Title: The effects of chronic endometritis on pregnancy outcomes.

Short running title; Endometritis on pregnancy.

Full names of the authors;

Authors' names: Aina Morimune[1], Fuminori Kimura[1], Akiko Nakamura[1], Jun Kitazawa[1], Akiko Takashima[1], Tsukuru Amano[1], Shoji Kaku[1], Suzuko Moritani[2], Ryoji Kushima[2], and Takashi Murakami[1]

Author's institutional affiliations;

[1]Department of Obstetrics and Gynecology, Shiga University of Medical Science
Seta Tsukinowa-cho, Otsu, Shiga 520-2192, Japan

[2]Department of Clinical Laboratory Medicine and Division of Diagnostic Pathology, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga 520-2192, Japan

Corresponding author:

Fuminori Kimura, M.D., Ph.D.
Department of Obstetrics and Gynecology, Shiga University of Medical Science
Seta Tsukinowa-cho, Otsu, Shiga 520-2192, Japan
Tel: +81-77-548-2267; Fax: +81-77-548-2406
E-mail: kimurafu@belle.shiga-med.ac.jp

Aina Morimune: aina@belle.shiga-med.ac.jp

Fuminori Kimura: kimurafu@belle.shiga-med.ac.jp

Akiko Nakamura: akikon@belle.shiga-med.ac.jp

Jun Kitazawa: jk110530@belle.shiga-med.ac.jp

Akiko Takashima: akkmrt@belle.shiga-med.ac.jp

Tsukuru Amano: tsukuru@belle.shiga-med.ac.jp

Shoji Kaku: kaku@belle.shiga-med.ac.jp

Suzuko Moritani: mori@belle.shiga-med.ac.jp

RyojiKushima: kushima@belle.shiga-med.ac.jp

Takashi Murakami: tm@belle.shiga-med.ac.jp

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Abstract 250 words

Problem: CE impacts implantation process and clinical outcomes of assisted reproductive technology. However, the effect of CE on pregnancy outcome is still unknown.

Method of Study: A retrospective case control study was conducted in a single university. Subjects who conceived by IVF within a year after a histological diagnosis of CE under 40 years of age from September 2013 to December 2107 were extracted. The rates of miscarriage, preterm birth, term birth and live birth per pregnancy according to the presence or absence of CE were analyzed. Logistic regression analysis was performed for miscarriage, preterm birth, term birth and live birth for 8 explanatory variables of 7 infertility factors and CE.

Results: A total of 39 pregnancies of 38 subjects with Non-CE and 35 pregnancies of 32 subjects with CE were finally analyzed. The rates of miscarriage, preterm birth, term birth and live birth per pregnancy were 12.8% vs 40.0% ($P<0.03$), 2.6% vs 14.3% ($P=0.1$), 84.6% and 45.7% ($P<0.001$) and 84.6% and 57.1% ($P<0.03$) in the Non-CE group and the CE group, respectively, although only the analysis for term birth rate had sufficient power to exclude Type II error. On logistic analysis, CE was a factor affecting objective variables of miscarriage, term birth and live birth.

Conclusions: The term birth rate per pregnancy became lower mainly due to an increase in miscarriages when CE was detected before pregnancy in the patients treated with IVF. A histopathological diagnosis of CE adversely affected pregnancy outcomes.

Keywords: Chronic endometritis, Infertility, Implantation failure, Pregnancy outcomes

Main text

Introduction

Chronic endometritis (CE) is a slight inflammation of the endometrium that is histologically diagnosed mainly by the presence of plasma cells in the endometrial stroma (1-4). There have been several recent reports that CE is associated with infertility and implantation failure (5-7). CE is known to histologically show various inflammatory features such as neutrophil infiltration, and decidua-like changes are often seen in addition to plasma cells (1-4). Furthermore, it has been reported that inflammatory cytokines such as TNFα, IL1, and IL6 are elevated in menstrual blood (8). Thus, it is thought that inflammation is present biochemically, as well as histologically, within the endometrium, though the degree may be low. Inflammation has been reported as a factor adversely affecting obstetric outcomes causing miscarriage and preterm birth (9, 10). CE has also been reported to affect decidualization and the distribution of NK cells in the implantation phase of the endometrium (11, 12). These facts suggest that CE not only affects implantation, but it may also affect pregnancy outcomes when patients conceive.

Until now, clinical research on CE has mainly involved patients with recurrent implantation failure (RIF) (3, 6, 7, 13, 14). The subsequent pregnancy rate after diagnosis of RIF is lower even without CE, since it is thought that there are other underlying diseases causing implantation failure (15, 16). These diseases such as severe adenomyosis, fibroid, and the presence of specific autoantibodies, which are causes of implantation failure, are potential factors that affect pregnancy outcomes. Thus, RIF without CE cannot be used as the control to study the effects of CE on fertility and pregnancy outcomes. We

think that this is one of the reasons there have been no reports studying the effects of CE on pregnancy outcomes. In the present study, subjects who had RIF, recurrent pregnancy loss (RPL), and diseases suspected to cause implantation failure were excluded. The aim of the study is to evaluate the impact of CE on pregnancy outcomes. This strategy made it possible to examine the effects of CE on pregnancy outcomes. To the best of our knowledge, this study is the first report to examine the effects of histologically confirmed CE on pregnancy outcomes in IVF patients without RIF, RPL and diseases suspected to cause implantation failure.

Methods

Ethics

This research was approved by the Ethics Committee of Shiga University of Medical Science. Informed consent was obtained from all patients (registration number R2014-090). All clinical studies were conducted according to the Declaration of Helsinki for Medical Research involving Human Subjects.

