


Endometrial polyps with increased plasma cells are associated with chronic endometritis in infertility patients: Hysteroscopic findings and post-polypectomy pregnancy rates

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Abstract

Purpose: The relationship between endometrial polyps (EPs), chronic endometritis (CE), hysteroscopic findings, and antimicrobial in infertility patients was determined.

Methods: We retrospectively enrolled 115 infertility patients with suspected EPs who underwent office hysteroscopy. Patients were divided into 3 groups: 38 with increased plasma cells in EPs (group 1); 31 without increased plasma cells in EPs (group 2); and 46 without EPs (group 3). The 3 groups underwent hysteroscopy with or without polypectomies, and immediately thereafter, an endometrial aspiration biopsy (EAB) was performed. CE was diagnosed based on plasma cell infiltration in the non-polypoid endometrium obtained by EAB.

Results: The percentage of CE was 68.4%, 32.2%, and 28.3% in groups 1, 2, and 3, respectively. CE was more frequent in group 1 than group 2 or 3 ($P = .01$ and $P = .002$, respectively). The number of polyps was higher in group 1 than group 2. After adjustment for age and assisted reproductive technology, antibiotic therapy was not associated with pregnancy (adjusted odds ratio, 0.44; 95% confidence interval, 0.05–3.57) in patients with EPs and CE.

Conclusions: Group 1 was associated with CE, and hysteroscopic findings were different from group 2. Antibiotic therapy after polypectomy for EPs with CE may not always be necessary.

KEYWORDS

endometritis, hysteroscopy, infertility, plasma cell, polyp

1 | INTRODUCTION

The 2010 WHO Global Burden of Disease Study estimated that 120 million couples experience infertility worldwide.¹ In 2011,

approximately 2 million assisted reproductive technology (ART) cycles were performed, resulting in an estimated 500 000 newborns according to the latest report of the International Committee for Monitoring Assisted Reproductive Technology (ICMART). The cumulative live birth rate per ART cycle increased from 25.2% in 2006

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to 28.0% in 2011, but patients still have a considerable physical, mental, and financial burden associated with the inability to become pregnant.^{2,3} It is important to eliminate factors preventing implantation before in vitro fertilization and embryo transfer (IVF-ET) so patients can successfully achieve pregnancy.^{4,5} Although fertilization rates have improved with intracytoplasmic sperm injection (ICSI), implantation rates have remained unchanged.^{5,6}

Recently, chronic endometritis (CE) has gained attention as a cause of recurrent implantation failure after ART and recurrent miscarriage.⁷⁻¹² Poor reproductive outcomes improve after CE resolution with antibiotic treatment.^{9,12}

A recent study, albeit conducted in premenopausal women, demonstrated an association between endometrial polyps (EPs) and CE.¹³ The study¹³ showed that CD138-positive EPs have a higher rate of co-existing CE than CD138-negative polyps. Indeed, there are features of CE that have not been elucidated, such as the notion that EPs are the result of CE and whether CE occurs because the inflammatory response to polyps spreads to the surrounding endometrium. EPs have conventionally been thought to occur due to multiple causes.¹⁴ The relationship between CE and EPs may change depending on the underlying cause of polyps.

EPs occur in up to 35% of infertility patients.^{15,16} An improved pregnancy rate has been reported after endometrial polypectomy in infertility patients;^{17,18} however, there are patients who do not become pregnant after polypectomy or in whom polyps recur soon thereafter. It is essential to elucidate how EPs affect the implantation environment to facilitate a deeper understanding of implantation failure.¹⁶

The objectives of the current study involving infertility patients were to enumerate the plasma cells in EPs, elucidate the relationship between CE and EPs, and detail the hysteroscopic findings of EPs with an increased number of plasma cells.

2 | METHODS

2.1 | Patients

The study participants were 245 infertility patients with suspected EPs on transvaginal ultrasound or hysterosalpingography (TV-US/HSG) who underwent office hysteroscopy (Figure 1). We conducted a retrospective cohort study. The patients had undergone the procedures mentioned below in the Department of Obstetrics and Gynecology outpatient clinic (Takagi Hospital, Okawa, Japan) between July 2017 and October 2019. Polypectomy was performed at the same time if an EP was present. Immediately thereafter, an endometrial aspiration biopsy (EAB) was performed. Plasma cell infiltration in each of the EPs and EAB was diagnosed by syndecan-1 (CD138) immunostaining. The presence or absence of CE was diagnosed based on the number of plasma cells in the non-polypoid endometrium obtained by EAB. A complete polyp resection could not be performed in 3 patients; complete polypectomies were performed in 72 patients. Of the 115 patients, 38 had an increased

number of plasma cells in the EP (group 1), 31 patients did not have an increased number of plasma cells in the EP (group 2), and 46 patients did not have EPs (group 3).

The exclusion criteria were as follows: previous ET; ≥ 3 miscarriages; ≥ 2 deliveries; previous hysteroscopic surgery; a history of CE or a sexually transmitted disease; presence of retained products of conception; endometrial carcinoma; endometrial hyperplasia; amenorrhea or uterine bleeding during the study. Patients were also excluded for use of antibiotics >3 days within 3 months, no immunosuppressive therapy, hormonal therapy during the cycle, or declining a polypectomy in the office, EAB or study participation.

2.2 | Study measures

The primary aim of our study was to determine the incidence of CE in groups 1, 2, and 3. The secondary measures included the percentage of micro-polyps in the endometrial non-polypoid areas, and the number of items shown to be positive by hysteroscopy (micro-polyps, hyperemia, and stromal edema). Sixty-nine patients with EPs were further examined by comparing groups 1 and 2 with respect to the number of EPs, maximum diameter of polyps (mm), and percentage of patients with an irregular polyp surface. We also compared pregnancy rates in the three groups and determined whether adding antibiotic therapy after polypectomy improved the pregnancy rates in 36 patients with CE. In women with CE in groups 1 and 2, reproductive outcomes were compared during the year after antibiotic therapy or polypectomy.

