ± 0.4 (0–2)
Spontaneous aborsion	0.3 ± 0.6 (0–2)
Induced aborsion	0.1 ± 0.3 (0–1)
Number of dilation and curettage	0.1 ± 0.4 (0–2)
Cycles of embryo transfer	3.2 ± 1.6 (2–8)
Adenomyosis	17.8% (n = 13/73)
Leiomyoma	27.4% (n = 20/73)
Ovarian endometrioma	23.3% (n = 17/73)
Results of three tests	
Abnormal hysteroscopic findings	56.2% (n = 41/73)
Endometrial CD138 positivity	49.3% (n = 36/73)
Endometrial dysbiosis	53.4% (n = 39/73)

Table 1. Characteristics of the 73 patients with implantation failure of the patients with adenomyosis or leiomyomas, those with a uterus larger than the small pelvis or those with FIGO classification II leiomyomas of grade 0–3 were excluded from this study. SD, standard deviation.

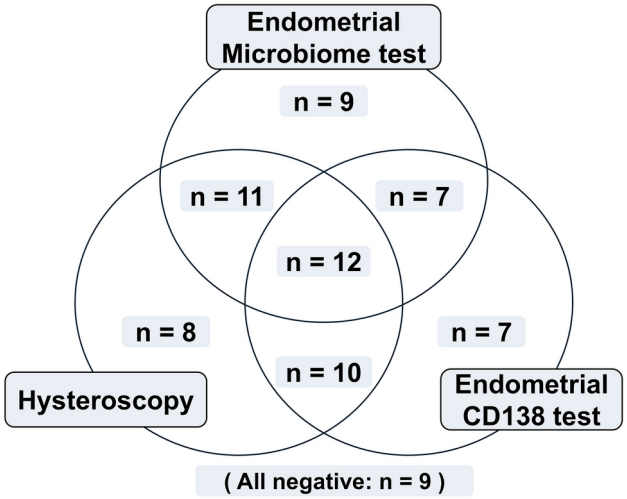

Fig. 2. Venn diagram of hysteroscopic, endometrial CD138 test, and endometrial microbiome test findings in 73 patients with RIF.

		Endometrial CD138 test			
		Positive	**Negative**	**Total**	**p-value**
Hysteroscopy	Positive	22	19	41	0.384
	Negative	14	18	32	
Total		36	37	73	
		Endometrial microbiome test			
		Positive	**Negative**	**Total**	**p-value**
Hysteroscopy	Positive	23	18	41	0.732
	Negative	16	16	32	
Total		39	34	73	
		Endometrial microbiome test			
		Positive	**Negative**	**Total**	**p-value**
Endometrial CD138 test	Positive	19	17	36	0.622
	Negative	20	17	37	
Total		39	34	73	

Table 2. Correlations among the test-positive individuals in the three tests.'Positive' means abnormal findings, and 'Negative' means normal findings.

hysteroscopy and endometrial microbiome test, p = 0.732; endometrial CD138 test and endometrial microbiome test, p = 0.622).

Patients with endometrial dysbiosis had a significantly high rate of clinical pregnancy after treatment

Of the 43 patients whose pregnancy outcomes were checked 1 year after treatment, 30 (69.8%) achieved clinical pregnancy (Table 3). Of the 30 patients who achieved clinical pregnancy, four patients had a natural pregnancy, while 26 patients became pregnant via embryo transfer. The percentage of double-embryo transfers did not significantly differ between the pregnant and non-pregnant groups ($p = 0.238$). Compared with the pregnancy group, patients in the non-pregnant group experienced significantly longer embryo transfer times ($p = 0.050$). Patients with ED detected using the endometrial microbiome test showed a significantly higher rate of clinical pregnancy after treatment than those without ED (88.9% vs. 56.0%, p = 0.021). Moreover, when comparing treatment strategies, those treated with antibiotics and *Lactobacillus* supplementation showed a significantly higher rate of clinical pregnancy after treatment than those treated with antibiotics and without *Lactobacillus* supplementation (88.9% vs. 60.0%, respectively; $p = 0.045$).

