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ORIGINAL ARTICLE

Therapeutic efficacy of gentle endometrial curettage on antibiotic-resistant chronic endometritis in infertile women

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Abstract

Purpose: To identify the efficacy of endometrial curettage on antibiotic-resistant chronic endometritis (CE) in infertile women.

Methods: Of 1580 women with CE, 87 with antibiotic-resistant CE after two to five cycles of antibiotic treatment were recruited between 2019 and 2021. The women who underwent endometrial curettage without applying any force and, in the subsequent menstrual cycle, endometrial sampling for CD138 immunostaining without antibiotic use. Pregnancy outcomes after in vitro fertilization treatment were analyzed in women who did not desire endometrial curettage and in those with cured and persistent CE after endometrial curettage.

Results: In 64 women who underwent endometrial curettage, the number of CD138-positive cells decreased from 28.0 ± 35.3 to 7.7 ± 14.0 ($p < 0.0001$), and CE in 41 women (64.1%) was cured (<5 CD138-positive cells). The pathological findings detected 3.1% of endometrial hyperplasia and 1.6% of endometrial cancer. The ongoing pregnancy rates in women aged ≤42 without endometrial curettage were significantly lower than those of women with cured and persistent CE (26.7%, 67.6%, and 57.1%, respectively, $p = 0.03$).

Conclusions: Gentle endometrial curettage for antibiotic-resistant CE significantly decreased the number of CD138-positive cells, resulting in improved pregnancy outcomes regardless of remaining CE. Endometrial curettage is also important as a screening for endometrial malignancy.

KEYWORDS
antibiotic resistance, CD138, chronic endometritis, endometrial curettage, infertility

Keiji Kuroda and Shunsuke Ishiyama contributed equally to this work.

1 | INTRODUCTION

Chronic endometritis (CE) is defined as a local endometrial inflammatory status with plasma cells across different menstrual cycles. CE has a harmful influence on the decidual transformation of human endometrium, resulting in a deviating or disappearing window of implantation and a decrease in the opportunity for embryo implantation.[1–3] Therefore, CE is detected in 30%–57% of infertile women with a history of implantation failure after multiple embryo transfer (ET) cycles.[4–6] Furthermore, CE is primarily caused by intrauterine infection with a wide variety of microorganisms, with the current treatment protocol for CE being broad-spectrum antibiotic therapy,[4,7] and is associated with an increased risk of complications during pregnancy, including miscarriage[8]; thus, the incidence of CE in women with recurrent pregnancy loss is also high, ranging from 24% to 56%.[9–12] Basic first-line treatment is oral doxycycline for 2 weeks due to its high recovery rate from CE (66%–93%).[4,7] The healing of CE can improve subsequent pregnancy outcomes during in vitro fertilization (IVF) treatment.[3,6,7,13,14]

Nevertheless, not all cases of CE are caused by microorganism infection.[15] Based on data obtained from an endometrial microbiome analysis, most infertile women with CE had less *Lactobacillus*, although some had a *Lactobacillus*-dominant endometrium.[16] Therefore, antibiotic therapy cannot overcome all CE cases. According to previous studies, 1%–25% of cases of CE were not cured after two or more cycles of broad-spectrum antibiotic therapy, including doxycycline.[7,13,17]

In our previous study, we found that most CE cases with intrauterine disorders could be cured via hysteroscopic surgery without antibiotic therapy.[18,19] During surgery, the endometrium with typical CE findings, including an erythrogenic surface, stromal edema, and micropolyps, was removed gently using a resecting loop without electrode application. The finding suggests that the artificial removal of the endometrium with a persistent inflammatory condition can be a potential treatment for antibiotic-resistant CE.

However, hysteroscopic surgery requires specialized equipment and the skills of the surgeon. Therefore, to enable every doctor to treat intractable CE without specialized equipment, we evaluated the therapeutic efficacy of endometrial curettage for antibiotic-resistant CE.