Subject

The purpose of this study was to investigate whether the presence of CE affects the pregnancy outcomes. We chose only the patients treated with IVF because pregnancy by IVF has been shown to have pregnancy outcome worse comparing with spontaneous pregnancy. Then, after the extraction of the patients conceived within a year after the diagnosis of presence or absence of CE, we excluded RIF, RPL and diseases that were likely to have an impact on the outcome of pregnancy before the analysis in order to achieve the purpose of present study. After that, the patients treated with antibiotics were

excluded. After these exclusions, pregnancy outcome was analyzed according to the presence or absence of CE. These methods are described in detail below.

At our institute, hysteroscopy has been performed in principle for the purpose of rule out of abnormal findings in the uterine cavity such as morphological abnormalities, submucosal fibroid, endometrial polyp and so on when the embryos are frozen or before in vitro fertilization. Endometrial tissue sampling has been performed for patients who desire the assessment of an endometrial dating and/or an examination for the presence or absence of CE.

A registration list of the patients performed hysteroscopy and endometrial sampling was used to identify research subjects. The patients who performed under 40 years of age were target in the present study. The subjects were extracted when the patients were conceived with IVF at hour hospital within a year after the histological diagnosis with the presence or absence of CE using CD138 immunostaining from September 2013 to December 2017. RIF, RPL, and patients with disease suspected to have a cause of implantation failure were excluded. RIF was defined as the failure of clinical pregnancy after 4 good quality embryo transfers, with at least three fresh or frozen IVF cycles, as per Coughlan et al (15). RPL was defined as the patient with 3 or more miscarriage (17). Patients suffering from endocrine and autoimmune diseases, uterine malformation such as septate uterus, multiple myoma, endometrial polyp detected by hysteroscopy, hydrosalpinx detected by ultrasonography, or adenomyosis with over 2.5cm thickness in uterine wall were to be excluded (18-25), when the hysteroscopy was detected. Patients were given 7 or more days of antibiotics for the purpose of CE treatment were also excluded.

Diagnosis of CE

When the endometrium was collected, the ovulation date was identified using a urine ovulation test and ultrasonography for the subjects, and the tissue around the center of the anterior endometrium was collected with 4.5 J.A.M.W Type Uterine Curettes 5-9 days after ovulation.

CD138 immunostaining of endometrial tissue was performed according to previous reports (11, 26). One of the pathologists examined the specimens and made the diagnosis. When one or more plasma cells stained with CD138 were found in 10 high-power fields (HPF; a field magnified 400 times with a microscope), the patient was diagnosed with CE.

Data collection

Pregnancy was defined as presence of the gestational sac in the uterine cavity. When a subject had been pregnant multiple times in a year, all data were counted. The data of patients' characteristics including age, gravidity, parity, body mass index (BMI), smoking status, number of previous ova pick-up cycles, cause of infertility, the date of serum follicle-stimulating hormone (FSH) level measured within 12 months before the diagnosis of the pregnancy and the data of quality of embryo transferred at the time of diagnosis of the pregnancy were extracted from patients' records.

Percentages of miscarriages, preterm births, term births, and live births in pregnancy and percentages of preterm births, term births, and live births in ongoing pregnancy were also analyzed. In addition, in the case of preterm birth, detailed information on the clinical course was also gathered.

Statics

The target number of participants in the present study was calculated based on a meta-analysis reported by Vitagliano, et al (27). The miscarriage rates of cured CE and persistent CE were calculated from this article, although the data in this article depended on the patients with RIF. According to the report, when CE was cured with antibiotics, the miscarriage rate was 14.1% (13/92), but when it persists the rate was 50.0% (4/8). They showed a statistical difference between them. Based on these results, we calculated the number of patients required for enrollment using software provided by the Department of Biostatistics, Vanderbilt University (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Independent, case-control, two proportion and Fisher's exact test were selected to measure the sample size in the section of Dichotomous. We selected 0.05 for α: the probability that we will falsely reject the null hypothesis, 0.8 for *power*: the probability of always rejecting the null hypothesis if the null hypothesis is false in the statistical hypothesis test, 0.141 for *P0*: the probability of the outcome for a control patient in prospective studies, 0.5 for *P1*: the probability of the outcome in an experimental subject in prospective studies. When we chose 0.9 for *m*: the ratio of control to experimental subjects for independent prospective studies, it was calculated that registration of 33 cases for control and 30 cases for case was necessary.

Statistical analysis was performed using Graph Pad Prism 5 (GraphPad Software Inc., La Jolla, CA). Each dataset was checked for a normal distribution using the Kolmogorov-Smirnov test, and Student's *t*-test or the non-parametric Mann-Whitney U test was used depending on the distribution pattern. The significance of differences such as rates of miscarriage, preterm birth, term birth and live birth between the Non-CE group and CE

group was examined using Fisher analysis and chi-square test was performed for cause of infertility and embryo quality. A significant difference was considered present at a P value less than 0.05. A power analysis was also performed with respect to the numbers of subjects to analyze the differences such as rates of miscarriage, preterm birth, term birth and live birth between the Non-CE group and CE group by the same software provided by the Department of Biostatistics, Vanderbilt University.

In addition, since this was a retrospective study, logistic regression analysis was performed for 8 explanatory variables of 7 infertility factors (male factor, oviduct factor, endometriosis, ovarian factor, anti-sperm antibody-positive, fertilization failure, unexplained infertility) and CE with respect to 4 objective variables of miscarriage, preterm birth, term birth, and live birth in the pregnancy group, and 3 objective variables of preterm birth, term birth, and live birth in the ongoing pregnancy groups. SSPS statistics version 25 was used for this analysis. Odds ratios and P values were calculated. A significant difference was defined as a P value less than 0.05.

