Received: 21 June 2020 | Revised: 17 October 2020 | Accepted: 27 October 2020

DOI: 10.1111/aji.13369

ORIGINAL ARTICLE

AJRI American Journal of Reproductive Immunology | WILEY

Diagnosis of chronic endometritis: How many CD138$^+$ cells/HPF in endometrial stroma affect pregnancy outcome of infertile women?

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Funding information
Supported by National Key Research &Developmental Program of China (2018YFC1003900, 2018YFC1003904), National Natural Science Foundation of China Grant (81601279, 81871128),, Sanming Project of Medicine in Shenzhen (SZSM201502035) and Basic Research Program of Shenzhen (JCYJ20180228164631121).

Abstract

Problem: The definition of chronic endometritis (CE) differs among studies, and currently, there is no accepted consensus. This study aimed to establish the minimum number of immunohistochemical analysis of CD138$^+$ plasma cells to identify a clinically relevant CE.

Method of study: We performed a retrospective study on 716 infertile patients who never did CE analysis and respective antibiotic treatment before. Samples were obtained by endometrial scratching in the mid-luteal phase before IVF-ET treatment. The number and distribution of CD138$^+$ cells were analyzed by immunohistochemistry. Thirty high-power fields (HPF) were evaluated for each sample. Patients were classified in 2 main groups: (a) CD138^{low} (<5 CD138$^+$ cells in all HPFs), (b) CD138^{high} (≥5 CD138$^+$ cells in at least one HPF). Pregnancy outcome was compared among the groups.

Results: In the CD138^{high} group, β-hCG positive rate, clinical pregnancy rate and live birth rate were significantly decreased ($P = .04$, $P = .01$, $P = .04$, respectively). Also after adjusting for patient age, body mass index (BMI), and clinical characteristics, the β-hCG positive rate ($P = .05$), clinical pregnancy rate ($P = .01$) and live birth rate ($P = .02$) were significantly lower in the CD138^{high} than those in the CD138^{low} group. Within the CD138^{low} group, these parameters were not significantly different between patients without any plasma cells and patients with up to 4 plasma cells/HPF.

Yuye Li and Shiru Xu are similar in the author order.

Conclusion: We conclude that immunohistochemical analysis of CD138$^+$ cells is a reliable method to detect CE which can be identified by the presence of ≥5 plasma cells in at least one out of 30 HPF.

KEYWORDS

CD138, chronic endometritis, infertile women, pregnancy outcome

1 | INTRODUCTION

Approximately 10%-12% of the reproductive-aged couples experience fertility problems and have trouble achieving pregnancy.[1] In vitro fertilization-embryo transfer (IVF-ET) has been increasingly recognized as an effective treatment of infertility. Despite significant advances in blastocyst culture and implication of preimplantation genetic diagnosis/screening (PGD/PGS) and technologies, embryo implantation failure rates remain a major limiting factor of IVF procedures. Impaired endometrial receptivity is thought to be one of the major contributing factors to embryo implantation failure.[2] Chronic endometritis (CE), a disease of persistent inflammation of the endometrium, is frequently associated with infertility and recurrent implantation failure as it may affect uterine receptivity and reduce the success rates of IVF-ET treatment.[3,4] Histopathologically, CE is predominantly characterized by plasma cell infiltration in the endometrial stroma.[5,6] CE is often caused by infection with the most prevalent pathogens, including *Escherichia coli*, *Streptococcus* spp., *Staphylococcus* spp., *Chlamydia*, *Mycoplasma*, *Ureaplasma* spp., *Yeast*[7-11] and some viruses.[11-13] Nonetheless, a clear association between the presence of a specific pathogen and the development of CE has not been demonstrated yet.[35] CE patients present no noticeable signs or mild symptoms, including spontaneous pelvic pain, atypical uterine bleeding, dyspareunia, and increased leukorrhea.[5,6,14] Accumulating pieces of evidence have suggested a pathological role of CE by inducing infertility and adversely affecting outcome in women undergoing IVF-ET treatment. The prevalence of CE in infertile women varies considerably among different studies from 2.8% to 56.8%,[3,15-17] in recurrent miscarriage (RM) from 9.3% to 67.6%,[18-21] and in repeated implantation failure (RIF) from 7.7% to 67.5%.[7,22-25] This large variance might be attributed to several factors, including differences in the diagnostic methods to identify and quantify plasma cells in CE.

