A comparison of the prevalence of chronic endometritis determined by the use of different diagnostic methods in women with and without reproductive failure

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Prevalence of chronic endometritis

A comparison of the prevalence of chronic endometritis determined by the use of different diagnostic methods in women with and without reproductive failure

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**Conflict of interest**

The authors declare no conflict of interest.

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**Capsule**

Based on new methods of plasma cell assessment, the prevalence of chronic endometritis in women with various categories of reproductive failure may have been over-estimated in earlier studies.
**ABSTRACT**

**Objective:** To compare the prevalence of chronic endometritis (CE) when different diagnostic methods are used.

**Design:** Prospective observational study.

**Setting:** University affiliated hospital.

**Patient(s):** Four groups of women were studied, including women with proven fertility (Fertile, n=40), unexplained recurrent miscarriage (RM, n=93), recurrent implantation failure (RIF, n=39) and infertile subjects undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer (Infertility, n=48).

**Intervention(s):** Endometrial biopsy was performed precisely seven days following LH surge (LH+7). Plasma cells were identified by traditional H&E staining and immunohistochemistry (IHC) for Syndecan-1 (CD138).

**Main Outcome Measure(s):** The prevalence of chronic endometritis.

**Result(s):** The use of CD138 epitope is more sensitive than H&E staining in identifying plasma cells. The use of plasma cell count per unit area had the lowest observer variability than that of cell count per 10 randomly chosen HPF or cell count per section. Using this method, the prevalence of CE in women with RM, RIF and Infertility were 10.8%, 7.7%, and 10.4%, respectively, not significantly higher than that of fertile subjects (5.0%).

**Conclusion(s):** Using what may be a new method of plasma cell assessment, it appears that the prevalence rates of CE reported in many earlier studies may have been over-estimated.

**Chinese Clinical Trials Registry Number:** ChiCTR-IOC-16007882
Key Words: chronic endometritis; plasma cell; prevalence; reference range; reproductive failure
INTRODUCTION

Chronic endometritis (CE) refers to local persistent inflammation of the endometrium. CE has been reported to be associated with various subgroups of reproductive failure, including infertility (1-3), recurrent miscarriage (4-8), or recurrent implantation failure (4, 9-11).

The presence of plasma cells in endometrial stroma has been accepted as the gold standard method to establish a diagnosis of CE (12). Nevertheless, the reported prevalence of CE in endometrial biopsy specimens varied considerably, ranging from 3 to 60% (Table 1). There are several possible explanations to account for the wide variation reported. Firstly, there are two different methods used to identify plasma cells. Traditionally, plasma cells are identified in H&E stained specimens. However, the identification of plasma cells in H&E sections requires experience coupled with diligent search, without which they can be easily missed. A more recently introduced method is immunohistochemistry (IHC) staining for Syndecan-1 (CD138), a proteoglycan found on the cell surface of plasma cells and keratinocytes. This has been found to improve the sensitivity and accuracy for identifying plasma cells, essential for the diagnosis of CE (13-15).

Secondly, various investigators have used different approaches to quantify CD138+ cell count (Table 1). In the first approach, the number of plasma cell per whole section was measured. In the second approach, the plasma cell count per a defined number of randomly chosen high power field (HPF, say 10) was measured. There are rationales behind each of these two approaches. Some investigators advocated scrutinizing the entire specimen as they believed that plasma cells are not normally present in the endometrium and the finding of one or more plasma cells is indicative of a diagnosis of CE (12, 16, 17). One shortcoming of such
an approach is that it does not take into account the size of the specimen. One would expect
that, other things being equal, the larger is the specimen size, the more likely it is to find
plasma cells, and vice versa. Consequently, other authors introduced the concept of plasma
cell density to correct for the size of the specimen examined; they advocated examining 10 or
more chosen HPFs and expressed the number of plasma cell detected per HPF or per 10 HPF,
as each HPF is equivalent to a defined area (4, 6, 10, 11, 15, 18, 19). To avoid bias in
selecting the HPFs to be examined and to improve objectivity, it is desirable to have
randomly chosen fields. However, the potential disadvantage of such an approach is that
plasma cells are usually present in low numbers and so the inclusion of only 10 selected
HPFs may not be sufficient to produce a consistently reproducible result. We postulate that a
new method of plasma cell assessment which combines the positive attributes of the two
above-mentioned methods would be to count all CD138+ cells in the entire section, then
measure the area of the examined tissue section and express the result as plasma cell count
per unit area. In this way, it would overcome the problem of local fluctuation of plasma cell
count as well as correcting for the variation in results due to sample size difference.