2 | MATERIALS AND METHODS

2.1 | Participant selection

The study protocol of this cross-sectional study was approved by the Ethics Committee of Sugiyama Clinic, Tokyo, Japan (No. 18-004 and 22-007). Of 1580 infertile women diagnosed histologically with CE between January 2019 and June 2021, CE in 1199 women (75.9%) was cured by one cycle of antibiotic therapy, including doxycycline (Figure 1). Of the remaining 381 women (24.1%), 34 chose not to undergo an endometrial biopsy for CD138 immunostaining. An additional one to four cycles of antibiotic therapy cured CE in 260 women (16.5%); however, the other women did not recover from CE. In this study, we recruited 87 (5.5%) women with antibiotic-resistant CE and recommended dilation and curettage (D&C) as a CE treatment. Written informed consent, including complications of D&C such as risks of uterine perforation and thinning of endometrium was obtained from all the women before the surgery was performed. Twenty-two women did not desire to undergo D&C, and 65 underwent D&C. We compared the three groups, including the patients who did not desire D&C (non-D&C group) and patients with and without CE after D&C (the persistent CE group and the cured CE group, respectively). Of the recruited women, we evaluated the pregnancy outcomes of 72 women aged 42 years or less with antibiotic-resistant CE who underwent fertility treatment in our clinic.

2.2 | Tests, diagnosis, and treatment for CE

To confirm the intrauterine circumstance, we performed a hysteroscopy and endometrial biopsy for the plasmacyte marker, CD138 immunohistochemistry staining, and endometrial bacterial culture with antibiotic sensitivity testing in all recruited women. When we found intrauterine disorders, including endometrial polyps, submucosal myomas, and intrauterine adhesion via hysteroscopy, we excluded them from this study. Endometrial sampling was conducted using an endometrial suction curette (Pipet Curet; Fuji Medical Corporation) during the luteal phase after the vagina was rinsed thoroughly with physiological saline to prevent sample contamination. The endometrial samples were divided into two: one was fixed in 10% formaldehyde for histological examination with CD138 immunostaining, and the other was kept in the tube for endometrial bacterial culture with antibiotic sensitivity testing. We sent both samples to BML, Inc. Pathologists stained the specimens using anti-CD138 antibodies (M7228; Dako, Agilent Technologies Japan, Ltd.) and counted the CD138-positive plasma cells in 10 nonoverlapping random stromal areas visualized at 400-fold magnification. CE was diagnosed in cases with five or more CD138-positive plasma cells.

When CE was diagnosed and specific bacteria were found, except for *Lactobacillus* spp. or *Bifidobacterium* spp., patients received bacterium-sensitive antibiotics for 2 weeks. If CE was found without specific bacteria, oral doxycycline (Vibramycin® tablets; Pfizer Japan Inc.), 100 mg twice a day for 2 weeks, was administered as the first-line treatment. If CE was not cured with or without specific bacteria, second-line therapy consisted of bacterium-sensitive antibiotics for 2 weeks or ciprofloxacin (Ciproxan®, 200 mg; Bayer Yakuhin, Ltd.) and metronidazole (Flagyl®, 250 mg; Shionogi & Co., Ltd.) twice daily for 2 weeks, respectively. When CE with specific bacteria was detected after 2 or more cycles of antibiotics, bacterium-sensitive antibiotics were given for 2 weeks. When CE was not cured by 2 or more cycles of antibiotic treatment, the inflammatory endometrium with plasma cells was artificially removed by D&C.