Treatment of endometrial dysbiosis is related to the achievement of clinical pregnancy

The treatment of ED was the only factor that influenced clinical pregnancy in multivariate analysis (OR 6.29, 95% CIs 1.18–33.3, p = 0.031) (Table 4). Conversely, the other factors including age, nulliparity, history of ≥ 4

		Clinical pregnancy (%)	p-value
All patients		69.8% (n = 30/43)	
Hysteroscopy	Positive	70.0% (n = 19/27)	0.911
	Negative	70.6% (n = 11/16)	
Endometrial CD138 test	Positive	65.0% (n = 13/20)	0.526
	Negative	73.9% (n = 17/23)	
Endometrial microbiome test	Positive	88.9% (n = 16/18)	0.021
	Negative	56.0% (n = 14/25)	

Table 3. Post-treatment clinical pregnancy outcomes for the three tests.

	Clinical pregnancy	
	OR (95% CIs)	p-value
Age > 40 years	1.270 (0.0670–23.9)	0.875
Nulliparity	0.498 (0.0394–6.30)	0.590
Embryo transfer ≧ 4 cycles	4.39 (0.462–41.7)	0.198
Leiomyoma	2.44 (0.444–13.4)	0.305
Adenomyosis	0.505 (0.0726–3.51)	0.490
Abnormal hysteroscopic findings	1.090 (0.217–5.46)	0.919
Endometrial CD138 positivity	0.813 (0.182–3.63)	0.786
Endometrial dysbiosis	6.29 (1.18–33.3)	0.031

Table 4. Multivariate analysis of the factors influencing clinical pregnancy.

cycles of embryo transfer, presence of leiomyoma or adenomyosis, positivity on hysteroscopy, and positivity in the endometrial CD138 test did not significantly affect clinical pregnancy.

Discussion

In this study, we analyzed the data of 73 patients with RIF who underwent three tests (hysteroscopy, the endometrial CD138 test, and the endometrial microbiome test). No correlations were observed among the hysteroscopic findings of CE, endometrial CD138 positivity, and endometrial microbiome testing in these patients. Only the ED group detected by endometrial microbiome testing showed significantly better post-treatment clinical pregnancy outcomes.

First, no correlations were observed among the hysteroscopic findings of CE, endometrial CD138 positivity, and endometrial microbiome testing in patients with RIF. Discordance between patient groups positive on hysteroscopy and the endometrial CD138 test has been previously reported[12], with a concordance rate of 42%[6]. This discrepancy may be related to differences in hysteroscopic skills, and lesions that are not captured on hysteroscopic imaging. The criteria for positivity in the endometrial CD138 test are also controversial[13]. Furthermore, in this study, the group of patients with ED differed from those positive for hysteroscopy and the endometrial CD138 test. Although patients with CE have a high probability of having ED[9], some patients with CE do not develop ED. CE is more common in patients with endometriosis and adenomyosis, and parturition is associated with a reduced incidence of CE[14,15]. This is categorized as immune-related CE, differing in pathogenesis from bacterial chronic endometritis with ED. The lack of correlation between the three tests in this study may have occurred for the above reasons. Thus, it is likely that the endometrial microbiome test selects a different patient population from the hysteroscopy and endometrial CD138 tests.

Second, the pregnancy outcomes in each of the test-positive patient groups were evaluated. In this study, 69.8% of patients subsequently achieved clinical pregnancy. This result suggests that interventions for CE and ED in patients with RIF may be meaningful. Our study also showed that 88.9% of patients with ED, confirmed using endometrial microbiome testing and treated with antibiotics and *Lactobacillus* supplementation, achieved clinical pregnancy after treatment, and only ED was associated with good subsequent pregnancy outcomes in the multivariate analysis. This association has two possible implications, namely, that endometrial microbiome testing is useful for detecting a specific population in terms of favorable pregnancy outcomes, and that treatment of ED with antibiotic therapy and supplementation with *Lactobacillus* vaginal tablets is promising. Although there have been reports of endometrial microbiomes specific to patients with RIF[16,17], this is the first study to show that the endometrial microbiome test is superior to the other two tests. It has been reported that it is unclear whether ED treatment leads to good pregnancy outcomes[18]. We observed that the diagnosis and treatment of ED led to good pregnancy outcomes in patients with RIF, suggesting that antibiotic therapy and supplementation with *Lactobacillus* vaginal tablets may have contributed to the improvement of the endometrial environment in these patients.