Different methods have been applied for the diagnosis of CE, including hysteroscopy, histology, microbial culture, and immunohistochemistry (IHC) for quantification of CD138$^+$ plasma cells.[8,10] As CE is frequently asymptomatic, hysteroscopy is a useful tool based on the identification of the specific visual signs of endometrial inflammation, but it may be perceived differently by different observers. In this context, Song et al described an overall accuracy of hysteroscopic CE diagnosis of only 67% and concluded that hysteroscopy should not replace histologic examination.[26] Furthermore, endometrial bacterial culture cannot be routinely performed because it has a long turnaround time and the fact that not all bacteria responsible for CE are culturable.[27,28] Therefore, a bacterial culture is generally not recommended to detect CE.

An increasing number of studies suggest that the diagnosis of CE should be based on the presence of plasma cells in the endometrial stroma. CD138, a transmembrane heparin sulfate proteoglycan syndecan-1, is the most specific molecule for identifying of plasma cells. Moreover, CD138 detection by IHC has the potential to improve the accuracy and sensitivity of diagnosis.[23,29-31]

Although CD138$^+$ plasma cell detection by IHC has been globally recognized as the most valuable marker for the diagnosis of CE, there is no consensus for a cutoff value for the definition of a clinically relevant CE. The main reason may be different approaches to quantify CD138$^+$ cells. An early study has counted CD138$^+$ cells in the whole tissue section and the presence of one single plasma cell was sufficient for the diagnosis of CE.[30] However, the specimen size was not taken into account. Several subsequent studies pointed out that the presence of a low number of plasma cells should not be sufficient to diagnose CE, because also healthy endometrial stroma may contain a limited number of plasma cells or indicate a slight inflammation without clinical relevance.[32,33] Because of low cutoff values of endometrial CD138$^+$ cells, the prevalence of CE was overestimated. Following, a more exact quantification of CD138$^+$ cells was introduced as per mm^2. Liu et al established diagnostic criteria based on the reference range of endometrial CD138$^+$ cells in 40 fertile women and defined CE as the level of CD138$^+$ cells above the 95th percentile. A density of <5.15 cells/0.1 mm^2 was defined as normal.[23] However, the relevance of this cutoff value for pregnancy success was not verified. Alternatively, the CD138$^+$ cells were counted in selected high-power fields (HPF). Currently, this approach is the most extensively reported. According to a study by Jonhston et al, more than one CD138$^+$ cell in one HPF was suggestive of CE.[22] In contrast, Chen et al recommended a cutoff of 5 or more plasma cells in one HPF for CE diagnosis.[34] Bouet PE et al proposed a minimum of 5 plasma cells in 10 non-overlapping HPFs for the diagnosis of CE.[25] Subsequently, Kitaya et al suggested 5 or more CD138$^+$ cells in 20 non-overlapping HPFs as indicative of CE.[35]

The evaluation of the published study does not lead to a clear and stable definition of diagnostic criteria for a pathologically relevant CE. As plasma cell numbers are low, the evaluation of a limited number of non-overlapping HPFs may be not representative, and thus, not yield a consistent and reproducible result. Furthermore, the size of HPF is mostly not provided and may differ greatly among studies. Besides, the criteria in different studies to establish definitions of

cutoff values for endometrial CD138$^+$ plasma cells for the diagnosis of CE remain elusive.

Therefore, the present study aimed to develop novel diagnostic criteria for CE based on immunohistochemical CD138$^+$ staining and cell number using a large sample size. Also, we analyzed the co-relationship between CD138$^+$ cell count/HPF and pregnancy outcome in infertile women and identified the minimum number of CD138$^+$ cell for CE diagnosis that had a significant impact on pregnancy outcome.

2 | MATERIAL AND METHODS

This retrospective study was approved by the Ethics Committee of Shenzhen Zhongshan Urology Hospital (SZZSECHU-F-2019055). All endometrial biopsies were collected with written informed consent from each participant.

2.1 | Subjects

We retrospectively analyzed 716 infertile patients who received IVF-ET treatment at the Fertility Centers of Shenzhen Zhongshan Urology Hospital between 2017 and 2018.

Included patients underwent endometrial curettage in the mid-luteal phase, followed by IVF-ET. All endometrial tissues were paraffin embedded for subsequent study. All included patients had a successful ovarian stimulation and subsequent embryo transfer.

The inclusion criteria were as follows: 1. infertile patients under the age of 45 years; 2. endometrial tissue biopsies were obtained during the mid-luteal phase of the menstrual cycle. We excluded patients who were previously diagnosed with CE by hysteroscopy or bacterial culture and received antibiotic treatment, and patients who did not receive IVF-ET treatment within 6 months after endometrial scratching, or those who were lost to follow-up.