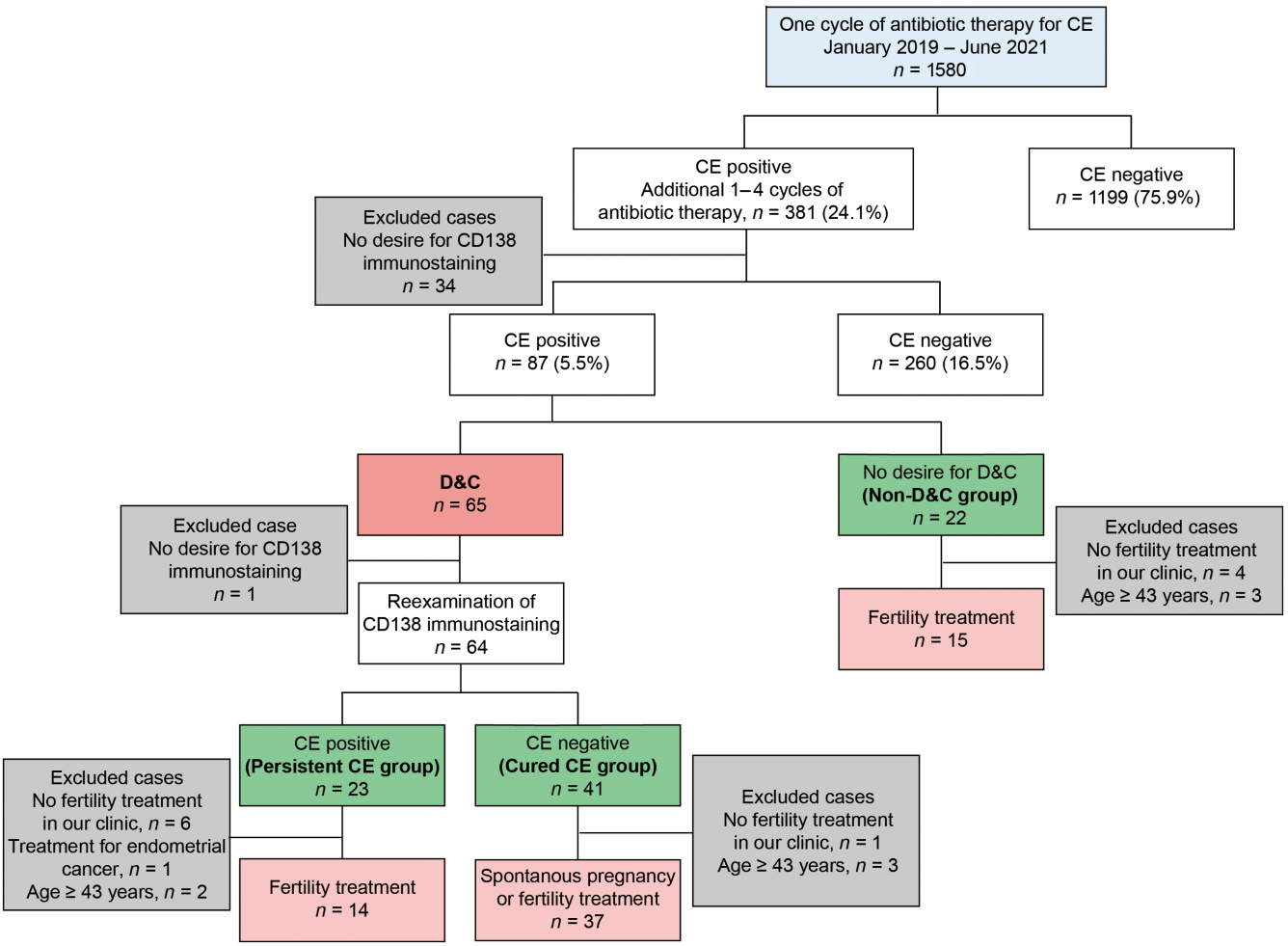

FIGURE 1 Patient selection. Flow chart showing participant recruitment. Of 1580 infertile women diagnosed histologically with CE, one cycle of antibiotic therapy cured CE in 1199 women (75.9%) between January 2019 and June 2021. Of the remaining 357 women (24.1%), after excluding 34 women who did not desire reexamination of endometrial biopsy, an additional one to four cycles of antibiotic therapy cured CE in 260 women (16.5%). We recruited 87 (5.5%) women with antibiotic-resistant CE for this study and performed dilation and curettage (D&C) on 65 of them. D&C led to recovery from CE in 41 women (the cured CE group) but not in 23 women (the persistent CE group). Twenty-two women chose not to undergo D&C (the non-D&C group). Of the recruited women, we evaluated pregnancy outcomes in 66 women aged ≤42 years with antibiotic-resistant CE who underwent fertility treatment in our clinic.

2.3 | Endometrial curettage and restarting fertility treatment

Endometrial curettage was performed for women with uncured CE. Before D&C, the vagina was washed sufficiently with physiological saline, and endometrial sampling was performed using an endometrial suction curette for endometrial microbiome testing (Varinos Inc.). Under intravenous anesthesia, the cervical canal was dilated to Hegar No. 13, and the endometrium was gently removed from the entire inside of the uterus without applying any force using a blunt uterine curette. The endometrium specimen was sent to BML for histopathologic examination.

An endometrial biopsy for CD138 immunostaining and intrauterine bacterial culture was performed without antibiotic use in the subsequent menstrual cycle after D&C, during the luteal phase. Based on the results of the bacterial culture and/or endometrial microbiome testing, one cycle of bacterium-sensitive antibiotics was administered to 21 patients after D&C. Fertility treatment was restarted after D&C, regardless of the remaining CE.

In endometrial microbiome testing, dysbiosis was defined as <80% of *Lactobacillus* and *Bifidobacterium*-dominant microbiota, based on a previous report.[20,21]

2.4 | Pregnancy outcomes in patients with and without endometrial curettage

Sixty-six women aged 42 years or younger with antibiotic-resistant CE restarted fertility treatment in our clinic. We compared pregnancy outcomes after CE treatment in the non-D&C (15 women), cured CE (37 women), and persistent CE groups (14 women). Clinical pregnancy is defined as an intrauterine gestational sac confirmed

via transvaginal ultrasound. A miscarriage is defined as a pregnancy loss in clinical pregnancy. An ongoing pregnancy was defined as a pregnancy that had completed 12 weeks of gestation.

