Chronic endometritis diagnosed using a cut-off of ≥5 CD138 plasma cells significantly affects the reproductive outcomes of frozen embryo transfer: a case-control study

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Abstract

Objective: To investigate the clinical significance of a diagnosis of chronic endometritis (CE) made using a diagnostic cut-off of ≥1 or ≥5 CD138 plasma cells per high power field (HPF) in asymptomatic patients undergoing in vitro fertilization (IVF) with frozen embryo transfer (FET).

Material and Methods: In this retrospective case-control study, 1,865 patients underwent freeze-all-IVF treatment between January and December 2019, with 419 undergoing endometrial biopsies at oocyte retrieval. Of the 419 biopsy-patients, 301 have since undergone first FET. The processed endometrial biopsies of the 301 patients underwent immunohistochemical (IHC) examination with anti-CD138 to count CD138+ plasma cells per HPF. CE diagnosis was defined as 0 CD138 plasma cells (control-group), ≥1 CD138 plasma cells ($CE^{control}$-group) or ≥5 CD138 plasma cells ($CE^{disease}$-group) per HPF.

Results: Twenty-six (8.6%) patients were retrospectively diagnosed having ≥1 CD138 plasma cells, and five patients (1.7%) having ≥5 CD138 plasma cells ($CE^{disease}$-group) per HPF. The live birth and pregnancy loss rates of the three groups were 52.7% and 27.9%, 53.8% and 26.3% and 20.0% and 66.7%, respectively. The antral follicle count (AFC) of the three groups were 15.0 (9.0-22.0), 10.5 (7.75-15.25), and 6.0 (5.0-14.0), respectively.

Conclusion: Asymptomatic patients diagnosed with CE with ≥5 CD138 plasma cells per HPF, had the lowest live birth and highest pregnancy loss rates, with these patients also having significantly reduced AFC. (J Turk Ger Gynecol Assoc 2023; 24: 165-71)

Keywords: Chronic endometritis, CD138, frozen embryo transfer, asymptomatic, live birth

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Introduction

Fertility work-ups performed before patients commence in vitro fertilization (IVF) treatment should include diagnostic tests that identify all factors with the potential to affect implantation. Implantation remains a perplexing issue, despite recent innovations in assisted reproductive technologies. Of clinical importance is the identification of all intrauterine anomalies, as these may affect endometrial receptivity. Chronic endometritis (CE) is a poorly understood and often underdiagnosed endometrial inflammatory disease, with microbial infection believed to result in the persistent inflammatory condition (1,2). CE, therefore, is often first diagnosed at the patient's fertility workup if there are indications to suggest the need for diagnostic hysteroscopy. Hysteroscopy may reveal anomalies characteristic of $CE^{disease}$, such as focal or diffuse micropolyps, stromal oedema, focal hyperemia, strawberry aspect, and endometrial haemorrhagic spots, or there may be other

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endometrial features indicating the possibility of endometrial dysfunction, which suggest the need for endometrial biopsy and histological assessment (3).

Implantation is a complex process, including the immunological regulation of blastocyst and endometrial interactions, with synchronized interactive dialogue mediated by steroid hormones, including estrogen and progesterone, cytokines, growth factors, adhesion molecules, prostaglandins, matrix-degrading enzymes and inhibitors (4). The CE hypothesis suggests that the presence of microorganisms and subsequent inflammation results in CE, which then affects implantation by altering local immunological regulation and time-dependent endometrial changes critical to successful reproduction (2,5). Modified immunological conditions may inhibit the ability of the endometrium to develop the critical inflammatory and immune-tolerant responses required for early pregnancy processes, including implantation, placentation and fetal protection (6). CE-related conditions may also affect the ability of the endometrium to respond to sex steroid hormones, impairing hormone-regulated morphological changes, such as endometrial cell proliferation, decidualization, and vascularization, as well as intrauterine contractility (7,8).