2.3 | Hysteroscopy

Outpatient hysteroscopy was performed without anesthesia and antibiotics during the follicular phase after menstruation. We used a 5-mm Bettocchi® hysteroscopy set (KARL STORZ, Tuttlingen, Germany), which has a rigid scope with a 30°-angle lens, a 2.9-mm outer diameter, and a 3-CCD Full HD "IMAGE1S" camera system (KARL STORZ). A 175-W LED light source (KARL STORZ) and a 26-inch Full HD monitor (KARL STORZ) were used. Videos were taken and recorded in digital format. Saline hanging >1 meter above the patient was used as the distending medium.

If an EP was demonstrated by hysteroscopy, a polypectomy was performed during the hysteroscopic examination. Scissor forceps were used to completely resect the polyp from the same horizontal plane as the normal endometrium. The resected tissue passed spontaneously with irrigation fluid or was removed using grasping forceps. If multiple polyps were present, the same resection procedure was repeated for each polyp. Uterine lavage was performed after complete polypectomy, and the absence of residual polyps was confirmed. Irrigation was stopped before removal of the hysteroscope. A syringe was connected to the drainage opening of the sheath, and the hysteroscope was withdrawn after the intrauterine irrigation

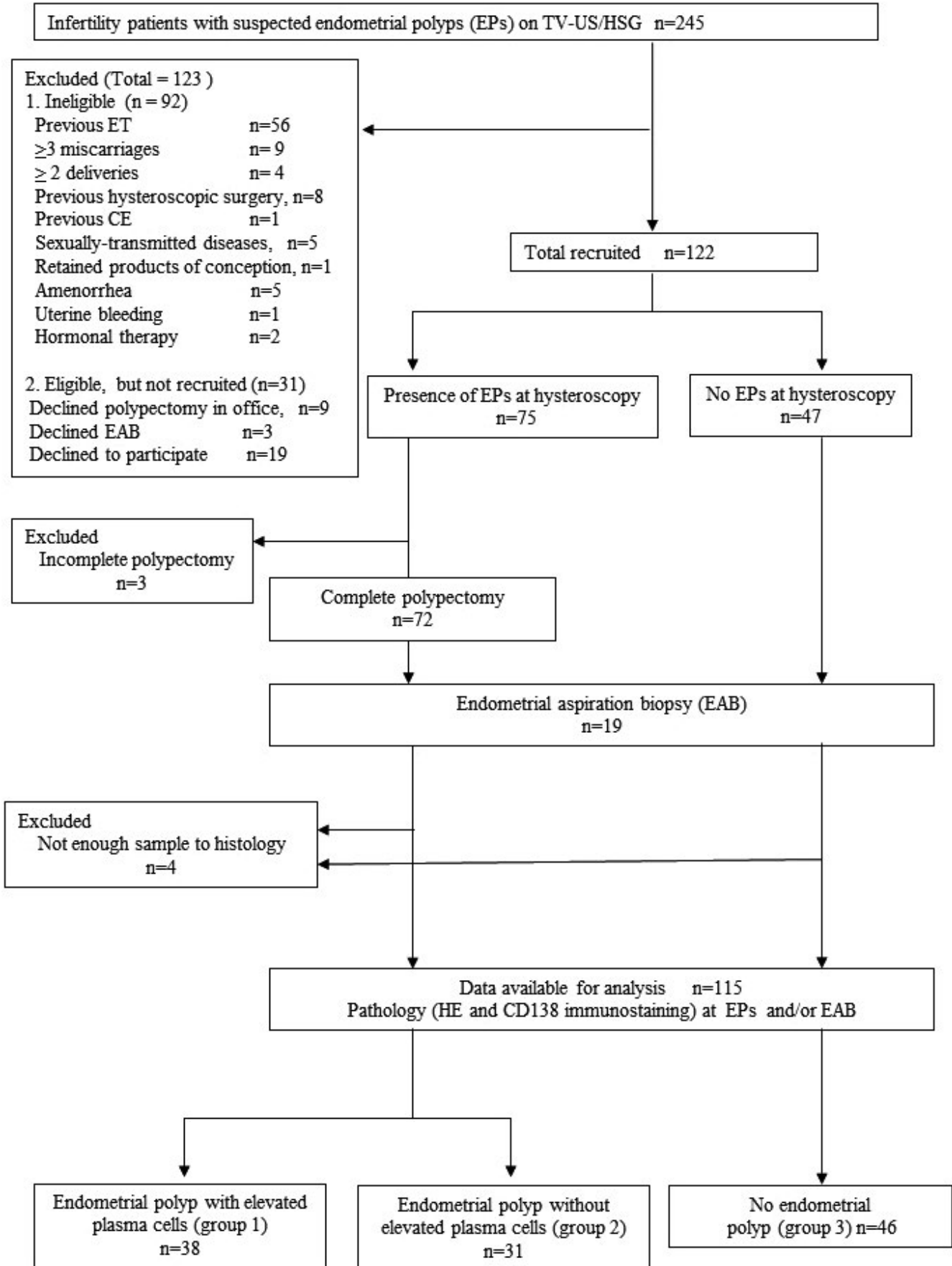


FIGURE 1 Diagram of study and distribution of the patients investigated. TV-US/HSG, transvaginal ultrasound or hysterosalpingography; ET, embryo transfer; HE, hematoxylin and eosin staining; CD138, syndecan-1 (CD138) immunostaining

fluid was completely aspirated. The maximum diameter of the EP was measured after excision.