2.5 | Statistical analysis

We conducted the statistical analysis using GraphPad Prism 5 (GraphPad Software Inc.). Differences in variables among two or three groups were analyzed using the Mann–Whitney *U*-test, Kruskal–Wallis test, or Fisher's exact test, as appropriate. The level of significance was defined as a p value of <0.05.

3 | RESULTS

3.1 | Therapeutic effects of endometrial curettage for antibiotic-resistant CE

In our study, two to five cycles of antibiotic therapy cured CE in 94.5% of the women with histologically diagnosed CE, yet 5.5% (87/1580 women) did not recover from CE (Figure 1). Among the 64 women who underwent D&C and reexamination of CD138 immunostaining, the antibiotic treatments could not significantly reduce the number of CD138-positive cells (average ± SEM) from 35.8 ± 8.3 to 28.0 ± 4.4 ($p = 0.93$; Figure 2). However, endometrial curettage decreased endometrial plasmacytes in 85.9% (55/64 women), with 7.7 ± 1.7 of the plasma cells after D&C ($p < 0.0001$), resulting in 41 women (64.1%) who recovered from intractable CE. There were no cases of complications after D&C.

Table 1 summarizes the characteristics of the recruited patients. There were no significant differences in patient age, duration of infertility, history of pregnancy, causes of infertility, serum anti-Müllerian hormone level, or number of previous ET cycles among the three groups. In the non-D&C, cured CE, and persistent CE groups, the number of CD138-positive plasma cells (average ± SD) at the first biopsy was 29.5 ± 29.2, 33.1 ± 73.8, and 40.7 ± 54.2, respectively, per 10 nonoverlapping random stromal areas ($p = 0.20$). After two to five cycles of antibiotic therapy, the number of plasma cells decreased by 9.6 ± 11.0, 26.2 ± 34.0, and 31.0 ± 38.1 in the non-D&C, cured CE, and persistent CE groups, respectively. Some women in the non-D&C group did not desire D&C because antibiotics reduced the number of CD138-positive cells to a certain degree; therefore, the number of plasma cells in the non-D&C group was significantly lower than that in the cured and persistent CE groups ($p < 0.0001$).

At D&C, the pathological findings detected 3.1% of endometrial polyps, 3.1% of endometrial hyperplasia, and 1.6% of endometrial cancer (G1; Table 2). In intrauterine bacterial culture testing, bacteria except *Lactobacillus* spp. and *Bifidobacterium* spp. were detected in approximately 30% of both cured and persistent CE groups ($p = 0.21$). Endometrial microbiome analysis indicated that 70%–80% of the microbiotas are *Lactobacillus* spp. and *Bifidobacterium* spp.; therefore, dysbiosis without *Lactobacillus* and *Bifidobacterium*-dominant microbiotas occurred in approximately 25% of cases in both groups ($p = 1.00$).

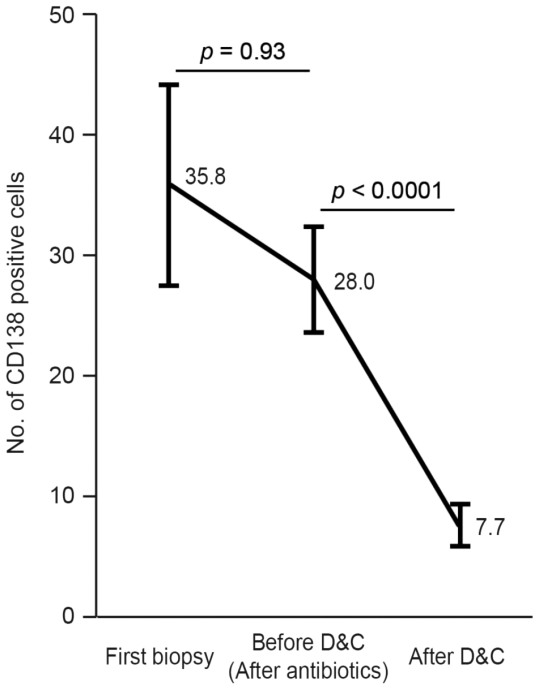

FIGURE 2 Change in the number of CD138-positive cells before and after D&C. Among the 64 women who underwent D&C and reexamination of CD138 immunostaining, antibiotic treatments could not significantly reduce the number of CD138-positive cells (average ± SEM) from 35.8 ± 8.3 to 28.0 ± 4.4 ($p = 0.93$); however, D&C decreased to 7.7 ± 1.7 ($p < 0.0001$).