Results

A total of 93 patients were extracted for the present study (Figure 1). Nine RIF and 3 RPL patients were excluded. A patient with multiple myomas was excluded. None of the study participants had endocrine and autoimmune diseases, uterine malformation such as septate uterus, endometrial polyp, hydrosalpinx, or adenomyosis, although they were described as an exclusion criterion, and, although 3 patients had past history to treat endometrial polyp and a patient had past history to treat hydrosalpinx, they were completely cured at the time of hysteroscopy performed before embryo transfer. Nine patients who received antibiotics were excluded. Seventy patients were eligible for the

present study and then included for the analysis of pregnancy outcome. Thirty-eight were Non-CE and 32 were CE. There were 4 patients, one in the Non-CE group and three in the CE group, who became pregnant twice within a year. In the Non-CE group, a patient gave birth at term after miscarriage. In the CE group, one patient gave birth at preterm and two patients gave birth at term after miscarriages. As a result, 39 pregnancies of Non-CE patients and 35 pregnancies of CE patients were analyzed.

In the cases of pregnancy, there were no differences in age, gravidity, parity, BMI, smoking status, and the number of previous ova pick-up cycles between the Non-CE and CE groups at the time of diagnosis of the pregnancy, though the serum FSH level tended to be higher in the CE group (Table 1). Percentages of miscarriage, preterm birth, term birth, and live birth in the pregnancy were: 12.8% (5/39) vs. 40.0% (14/35) $P<0.03$; 2.6% (1/39) vs. 14.3% (5/35) $P=0.095$; 84.6% (33/39) vs. 45.7% (16/35) $P<0.001$; and 84.6% (33/39) vs. 57.1% (20/35) $P<0.03$ in Non-CE vs. CE, respectively (Table 1). The rate of term birth per pregnancy was found to be adequate by the power analysis (Power = 0.923), although the rates of miscarriage per pregnancy, preterm birth per pregnancy and live birth per pregnancy were not. There were no differences in cause of infertility and embryo quality.

In ongoing pregnancy, there were no differences in age, gravidity, parity, serum FSH level, BMI, smoking status, and the number of previous ova pick-up cycles between the Non-CE and CE groups (Table 2). Percentages of preterm birth, term birth, and live birth in the ongoing pregnancy were: 2.9% (1/34) vs. 23.8% (5/21) $P<0.03$; 97.1% (33/34) vs. 76.2% (16/21) $P<0.03$; and 100% (34/34) vs. 95.2% (20/21) P=NS, respectively (Table 2). The rates of preterm birth per ongoing pregnancy and term birth per ongoing pregnancy were not found to be adequate by the power analysis. There was no difference

239 in cause of infertility, though the embryo quality tended to be lower in the Non-CE group.
240 Table 3 shows a list of preterm births. One case in the Non-CE group and 5 cases in the
241 CE group resulted in preterm births. All of these cases had pregnancy check-ups at our
242 hospital. In the Non-CE group, one case underwent a caesarean section due to uterine
243 bleeding caused by total placental previa at a gestational age of 36 weeks and 2 days. On
244 the other hand, 2 of the 5 CE cases had preterm premature rupture of the membranes
245 (pPROM). One of them developed pPROM at 22 weeks and 5 days of gestation, resulting
246 in stillbirth. The other was one case with pPROM at 35 weeks and 4 days who gave birth
247 at 36 days and 0 days of gestation. One case was admitted due to preterm labor at 33
248 weeks of gestation, and cesarean delivery was performed at 36 weeks and 6 days of
249 gestation because of difficulty controlling uterine contractions and a previous cesarean
250 section. There was also one case of cesarean section due to a severe hypertensive disorder
251 of pregnancy (HDP) at 36 weeks and 2 days of gestation. In addition, there was one case
252 that was delivered by cesarean section due to a refractory fetal pleural effusion at 36
253 weeks and 3 days of gestation.
254 On logistic analysis, CE was a factor that affected the objective variables of miscarriage,
255 term birth, and live birth in the pregnancy group, and preterm birth and term birth in the
256 ongoing pregnancy group. The odds ratio and P values for each were 4.8 ($P<0.03$), 0.11
257 ($P<0.001$), and 0.18 ($P<0.01$) in the pregnancy group (Table 4), and 16.3 ($P<0.05$) and
258 0.61 ($P<0.05$) in the ongoing pregnancy group (Table 5).
259
260 **Discussion**
261 In the present study, the pregnancy outcomes of patients who became pregnant within a
262 year after histological examination for the presence of CE were retrospectively

investigated. In addition, the correlations between 4 objective variables of miscarriage, preterm birth, term birth, and live birth and eight infertility factors including CE as explanatory variables were analyzed by logistic analysis. The results suggested that the term birth rate was lower and the miscarriage rate was higher when the patients became pregnant, and the preterm birth rate was higher even when pregnancy continued although only the rate of term birth per pregnancy was found to be adequate by the power analysis. CE was a factor adversely affecting objective variables of miscarriage and preterm birth.There are no difference regarding the patients' characteristics, infertility cause and embryo quality between the groups. Thus, these differences did not affect the poor outcome of pregnancy in the CE patients.