There is still no consensus regarding the clinical significance of CE, with no definitive evidence to support the hypothesis that CE is always associated with adverse reproductive outcomes (9,10). The higher prevalence of CE in patients with recurrent implantation failure (RIF), recurrent pregnancy loss (RPL), and preterm delivery, however, does support the belief that CE may adversely affect some reproductive outcomes in IVF (11). The controversy surrounding the significance of CE arises mainly because of the various criteria used in the diagnosis of CE, as reported from the analysis of surveys completed by pathologists (12). Moreover, basic histological assessment of hematoxylin & eosin (H&E) stained endometrial biopsies is generally accepted to be the gold standard for CE diagnosis, when coupled with immunohistochemical (IHC) staining using anti-Syndecan-1 (CD138) that increases the accuracy of plasma cell identification (13). Histopathologically, CE is described as the infiltration of the endometrial stroma by plasma cells, with some pathologists considering a single plasma cell per unit measure a diagnosis of CE (12). Moreover, emerging evidence suggests that ≥5 plasma cells per high-power field (HPF) may better define a diagnosis of CE with significant clinical implications (14,15).

At the IVF centre where the present study was conducted, freeze-all-IVF is performed routinely, which provides the opportunity to safely perform diagnostic endometrial biopsies at the time of the oocyte retrieval procedure (16). In the present study, the clinical significance of CE diagnosed using the criteria of either ≥1 or ≥5 plasma cells per HPF was investigated in asymptomatic patients undergoing freeze-all-IVF with frozen embryo transfer (FET).

Materials and Methods

Patients

This retrospective, case-control study was performed at a single IVF centre, with cycles of patients who had undergone endometrial biopsies at oocyte retrieval for freeze-all-IVF treatments performed between January and December 2019 being investigated. The original endometrial biopsies were performed to benefit from the rejuvenating effect of endometrial scratching (17). In the present study, the previously processed endometrial biopsy tissues were stained with CD138 IHC, to perform plasma cell counts, after patients had undergone their first FET. Routine fertility workup included 2-D transvaginal ultrasound scans, with saline-infused sonography, hysterosalpingography, and hysteroscopy performed if intrauterine anomalies were suspected. Importantly, freeze-all-IVF treatments provide the opportunity to perform therapeutic or diagnostic intrauterine procedures, including endometrial biopsy, at oocyte retrieval without adversely affecting the pregnancy prognosis of patients (16). Cycles were included according to the following criteria: if an endometrial biopsy was performed at oocyte retrieval; female age was ≤42 years; and female body mass index (BMI) was ≤35 kg/m^2. Exclusion criteria were: intrauterine or tubal anomalies not corrected; RIF or RPL diagnosed; corticosteroid treatment; or if the female patient had autoimmune disease, antiphospholipid syndrome, or thrombophilia. However, cycles in which hysteroscopic surgery for intrauterine or tubal anomalies were performed at the time of the oocyte retrieval and endometrial biopsy were included, with the FET of these patients delayed according to the type of surgery (16).

This work was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (approval number: KAEK-970, date: 22.12.2021).

Procedures

Endometrial biopsy

All endometrial biopsies were taken on ovarian cycle days 8-16, with all endometrial biopsies taken using 3.0 mm endometrial suction curettes (gynaecological sampler, Medbar, İzmir, Turkey) immediately following the oocyte retrieval. The endometrial biopsies were washed in phosphate buffered-saline and fixed overnight in vials containing fixative (10% phosphate-buffered formaldehyde).

Endometrial tissue assessment

The endometrial biopsies were transferred to an independent histopathology laboratory, where tissue preparation and assessment was performed by experienced gynecological histopathologists. Previously processed endometrial tissue sections were stained with anti-CD138 (EP201, cell-marque, Merck KGaA, Darmstadt, Germany) using Dako Autostainer Link48 (Dako Colorado Inc. Ft Collins, CO, USA), with tissue sections examined by light microscopy (400x magnification). The mean number of CD138 plasma cells counted per HPF (in 10 HPF) were recorded. CE diagnosis was reported according to the mean number of plasma cells counted per HPF, with CE diagnoses grouped as follows: 0 CD138 plasma cells (control-group); ≥1 CD138 plasma cells ($CE^{control}$-group); and ≥5 CD138 plasma cells ($CE^{disease}$-group).