2.2 | Endometrial biopsy

Endometrial tissue samples were collected during the mid-luteal phase (LH day 7-9) of the menstrual cycle. All endometrial biopsies were obtained by using an endometrial curette (Gynetics, Lommel, Belgium). The specimens were incubated overnight with 10% neutral buffered formalin at room temperature and then embedded in paraffin wax.

2.3 | Immunohistochemistry staining

Four micrometre slices were cut from the paraffin blocks, deparaffinized in xylene and dehydrated through the application of a series of alcohol. IHC staining was performed using an automated Leica Bond III immunostainer (Leica Biosystems, Newcastle, UK) following the manufacturer's protocol. Briefly, for antigen retrieval, slides were heated for 20 minutes in a microwave in 10 mmol/L sodium citrate. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 10 minutes. The sections were then incubated with a 1:250 dilution of mouse anti-CD138 antibody (Gene Tech, clone B-A38, Shanghai, China). Subsequently, the slides were washed and incubated with a horseradish peroxidase-conjugated secondary antibody for 30 minutes. The immunostaining was carried out with 3, 3′-diaminobenzidine chromogen (DAB), and counter-staining with hematoxylin. The sections were observed by an experienced pathologist under a light microscope (400× magnification).

2.4 | Identification and counting of CD138$^+$ plasma cells

For the diagnosis of CE, the total number of plasma cells was determined by counting CD138$^+$ cells in endometrial stroma under a light microscope (Nikon Microscope 50i, Melville, New York) in 30 selected HPF with a tissue coverage of at least 80% (magnification 400× 0.2375 mm^2). Our preliminary results showed that the density of CD138$^+$ cells in the selected 30 HPFs was consistent with that of the entire slide (unpublished data).

Cells were considered to be CD138$^+$ plasma cells when the plasma cell membrane exhibited a strong immunopositivity, the cytoplasm exhibited weak positive immunostaining, the nucleus was round and positioned on one side of the cell, and the condensed chromatin within the nucleus was organized radially along the nuclear membrane to form a wheel pattern.

Two experienced pathologists independently performed the identification and counting of CD138$^+$ cells. Any disagreement between the two pathologists was resolved through discussions or a panel discussion with a senior pathologist.

According to the density of CD138$^+$ cells in the stroma in one HPF, the patients were categorized into six groups: group a contained 0 CD138$^+$ cells in one HPF in all of the 30 selected HPFs (0/HPF) and the remaining HPFs had no CD138$^+$ cell; group b had 1 CD138$^+$ cell in at least 1 out of 30 selected HPFs (1/HPF), and the remaining HPFs had ≤1 CD138$^+$ cells; group c contained 2 CD138$^+$ cells in at least 1 out of 30 selected HPFs (2/HPF) and the remaining HPFs had ≤2 CD138$^+$ cells; group d contained 3 CD138$^+$ cells in at least 1 out of 30 selected HPFs (3/HPF) and the remaining HPFs had ≤3 CD138$^+$ cells; group e contained 4 CD138$^+$ cells in at least 1 out of 30 selected HPFs (4/HPF) and the remaining HPFs had ≤4 CD138$^+$ cells; group f contained five or more CD138$^+$ cells in at least 1 out of 30 selected HPFs.

2.5 | Statistical analysis

All statistical analyses were performed with SPSS Statistics version 20.0 (SPSS Inc, Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD) (for normally distributed data) or

median with inter-quartile ranges (for not normally distributed data). The correlation between CD138$^+$ expression and the clinicopathological characteristics was analyzed by using the Chi-square test.

Multivariable logistic regression models were used to identify the prognostic significance of CD138$^+$ cells for pregnancy outcome. The odds ratios (ORs), adjusted odds ratios (aORs) and their corresponding 95% confidence intervals (95% CIs) for HCG positive rate, clinical pregnancy rate and live birth rate were calculated for patients with by less than five CD138$^+$ cells/HPF and five or more CD138$^+$ cells/HPF after adjusting for confounding factors including, patients age, body mass index (BMI), number of embryos transferred, and characteristics of the embryo (percentage of frozen embryo transfer, blastocysts, good-quality embryo). A *P*-value of ≤ .05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics of infertile patients

A total of 716 infertile patients were included in this retrospective study between January 2017 and December 2018 (Figure 1). As illustrated in Figure 2, the CD138$^+$ cells in the endometrial stroma were frequently aggregated and formed clusters. For a first orientation, according to the distribution of CD138$^+$ cells in the stroma in 30 analyzed HPF, patients were categorized into six groups: patients with (a) no CD138$^+$ cells in any HPF (n = 443); (b) 1 CD138$^+$ cell in at least 1 HPF (n = 178); (c) 2 CD138$^+$ cell in at least 1 HPF (n = 33); (d) 3 CD138$^+$ cell in at least 1 HPF (n = 18); (e) 4 CD138$^+$ cell in at least 1 HPF (n = 6); (f) ≥5 CD138$^+$ cell in at least 1 HPF (n = 38). The demographic characteristics of the six groups have been summarized in Table 1. No significant differences in the clinical characteristics of patients were observed among these groups.