The previous clinical studies of CE mainly targeted in patients with implantation failure. In these reports, CE was shown to be a cause of implantation failure, because the pregnancy rate improved in the cured group compared with the persistent CE group after the administration of antibiotics. In the present study, the effects of CE on pregnancy outcomes were examined in pregnant patients by undergoing IVF, who had not been diagnosed with RIF, RPL, or a disease suspected to cause implantation failure at the time of selecting patients for the present study. The pregnancy outcome of CE patients achieving pregnant by IVF within a year were compared with those in the Non-CE group. There have been no reports regarding the effect of CE on pregnancy outcomes with complete follow-up after the diagnosis of CE. Although this study was retrospective, the results suggested that CE patients were more likely to have a miscarriage when they became pregnant and to proceed to preterm birth even when pregnancy continued.

The rates of miscarriage per pregnancy were approximately 12.8% and 40% in the Non-CE and CE groups, respectively. The miscarriage rate of IVF not receiving a

preimplantation genetic diagnosis is generally reported to be about 15-20% (28, 29). This study suggested that the rate in the Non-CE group was lower, and that in the CE group was considerably higher. Recently, we have reported that chronic decidualitis was found more frequently in miscarriage cases of the CE patients (30). When CE patients become pregnant, this inflammation may persist at a high rate. It is thought that microbiome itself and/or inflammatory cytokines induced by this bacterium may affect embryonic development and cause miscarriage (31, 32). On the other hand, it has been reported that CE modifies decidualization (11). The CE may have a higher miscarriage rate by delaying the window of implantation due to the modification of decidualization, allowing it to accept only weak embryos to be miscarried (33, 34). Further research is needed on the mechanism why CE causes miscarriage. We think that it may be a clue to reduce miscarriage.

Analysis of the clinical course of preterm birth patients showed one case of placenta previa in the Non-CE group and 5 cases in the CE group, including 2 cases of pPROM, one case of preterm labor with difficulty regulating uterine contractions, one case of severe gestational hypertension, and one case of fetal anomaly (Table 3). The rate of preterm birth was significantly higher in the CE group than in the Non-CE group in ongoing pregnancy. Placental evaluation was performed only in 3 of 6 cases of preterm birth; a case of pPROM at 24 weeks of pregnancy, a case of preterm labor and a case of fetal pleural effusion. All were diagnosed with chorioamnionitis class I in Blanc's classification (35).

When focusing on pPROM and preterm labor, onset of those diseases directly seems to be associated with inflammation, there were no cases in Non-CE and 2 cases of pPROM and one case of preterm labor that was difficult to control in CE. The rate of preterm birth due to pPROM or preterm labor in ongoing pregnancy tended to be higher in CE (0% 0/34 vs. 14.3% 3/21, P=0.05). Two of three preterm deliveries occurred at 36 weeks of pregnancy and this result gives us the impression that the relationship between CE and preterm birth is weakened, but if we take a closer look at the cases, one had been hospitalized and treated with long term tocolysis for threatened preterm delivery from 33 weeks of pregnancy and the other had pPROM at 35 weeks of pregnancy. On the other hand, there was not any patient hospitalized due to threatened preterm delivery or pPROM in the Non-CE. Based on these results, we think it is possible there is a relationship between CE and preterm birth and speculate that a few CE patients may persist inflammation in the uterus until late pregnancy or that patients diagnosed with CE are more likely to have microbiome easily supplied to the uterus from other organs such as oral cavity and gut during the pregnancy (4, 36-38).

Although HDP in one case with preterm delivery was found only in the CE group, it was found in one case of Non-CE and 2 cases of CE with term delivery (data not shown), and a case with fetal anomaly was found in the CE group. There was no significant difference in these conditions between the two groups (data not shown). It has been shown that inflammation impaired the ability of extra villous trophoblast (EVT) to invade in vitro, that may be involved in HDP(39, 40). Also, there has been reports of endometritis caused by cytomegalovirus (41, 42), which is a famous pathogen causing fetal defects (43).

Theoretically, CE could be a cause of these disease and might be shown to be a risk factor for HDP and fetal defects in the future. Because of the reason, the results are presented in the present article.

When we focus on the analysis of the treatment outcomes of infertility patients, 84 subjects of 40 Non-CE and 44 CE, who had been treated with IVF for 1 year at this hospital after histological diagnosis of CE using CD138 immunostaining from September 2013 to December 2017, were eligible after the exclusion of the patients who met exclusion criteria similar to the analysis of pregnancy outcomes per pregnancy (Supplemental figure 1.)

The results were shown in Supplemental table 1. The percentages of pregnancies, miscarriages, preterm births, term births, and live births per total patients were not presented as the main results in the present study, as these results were led under such special conditions stated in the footnote of supplemental table 1. However, the presence of CE seems to have adversely affected the rates of patients who can become pregnant per total patients, patients who have had a miscarriage per total patients, patients who have experienced term delivery per total patients and patients who have experienced live birth per total patients although only the rates of patients who have experienced term delivery per total patients and patients who have experienced live birth per total patients. Also, CE was a factor that affected the objective variables on the logistic analysis (Supplemental table 2). CE is a problem to be solved to improve both infertility and pregnancy outcomes. These findings suggest that patients undergoing IVF should be examined for CE.

The numbers of CD138 positive cells in 10 HPFs were compared among miscarriage,

preterm birth, and term birth in the CE group. There was no difference found in those numbers. It has been reported that there is a correlation between plasma cell survive and/or density and persistence and severity of inflammation in the tissue (44-48). Applying this theory to CE, the high density of plasma cell indicates a high degree of endometrial inflammation and dysfunction. However, our results indicate that there is no association between the intrauterine inflammation before pregnancy and the induction of miscarriage and preterm birth.