There is also no consensus on the diagnostic criteria used to define what constitutes CE. At
least seven criteria have been reported in the literature, which included ≥1 plasma cell/section
(20), ≥1 plasma cell/HPF (10), ≥1 plasma cell/10 HPF (3), ≥5 plasma cells /10 HPF (4), ≥5
plasma cells/20 HPF (22), and the presence of 1-5 plasma cells/HPF or discrete clusters <20
plasma cells (7), ≥0.25 endometrial stromal plasmacyte density index (ESPDI, the sum of the
stromal CD138+ cell counts divided by the number of the HPFs evaluated) (11) (see Table 1).
The proposed criteria all appeared rather arbitrarily chosen, not based on reference ranges
derived from normal fertile populations.

In this study, our aim was to establish a reference range of plasma cell count in the
endometrium of fertile subjects by using two different methods of identification and three different methods of quantification, as discussed above, followed by a comparison of the performance of these methods. The prevalence rates of CE so derived among women with reproductive failure was then determined, using this methodology, with a view to determining the optimal strategy to identify and quantify plasma cell and to diagnose CE.

MATERIALS AND METHODS

Participants

Subjects were recruited from women attending the Department of Obstetrics and Gynecology, Prince of Wales Hospital, The Chinese University of Hong Kong. Women were recruited from four groups: (1) fertile control group: healthy women with at least one live birth within 2 years (n=40); (2) unexplained RM group: women with recurrent miscarriage, which was defined as the loss of three or more consecutive pregnancies before 24 weeks gestation (23) (n=93); (3) RIF group: women with recurrent implantation failure which was defined as failure to achieve a clinical pregnancy after transfer of at least four good quality embryos in three or more transfer cycles in women under the age of 40 years (24) (n=39); and (4) infertile group: women with infertility undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer using non donor oocytes (n=48).

The inclusion criteria were women who were between 20 to 40 years of age with regular menstrual cycle (25-35 days), normal pelvic ultrasonography, and had not used any antibiotics, oestrogen or progestogen hormonal therapy, steroid treatment or intrauterine contraceptive device within 2 months of recruitment. The exclusion criteria included the presence of hydrosalpinx, structural uterine abnormalities, parental chromosomal abnormalities and significant medical conditions such as systemic lupus erythematosus.
All subjects in this study had daily urine dipstick test from day 9 of the menstrual cycle onwards to identify the LH surge (ovulation), which was used to precisely time the endometrial biopsies to seven days after the LH surge (day LH+7). All biopsies were obtained using a Pipelle sampler (Prodimed, France) or Pipet Curettage (Cooper Surgical, USA). The specimens were immediately placed into 10% neutral buffered formalin for overnight fixation at room temperature and then embedded into paraffin wax.

**Processing of Specimen**

The paraffin-embedded human endometrial tissues were cut into sections (4 μm) and then dewaxed in xylene and then rehydrated through descending ethanol to phosphate-buffered saline.

*Haematoxylin-Eosin (H&E) staining.* Paraffin-embedded human endometrial tissue sections were examined with routine H&E staining.