3.2 | Association of CD138$^+$ cells with pregnancy outcome of infertile women

We have assessed the association of the above described patient categories with pregnancy outcome. There was no significant difference in rates of β-hCG positive, clinical pregnancy, early miscarriage, and live birth among the groups, except for group f which the clinical pregnancy rate was significantly lower than in group a (*P* = .01; Table 2 and Figure 3). A trend toward lower rates of β-hCG positive

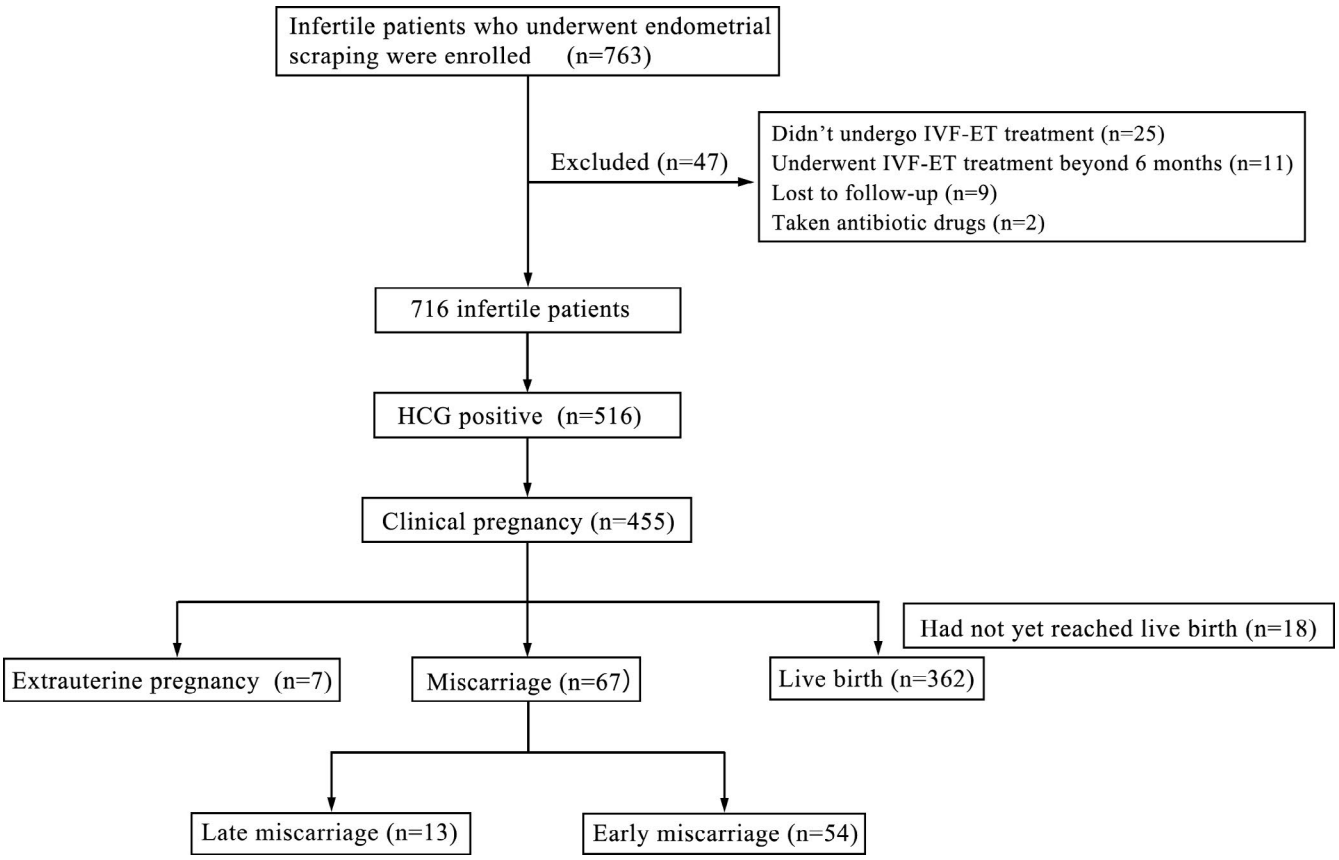

FIGURE 1 Number of infertile women enrolled and pregnancy outcomes in the study. A total of 763 infertile patients who underwent endometrial scraping between 2017 and 2018 were retrospectively analyzed in this study. Based on the inclusion criteria, 47 patients were excluded from the study, a total of 716 infertile patients were included in the study. The included patients were followed-up to determine pregnancy outcomes