In 21 patients after D&C, nine women in the persistent CE group underwent reexamination of endometrial CD138 immunostaining after antibiotic treatment, yet cured CE was confirmed in only one case (11.1%), with additional bacterium-sensitive antibiotics being administered based on the results of bacterial culture and/or endometrial microbiome testing.

3.2 | Pregnancy outcomes

Table 3 shows the pregnancy outcomes of the recruited women. Suprisingly after D&C, three women (7.9%) conceived spontaneously in the cured CE group. The other 63 women underwent IVF treatment. There were no significant differences in the number of transferred embryos or endometrial thickness at ET among the three groups. The cumulative pregnancy rates within two ET cycles in the women in the non-D&C, cured CE, and persistent CE groups were 33.3%, 78.4%, and 57.1%, respectively. The cumulative pregnancy outcome in the women with cured CE after D&C was significantly higher than that of the other two groups ($p = 0.007$). There was no significant difference in the miscarriage rates in the three groups ($p = 0.60$). The cumulative ongoing pregnancy rates in women without D&C were

TABLE 1 Patient characteristics.

		D&C		
	Non-D&C group	Cured CE group	Persistent CE group	
	n = 22	*n* = 41	*n* = 23	*p* Value
Age, years	38.2 ± 4.9	36.3 ± 4.8	38.3 ± 3.7	0.10
Duration of infertility, years	2.5 ± 1.4	2.7 ± 1.6	3.0 ± 3.0	0.87
History of pregnancy				
Gravida	0.5 (0–3)	0 (0–4)	1 (0–4)	0.29
Para	0 (0–1)	0 (0–2)	0 (0–2)	0.51
Rate of recurrent pregnancy loss	1 (4.5)	1 (2.4)	1 (4.3)	1.00
Causes for infertility				
Tubal factors	3 (13.6)	4 (9.8)	1 (4.3)	0.50
Endometriosis	2 (9.1)	2 (4.9)	4 (17.4)	0.27
Ovarian factors	7 (31.8)	11 (26.8)	12 (52.2)	0.14
Male factors	4 (18.2)	7 (17.1)	5 (21.7)	0.94
Unexplained infertility	9 (40.9)	20 (48.8)	7 (30.4)	0.36
Serum AMH level, ng/ml	2.5 ± 2.6	2.8 ± 2.4	2.7 ± 3.4	0.47
No. of previous embryo transfer	2 (0–6)	2 (0–11)	1 (0–5)	0.15
Rate of repeated implantation failure[a]	3 (13.6)	15 (35.7)	7 (30.4)	0.16
No. of CD138-positive cells at first biopsy[b]	29.5 ± 29.2	33.1 ± 73.8	40.7 ± 54.2	0.20
No. of treatment cycles using antibiotics before D&C	2 (2–4)	2 (2–4)	2 (2–5)	0.38
No. of CD138-positive cells after antibiotics[b]	9.6 ± 11.0	26.2 ± 34.0	31.0 ± 38.1	<0.0001*

Abbreviations: AMH, anti-Müllerian hormone; CE, chronic endometritis; D&C, dilatation and curettage.

[a]Repeated implantation failure was diagnosed as history of implantation failure after three or more embryo transfer cycles.

[b]Immunohistochemistry for CD138-positive cells was counted in 10 nonoverlapping random stromal areas visualized at 400-fold magnification. Chronic endometritis was diagnosed as five or more CD138-positive cells. Data are presented as *n* (%) or mean ± SD or median (range).

*$p < 0.05$.

significantly lower than those of women with cured and persistent CE (26.7%, 67.6%, and 57.1%, respectively, $p = 0.03$; Figure 3).

To identify the impact factors on pregnancy outcomes among three groups, we compared 38 women with ongoing pregnancy to 28 women with pregnancy failure, including implantation failure and miscarriage (Table S1). Patient age was significantly younger in the ongoing pregnancy group as compared with the pregnancy failure group ($p = 0.02$). Furthermore, the D&C rate and the number of CD138-positive cells before first ET were significantly higher and lower, respectively, in the ongoing pregnancy group as compared with the pregnancy failure group (both $p = 0.04$).