*Immunohistochemistry (IHC) staining.* Slides were pre-treated with microwave heating for 20 minutes in sodium citrate buffer for antigen retrieval, and then quenched with 0.3%v/v hydrogen peroxidase in methanol to block endogenous peroxidase activity. Then the sections were blocked with rabbit serum to prevent non-specific binding and then incubated with a 1:50 dilution of mouse monoclonal antibody against human Syndecan-1 (clone B-A38; Cell Marque, Rocklin, CA, USA) overnight at 4°C. After incubation, the sections were washed in phosphate-buffered saline Tween 20 and incubated with secondary rabbit anti-mouse horseradish peroxidase (HRP) labelled antibody (1:100, ab97046, Abcam, Cambridge, MA, USA) for one hour, followed by colour development with 3, 3-diaminobenzidine (DAB; DAKO, CA, USA), counterstained with haematoxylin, dehydrated with ethanol, cleared in
Image analysis was performed by one operator who firstly scanned the slides at lower magnification, and then captured images (×400) of all the fields of CD138+ plasma cells using the Leica DM6000B system (Leica Microsystems Ltd., Wetzlar, Germany). Then the whole section of each sample was tile scanned under ×50 magnifications by the same system, which was able to merge separate images into one image covering all the tissue. Cell counts and section area were analysed using Image J (Version 1.51a, Wayne Rasband, NIH, USA). The total number of plasma cells was determined by counting immune-positive cells in the entire specimen. Cells were considered to be likely CD138+ plasma cells, if they exhibited unambiguous complete brown staining with intact cell membrane, a clearly defined nucleus typical of a plasma cell, and occurring singly or as small clusters of cells, excluding background stroma, glands and other confounders. The identification and counting of the CD138+ cells was performed manually under the microscope, whereas the measurement of the specimen area was made on images captured in the computer by Image J software entirely.

Determination of Observer Variability

The intra-observer variability of CD138+ cell count was determined by asking a single observer to repeat the measurement of 20 randomly chosen specimens on two separate occasions, without knowledge of the results of the first count. The observer utilised three different quantification methods to do the measurement, namely method I: CD138+ cell count per 10 randomly chosen HPF; method II: CD138+ cell count per whole section; method III: CD138+ cell count per unit area (cell density). For the third method, Image J software was used to measure the area of the tissue section.
The inter-observer variability was determined by asking two observers (Y.L. and X.C.) to perform the measurement of CD138+ cell count on the same set of 20 randomly chosen specimens, independently of each other, also using the three different quantification methods as in the case of intra-observer variability study.

Reference Range

In this study, the reference range of plasma cell count or density was derived from the 40 fertile control subjects. Values below the 95\textsuperscript{th} percentile were considered as normal, whereas values above the 95\textsuperscript{th} percentile were considered as abnormal and indicative of a diagnosis of CE.

Definition of CE

Chronic endometritis was defined as the presence of CD138+ plasma cell count or density above the established reference ranges (95\textsuperscript{th} percentile), whatever the quantification method was used.

Ethical Considerations

This study was approved by the local hospital ethics committee (CREC Ref. No.: 2015.477). Written informed consents were obtained from all participants.

Statistical Analysis

We have previously analysed the distribution of our data and confirm that results in the control population was not normally distributed which was the reason why we adopted non-parametric method to analyse the data and used 95\textsuperscript{th} centile as the cut-off instead of 2SD above the mean (parametric method) as the cut-off. The maternal age and body mass index
(BMI) of the women in the four groups were compared by ANOVA analysis. Intra- and inter-class correlation coefficients (intra- and inter-CC) with 95% confidence interval (95% CI) were used to assess the intra- and inter-observer agreement in the calculation of plasma cell count, area of tissue examined and plasma cell density (intra- and inter- CC values <0.40 were considered as poor agreement, between 0.40 and 0.75 as moderate, and >0.75 as excellent agreement). Chi-Square test was used to compare the prevalence of CE between subgroups. Statistical analysis was performed by using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) and P value with <0.05 was considered as a statistical significance.

RESULTS

From December 2014 to June 2017, 229 subjects underwent endometrial biopsy. Nine subjects were excluded because of insufficient tissue obtained. In total, 220 subjects were included in the study.