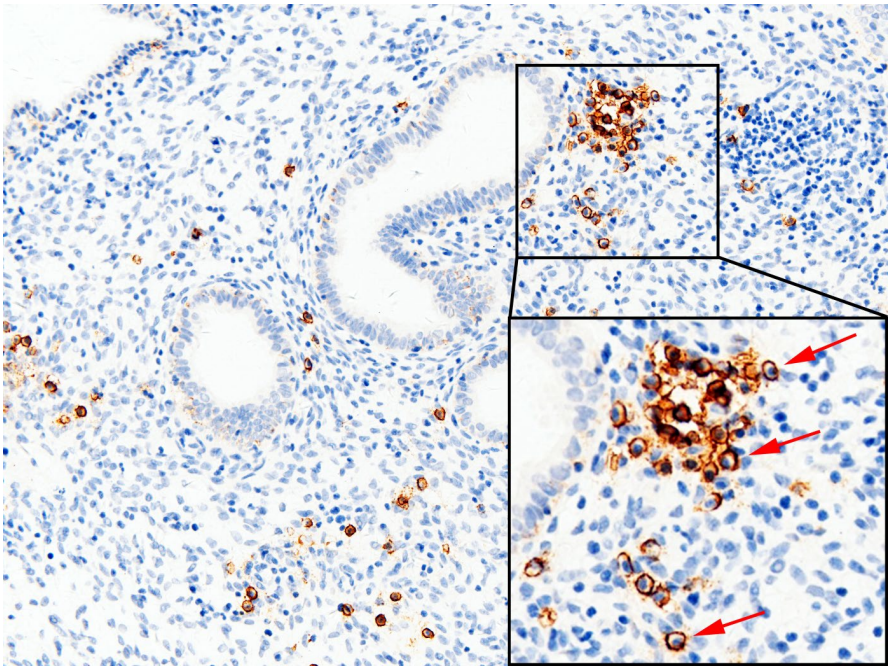

FIGURE 2 CD138$^+$ plasma cells in endometrial stroma. Plasma cells were positively stained with anti-CD138 (brown color), and nuclei were stained with DAB (blue). Magnification 400×, 0.375mm^2/fields

TABLE 1 Demographic and clinical characteristics of infertile patients

	a	b	c	d	e	f
Groups	**0/HPF**	**1/HPF**	**2/HPF**	**3/HPF**	**4/HPF**	**≥5/HPF**
Number	443	178	33	18	6	38
Age (Year)	31 (29, 34)	31 (28, 34)	32 (24,42)	32 (27, 40)	34 (29, 39)	33 (23, 43)
BMI (Kg/m^2)	20.70 (19.38, 22.68)	21.10 (19.52, 23.25)	21.50 (17.15, 27.99)	20.73 (18.07,31.11)	21.24 (18.49,24.03)	20.90 (16.80,29.38)
Duration of infertility	3.00 (1.50, 4.00)	3.00 (2.00,4.25)	3.00 (0.50, 18.00)	3.50 (0.50, 11.00)	3.50 (2.00, 9.00)	3.00 (1.00,10.00)
Type of infertility-No. (%)						
Primary	217 (48.98%)	101 (56.74%)	16 (48.48%)	8 (44.44%)	3 (50.00%)	13 (34.21%)
Secondary	226 (51.02%)	77 (43.26%)	17 (51.52%)	10 (55.56%)	3 (50.00%)	25 (65.79%)
Percentage of blastocyst transferred-No. (%)	359 (81.04%)	139 (78.09%)	27 (81.82%)	12 (66.67%)	3 (50.00%)	29 (79.32%)
No. of embryos transferred	1.00 (1.00, 2.00)	1.00 (1.00, 2.00)	1.00 (1.00, 3.00)	1.00 (1.00, 2.00)	2.00 (1.00, 2.00)	1.00 (1.00, 3.00)
Percentage of good-quality embryo transferred -No. (%)	388 (87.58%)	149 (83.71%)	30 (90.91%)	14 (77.78%)	5 (83.33%)	32 (84.21%)
Percentage of frozen embryo transferred-No. (%)	275 (62.08%)	101 (56.74%)	21 (63.64%)	8 (44.44%)	2 (33.33%)	24 (63.16%)

Abbreviations: BMI, Body Mass Index; HPF, high-power fields.

and live birth was detectable in group f compared with group a ($P = .06$ and $P = .05$, respectively). Also group d and e showed a tendentially, but not significantly reduced pregnancy success.