4 | DISCUSSION

This is the first study to identify the therapeutic effects of gentle endometrial curettage on antibiotic-resistant CE. Of CE cases, 94.5% in this study were cured by antibiotic treatment alone, where the main cause of CE is intrauterine infection with a wide variety of microorganisms.[22] However, there have been no reports of treatment protocols without antibiotic use for CE in the absence of intrauterine disorders. In this study, an improvement in CE was observed in two-thirds of the cases of antibiotic-resistant CE after gentle endometrial curettage. Surprisingly, three of the infertile women in the cured CE group who had undergone IVF treatment had spontaneous pregnancies before ET. In addition, even if CE had not decreased to <5 CD138-positive cells, the patients who had undergone D&C had pregnancy outcomes as good as those in the cured CE group, and they had significantly higher ongoing pregnancy rates compared with the patients without D&C.

The human endometrium is cyclically remodeled through proliferation, decidualization, and menstruation. Cyclic regeneration with the recruitment of endometrial stem cells plays a significant role in successful pregnancy.[23] D&C may support the removal of inflammatory endometrium across different menstrual cycles and regenerate a new functional layer. Gentle endometrial curettage has beneficial

TABLE 2 Findings of endometrial biopsies before and after D&C.

	Cured CE group	Persistent CE group		Total
	n = 41	*n* = 23	*p* Value	*n* = 64
Pathological findings at D&C				
Normal endometrium	38 (92.7)	21 (91.3)	0.44	60 (93.8)
Endometrial polyp	2 (4.9)	0 (0)		2 (3.1)
Endometrial hyperplasia	1 (2.4)	1 (4.3)		2 (3.1)
Endometrial cancer	0 (0)	1 (4.3)		1 (1.6)
No. of CD138-positive plasma cells after D&C[a]	1.3 ± 1.3	19.0 ± 18.8	<0.0001*	7.7 ± 14.0
Detection rate of intrauterine bacterial culture				
Lactobacillus and *Bifidobacterium*	5 (12.2)	3 (13.0)	1.00	8 (12.5)
Other bacteria	12 (29.3)	8 (34.8)	0.21	20 (31.3)
No detection	24 (58.5)	13 (56.5)	1.00	37 (57.8)
Endometrial microbiome analysis	***n* = 15**	***n* = 12**		***n* = 27**
Lactobacillus and *Bifidobacterium*, %	69.6 ± 43.3	76.2 ± 43.3	0.36	72.3 ± 42.2
Other bacteria, %	30.4 ± 43.3	23.8 ± 43.3	0.36	27.7 ± 42.2
Dysbiosis[b]	4 (26.7)	3 (25.0)	1.00	7 (25.9)
Rate of additional antibiotic treatment	12 (29.3)	9 (39.1)	0.58	21 (32.8)
		***n* = 9**		
No. of CD138-positive cells after antibiotics[a]	–	18.7 ± 25.5	–	–
Recovery rate from CE after additional antibiotics	–	1 (11.1)	–	–

Note: Data are presented as *n* (%) or mean ± SD.

Abbreviations: CE, chronic endometritis; D&C, dilatation, and curettage.

[a]Immunohistochemistry for CD138-positive cells was counted in ten nonoverlapping random stromal areas visualized at 400-fold magnification. Chronic endometritis was diagnosed as five or more CD138-positive cells.

[b]Dysbiosis was defined as <80% of *Lactobacillus* and *Bifidobacterium*-dominant microbiota.

*$p < 0.05$.

effects on subsequent pregnancy outcomes, regardless of remaining CE.

Specific bacteria and dysbiosis were not found in the results of the endometrial bacteria culture and microbiome testing in 65%–75% of women who underwent D&C. Furthermore, additional antibiotic therapy after D&C is almost ineffective for antibiotic-resistant CE. Endometrial microbiome analysis can only show the rate of endometrial microbiota, not the number of microorganisms. Therefore, most CE cases after multiple antibiotics are not cured by additional antibiotic use based on the microbiome analysis results. Although the use of multiple antibiotics might have lowered the likelihood of detecting specific bacteria in this study, antibiotic-resistant CE may include noninfectious CE. In our previous study, unnecessary antibiotic use after hysteroscopic surgery for endometrial polyps with CE was associated with delayed CE healing and impaired pregnancy outcomes as compared with cases without additional doxycycline treatment after surgery.[18] Therefore, unnecessary antibiotic therapy for CE may have an adverse effect on subsequent pregnancy outcomes. Furthermore, inappropriate systemic antibiotic treatment may increase the occurrence of antibiotic-resistant bacteria, which has been increasing globally in infectious diseases.[24,25] Therefore, the inappropriate use of broad-spectrum antibiotics for CE should be avoided. Noting that infectious CE is also included in the women with antibiotic-resistant CE. Removal of the source of the infection advances the healing of an infectious disease. Thus, D&C can be efficacious for infectious CE.