To date, no reports of the effects of CE on pregnancy outcomes have been found. A meta-analysis studied by Vitagliano A, et al. only reported that four out of eight pregnant women had a miscarriage when CE persisted after antibiotic treatment for RIF (27) . At present, when diagnosed as CE, the patient is usually treated with antibiotics and then undergoes embryo transfer. When the present study was conducted, it was already beginning to be thought that antibiotics might be effective for the improvement of clinical outcomes. When embryo transfer was performed to subjects with a diagnosis of CE in out hospital, they were asked if they would be treated with antibiotics. The patients of the CE group extracted to the present study were those who did not want antibiotic treatment. In this sense, the cases who became pregnant following their diagnosis with or without CE are few and extremely valuable. It has been assumed that the in utero environment at the time of pregnancy establishment influences the prognosis of pregnancy (49). The results will provide new evidence to support this assumption. To the best of our knowledge, there are no studies regarding the effect of CE on the prognosis of pregnancy, and this is the first report in the world. These are the strength of the present study.

On the other hand, this study was performed retrospectively. Although the numbers of

the case and controls were satisfied with power analysis to analyze miscarriage rate, they were relatively small, and, based on the power analysis on the actual numbers of subjects, only the term birth rate met the number of subject and the miscarriage rate and preterm birth rate did not. In this sense, we can definitively conclude that the term birth rate per pregnancy becomes lower due to increases in miscarriages and preterm births in CE patients. Also, patients who sought antibiotic treatment was treated with them and were excluded from the study. This attitude is morally correct, but this means that the CE group did not reflect all CE patients in the study period. This causes selection bias. These are the limitation in the present study.

Based on the present study, it is important to investigate the effects of antibiotic treatment whether cure of CE improves obstetric outcome in the future. This result is very important in considering the pathophysiology of CE.

In conclusion, the term birth rate per pregnancy became lower mainly due to an increase in miscarriages when CE was detected before pregnancy in the patients treated with IVF. A histopathological diagnosis of CE adversely affected term birth rate per pregnancy.

Conflict of interest:

No authors have any conflict of interest to disclose.

Authors' contributions: Conception and design: FK; acquisition of data: AM, FK, AN, JK, TH, AT, AT, TA, SK; analyzed the data: HK, FK, TA, ST, SK, MS, RK; drafting the manuscript: AM, FK; substantively revised it: RK; final approval of the version: TM. All authors read and approved the final manuscript.

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Figure Legends

Figure 1. Subjects of this study

A total of 93 patients were extracted for the present study (Figure 1). Nine RIF and 3 RPL patients were excluded. A patient with multiple myomas was excluded. Nine patients who received antibiotics were excluded. Seventy patients were included for the analysis of pregnancy outcome. Thirty-eight were Non-CE and 32 were CE. There were 4 patients, one in the Non-CE group and three in the CE group, who became pregnant twice within a year. In the Non-CE group, a patient gave birth at term after miscarriage. In the CE group, one patient gave birth at preterm and two patients gave birth at term after miscarriages. As a result, 39 pregnancies of Non-CE patients and 35 pregnancies of CE patients were analyzed.

Figure 2. The number of CD138 positive cell per 10HPFs in miscarriage, preterm birth and term birth

There was no difference found in the number of CD138 positive cell per 10HPFs among miscarriage, preterm birth and term birth.

Supplemental figure 1

A total of 115 patients were extracted for the present study. Eleven RIF and 4 RPL patients were excluded. A patient with hypothyroidism with positive anti-thyroid peroxidase antibody, 2 patients with multiple myomas and a patient with adenomyosis were excluded. None of the study participants had uterine malformation, although it was not described as an exclusion criterion. Twelve patients who received antibiotics were excluded.

Table legends

Supplemental table 1.

Forty patients of Non-CE patients and 44 patients of CE patients were analyzed. There were no differences in age, gravidity, parity, BMI, smoking status, and the number of previous ova pick-up cycles between the Non-CE and CE groups at the time of diagnosis of the pregnancy, although the serum FSH level tended to be higher in the CE group. Percentages of pregnancy, miscarriage, preterm birth, term birth, and live birth in the pregnancy were: 95% (38/40) vs. 72.7% (32/44) P<0.01; 12.6% (5/40) vs. 31.8% (14/44) P<0.03; 2.5% (1/40) vs. 11.3% (5/44) P=NS; 82.5% (33/40) vs. 36.4% (16/44) P<0.0001; and 85% (34/40) vs. 45.4% (20/44) P<0.0003 in Non-CE vs. CE, respectively. The rate of term birth per patient and live birth per patient were found to be adequate by the power analysis (Powers; 0.992 and 0.962), although the rates of miscarriage per patient and preterm birth per patient were not. There were no differences in cause of infertility and embryo quality.

The number of patients achieving pregnant is very high in view of general IVF outcomes. We think this result is due to the following reasons. 1) It is assumed that “freeze all blastocysts strategy” was used and CE test was mainly performed after frozen blastocyst was completed. When the patients were examined whether CE was present or not, at least an embryo had been frozen in 83 of 84 patients and multiple embryos had been frozen in 63 of 84 patients. 2) It is also possible that endometrial tissue sampling for CE testing might have a scratching effect (50) . 3) Average patient age is approximately 35 years old, excluding those over 40 years old when the presence of CE was examined. 4) In addition,

RIF, RPL and patients with diseases likely to affect implantation and obstetrics outcomes were excluded. We think that these results suggested that CE is a factor that affects both fertility and obstetric outcome.