Based on these findings, we assumed that the expression of at least 5 CD138$^+$ cells in one HPF might be crucial for influencing pregnancy outcomes. Thus, all infertile patients of this study were divided into two groups: in one group, all patients had less than 5 CD138$^+$ cells/HPF in each HPF (CD138^{low}; n = 678). In the second group, patients had 5 or more than CD138$^+$ cells/HPF in at least 1 HPF (CD138^{high}; n = 38). The pregnancy outcome has been compared between both groups. As presented in Table 3, the data revealed that the β-hCG positive rate, clinical pregnancy rate, and live birth rate in the CD138^{high} group were significantly decreased ($P = .04$, $P = .01$, and $P = .04$, respectively).

TABLE 2 Association between pregnancy outcome and the number of CD138$^+$ cell/HPF in the stroma

	a	b	c	d	e	f
Groups	**0/HPF**	**1/HPF**	**2/HPF**	**3/HPF**	**4/HPF**	**≥5/HPF**
Number	443	178	33	18	6	38
HCG positive rate (%)	320/443 (72.33%)	128/178 (71.91%)	24/33 (72.73%)	17/18 (94.44%)	5/6 (83.33%)	22/38 (57.89%)
Clinical pregnancy rate (%)	283/443 (63.88%)	115/178 (64.61%)	20/33 (60.61%)	15/18 (83.33%)	5/6 (83.33%)	17/38 (44.74%)*
Early miscarriage rate (%)	36/278 (12.95%)	16/113 (14.16%)	1/20 (5.00%)	0/15 (0.00%)	0/5 (0.00%)	1/17 (5.88%)
Live birth rate (%)	221/381 (58.01%)	90/153 (58.82%)	19/31 (61/29%)	15/18 (83.33%)	3/4 (75.55%)	15/36 (41.67%)
Preterm birth rate (%)	23/221 (10.41%)	11/90 (12.22%)	3/19 (15.79%)	2/15 (13.33%)	1/3 (33.33%)	2/15 (13.33%)

Note: Villous chromosome testing was not performed in patients with early miscarriage, and early miscarriage could not be ruled out due to chromosomal abnormalities.

*P = .01, ≥5/HPF versus 0/HPF, Because of multiple comparisons, groups with an adjusted $P \leq .01$ were considered statistically significant.

3.3 | Correlation of ≥5 CD138$^+$ cells in at least 1 HPF with pregnancy outcome

To further demonstrate whether five or more CD138$^+$ plasma cells in at least one HPF were an independent factor affecting pregnancy outcome, a multivariate logistic regression analysis was performed. As presented in Table 4, the analysis revealed a negative correlation between the CD138^{high} group and pregnancy outcome. After adjusting for patient age, BMI, number of embryos transferred, and the characteristics of the embryo (percentage of transfer of frozen embryo, blastocysts, and good-quality embryo), the following parameters were significantly lower in the CD138^{high} than in the CD138^{low} group: β-hCG positive rate (aOR 1.95; 95% CI, 0.99-4.02; P = .05), clinical pregnancy rate (aOR, 2.50; 95% CI, 1.25-5.01; P = .01), and live birth rate (aOR, 2.32; 95% CI, 1.12-4.49; P = .02). However, the differences in early miscarriage rate (aOR, 0.19; 95% CI, 0.03-1.46; P = .11) and preterm birth rate (aOR, 1.04; 95% CI 0.21-5.02; P = .97) were not significant between the two groups. Overall, the findings revealed that ≥5 CD138$^+$ cells in at least 1 out of 30 analyzed HPF were an independent risk factor affecting pregnancy outcome.

Based on the above findings, the minimum number of plasma cells in stroma suggestive for a clinically relevant CE was considered to be 5 CD138$^+$ cells in at least 1 out of 30 selected HPF. Less than five CD138$^+$ plasma cells per HPF in each of 30 selected HPF indicated the absence of a clinically relevant CE. Based on this definition, the prevalence of CE in the infertile women in this study was 5.3% (38/716).

4 | DISCUSSION

In previous studies, CE has been characterized by the infiltration of plasma cells in the endometrium. However, thus far, unified diagnostic criteria for CE have not been precisely defined. This study has investigated the association between the number of CD138$^+$ cells in the endometrial stroma and pregnancy outcome to provide clinically relevant diagnostic criteria for CE. A novelty of this study is that not only the maximum but not the mean CD138$^+$ cell concentration in a tissue area has been evaluated. For this approach, the analyzed tissue section has been divided into 30 HPF, but only the CD138$^+$ cells with the highest concentration have been used for statistical evaluation.