Based on the results of this study, combining these three tests to investigate the uterine factors of RIF could provide the necessary therapeutic intervention for a wide patient population with CE and ED. The significantly

better post-treatment pregnancy outcomes in patients with ED is a strength of this study and may provide important evidence for future practice in patients with RIF. The endometrial microbiome test may be the most beneficial if not all tests can be performed owing to cost or patient invasiveness.

However, this study had some limitations. First, it was a retrospective study. In this study, all the patients were East Asian ancestry living in Japan and the endometrial CD138 and endometrial microbiome tests were performed simultaneously during the secretory phase of the hormone replacement cycle; therefore, the patient conditions were somewhat consistent. However, data on the number of post-treatment transplants, whether pre-implantation genetic testing for embryo aneuploidy was performed, whether oocytes were collected again, and the rate of live birth were unavailable. In addition, one prospective study showed that the abnormal vaginal microbiota was not improved by treatment with *Lactobacillus* vaginal supplementation[19]. Further prospective studies of the endometrial microbiome should be conducted to obtain high-quality evidence. Second, retesting was not performed after treatment for CE and ED. Although it is important to confirm the improvement of CE or ED in posttreatment reexaminations, the majority of patients did not wish to be reexamined because of the invasiveness of reexamination, the prolonged period of reexamination, and the desire to perform embryo transfer immediately after treatment. Because this study did not have retesting data, it was challenging to conclude that the treatment of ED was completely effective. However, the findings clearly indicate that endometrial microbiome testing is useful for detecting a specific population in terms of favorable pregnancy outcomes, and that treatment of ED with antibiotic therapy and supplementation with *Lactobacillus* vaginal tablets is promising. Although few patients underwent reexamination in retrospective studies, it is necessary to continue reexamination of patients who wish to retest before the post-treatment embryo transfer or those who multiple good embryo transfers did not result in pregnancy after the treatment, and to accumulate cases. Third, the optimal duration of *Lactobacillus* vaginal tablet supplementation has not yet been established. Currently, three options are available for the treatment of bacterial flora: oral lactoferrin (prebiotics), oral *Lactobacillus* (probiotics), and *Lactobacillus* vaginal tablets (probiotics). *Lactobacillus* vaginal tablets were used in this study for their potentially direct effect on the endometrial microbiome[11,20]. Tablets containing at least 2 billion *Lactobacillus* were used once a day from one month before embryo transfer to confirmation of the gestational sac, but the actual duration and dosage may vary for each patient. Additionally, the standard antibiotic therapy used in this study was 2 weeks of doxycycline (200 mg on the first day and 100 mg on subsequent days). Ciprofloxacin and metronidazole may be used as second-line treatments[3]. Further studies on the appropriate duration and type of antibiotic therapy are warranted.

In conclusion, no correlations were observed among the hysteroscopic findings of chronic endometritis, positive endometrial CD138, and ED in patients with RIF, suggesting that different patient groups were detected by each test. Of the three tests, only the ED group detected by endometrial microbiome testing had significantly better post-treatment pregnancy outcomes.

Data availability

The data and materials underlying this article are available upon reasonable request from the corresponding authors.

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Author contributions
DH, MM, CI, RI, YF, SA, TH, and YH collected data. DH, MM, KK, MI, SA, YF, MH, OWH and YO discussed and interpreted the results. DH drafted the manuscript, edited by YH. MM and YH supervised the study.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to Y.H.

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