References

1. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observations. Obstet Gynecol. 1981;58(2):176-84.
2. Bayer-Garner IB, Korourian S. Plasma cells in chronic endometritis are easily identified when stained with syndecan-1. Mod Pathol. 2001;14(9):877-9.
3. Kasius JC, Fatemi HM, Bourgain C, Sie-Go DM, Eijkemans RJ, Fauser BC, et al. The impact of chronic endometritis on reproductive outcome. Fertil Steril. 2011;96(6):1451-6.
4. Kimura F, Takebayashi A, Ishida M, Nakamura A, Kitazawa J, Morimune A, et al. Review: Chronic endometritis and its effect on reproduction. J Obstet Gynaecol Res. 2019;45(5):951-60.
5. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. Fertil Steril. 2014;101(4):1026-30.
6. Bouet PE, El Hachem H, Monceau E, Gariepy G, Kadoch IJ, Sylvestre C. Chronic endometritis in women with recurrent pregnancy loss and recurrent implantation failure: prevalence and role of office hysteroscopy and immunohistochemistry in diagnosis. Fertil Steril. 2016;105(1):106-10.
7. Liu Y, Chen X, Huang J, Wang CC, Yu MY, Laird S, et al. Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure. Fertil Steril. 2018;109(5):832-9.
8. Tortorella C, Piazzolla G, Matteo M, Pinto V, Tinelli R, Sabba C, et al. Interleukin-6, interleukin-1beta, and tumor necrosis factor alpha in menstrual effluents as biomarkers of chronic endometritis. Fertil Steril. 2014;101(1):242-7.
9. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345(6198):760-5.
10. Boyle AK, Rinaldi SF, Norman JE, Stock SJ. Preterm birth: Inflammation, fetal

injury and treatment strategies. J Reprod Immunol. 2017;119:62-6.
11. Wu D, Kimura F, Zheng L, Ishida M, Niwa Y, Hirata K, et al. Chronic endometritis modifies decidualization in human endometrial stromal cells. Reprod Biol Endocrinol. 2017;15(1):16.
12. Matteo M, Cicinelli E, Greco P, Massenzio F, Baldini D, Falagario T, et al. Abnormal pattern of lymphocyte subpopulations in the endometrium of infertile women with chronic endometritis. Am J Reprod Immunol. 2009;61(5):322-9.
13. Johnston-MacAnanny EB, Hartnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil Steril. 2010;93(2):437-41.
14. Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod. 2015;30(2):323-30.
15. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online. 2014;28(1):14-38.
16. Laufer N, Simon A. Recurrent implantation failure: current update and clinical approach to an ongoing challenge. Fertil Steril. 2012;97(5):1019-20.
17. van Dijk MM, Kolte AM, Limpens J, Kirk E, Quenby S, van Wely M, et al. Recurrent pregnancy loss: diagnostic workup after two or three pregnancy losses? A systematic review of the literature and meta-analysis. Hum Reprod Update. 2020;26(3):356-67.
18. Busnelli A, Paffoni A, Fedele L, Somigliana E. The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis. Hum Reprod Update. 2016;22(6):793-4.
19. Klatsky PC, Tran ND, Caughey AB, Fujimoto VY. Fibroids and reproductive outcomes: a systematic literature review from conception to delivery. Am J Obstet Gynecol. 2008;198(4):357-66.
20. Youm HS, Choi YS, Han HD. In vitro fertilization and embryo transfer outcomes in relation to myometrial thickness. J Assist Reprod Genet. 2011;28(11):1135-40.
21. Chan YY, Jayaprakasan K, Tan A, Thornton JG, Coomarasamy A, Raine-Fenning NJ. Reproductive outcomes in women with congenital uterine anomalies: a systematic review. Ultrasound Obstet Gynecol. 2011;38(4):371-82.
22. Alansari LM, Wardle P. Endometrial polyps and subfertility. Hum Fertil (Camb). 2012;15(3):129-33.
23. Strandell A, Lindhard A, Waldenstrom U, Thorburn J, Janson PO, Hamberger L.

Hydrosalpinx and IVF outcome: a prospective, randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. Hum Reprod. 1999;14(11):2762-9.
24. Coughlan C. What to do when good-quality embryos repeatedly fail to implant. Best Pract Res Clin Obstet Gynaecol. 2018;53:48-59.
25. Simon A, Laufer N. Assessment and treatment of repeated implantation failure (RIF). J Assist Reprod Genet. 2012;29(11):1227-39.
26. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. The association between endometriosis and chronic endometritis. PLoS One. 2014;9(2):e88354.
27. Vitagliano A, Saccardi C, Noventa M, Di Spiezio Sardo A, Saccone G, Cicinelli E, et al. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. Fertil Steril. 2018;110(1):103-12 e1.
28. Tummers P, De Sutter P, Dhont M. Risk of spontaneous abortion in singleton and twin pregnancies after IVF/ICSI. Hum Reprod. 2003;18(8):1720-3.
29. Hipp H, Crawford S, Kawwass JF, Chang J, Kissin DM, Jamieson DJ. First trimester pregnancy loss after fresh and frozen in vitro fertilization cycles. Fertil Steril. 2016;105(3):722-8.
30. Kaku S, Kubo T, Kimura F, Nakamura A, Kitazawa J, Morimune A, et al. Relationship of chronic endometritis with chronic deciduitis in cases of miscarriage. BMC Womens Health. 2020;20(1):114.
31. Villa P, Cipolla C, D'Ippolito S, Amar ID, Shachor M, Ingravalle F, et al. The interplay between immune system and microbiota in gynecological diseases: a narrative review. Eur Rev Med Pharmacol Sci. 2020;24(10):5676-90.
32. Kitaya K, Yamada H. Pathophysiological roles of chemokines in human reproduction: an overview. Am J Reprod Immunol. 2011;65(5):449-59.
33. Coulam C. What about superfertility, decidualization, and natural selection? J Assist Reprod Genet. 2016;33(5):577-80.
34. Teklenburg G, Salker M, Heijnen C, Macklon NS, Brosens JJ. The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. Mol Hum Reprod. 2010;16(12):886-95.
35. Blanc WA. Pathology of the placenta, membranes, and umbilical cord in bacterial, fungal, and viral infections in man. Monogr Pathol. 1981(22):67-132.
36. Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. Maternal Microbiome and Pregnancy Outcomes That Impact Infant Health: A Review. Adv Neonatal Care. 2015;15(6):377-85.