Based on the highest density of CD138$^+$ cells in one out of 30 HPF, patients were divided into two groups (CD138^{low} with <5 plasma cells in each analyzed HPF, and CD138^{high} with ≥5 plasma cells in at least 1 out of 30 analyzed HPF). In the CD138^{high} group,

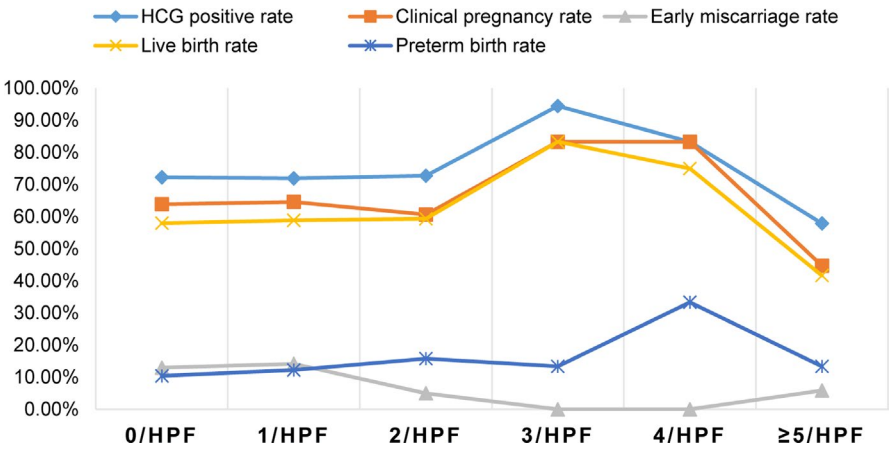

FIGURE 3 Pregnancy outcomes among patients with different number of CD138$^+$ cells/HPF. Based on the number of CD138$^+$ cells in one HPF, the patients were divided into six groups (0/HPF, 1/HPF, 2/HPF, 3/HPF, 4/HPF, and ≥5/HPF). The β-hCG positive rate, clinical pregnancy rate, early miscarriage rate, live birth rate, and preterm birth rate were determined

TABLE 3 Association of pregnancy outcome between <5/HPF and ≥5/HPF groups

Outcomes	<5/HPF	≥5/HPF	*P*-value
Number	678	38	
HCG positive rate (%)	494/678 (72.86%)	22/38 (57.89%)	$P = .04^*$
Clinical pregnancy rate (%)	438/678 (64.60%)	17/38 (44.74%)	$P = .01^*$
Early miscarriage rate (%)	53/431 (12.30%)	1/17 (5.88%)	$P = .42$
Live birth rate (%)	348/588 (59.18%)	15/36 (41.67%)	$P = .04^*$
Preterm birth rate (%)	40/348 (11.49%)	2/15 (13.33%)	$P = .827$

Note: The *P*-value of ≤0.05 was considered statistically significant.
*$P<.05$.

TABLE 4 Multinomial regression analysis for association of pregnancy outcomes and CE

Outcomes	Non-CE	CE	OR (95% CI)	aOR (95% CI)	*P*-value
HCG positive rate (%)	494/678 (72.86%)	22/38 (57.89%)	1.95 (1.00, 3.80)	1.99 (0.99, 4.02)	$P = .05^*$
Clinical pregnancy rate (%)	438/678 (64.60%)	17/38 (44.74%)	2.25 (1.17, 4.35)	2.50 (1.25, 5.01)	$P = .01^*$
Early miscarriage rate (%)	53/431 (12.30%)	1/17 (5.88%)	0.19 (0.03, 1.43)	0.19 (0.03, 1.46)	$P = .11$
Live birth rate (%)	348/588 (59.18%)	15/36 (41.67%)	2.03 (1.03, 4.02)	2.32 (1.12,4.79)	$P = .02^*$
Preterm birth rate (%)	40/348 (11.49%)	2/15 (13.33%)	0.84 (0.18, 3.88)	1.04 (0.21, 5.02)	$P = .97$

Note: The *P*-values were adjusted for patients' age, BMI, number of embryos transferred, and the embryo characteristics (percentages of transfer of frozen embryo, blastocysts, high-quality embryo). Non-CE: cases with less than five $CD138^+$ or no plasma cells per HPF in all 30 selected HPF ; CE: cases with five or more $CD138^+$ plasma cells in the endometrial stroma in at least 1 out of 30 selected HPF. The *P*-value of ≤.05 was considered statistically significant.
aOR, adjusted Odds Ratio, CI, Confidence Interval; OR, Odds Ratio.
*$P<.05$.

the β-hCG positive rate, clinical pregnancy rate, and live birth rate were significantly reduced. As illustrated in Figure 2, $CD138^+$ cells aggregated and formed local clusters, especially in the $CD138^{high}$ group. These clusters may form inflammatory lesions which affect the endometrial immune micro-environment[36] and endometrial receptivity, potentially leading to adverse pregnancy outcome.