Demographics

The demographic details of the subjects are summarized in Supplementary Table 1. The mean age of all the subjects was 34.4 (range from 21 to 40) years. The mean age in reproductive failure group (35.4 ± 3.1 years) was significantly (P<0.01) higher than that of control group (29.6 ± 3.4 years), whilst there was no significant difference between RM, RIF and Infertility subgroups. There was also no significant difference in body mass index between groups.

Identification of Plasma Cell

No classical plasma cells were identified in any of the routing H&E stained sections (n=220) examined in this study while the use of CD138 IHC staining identified presence of one or
more plasma cells in 95/220 (43.2%) of the specimens examined (Figure 1). In most cases, the distribution of plasma cells within the endometrium was not uniform, being localized focally or widely dispersed in the stroma.

**Intra-observer Variability of Quantification Methods**

The results of the intra-observer variability of the three different quantification methods of measurement, namely method I: CD138+ cell count per 10 randomly chosen HPF; method II: CD138+ cell count per whole section; method III: CD138+ cell count per unit area (cell density) are compared in Supplementary Table 2. The intra-class correlation coefficient (intra-CC) value of cell count per 10 randomly chosen HPF was 0.46, which was considered as moderate. The intra-CC of cell count per whole section and cell count per unit area of whole section were 0.90 and 0.84, respectively, both considered as excellent.

**Inter-observer Variability of Quantification Methods**

The results of the inter-observer variability of the three different methods of measurement are compared in Supplementary Table 3. The intra-CC of plasma cell count per 10 randomly chosen HPF was 0.39, which was considered as poor. The intra-CC of cell count per whole section and cell count per unit area of whole section were 0.88 and 0.83, respectively, both considered as excellent.

**Reference Range**

In establishing the reference range of the plasma cell count or density, the specimens from fertile control subjects were examined and the 95th percentile of the results was used to define the upper limit of the reference range. The reference range for three different methods of
quantification were: (I) 1.95 CD138+ cells per 10 randomly chosen HPF; (II) 2.95 CD138+ cells per section; and (III) 5.15 CD138+ cells per 0.1 mm² (Table 2).

254 **Prevalence of CE**

255 The prevalence of CE in the various subgroups in this study as determined by the three different methods of CD138+ cell quantification, in conjunction with two different diagnostic criteria selected (one based on a previously literature report and the other based on reference range derived from fertile women) was analysed and compared in Table 2. Quantification using method I and method II consistently produced higher prevalence rates than quantification using method III. The application of previously published criteria consistently produced higher prevalence rates than those produced by the application of criteria based on reference range derived from fertile population. The prevalence of CE in women with reproductive failure as determined by quantification methods I or II, regardless of the diagnostic criteria used, was significantly higher than fertile subjects in three out of the four chosen criteria used (Table 2). However, the prevalence of CE in women with reproductive failure and its subgroups, determined by using quantification method III and diagnostic criteria based on established reference range, was not significantly (p>0.05) higher than that of fertile subjects.

269 **Confounding Variable**

270 The possible impact of age as a confounding on the results of the expression of CD138 was examined with the use of regression analysis. There was no significant association between CD138+ cell density and age.

**DISCUSSION**
In this prospective observational study, we have used different methods to identify and quantify plasma cell count and apply different criteria to diagnose CE; using our proposed new methods of plasma cell assessment, we found that the prevalence rates of CE reported in earlier studies of women with reproductive failure may have been over-estimated.

**Identification of Plasma Cells**

The identification of plasma cells in endometrial biopsy specimen continues to be considered as the gold standard method for the diagnosis of CE (12, 13, 25). Typical plasma cells have a large cell body, high nuclei/cytoplasm ratio, basophilic cytoplasm, and nuclei with heterochromatin in a unique arrangement called “spoke-wheel” or “clock-face” pattern (13, 17). However, plasma cells may from time to time be missed in routine histological examination. The results from this study agree with earlier reports (1, 13-15) on the usefulness of CD138 immunostaining in the identification of plasma cells, which has been shown to be a more sensitive and accurate method to identify plasma cells compared with the conventional H&E staining method (13-15). In accordance with previous publications, we could not find any classic plasma cells in any of the H&E stained specimens, whilst the use of CD138 staining led to the detection of one or more plasma cells in 46.1% (83/180) of samples from women with reproductive failure, and 95/220 (43.2%) for all the subjects in this study.