559 37. Bearfield C, Davenport ES, Sivapathasundaram V, Allaker RP. Possible
560 association between amniotic fluid micro-organism infection and microflora in the mouth.
561 BJOG. 2002;109(5):527-33.
562 38. DiGiulio DB, Romero R, Kusanovic JP, Gomez R, Kim CJ, Seok KS, et al.
563 Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory
564 response, and pregnancy outcome in women with preterm pre-labor rupture of
565 membranes. Am J Reprod Immunol. 2010;64(1):38-57.
566 39. Anton L, Brown AG, Parry S, Elovitz MA. Lipopolysaccharide induces cytokine
567 production and decreases extravillous trophoblast invasion through a mitogen-activated
568 protein kinase-mediated pathway: possible mechanisms of first trimester placental
569 dysfunction. Hum Reprod. 2012;27(1):61-72.
570 40. Gomez LM, Anton L, Srinivas SK, Elovitz MA, Parry S. Low-Dose Aspirin May
571 Prevent Trophoblast Dysfunction in Women With Chlamydia Pneumoniae Infection.
572 Reprod Sci. 2019;26(11):1449-59.
573 41. Dehner LP, Askin FB. Cytomegalovirus endometritis: report of a case associated
574 with spontaneous abortion. Obstet Gynecol. 1975;45(2):211-4.
575 42. Giraldo-Isaza MA, Jaspan D, Cohen AW. Postpartum endometritis caused by
576 herpes and cytomegaloviruses. Obstet Gynecol. 2011;117(2 Pt 2):466-7.
577 43. Kawasaki H. Pluripotent stem cells are protected from cytomegalovirus infection
578 at multiple points: implications of a new pathogenesis for congenital anomaly caused by
579 cytomegalovirus. Congenit Anom (Kyoto). 2012;52(3):147-54.
580 44. Cassese G, Arce S, Hauser AE, Lehnert K, Moewes B, Mostarac M, et al. Plasma
581 cell survival is mediated by synergistic effects of cytokines and adhesion-dependent
582 signals. J Immunol. 2003;171(4):1684-90.
583 45. Slocombe T, Brown S, Miles K, Gray M, Barr TA, Gray D. Plasma cell
584 homeostasis: the effects of chronic antigen stimulation and inflammation. J Immunol.
585 2013;191(6):3128-38.
586 46. Mallison SM, 3rd, Smith JP, Schenkein HA, Tew JG. Accumulation of plasma
587 cells in inflamed sites: effects of antigen, nonspecific microbial activators, and chronic
588 inflammation. Infect Immun. 1991;59(11):4019-25.
589 47. Scott BB, Goodall A, Stephenson P, Jenkins D. Rectal mucosal plasma cells in
590 inflammatory bowel disease. Gut. 1983;24(6):519-24.
591 48. Tiniakos DG, Brain JG, Bury YA. Role of Histopathology in Autoimmune
592 Hepatitis. Dig Dis. 2015;33 Suppl 2:53-64.
593 49. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, et al.
594 Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms

595 on pregnancy outcome. Hum Reprod Update. 2016;22(1):104-15.
596 50. Vitagliano A, Andrisani A, Alviggi C, Vitale SG, Valenti G, Sapia F, et al.
597 Endometrial scratching for infertile women undergoing a first embryo transfer: a
598 systematic review and meta-analysis of published and unpublished data from randomized
599 controlled trials. Fertil Steril. 2019;111(4):734-46 e2.
600

Table 1. Characteristics and pregnancy outcomes of pregnancy

	Non-CE N=39	CE N=35	P value	Power
Age (y)	35.2±0.51	34.3±0.55	NS	
Gravidity	0 (0-4)	0 (0-3)	NS	
Parity	0 (0-1)	0 (0-1)	NS	
The level of serum FSH	7.1 (4.3-18.4)	8.8 (5.4-14.8)	.06	
Previous OPU cycles	1 (1-9)	1 (1-6)	NS	
BMI (kg/m^2)	20.7 (16.91-32.81)	21.82 (17.0-32.81)	NS	
Smoking	0	0	NS	
Miscarriage / pregnancy	12.8% (5/39)	40.0% (14/35)	<.03	.674
Preterm birth / pregnancy	2.6% (1/39)	14.3% (5/35)	.095	.29
Term birth / pregnancy	84.6% (33/39)	45.7% (16/35)	<.001	.922
Live birth / pregnancy	84.6% (33/39)	57.1% (20/35)	<.03	.657
Cause of infertility			NS	
Male factor	7	6		
Tubal factor	6	11		
Endometriosis	10	10		
Ovarian factor	6	6		
Antisperm antibody	1	1		
Fertilization failure	0	1		
Unknown	15	6		
Embryo quality			NS	
Good quality blastocyst	65% (26/40)	68.4% (26/38)		
Poor quality blastocyst	32.5% (13/40)	23.7% (9/38)		
Good cleaved embryo	2.5% (1/40)	7.9% (3/38)		

BMI: body mass index, CE: Chronic endometritis, FSH: follicle-stimulating hormone, OPU: ova pick up, NS: not significance