Hysteroscopic CE findings were evaluated based on the diagnostic criteria proposed by Cicinelli et al.¹⁹ Specifically, the criteria defined micro-polyps as small intrauterine new growths <1 mm in size with a distinct connective vascular axis distributed in focal areas or the entire endometrial surface. Using this definition, we excluded micro-polyps from EPs. Our findings were also evaluated based on definitions of hyperemia, including hemorrhagic spots and stromal edema in the aforementioned report.¹⁹

2.4 | Endometrial aspiration biopsy

An Endosuction[®] catheter (Hakko Co., Ltd., Chikuma-shi, Japan) was inserted into the uterus immediately after withdrawing the hysteroscope. This catheter has a 3-mm outer diameter and consists of a stylet and a sheath with one opening at the tip and two openings on the sides. Circumferential aspiration sampling of the endometrium (EAB) was performed by slowly withdrawing the stylet, and while applying negative pressure, rotating the sheath as the catheter was moved in and out in the uterine cavity. The EPs removed by hysteroscopy and endometrial tissues collected by EAB were placed in separate containers, fixed with formalin, and histopathologic examination was performed.

2.5 | Hematoxylin-eosin (HE) staining

We defined an EP based on histopathologic diagnosis. Specifically, an EP was defined as a nodule composed of endometrial glands, blood vessels, and stroma protruding from the endometrial surface.^{14,20}

2.6 | CD138 immunostaining

Clone MI15 antibody (Dako, Glostrup, Denmark) was diluted 50-fold and used for CD138 immunostaining. Four μm -thick sections of the unstained specimens were prepared, deparaffinized, and microwaved for antigen retrieval. Target retrieval solution at pH 9.0 (Dako) was diluted 10-fold with distilled water and used. This solution was placed in a heat-resistant container and microwaved for 5 minutes. A specimen was immersed and microwaved for 5 minutes. The specimen with the container was cooled slowly. Blocking was performed using drops of a peroxidase blocking solution (Dako) to completely cover tissue sections, which were subsequently allowed to stand in a moisture chamber for 10 minutes. Then, tissue sections were thrice-washed with a phosphate-buffered solution (PBS) for 5 minutes each. The primary antibody was prepared using clone MI15 antibody (Dako) diluted 50-fold with antibody dilution buffer. Drops of the antibody were used to completely cover the sections, which were allowed to stand in the moisture chamber at room temperature for 60 minutes, then thrice-washed with PBS for 5 minutes each.

Secondary antibody was prepared using drops of a polymer reagent (Dako) to completely cover the sections, which were then allowed to stand in the moisture chamber for 30 minutes and subsequently thrice-washed with PBS for 5 minutes each. The Real Envision[™] detection system (K5007; Dako) was used for DAB color development. The sections were immersed in a hematoxylin solution for 10-20 seconds for color development, then in warm water for 10-20 minutes for washing for nuclear staining, color development, and mounting. An alcohol solution was used for dehydration, a xylene solution for clearing, and a mounting medium for mounting.

2.7 | Definition of CE

Total numbers of plasma cells infiltrating the endometrial stroma were measured in hot spot areas, which were 20 non-overlapping fields with the greatest plasma cell infiltration at 400x high-power magnification. The cutoff value for CE diagnosis was a total of ≥ 10 plasma cells in 20 fields in the non-polypoid endometrium obtained by EAB (21). An increase in the number of plasma cells in an EP was similarly defined as ≥ 10 plasma cells in 20 fields.¹³

2.8 | Management of patients diagnosed with CE

Doxycycline was prescribed for 14 days in patients with CE. EABs were repeated during the follicular phase in the following treatment cycle. In women with persistent CE, other antibiotics, such as Levofloxacin Hydrate or Metronidazole, were prescribed for up to three cycles until the histopathologic findings were normalized. If the patient did not agree to follow the above protocol, no antibiotics were administered. For patients who were lost to follow-up, reproductive outcomes were determined by telephone interview 1 year after hysteroscopy or antibiotic therapy.

2.9 | Statistical analysis

Statistical processing was performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (the R Foundation for Statistical Computing, Vienna, Austria²²). One-way ANOVA was used to analyze differences among the three groups for age, body mass index (BMI), duration of infertility, and number of past intrauterine inseminations (IUIs). Fisher's exact test and a chi-square test were used to compare the percentage of patients with the following: a history of miscarriage; a previous delivery; a uterine submucosal myoma or uterine adenomyosis protruding into the uterine cavity; and infertility caused by endometriosis, tubal factors, male factors, unknown cause, premature ovarian failure, and/or polycystic ovarian syndrome. Fisher's exact test was used to analyze differences among the three groups for CE rate and percentage of patients with micro-polyps in the endometrial non-polypoid areas, and Holm's post hoc test was used to establish

the statistical significance between groups. One-way ANOVA was used for statistical analysis of the number of hysteroscopic CE findings in the endometrial non-polypoid areas, and Tukey's post hoc test was used for comparisons between groups. The Mann-Whitney *U* test was used to analyze differences in the number of EPs between groups 1 and 2. Student's *t* test was used for the maximum diameter of EPs and a chi-square test was used for the percentage of patients with irregularly surfaced polyps.

Next, Fisher's exact test was used to compare the pregnancy rates in the three groups and a simple logistic regression model was used to analyze the crude odds ratios of antibiotic treatment and other variables for pregnancy in patients with EPs and CE. To control for age and ART therapy as known confounding variables, a multivariate logistic regression model was used to determine the adjusted odds ratio of antibiotic therapy for pregnancy. The statistical level of confidence was 95%, and a probability value $<.05$ was accepted as statistically significant.

2.10 | Ethical considerations

Written informed consent was obtained from patients before hysteroscopic examination and treatment, regarding hysteroscopic image storage and use for our study, collection of endometrial tissues after the examination and treatment, performance of histopathologic examinations using HE staining and CD138 immunostaining, and use of results and images from histopathologic examinations for our study.

This study was conducted according to the Helsinki Declaration. The proposed study underwent an ethical review and was approved by our hospital Ethics Committee (Kouhoukai Ethics Committee, approval number 346).

3 | RESULTS

Table 1 shows the clinical characteristics of the three groups. There was no significant difference in clinical characteristic among the three groups.