**Quantification of Plasma Cell**

In this study, we have based the diagnosis of CE on the stromal plasma cell count only; there are a number of morphological features which have been reported to be associated with chronic endometritis, namely superficial stroma edema, stromal inflammatory infiltrate, increased stromal density, focal stromal haemorrhage and spindling of stroma, most notably
in the upper half of the mucosa (12). Greenwood & Morgan (12) argued for the inclusion of these additional morphologic features in the definition of chronic endometritis, which was supported by Bayer-Garner and Korourian (13), and Cicinelli et al. (32), but some investigators based the diagnosis on plasma cell count only (19, 27). We have not included the additional morphological features proposed by Greenwood & Morgan in our analysis partly because there is as yet no consensus on the diagnostic value of these features nor are they easily quantifiable.

It should be noted that the proposed “plasma cell density” measurement in this study referred to plasma cell count per unit area, calculated from the entire area of the specimen, consisting of all fields whether complete or not, which is different from the endometrial stromal plasmacyte density index (ESPDI) which was calculated as the sum of the stromal CD138+ cell counts divided by the number of the high power fields evaluated (11), which is in essence plasma count per selected number of complete HPFs.

**Observer Variability**

Observer variability is a measure of how reproducible the results are. In this study we directly compare the observer variability of three different quantification methods and found that the coefficient of variation of results obtained from the cell count per HPF method was considerably higher than that of the cell count per section method and the cell density method. It is likely that the source of variation of the cell count per HPF method comes from the random nature of selection of the HPF, primarily because the (plasma) cell count is low. Missing one or two positively stained cells could make a significant difference to the results. The other two methods, both had intra-observer and inter-observer coefficient of correlation in the excellent category. The intra and inter-observer variability for cell density appeared slighter higher than that of cell count per section, which is to be expected, as the
measurement of cell density requires an additional measurement of area and so introduces an
additional source of variation.

**Reference Range**

In this study we have chosen to establish the normal ranges derived from a fertile population
and define results above the 95th centile as abnormal (basis for the diagnosis of chronic endometritis). This approach is commonly used for establishing clinical laboratory values.

The sample size of 40 in the control group is somewhat small and may well be a limitation of our study. On the other hand, a particularly strength of our study is the special efforts made to ensure the homogeneity of the specimens (including control group) by collecting them precisely on day LH+7, which would have reduced the variance of results.

Our finding agrees with that of Achilles and co-workers (26) who found plasma cells were commonly present in the endometrium of asymptomatic, fertile and healthy women and their presence alone, in small numbers, may not signify upper genital tract inflammation. Together, these studies suggest that the hitherto held view that the finding of one or more plasma cell in the endometrium is abnormal and diagnostic of CE may lead to over diagnosis of the condition. Some previous studies used hysterectomy specimen as control (21, 22) which called into question their validity as many of these subjects could well have uterine pathology.

**Prevalence of CE in Women with Reproductive Failure**

Using the reference range established and based on the CD138+ cell density quantification method, the prevalence of CE in each of the three subgroups of women with reproductive failure (10.8% for women with recurrent miscarriage, 7.7% in women with RIF and 10.4% in women with infertility) was not significantly different from that of the fertile group. In addition, the prevalence of CE among the entire group of reproductive failure (18/180, 10.0%)
was also not significantly different from the fertile group. This finding agrees with the earlier reports of Kasius et al. (27, 28) who observed in a randomized controlled trial that the prevalence of CE in a population of symptomatic infertile patients was low (2.8%) and that the contribution of CE to reproductive failure could have been over-estimated in earlier studies. Further analysis of data in Table 2 showed that the use of cell count per randomly chosen HPF quantification method or the cell count per section quantification methods both resulted in over-estimation of prevalence rates. Moreover, the application of arbitrarily chosen diagnostic criteria used in the literature also led to over-estimation of the prevalence rates. Our observation provided an explanation for the rather high prevalence rates (up to 60%) of CE reported in earlier studies (Table 1).