Although CE has received much attention since its association with repeated reproductive failures was revealed, it was first reported 100 or more years ago.[26] This is the first study of the therapeutic effect of D&C on CE; however, in 1972, Wallach[27] described one of the treatments for CE as follows: "dilatation and curettage is also immediately followed by the cyclic administration of and

TABLE 3 Pregnancy outcomes.

		D&C		
	Non-D&C group	Cured CE group	Persistent CE group	
	n = 15	*n* = 37	*n* = 14	*p* Value
Age, years	36.7 ± 4.1	35.5 ± 4.2	38.3 ± 3.3	0.07
Serum AMH level, ng/ml	2.4 ± 2.6	3.1 ± 2.4	2.2 ± 1.8	0.25
Spontaneous pregnancy	0 (0)	3 (7.9)	0 (0)	0.42
First embryo transfer	***n* = 15**	***n* = 34**	***n* = 14**	
Number of transferred embryos per cycle	1.1 ± 0.3	1.1 ± 0.2	1.2 ± 0.4	0.22
Endometrial thickness at embryo transfer, mm	10.6 ± 2.3	10.3 ± 2.4	10.5 ± 3.3	0.86
Vitrified-warmed embryo transfer	11 (73.3)	27 (79.4)	10 (71.4)	0.79
Cleavage stage embryos	4 (26.7)	7 (20.6)	4 (28.6)	
Morphologically poor blastocysts	2 (13.3)	4 (11.8)	2 (14.3)	0.94
Morphologically good blastocysts	9 (60.0)	2 (67.6)	8 (57.1)	
Clinical pregnancy rate	4 (26.7)	18 (52.9)	7 (50.0)	0.23
Miscarriage rate	1 (25.0)	3 (16.7)	0 (0)	0.58
Ongoing pregnancy rate	3 (20.0)	15 (44.1)	7 (50.0)	0.21
Second embryo transfer	***n* = 8**	***n* = 17**	***n* = 4**	
Number of transferred embryos per cycle	1.1 ± 0.4	1.2 ± 0.4	1.3 ± 0.5	0.87
Endometrial thickness at embryo transfer, mm	10.0 ± 2.2	10.3 ± 2.2	10.4 ± 3.4	0.94
Vitrified-warmed embryo transfer	8 (100)	15 (88.2)	4 (100)	1.00
Cleavage stage embryos	3 (37.5)	5 (29.4)	1 (25.0)	0.79
Morphologically poor blastocysts	0 (0)	3 (17.6)	1 (25.0)	
Morphologically good blastocysts	5 (62.5)	9 (52.9)	2 (50.0)	
Clinical pregnancy rate	1 (12.5)	8 (47.1)	1 (25.0)	0.26
Miscarriage rate	0 (0)	1 (12.5)	0 (0)	1.00
Ongoing pregnancy rate	1 (12.5)	7 (41.2)	1 (25.0)	0.40
Total clinical pregnancy rate within two ETs	5 (33.3)	29 (78.4)	8 (57.1)	0.007*
Total miscarriage rate within two ETs	1 (20.0)	4 (13.8)	0 (0)	0.60
Total ongoing pregnancy rate within two ETs	4 (26.7)	25 (67.6)	8 (57.1)	0.03*

Note: Data are presented as *n* (%) or mean ± SD.

Abbreviations: AMH, anti-Müllerian hormone; CE, chronic endometritis; ET, embryo transfer.

*$p < 0.05$.

estrogen in high dosage for 1 or 2 months to achieve a rapid shearing of regenerated tissue." Despite the lack of detailed data, Wallach noticed the therapeutic effects of the artificial removal of inflammatory endometrium on CE. D&C is associated with a subsequent thin endometrium, leading to decreased female fecundity.[28] Therefore, when removing the endometrium with CE using a uterine curette, it is important to perform it without applying any force. In this study, there was no significant difference in the endometrial thickness between the women with and without D&C.