CE existed in 26 of 38 patients (68.4%) in the group with EPs and increased number of plasma cells (group 1); 10 of 31 patients (32.2%) in the group with EPs, but without increased number of plasma cells (group 2); and 13 of 46 patients (28.3%) in the group without EPs (group 3). There was a statistically significant difference between groups 1 and 2 ($P = .01$) and groups 1 and 3 ($P = .002$; Figure 2A), but no difference between groups 2 and 3.

The percentage of patients with micro-polyps in the endometrial non-polypoid areas was 47.4% (18 of 38) in group 1, 29.0% (9 of 31) in group 2, and 13.0% (6 of 46) in group 3. There was a significant difference between groups 1 and 3 ($P = .002$; Figure 2B). The number of hysteroscopic findings suspicious for CE in the endometrial non-polypoid areas was 1.5 ± 1.0 in group 1, 1.4 ± 0.9 in group 2, and 0.8 ± 0.8 in group 3. There was a statistically significant difference between groups 1 and 3 ($P = .002$; Figure 2C) and groups 2 and 3 ($P = .02$).

TABLE 1 Clinical characteristics of patients in the 3 groups

Variable	Group 1: EPs with increased plasma cells n = 38	Group 2: EPs without increased plasma cells n = 31	Group 3: No EPs n = 46	P value
Age(y)	34.9 ± 3.8	34.1 ± 4.9	35.9 ± 3.4	.15
Body mass index (kg/m ²)	21.4 ± 3.1	21.5 ± 2.3	22.0 ± 2.4	.49
Infertility duration(y)	2.9 ± 1.7	3.8 ± 2.6	2.9 ± 1.9	.11
No previous miscarriages, n (%)	28(73.7)	19(61.3)	27 (58.7)	.33
Nulliparity, n (%)	34(89.5)	24(77.4)	35 (76.1)	.25
No. of previous IUI	0.4 ± 0.8	0.4 ± 0.9	0.9 ± 1.9	.19
Submucous myoma, n (%)	0	0	2(4.3)	.34
Adenomyosis, n (%)	0	0	2(4.3)	.34
Intrauterine adhesion, n (%)	0	0	1 (2.2)	1
Uterine septum, n (%)	0	0	1 (2.2)	1
Causes of infertility ^a				
Endometriosis, n (%)	6 (15.8)	1(3.2)	8(17.4)	.14
Tubal factor, n (%)	8(21.1)	7(22.6)	9(19.6)	.96
Male factor, n (%)	0	2(6.5)	2(4.3)	.37
Unexplained, n (%)	23(60.5)	19(61.3)	21 (45.7)	.29
Premature ovarian failure, n (%)	0	0	0	0
Polycystic ovarian syndrome, n (%)	2 (5.3)	1(3.2)	5(1.1)	.38

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

Abbreviations: EPs, endometrial polyps; IUI, intrauterine insemination.

^aThe total of each group exceeds 100% because some patients had multiple causes of infertility