Table 2. Characteristics and pregnancy outcomes of ongoing pregnancy

	Non-CE N=34	CE N=21	P value	Power
Age (y)	35.2±0.55	34.5±0.70	NS	
Gravity	0 (0-4)	0 (0-3)	NS	
Parity	0 (0-1)	0 (0-1)	NS	
The level of serum FSH	7.1 (4.3-18.4)	8.5 (5.4-11.9)	NS	
Previous OPU cycles	1 (1-9)	1 (1-6)	NS	
BMI (kg/m^2)	20.79 (16.91-32.81)	21.5 (17.0-32.81)	NS	
Smoking	0	0	NS	
Preterm birth / ongoing pregnancy	2.9% (1/34)	23.8% (5/21)	<.03	.505
Term birth / ongoing pregnancy	97.1% (33/34)	76.2% (16/21)	<.03	.625
Live birth / ongoing pregnancy	100% (34/34)	95.2% (20/21)	NS	.301
Cause of infertility			NS	
Male factor	5	4		
Tubal factor	6	6		
Endometriosis	9	5		
Ovarian factor	5	4		
Antisperm antibody	1	1		
Fertilization failure	0	1		
Unknown	14	4		
Embryo quality				
Good quality blastocyst	66.7% (24/36)	79.2% (19/24)	.08	
Poor quality blastocyst	30.1% (11/36)	8.3% (2/24)		
Good cleaved embryo	2.8% (1/36)	12.5% (3/24)		

BMI: body mass index, CE: Chronic endometritis, FSH: follicle-stimulating hormone, OPU: ova pick up, NS: not significance

Table 3. Clinical courses of preterm birth

Presence of CE	Gestational age at delivery	Causes and details of preterm birth	Delivery style	Birth weight (g)	Apgar score
Non-CE	36w2d	Placenta previa	CS	2452	8/9
CE	22w5d	PROM at 22 weeks 5 days of gestation	VD	566	Still birth
CE	36w0d	PROM at 35 weeks 4 days ofgestation	VD	2696	8/9
CE	36w6d	Preterm labor admitted for long-term tocolysis from 33 weeks of gestation	CS	3206	8/9
CE	36w2d	Severe hypertensive disorder of pregnancy	CS	2110	9/10
CE	36w3d	Fetal pleural effusion	CS	2568	8/9

CE: chronic endometritis, PROM: preterm rapture of membrane, CS: caesarian section, VD: vaginal delivery

Table 4. Logistic analysis of patients who became pregnant

	Variable	Odds ratio	95% CI	P value
Miscarriage	CE	4.8	1.4-16.3	<.03
Preterm birth	CE	9.6	0.77-121.0	.08
Term birth	CE	0.11	0.03-0.39	<.001
	Male factor	21.7	1.3-359.0	<.05
	Tubal factor	34.5	1.7-683.0	<.03
	Endometriosis	16.5	0.85-321.5	.07
	Unknown	54.2	2.3-1263.9	<.03
Live birth	CE	0.18	0.052-0.61	<.01
	Unknown	10	0.69-144.3	.09

CE: chronic endometritis, CI: confidential interval, NS: not significance

Table 5. Logistic analysis of patients with ongoing pregnancy

	Variable	Odds ratio	95% CI	P value
Preterm birth	CE	16.3	1.3-204.6	<.05
Term birth	CE	0.61	0.005-0.77	<.05
Live birth				NS

CE: chronic endometritis, NS: not significance

Supplemental table 1. Characteristics and fertility and pregnancy outcomes of all patients by group

	Non-CE N=40	CE N=44	P value	Power
Age (y)	35.2±0.48	34.8±0.49	NS	
Gravidity	0 (0-4)	0 (0-3)	NS	
Parity	0 (0-1)	0 (0-1)	NS	
The level of serum FSH	7.3 (4.3-18.4)	8.7 (5.4-14.8)	0.06	
Previous OPU cycles	1 (1-9)	1 (1-6)	NS	
BMI (kg/m^2)	20.79 (16.91-32.81)	21.85 (16.38-35.74)	NS	
Smoking	0	0	NS	
Pregnancy / total	95% (38/40)	72.7% (32/44)	<.01	0.700
Miscarriage / total	12.6% (5/40)	31.8% (14/44)	<.03	0.450
Preterm birth / total	2.5% (1/40)	11.3% (5/44)	NS	0.197
Term birth / total	82.5% (33/40)	36.4% (16/44)	<.0001	0.992
Live birth / total	85% (34/40)	45.4% (20/44)	<.0003	0.962
Cause of infertility			NS	
Male factor	9	10		
Tubal factor	6	13		
Endometriosis	10	12		
Ovarian factor	7	6		
Antisperm antibody	1	1		
Fertilization failure	0	1		
Unknown	13	7		
Embryo quality			NS	
Good quality blastocyst	37	50		
Poor quality blastocyst	15	25		
Good cleaved embryo	4	11		

BMI: body mass index, CE: Chronic endometritis, FSH: follicle-stimulating hormone, OPU: ova pick up, NS: not significance

Supplemental table 2. Logistic analysis of the total patient group

	Variable	Odds ratio	95% CI	P value
Pregnancy	CE	0.13	0.024-0.74	<.03
Miscarriage	CE	3.5	1.1-11.5	<.05
Preterm birth				NS
Term birth	CE	0.091	0.03-0.29	<.0001
	Tubal factor	7	1.05-103.4	<.05
	Endometriosis	10.4	1.36-108.0	<.03
	Unknown	36.2	2.8-470.0	<.01
Live birth	CE	0.11	0.034-0.39	<.0001
	Unknown	24.2	1.8-331.8	<.03

CE: chronic endometritis, CI: confidential interval, NS: not significance