Precise Timing of Specimen

A notable feature of our study was that we obtained all our specimens on a precise chronological date, that is exactly 7 days after the LH surge. Whilst is considered acceptable to have specimens collected on LH+7 +/- day, we have preferred to include only samples on a precise chronological date to reduce possible variance in results due to cyclic changes in the cell count. Several studies suggested that the prevalence of CE appeared to be higher when the biopsy was obtained in the proliferative phase than the secretory phase (29-31). It remains possible that timing of the biopsy in different stages of the cycle could be a confounding variable.

Other Diagnostic Method

In addition to the identification of plasma cell in endometrial biopsy specimen, hysteroscopy has been proposed as an alternative method of diagnosis for CE (32-34). Although hysteroscopy has an accuracy rate of 92.7% in the diagnosis of CE (35), histological
identification of plasma cell remains the gold standard of diagnosis of CE (12, 16, 17).

Although it is believed that CE is due to an underlying infection, routine microbial culture of endometrial secretion in women with CE is often negative and so precludes its use in clinical diagnosis (36). However, it is now possible to examine the entire microflora present in the endometrium by genomic testing (microbiome study). A recent study by Moreno and co-workers (37) demonstrated the existence of an endometrial microbiota that is highly stable around the time of implantation and changes in microbiota profile appeared to be associated with adverse reproductive outcomes. This finding adds a novel microbiological dimension to our understanding of CE. It would be of interest to establish what specific changes in microbiome induce the emergence of plasma cells in the endometrium.

Consensus and Clinical Significance

A review of the literature on the prevalence of CE, revealed that many investigators used different methods of quantification and applied different diagnostic criteria (see Table 1), often without justification. There was a lack of consensus in the diagnostic approach to define CE. In order to make progress in the field and to provide effective and appropriate treatment to women with reproductive failure a consensus on the quantification method and diagnostic criteria is essential. We hope that the findings in this study serve to highlight the importance of such a development. Whilst we have put forward data and argument to support that “plasma cell density” measurement is a more reliable method of plasma cell assessment for diagnosis of CE, ultimately, the proof of such a concept requires clinical studies to confirm that the measurement is of useful prognostic value and leads to effective treatment.

To conclude, we have found that plasma cells may be present in small number in the endometrium of fertile subjects, the quantification of plasma cell density improves the
accuracy of the diagnosis of CE and the prevalence of CE in women with reproductive failure was only 10%, lower than previously reported.
Acknowledgements

The authors would like to thank Research Nurses Lai-Yiu Tse and Wing-Ching Cheung for recruiting patients.
References:


19. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. Fertility and sterility 2014;101:1026-30.


Figure Legends

Figure 1. Expression of plasma cells using (A) H&E staining, and (B) immunohistochemistry staining for syndecan-1(CD138) in the same field of endometrial tissue from the same woman. Plasma cells identified by black solid arrow. GE: glandular epithelium; SC: stromal cell; LE: luminal epithelium. Magnification: ×400; Scale bar: 50 µm.

Figure 2. Scatter plot of CD138+ cell count per unit area (cell density) in four subgroups of women (Fertile: fertile control women; RM: unexplained recurrent miscarriage; RIF: recurrent implantation failure; Infertility: infertile women undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer). Reference range of CD138+ cell count per unit area is up to 5.15 CD138+ cells/0.1 mm² showing on the Y axis. There was no statistically significant difference between subgroups by chi-square test.
Table 1 Prevalence of chronic endometritis reported in the literature among three groups of women (I) infertility, (II) recurrent miscarriage, and (III) recurrent implantation failure, in relation to inclusion criteria, diagnostic criteria and timing of endometrial biopsy.