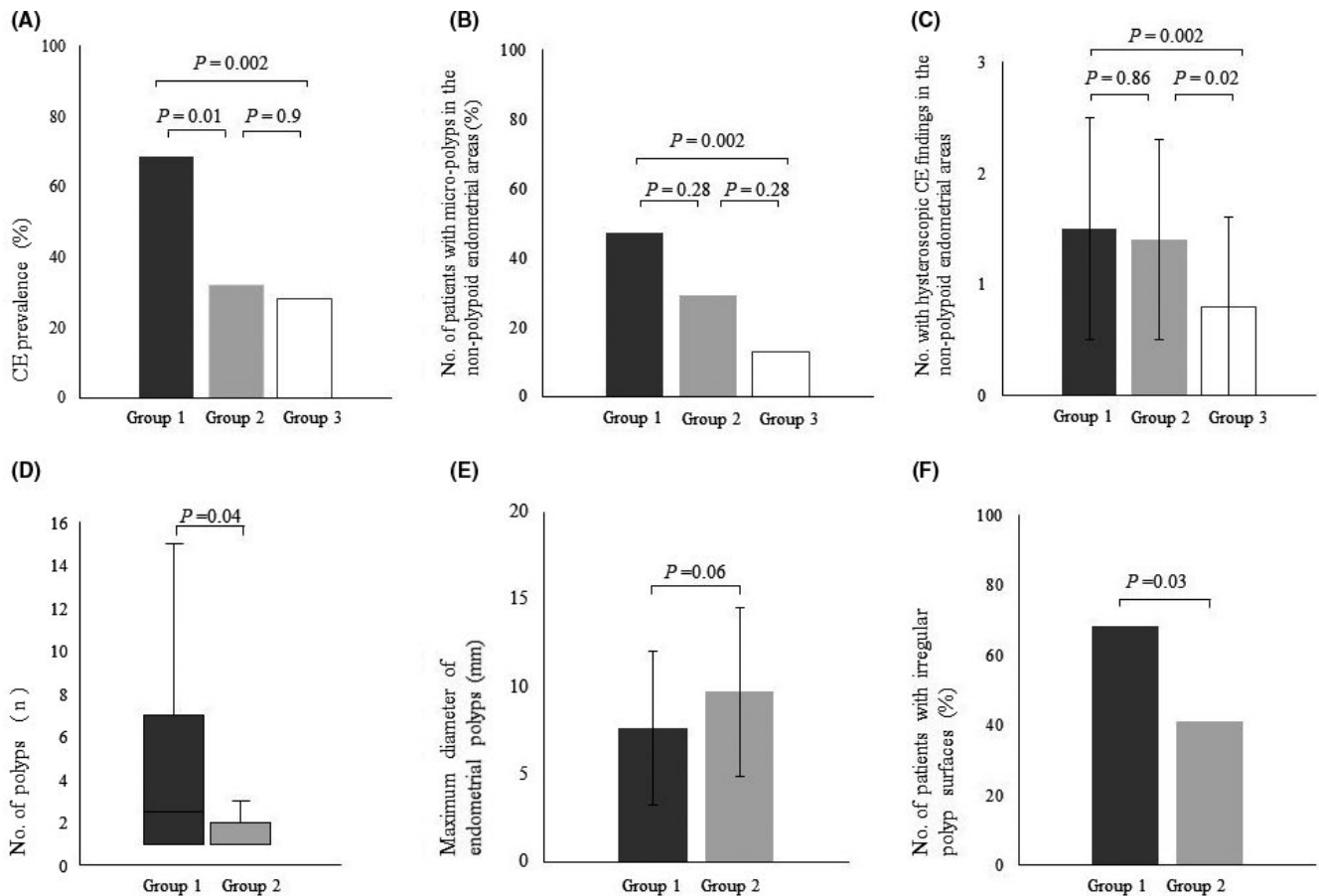


FIGURE 2 (A-F) Chronic endometritis and hysteroscopic findings of the 3 groups and characteristics of endometrial polyps (EPs). Patients were divided into the following 3 groups: 38 with increased plasma cells in EPs (group 1); 31 without increased plasma cells in EPs (group 2); and 46 without EPs (group 3). (A) The percentage of CE was 68.4% (26/38) in group 1, 32.2% (10/31) in group 2, and 28.3% (13/46) in group 3. There was a statistically significant difference between groups 1 and 2 ($P = .01$) and groups 1 and 3 ($P = .002$), but no difference between groups 2 and 3. (B) The percentage of patients with micro-polyps in the endometrial non-polypoid areas was higher in group 1 (47.4%, $n = 18$) than groups 2 (29.0%, $n = 9$) and 3 (13.0%, $n = 6$). There was a significant difference between groups 1 and 3 ($P = .002$). (C) The number of hysteroscopic findings suspicious for CE (number with hyperemia, micro-polyps, and stromal edema) in the endometrial non-polypoid areas was higher in group 1 (mean \pm standard deviation [SD]: 1.5 ± 1.0) than in group 2 (1.4 ± 0.9) and group 3 (0.8 ± 0.8 ; $P = .002$). There was a statistically significant difference between groups 1 and 3 ($P = .002$) and groups 2 and 3 ($P = .02$). (D) The median number of EPs [quartiles] was significantly higher in group 1 (2.5 [1-7]) than group 2 (1.0 [1-2]; $P = .04$). (E) The maximum diameter of EPs tended to be smaller in group 1 (mean \pm SD: 7.6 ± 4.4 mm) than group 2 (9.7 ± 4.8 mm), but there was no significant difference ($P = .06$). (F) The percentage of patients with irregular polyp surfaces was more frequent in group 1 (68.4%, $n = 26$) and group 2 (41.2%, $n = 13$; $P = .03$).

The median number of polyps was significantly higher in group 1 (2.5; quartiles, 1-7) than group 2 (1.0; quartiles, 1-2; $P = .04$; Figure 2D) based on the presence or absence of plasma cells in the EP. The percentage of patients with polyps with irregular surfaces was 68.4% in group 1 and 41.2% in group 2 ($P = .03$; Figure 2F). The maximum diameter of EPs was 7.6 ± 4.4 mm in group 1 and 9.7 ± 4.8 mm in group 2. This result indicated that diameter of the EPs in the former group was smaller than those in the latter group, but there was no significant difference ($P = .06$; Figure 2E).

Figures 3 and 4 are representative hysteroscopic and histologic images of EPs and non-polypoid areas in the same group 1 patient.

There was no difference in the clinical pregnancy rate within 1 year between the groups after eliminating 4 patients who were lost to follow-up (group 1: 67.6% [25/37], group 2: 51.7% [15/29], and group 3: 53.3% [24/45], $P = .34$). Figures 5-7 show the reproductive

outcomes among all patients in the three groups with or without CE who did and did not receive antibiotics. After adjustment for age and ART, antimicrobial treatment after polypectomy was not shown to be significantly associated with pregnancy (adjusted odds ratio, 0.44; 95% confidence interval, 0.05-3.57; Table 2).

4 | DISCUSSION

Our study retrospectively examined 115 infertility patients with suspected EPs on TV-US/HSG who had undergone outpatient mini-hysteroscopic examinations, EABs, HE staining, and CD138 immunostaining. If an EP was found, it was resected during the hysteroscopic procedure. The resected polyp and a specimen obtained from EAB were kept separately, and plasma cells were enumerated.