Antibiotic prophylaxis

Antibiotic prophylaxis therapy was administered twice during treatment. Firstly, a single dose of azithromycin (2x500 mg, Zitrotek, Pfizer İlaç Ltd. Şti. İstanbul, Turkey) was administered for empirical antibiotic prophylaxis for unsuspected sexual transmitted disease to the couple (both male and female patients) before proceeding with ovarian stimulation (OS). The second antibiotic treatment consisted of 200 mg of doxycycline (Tetradox, Actavis, İstanbul, Turkey) administered for four days after oocyte retrieval. The doxycycline prophylaxis was a precautionary measure against any possible procedure-related infections (18), with the total antibiotic prophylaxis therapy administered in the attempt to normalize patient semen and vaginal microbiomes (19).

Ovarian stimulation, embryo culture, and blastocyst vitrification

OS was performed using standard protocols, as previously described (20). In vitro oocyte collection and embryo cultures were performed using standard protocols, as previously described (20). Blastocysts were scored and selected for cryopreservation on days 4-6 of in vitro embryo culture (21), with all viable blastocysts cryopreserved using vitrification protocols and technologies (Cryotop, Kitazato BioPharma Co. Ltd, Fuji-city, Japan).

Artificial cycle frozen embryo transfer

Artificial cycle FET (AC-FET) was performed, as previously described (22), with the daily administration of vaginal progesterone (90 mg TID, Crinone® 8%, Merck Serono, İstanbul, Turkey) started on day-15 and continued in conjunction with oral estrogen (2 mg TID) until the day of the pregnancy tests. In addition, patients were given weekly intramuscular (IM) progesterone (Proluton® depot 500 mg/2 mL, Bayer Türk, İstanbul, Turkey), with the first IM progesterone administered on the third day of vaginal progesterone. Pregnant patients continued with all luteal phase support drugs until the 10th week of gestation.

Blastocyst transfer

The start date (day-15) of progesterone administration and the day of blastocyst cryopreservation were used to schedule FET. Blastocysts cryopreserved on days 5 and 6 were transferred on the sixth day of progesterone and blastocysts cryopreserved on day 4 on the fifth day of progesterone (21). All blastocysts to be transferred were rescored approximately 2 hours before the transfer, with the day of cryopreservation and blastocyst scores (including, expansion, inner cell mass, and trophectoderm scores) used to define a poor, fair, or good quality blastocyst. Blastocysts with inner mass and trophectoderm scores of AC, CA, BC, CB, and CC were defined as poor quality, irrespective of expansion grade. A maximum of two blastocysts were transferred.

Outcomes and statistical analysis

The primary outcome measure was live birth (LB) from the first FET of freeze-all-IVF cycles, with LB defined as the delivery of a live infant at ≥22 weeks of gestation. The secondary outcome measure was the mean number of CD138 plasma cells per HPF. RIF was defined as the failure of more than three previously performed fresh or FET to result in a clinical implantation, with female age and embryo quality taken into consideration. RPL was defined as the loss of more than three pregnancies before 22 weeks of gestation.

Statistical analysis

SPSS, version 11.5 (IBM Inc., Armonk, NY, USA) was used in all statistical analyses, with variables analysed as means ± standard deviation, medians (plus interquartile ranges 25% and 75%; interquartile range), or as rates (percentages). In univariate comparative analyses, Mann-Whitney Rank Sum tests and chi-square tests were performed, with significant difference indicated by a $p<0.05$.