From the histopathological findings, D&C revealed 3.1% of endometrial hyperplasia and 1.6% of endometrial cancer in patients. In reproductive-aged women, the total incidence of endometrial hyperplasia and cancer is <0.1%[29,30]; therefore, antibiotic-ineffective CE must be a risk factor for endometrial cancer. A chronic inflammation-mediated condition triggers the onset of neoplastic transformation and the development of cancer in the female reproductive tract.[31,32] In this study, we performed endometrial sampling several times prior to D&C; however, endometrial atypia, or cancer, was not detected at that time. The average number of CD138-positive cells at the first endometrial biopsy in the three women with endometrial hyperplasia and cancer was 27.3 ± 10.0, which did not significantly differ from 33.1 ± 73.8 to 40.7 ± 54.2 in the cured and persistent CE groups, respectively. Thus, D&C for antibiotic-resistant CE is also important for identifying the presence of atypical or malignant findings.

This study has some limitations. First, we cannot analyze the predictive factor of a successful pregnancy using multivariate analysis, as the number of recruited patients was small, with most CE being

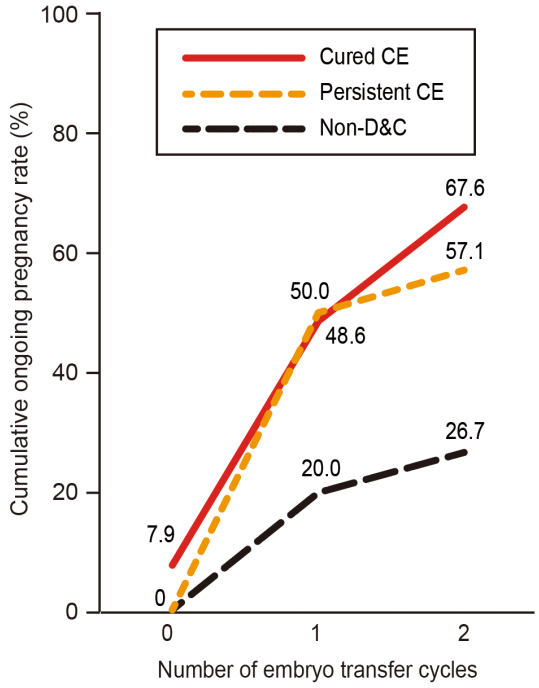

FIGURE 3 Cumulative ongoing pregnancy rates. Pregnancy outcomes in 15 women without D&C (non-D&C), in 37 women with cured CE, and in 14 women with persistent CE after D&C are shown. After D&C, three women (7.9%) in the cured CE group conceived spontaneously, and the others underwent IVF treatment. The cumulative ongoing pregnancy rates after one to two embryo transfer cycles in the women in the non-D&C, cured CE, and persistent CE groups were 26.7%, 67.6%, and 57.1%, respectively ($p = 0.03$).

cured by antibiotics. Our study results were preliminary and were an exploratory analysis. Although the sample size is not enough to draw a conclusion, we believe our study results deserve attention and have important clues for the future direction of the study. Second, we could not compare the therapeutic effects of further antibiotics and D&C on antibiotic-resistant CE, because one of the patients was hospitalized after four cycles of antibiotics due to pseudomembranous colitis; thus, the number of cases receiving ≥4 cycles of antibiotics was small. Third, global standard diagnostic criteria for CE remain undefined. In this study, five or more CD138-positive cells per 10 nonoverlapping random stromal areas were defined as a diagnosis of CE. Yet, different diagnostic criteria may yield different results. Fourth, data from endometrial microbiome testing could not be confirmed in all cases. Some of the patients who underwent D&C did not want to have the procedure due to the expensive test.

In conclusion, this is the first study to clarify the therapeutic effects of endometrial curettage on antibiotic-ineffective CE. Gentle endometrial curettage for antibiotic-resistant CE significantly decreased the number of CD138-positive cells, resulting in improved pregnancy outcomes regardless of the remaining CE. D&C for antibiotic-resistant CE may improve the intrauterine environment, and D&C for remaining CE after antibiotic use is also important as a screening tool for endometrial malignancy.

An old procedure, D&C, could be a novel treatment for CE. We used as many as two to five cycles of antibiotic therapy in this study, yet if CE is not cured after two cycles of broad-spectrum antibiotics, D&C could be chosen as a treatment protocol to avoid unnecessary antibiotic use and prevent emergent resistant bacterial threats. Noting that D&C is associated with subsequent thin endometrium; therefore, it is important to perform it gently. To establish the therapeutic effects of endometrial curettage for intractable CE, further clinical trials are warranted.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest to declare relevant to this study. Human rights statement and informed consent: This study was approved by the local ethics committee of Sugiyama Clinic (No. 18-004 and 22-007). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964 and its later amendments. The data that support the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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