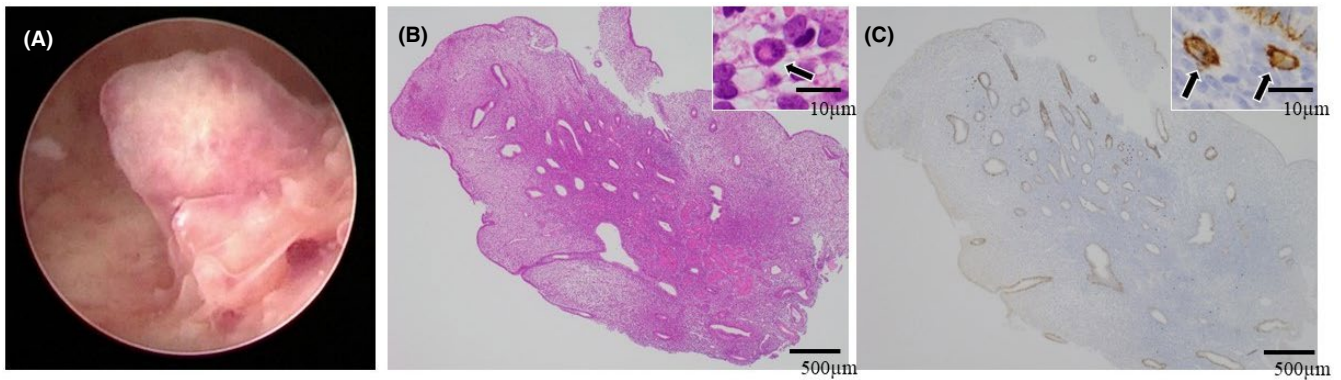


FIGURE 3 (A-C) Hysteroscopic and histologic findings of a chronic endometritis patient with an endometrial polyp and an increased number of plasma cells. (A) Hysteroscopic finding of an endometrial polyp with an irregular surface, micro-polyps, and hyperemia. (B) Hematoxylin and eosin (H&E) image of the excised endometrial polyp shown in A showing surface stromal edema and hyperemia. Endometrial polyp with increased infiltration of plasma cells (inset: higher magnification of plasma cells). (C) Syndecan-1 (CD138) immunostaining image of the endometrial polyp in B. Note the increased infiltration of plasma cells (brown staining) by CD138 in the endometrial stroma (inset: higher magnification of plasma cells) [Colour figure can be viewed at wileyonlinelibrary.com]

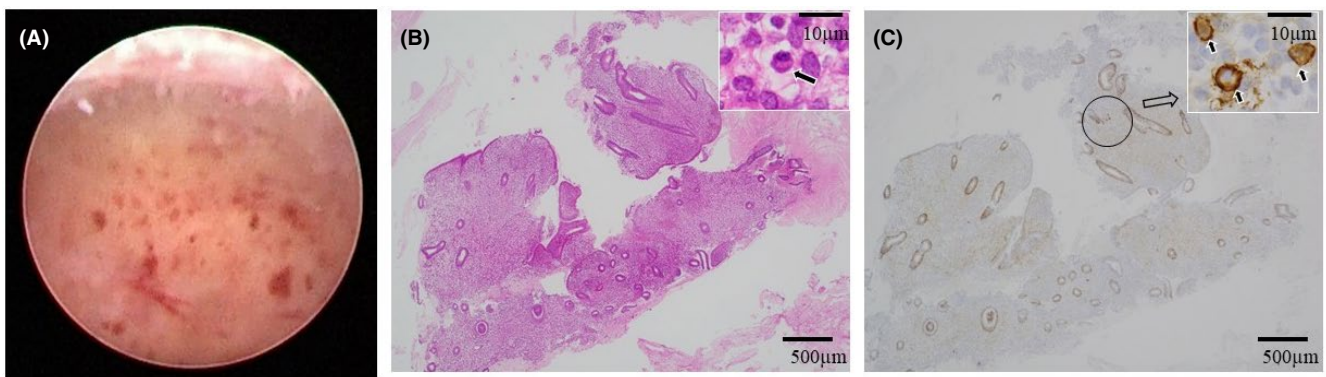


FIGURE 4 (A-C) Hysteroscopic and histologic findings of non-polypoid endometrium in the same patient represented in Figure 3. (A) Hysteroscopic finding of non-polypoid endometrium with diffuse hyperemia and multiple micro-polyps ≤ 1 mm in size in the same patient. (B) Histologic features of non-polypoid endometrium removed by endometrial aspiration biopsy after office hysteroscopic resection (inset: higher magnification of plasma cells). H&E staining image showing some plasma cells (inset: higher magnification of plasma cells). (C) Increased plasma cells were easily identified by CD138 immunostaining in endometrial stroma (inset: higher magnification of plasma cells) [Colour figure can be viewed at wileyonlinelibrary.com]

A diagnosis of CE was made by plasma cell infiltration and CD138 positivity in the stroma of the non-polypoid endometrium. When patients were divided based on the presence or absence of an increase in the number of plasma cells in the EPs, only patients with increased numbers had an association between CE and EPs. The polyps with increased numbers frequently had irregular surfaces and occurred in multiples. After adjustment for age and ART, antibiotic treatment after polypectomy was not significantly associated with pregnancy (adjusted odds ratio, 0.44; 95% confidence interval, 0.05-3.57) in 34 patients with EPs and CE.

Our literature search did not yield any reports indicating an association between CE and EPs in infertility patients; however, there is a study by Cicinelli et al¹³ involving patients with premenstrual abnormal uterine bleeding. After the initial hysteroscopic examination, they performed another hysteroscopy and collected endometrial tissue from the EP and non-polypoid areas using a resectoscope or morcellator. They then examined CD138 positivity in each area. The

rate of CE was 61.7% [148 of 240] in the non-polypoid endometrium in a group with EPs and 24.2% [58 of 240] in a group (including 57% with submucosal myomas) without EPs, indicating a significantly higher percentage in the former than the latter ($P < .0001$). Looking only at the group with polyps, they found that the CE rate was significantly higher when the number of plasma cells was increased in the polyp than when the number was not increased.

The results of our study showed an association between CE and EPs. This result is consistent with those of Cicinelli et al,¹³ although our study involved infertility patients, while their study involved patients with premenopausal abnormal uterine bleeding. In addition, we demonstrated that the CE rate did not differ between patients without EPs and those with EPs without an increased number of plasma cells. Thus, an association does not necessarily exist between CE and all EPs. Notably, morphologic differences exist between EPs with and without increased numbers of plasma cells. Considering the mechanism underlying the association between CE and EPs, and based