Results

In total 1865 patients aged 18-42 years (mean age: 33.2±6.01 years) underwent oocyte retrievals as part of freeze-all-IVF treatments in 2019, with 419 (22.6%) undergoing endometrial biopsies at oocyte retrievals (Table 1). Endometrial biopsies were done on ovarian cycle-day 12.3±2.14. One hundred and eighteen patients were excluded from the study, with the 301 patients who had undergone their first FET included. Forty-seven patients had delayed FET because of hysteroscopic surgery performed at oocyte retrieval, 43 (15.6%) were in the

control-group and 4 (15.4%) in the CE-group. The processed endometrial tissues of these patients were retrospectively stained with CD138 IHC to count the number of plasma cell present. Of the 301 patients, 26 (8.6%) were identified as having ≥1 CD138 plasma cells per HPF ($CE^{control}$-group) and 5 (1.7%) having ≥5 CD138 plasma cells per HPF ($CE^{disease}$-group).

In Table 2, the demographics of patients with 0 CD138 plasma cells (control-group) are compared with those of patients with ≥1 CD138 plasma cells ($CE^{control}$-group). Only the median AFCs of the two groups were significantly different, with patients in the $CE^{control}$-group having fewer AFCs. The majority of patients were diagnosed as having primary infertility, with unexplained and male infertility being the most prevalent of the infertility etiologies.

The overall LB rate from first FET of freeze-all-IVF treatments performed in 2019 was 47.9% (627/1308). The reproductive outcomes of the first FET in the different study groups are presented in Table 3. The pregnancy, clinical pregnancy, LB and pregnancy loss rates were similar in the control- and $CE^{control}$-groups. The pregnancy, clinical pregnancy, and LB rates were lower in the $CE^{disease}$-group, with a pregnancy loss rate of 66.7%. The patients in the $CE^{disease}$-group tended to have fewer AFC than patients with <5 plasma cell counts with a median of 6.0 (5.0-14.0) vs. 11.0 (8.5-17.0), respectively (p=0.161). The blastocyst quality of patients diagnosed with CE (either ≥1 or ≥5 plasma cells) was unaffected, with 96.2% of the primary blastocysts transferred defined as being of good quality. One patient in the $CE^{disease}$-group underwent a second FET treatment, which resulted in a clinical pregnancy loss.

Table 1. Patient inclusion and exclusion

n (endometrial biopsy cycles)	**419**
Excluded cycles	
Female age >42 years	17
Female BMI >35 kg/m²	9
RIF	3
RPL	3
Cycle outcome exclusions	
No oocytes retrieved	3
No mature oocytes retrieved	5
No fertilization	6
Embryo development arrest	63
No first FET	8
Unknown pregnancy outcome	1
n (cycles with first FET)	**301**

BMI: Body mass index, RIF: Recurrent implantation failure, RPL: Recurrent pregnancy loss, FET: Frozen embryo transfer, No FET: No first FET following oocyte retrieval

Discussion

While the cause-and-effect relationship between CE and adverse reproductive outcomes in IVF is clinically plausible, the impact of CE on the reproductive outcomes of IVF patients remain controversial (5). Patient selection in previous studies may have contributed to this controversy, with some previous studies investigating the effect of CE in patients

Table 2. Patient demographic comparisons

	Control-group (n=275)	**CE-group (n=26)**	**p**
Female age (years)	31.6 (27.8-36.6)	33.7 (27.8-36.8)	0.592
Female BMI (kg/m²)	25.0 (22.0-27.0)	24.0 (21.0-28.0)	0.517
AFC (n)	15.0 (9.0-22.0)	10.5 (7.75-15.25)	0.022
Infertility duration (years)	3.5 (2.0-6.0)	4.0 (1.50-5.25)	0.569
Primary infertility (%)	84.7 (233)	76.9 (20)	0.448
Patients with previous embryo transfers (%)	32.7 (90)	30.8 (8)	0.988
LB rate from previous embryo transfers (%)	8.3 (12/145)	7.1 (1/14)	0.717
Primary infertility etiologies (%)			
Unexplained	50.9 (140)	50.0 (13)	0.907
Male	27.3 (75)	30.8 (8)	0.879
DOR	7.6 (21)	11.5 (3)	
PCOS	7.3 (20)	3.8 (1)	
Tubal	6.2 (17)	3.8 (1)	
Endometrioma	0.7 (2)	0.0 (0)	