Based on $CD138^+$ cell count evaluation in those local areas (HPF) with their highest density (≥5 plasma cells), the prevalence of clinically relevant CE in infertile women was 5.3% (38/716). In previous studies, the prevalence of CE in RM was 10.4% (45/433), and in RIF was 10.5% (29/275).[36] In our study, the prevalence of CE in infertile patients was lower than reported in most previous studies.[3,15-25] The reason may be attributed to differences in sample size and the cycle phase of endometrium biopsy collection.

In our study, all endometrium biopsies have been obtained during the luteal phase. Several reports had indicated a higher prevalence of CE when the endometrial specimen was collected in the proliferative phase than in the secretory phase,[37-40] suggesting that taking the biopsy in different stages of the menstrual cycle may be a confounding factor. In our own unpublished analyses, in the proliferative phase, we found a far higher number of infertile patients with ≥5 plasma cells in at least 1 out of 30 analyzed HPF (203 out of 403 patients equivalent to 50.37%), but this was not associated with pregnancy outcome. It is suggested that the prevalence of CE in the proliferative phase could be incorrect and the CE diagnostic criteria here established in the mid-luteal phase may not be suitable for the proliferative phase. The CE diagnostic criteria in the proliferative phase need to be further investigated.

In a previous study, we found significantly elevated proportions of uterine $CD68^+$macrophages, $CD83^+$mature dendritic cells, $CD8^+$T cells, and $Foxp3^+$Treg cells in CE patients. We have concluded that CE induces embryo implantation failure and live birth by disrupting the balance of the endometrial immune microenvironment.[36] It is worth noting that in the current study, CE did not influence the early miscarriage rate and preterm rate of infertile patients. This is inconsistent with previous reports. In this context, Edmonson et al reported that the frequency of chronic deciduitis was significantly higher in patients with preterm labor than in a control group (41% vs. 15%; $P = .02$),[41] The study also suggested that chronic deciduitis plays a crucial role in the etiology of preterm labor in some cases.[42] This difference may be attributed to discrepancies in the definition of chronic deciduitis and chronic endometritis among studies.

The present study has several limitations. First, it was a retrospective, and relevant clinical data of the patients contributing to pregnancy outcome may have been missing, which may influence the conclusions of the study. Second, the results represented derive from a single-center experience. Further multicenter clinical studies are warranted to confirm the validity and clinical applicability of the proposed criteria.

Third, the hysteroscopy report of the patients has not been analyzed. In the future, we will analyze the consistency of hysteroscopy results with the $CD138^+$ cell count-based diagnosis.

In summary, the present study has developed and proposed reliable, accurate, and effective diagnostic criteria for CE based on the number of endometrial $CD138^+$ cells in the mid-luteal phase. Furthermore, using a large sample size, the study also indicated that locally elevated $CD138^+$ cells in the mid-luteal phase were significantly associated with adverse pregnancy outcomes in infertile women. Collectively, the proposed method may help to standardize histopathologic diagnostic criteria for CE in clinical practice.

CONFLICT OF INTEREST

All other authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

YY.L. involved in substantial contributions to conception and design, acquisition of data and interpretation of data, and drafting the article; SR.X involved in substantial contributions to collected the endometrium, acquisition of data and analysis of data, revising it critically for important intellectual content. SY.Y performed substantial contributions to doing the IHC experiment and acquisition of data, revising it critically for important intellectual content. CY.H and ML.M involved in substantial contributions to acquisition of data and revising it critically for important intellectual content. SL.L and WR.C involved in substantial contributions to doing the IHC experiment and acquisition of data. RC.L and LH.D involved in substantial contributions to revising it critically for important intellectual content. LJ, D and YZ involved in substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. All authors read and approved the version to be published.

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How to cite this article: Li Y, Xu S, Yu S, et al. Diagnosis of chronic endometritis: How many CD138$^+$ cells/HPF in endometrial stroma affect pregnancy outcome of infertile women?. *Am J Reprod Immunol*. 2021;85:e13369. https://doi.org/10.1111/aji.13369