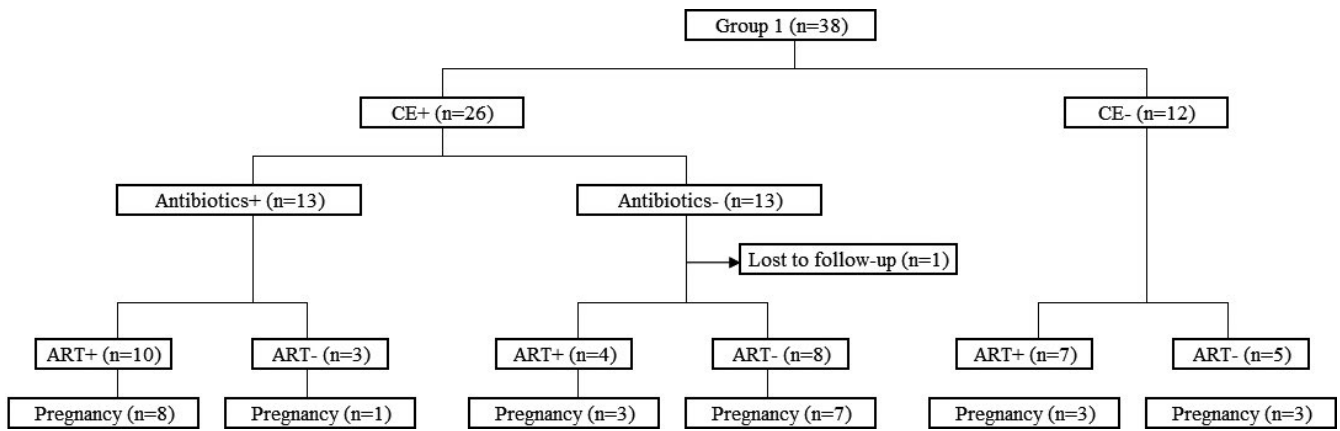


FIGURE 5 Clinical pregnancies after polypectomy among patients with an increase in the number of plasma cells in endometrial polyps (group 1). CE, chronic endometritis; ART, assisted reproductive technology; Pregnancy, clinical pregnancy defined by the presence of a gestational sac

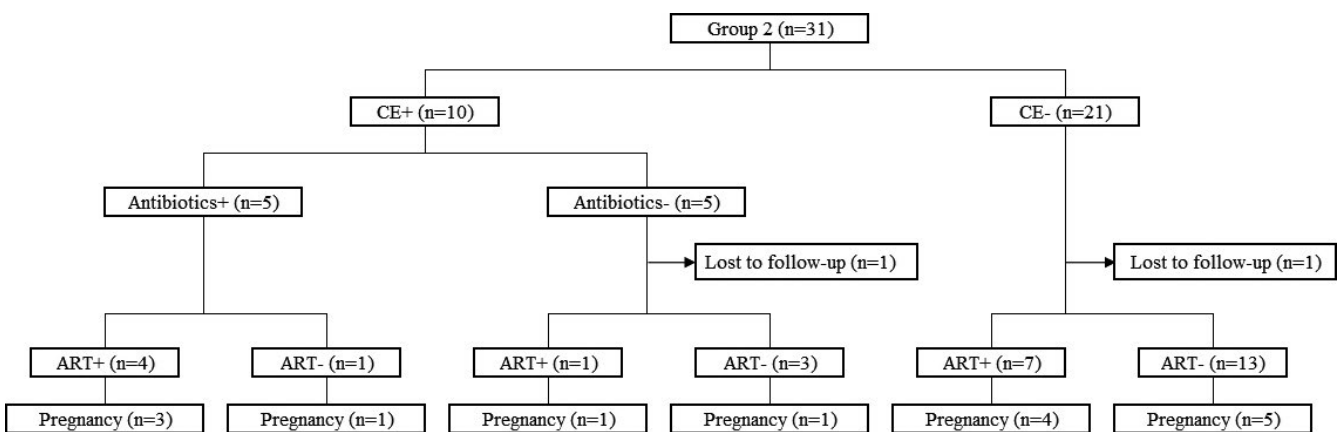


FIGURE 6 Reproductive outcomes after polypectomy among patients without an increase in the number of plasma cells in endometrial polyps (group 2). CE, chronic endometritis; ART, assisted reproductive technology. Pregnancy: clinical pregnancy defined by the presence of a gestational sac

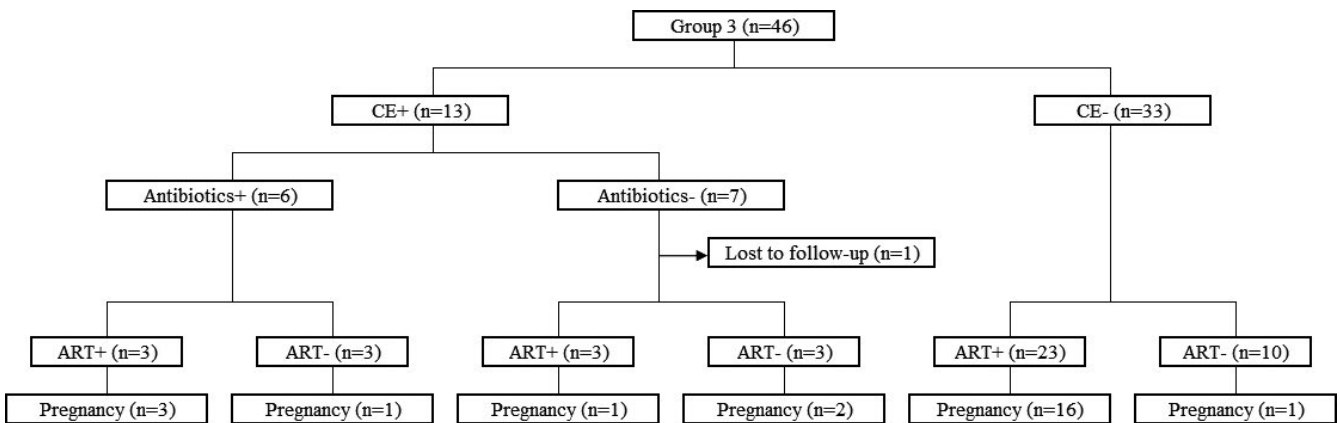


FIGURE 7 Reproductive outcomes among patients without endometrial polyps (group 3). CE, chronic endometritis; ART, assisted reproductive technology. Pregnancy: clinical pregnancy defined by the presence of a gestational sac

on the above findings, it is our opinion that EPs are highly unlikely to be the mechanical cause underlying CE and an increased number of plasma cells in polyps. Thus, an increased number of plasma cells in EPs is predicted to be associated with the cause of EPs. EPs

are thought to be caused by factors such as inflammation and estrogen dependency.²³⁻²⁶ A high estrogen state is known to have some involvement in the occurrence of premenopausal EPs, as has been reported in the increased occurrence of EPs with tamoxifen use.^{14,16}