Data presented as median (interquartile range) or percentage (number), with statistical significance of p<0.05 in Mann-Whitney Rank Sum and chi-square tests. BMI: Body mass index, CE: Chronic endometritis, AFC: Antral follicle count, LB: Live birth, PCOS: Polycystic ovary syndrome, DOR: Diminished ovarian reserve (defined as total AFC of ≤5)

Table 3. The reproductive outcomes from first FET of CE patients

		Control-group (0)[a]	CE^{control}-group (≥1)[b]	p-value[a vs. b]	CE^{disease}-group (≥5)
First FET (n)		275	26		5
Number transferred	n	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.281	1.0 (1.0-1.5)
Blastocyst quality poor	% (n)	2.5 (7)	0.0 (0)		
Blastocyst quality fair	% (n)	8.0 (22)	3.8 (1)		
Blastocyst quality good	% (n)	89.5 (246)	96.2 (25)	0.455	80.0 (4)
Pregnancy rate (%)	% (n)	73.1 (201)	73.1 (19)	0.818	60.0 (3)
Clinical pregnancy rate (%)	% (n)	61.5 (169)	61.5 (16)	0.840	40.0 (2)
Live birth rate (%)	% (n)	52.7 (145)	53.8 (14)	0.923	20.0 (1)
Total pregnancy loss rate (%)	% (n)	27.9 (56)	26.3 (5)	0.901	66.7 (2)

Data presented as median (interquartile range) or percentage (number), with statistical significance of $p<0.05$ in Mann-Whitney Rank Sum and chi-square tests. CE: Chronic endometritis, FET: Frozen embryo transfer, Number transferred: The number of blastocysts transferred, NB for blastocyst quality: Poor includes all blastocysts with inner mass and trophectoderm scores of AC, CA, BC, CB, and CC, with only the score of the primary blastocysts transferred analysed. Pregnancy rate, defined as a β-HCG level of >5 mIU/mL measured 9 days after blastocyst transfer. Clinical pregnancy rate, defined as a pregnancy with normal fetal heart activity confirmed on ultrasound after 5 weeks of gestation. Early pregnancy loss, defined as a pregnancy lost before normal fetal heart activity confirmation and miscarriage, defined as a clinical pregnancy lost before 22 weeks of gestation. Total pregnancy loss includes all pregnancy losses

having RIF or RPL. RIF and RPL are complex, multifactorial infertility etiologies, which may complicate cause-and-effect investigations (23,24). Moreover, the factor that has contributed most to the controversy is the absence of universally accepted criteria for defining CE. In the present study, the reproductive outcomes in asymptomatic patients who had undergone their first FET following blastocyst freeze-all-IVF were investigated, with endometrial biopsies taken at oocyte retrievals retrospectively stained with CD138 IHC for the diagnosis of CE, and sub-grouped as either ≥1 or ≥5 CD138 plasma cells per HPF.

In the present study, endometrial biopsies were taken mid-cycle (day-12.3), with less than 10% having ≥1 CD138 plasma cells and fewer than 2% having ≥5 CD138 plasma cells per HPF. Asymptomatic patients in the CE^{control}-group had reproductive outcomes not dissimilar to those with zero CD138 plasma cells who constituted the non-CE^{control}-group in the present study. These reproductive outcomes supported the assertion made in the study of Kasius et al. (25) that "CE diagnosed in asymptomatic patients had minimal clinical implications in IVF treatments". In the same study, CE diagnoses were qualitative, that is the CE group had "evident CE". In a more recent study, no significant differences between pregnancy outcomes in patients with 0 CD138+/HPF and those with 1-4 CD138+/HPF were reported (15). In the present study, patients in the CE-group were associated with lower AFC but this reduction in AFC did not have a negative effect on pregnancy outcomes in the CE^{control}-group, with similar rates of good quality (primary) blastocysts transferred.

Historically, plasma cell identification in endometrial tissue has been a difficult challenge, even for the most experienced histopathologists when conventional HE staining was used, with the main difficulty being the ability to accurately distinguish plasma cells from other morphologically similar endometrial and immunological cells (26). Moreover, diagnostic accuracy is not only dependent on the experience of the pathologist, but also on staining protocol, as well as the timing, method, size, and location of endometrial biopsy (26). In the present study, all endometrial biopsies were obtained mid-cycle (day-12.3), with endometrial tissues retrospectively stained with CD138 IHC. Even though CD138 IHC has markedly increased the accuracy of plasma cell counts, there is still no consensus on the criteria defining CE that has clinical consequences (5,12,13). The study of Liu et al. (13), suggested that plasma cell density quantified per unit area improved the accuracy of CE diagnosis. Hirata et al. (27) found that the sensitivity and accuracy were optimal if CE was diagnosed as ≥1 plasma cells in 10 HPF. The emerging evidence, however, suggests that criteria that include plasma cell counts of ≥5 may more accurately define clinical CE (14,15).

Patients diagnosed having CE, with the diagnosis defined as ≥5 plasma cells, were reported to have significantly reduced LB rates (14,15). Xiong et al. (15) found no differences in pregnancy outcomes between patients with 0 CD138+ cells/HPF and those with 1-4 CD138+ cells/HPF. In the present study, the majority (81%) of patients in the CE-group had plasma cell counts of <5. Interestingly, patients diagnosed having ≥1 plasma cell had reduced AFC, with patients diagnosed having ≥5 plasma cells having an even lower, but not significantly lower AFC. This decrease in AFC may be the clinical consequence of progressive CE^{disease}, with those diagnosed having ≥5 CD138 plasma cells particularly affected. Patients with ovarian endometriosis or endometrioma have also been reported to have significantly reduced AFCs (28). In the present study, asymptomatic patients

diagnosed with ≥5 CD138 plasma cells had the lowest LB rate and highest pregnancy loss rate.

While the origins of the microorganisms associated with CE are a matter for speculation, pharmacological therapies have been reported to be effective (70-90%) in the treatment of CE (26). These therapies have included broad-spectrum, microbe-specific, and multi-course antibiotic therapies (11,15,29). Patients cured of CE, as evidenced by restored endometrial health, using antibiotic therapy were reported to have reproductive outcomes similar to those of patients without CE (11,15), with the authors suggesting that $CE^{disease}$ resolution confirmation procedures should be performed routinely before patients were allowed to commence with IVF treatment (11,15). In the present study, patients were administered azithromycin and doxycycline prophylactically during the course of their treatment, with doxycycline a common antibiotic included in CE antibiotic therapies (15,23,30). Moreover, whereas the standard prescribed duration of doxycycline was fourteen days for $CE^{disease}$, doxycycline was only administered for four days in the present study. It is uncertain whether the low-intensity antibiotic prophylaxis therapy administered by patients had an effect on $CE^{disease}$ because follow-up endometrial biopsies were not performed in the present study. However, while the $CE^{control}$-group were observed to have normal pregnancy prognoses, the $CE^{disease}$-group continued to have poor pregnancy prognoses, with the latter indicating the possible persistence of high level $CE^{disease}$.

Study Limitations

The present study confirms the importance of having universally accepted criteria defining clinical CE, with the study adding to the growing evidence to support ≥5 plasma cells per unit measure as a defining criterion. In addition, because the greatest improvement in reproductive outcomes has been reported for patient groups with restored endometrial health, it might be advisable to routinely perform follow-up endometrial biopsies for all patients with a diagnosis of CE (15). Limitations such as the retrospective cycle analyses, the low number of patients diagnosed having CE, the routine use of precautionary antibiotic prophylaxis, and the uncertain status of $CE^{disease}$ at FET weakened the strength of evidence of the present study.

Conclusion

Asymptomatic patients diagnosed with CE, defined as a mean of ≥5 CD138 plasma cells per HPF, had the lowest LB and highest pregnancy loss rates, with these patients also having significantly reduced AFC compared to patients with zero CD138 plasma cells identified.

Ethics Committee Approval: *This work was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (approval number: KAEK-970, date: 22.12.2021).*

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