TABLE 2 Crude and Adjusted odds ratio of variables for clinical pregnancy within one year after polypectomy and/or antibiotic therapy in patients with chronic endometritis

	Crude odds ratio	95%CI	P value	Adjusted odds ratio	95%CI	P value
Body mass index (kg/m ²)	1.14	0.87-1.49	.33	1.26	0.91-1.74	.16
Infertility duration(y)	0.9	0.63-1.28	.55	0.86	0.58-1.27	.46
No previous miscarriages	1.25	0.19-8.23	.82	0.86	0.12-6.27	.88
Nulliparity	1.25	0.19-8.23	.82	0.53	0.06-4.87	.57
Group 1 (reference group 2)	2.53	0.51-12.6	.26	2.58	0.46-11.3	.31
Antibiotic therapy	0.67	0.15-2.98	.6	0.44	0.05-3.57	.44

Note: To control for age and assisted reproductive technology as a known confounding variable, a multivariate logistic regression model was used to determine the adjusted odds ratio of variables. Group 1: patients with increased plasma cell in endometrial polyps. Group 2: patients without increased plasma cell in endometrial polyps.

Abbreviation: CI, confidence interval.

EP formation may be the result of localized, chronic endometrial inflammation.¹⁶ It is known that mast cells promote secretion of cytokines and growth factors, causing an inflammatory response, resulting in the formation of new blood vessels and growth of tissues.²⁶ It is also known that EP tissues have significantly higher levels of the following than normal endometrial tissues: mast cells;²⁷ vascular endothelial growth factor (VEGF), an angiogenic growth factor;²⁸ transforming growth factor beta-1 (TGF beta-1), which is involved in the formation of fibrous tissue;²⁹ and Ki-67, the expression of which is associated with tissue proliferation.³⁰ These cellular and chemical mediators cause inflammation and an excessive inflammatory response, which can result in tissue injury and hyperplasia.^{16,31}

We typically perform outpatient 5-mm mini-hysteroscopy to diagnose EPs in all patients. Because 5-mm mini-hysteroscopy does not require cervical dilation or anesthesia, a hysteroscopic examination can be switched immediately to surgery. Generally, a resectoscope or morcellator is often used if surgery is necessary. Thus, patients are required to be hospitalized at a later date, undergo preoperative cervical dilation, and be anesthetized. Because mini-hysteroscopy, unlike conventional hysteroscopic surgery, can be performed without anesthesia or a power source, it can be performed as an office-based operative hysteroscopic procedure. This procedure, therefore, is very convenient, minimally invasive, and economical. In our study, there were only a few patients in whom EPs were not completely resectable. Thus, another advantage is the high success rate. After a complete polypectomy, we perform uterine lavage thoroughly to prevent contamination from polyp fragments, then aspirate and sample endometrial tissue (EAB). EAB sampling is done using a narrow straw-shaped instrument, which causes less pain and allows sampling of a wider area in a larger amount compared with a metal curettage.

Researchers have used different plasma cell measurements and reference values; however, there is a consensus on the presence of CE when there are ≥ 2 endometrial stromal plasma cells.³² Our cutoff value for CE diagnosis was based on that used by Boet et al,²⁴ that is,

≥ 5 plasma cells in 10 fields under high magnification. Specifically, our cutoff value was a total of ≥ 10 plasma cells in 20 non-overlapping fields, beginning with hot spots. A risk has been reported of undercounting plasma cells if a biopsy sample is too small.³³ Therefore, we counted plasma cells in hot spots, enabling us to more easily determine the presence of CE with a pattern of localized plasma cells. Using 20 fields, we detected CE with a pattern of plasma cells scattered in a relatively extensive area.

The results of our study suggest that in addition to mechanical factors caused by EPs, the mechanism underlying implantation failure may be due to co-existing CE. Whether CE improves after polypectomy or additional antibiotic agents are necessary is unknown. In our study, the pregnancy rate in patients with CE was the same as the pregnancy rate in the polypectomy-only and antibiotic groups. Thus, it is possible that a polypectomy resolved the CE. The involvement of CE in recurrent implantation failure after ART and recurrent pregnancy loss might warrant further investigation of the EP effect. In one report, antibiotics were administered to patients with an increased number of plasma cells in EPs after polypectomy, but the pregnancy rate was similar to that of patients without an increased number of plasma cells in EPs.³⁴ Of note, this report did not examine the presence or absence of CE. Whether or not antibiotic therapy is necessary after polypectomy in the presence of an increased number of plasma cells in EPs or CE requires further investigation, including a large number of patients.

Our study was retrospective and conducted on a relatively small number of patients. The non-polypoid endometrium was aspirated and sampled after complete hysteroscopic resection of EPs and subsequent thorough lavage; however, we cannot rule out the possibility of sample contamination with polyp tissue.

Our study showed that only EPs with an increased number of plasma cells were associated with CE, while EPs without an increased number of plasma cells were not associated with CE. The hysteroscopic findings of the EPs with an increased number of plasma cells were as follows: multiple polyps; irregular surface; and smaller in size than the EPs without an increased number of plasma

cells. Thus, antibiotic treatment after polypectomy for EPs with CE may not always be necessary.

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DISCLOSURES

Conflict of interest: The authors declare no conflicts of interest. **Human rights statements and informed consent:** All of the procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the principles of the 1964 Helsinki Declaration and subsequent amendments. This study was approved by the Institutional Review Board of Takagi Hospital. This was a retrospective study involving patients who submitted informed consent for undergoing fertility treatment in our Department. **Animal studies:** This article did not involve animal experimentation by any of the authors.

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