<table>
<thead>
<tr>
<th>References</th>
<th>Inclusion criteria</th>
<th>Diagnostic criteria (plasma cell count)</th>
<th>Timing of endometrial biopsy</th>
<th>Prevalence (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Infertility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cicinelli et al., 2005</td>
<td>Unexplained infertility</td>
<td>≥1/section</td>
<td>Follicular phase</td>
<td>30% (45/150)</td>
</tr>
<tr>
<td>Kitaya and Yasuo, 2010</td>
<td>Unexplained infertility</td>
<td>≥1/10 HPF</td>
<td>LH+6~8</td>
<td>29% (22/76)</td>
</tr>
<tr>
<td>Kasius et al., 2011</td>
<td>Infertility</td>
<td>≥1/section</td>
<td>Follicular phase</td>
<td>3% (17/606)</td>
</tr>
<tr>
<td>Kitaya et al., 2012</td>
<td>Infertility</td>
<td>≥5/20 HPF</td>
<td>Follicular phase</td>
<td>44% (23/52)</td>
</tr>
<tr>
<td><strong>II. Recurrent miscarriage, RM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kitaya, 2011</td>
<td>≥3 miscarriages</td>
<td>≥1/10 HPF</td>
<td>LH+6~8</td>
<td>9% (5/54)</td>
</tr>
<tr>
<td>Zolghadri et al., 2011</td>
<td>≥3 miscarriages</td>
<td>≥1/section</td>
<td>Follicular phase</td>
<td>43% (61/142)</td>
</tr>
<tr>
<td>Cicinelli et al., 2014</td>
<td>≥3 miscarriages</td>
<td>≥1/section</td>
<td>Follicular phase</td>
<td>53% (190/360)</td>
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<tr>
<td>McQueen et al., 2015</td>
<td>≥2 miscarriages</td>
<td>1-5/HPF or discrete clusters &lt;20</td>
<td>Not mentioned</td>
<td>56% (60/107)</td>
</tr>
<tr>
<td>Bouet et al., 2016</td>
<td>≥2 unexplained miscarriages</td>
<td>≥5/10 HPF</td>
<td>Follicular phase</td>
<td>27% (14/51)</td>
</tr>
<tr>
<td><strong>III. Recurrent implantation failure, RIF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johnston-MacAnanny et al., 2010</td>
<td>≥2 failed ET cycles or &gt;10 failed embryos transfer</td>
<td>≥1/HPF</td>
<td>Not mentioned</td>
<td>30% (10/33)</td>
</tr>
<tr>
<td>Kitaya et al., 2017</td>
<td>≥3 failed embryos transfer</td>
<td>≥0.25 ESPDI</td>
<td>Follicular phase</td>
<td>34% (142/421)</td>
</tr>
<tr>
<td>Cicinelli et al., 2015</td>
<td>≥3 failed ET cycles</td>
<td>≥1/section</td>
<td>Follicular phase</td>
<td>57% (61/106)</td>
</tr>
<tr>
<td>Bouet et al., 2016</td>
<td>≥3 failed embryos transfer</td>
<td>≥5/10 HPF</td>
<td>Follicular phase</td>
<td>14% (6/43)</td>
</tr>
</tbody>
</table>

Note: HPF = high power field, ×400 magnification. LH+6~8 = six to eight days after luteal hormonal surge. ET = embryo transfer. ESPDI = The endometrial stromal plasmacyte density index.
### Table 2
The prevalence of chronic endometritis using three different quantification methods of CD138+ cell (I) CD138+ cell count per 10 randomly chosen HPF; (II) CD138+ cell count per whole section; and (III) CD138+ cell count per unit area, in conjunction with selected diagnostic criteria.

<table>
<thead>
<tr>
<th>Population</th>
<th>(I) CD138+ cell count per 10 randomly chosen HPF</th>
<th>(II) CD138+ cell count per whole section</th>
<th>(III) CD138+ cell count per unit area</th>
<th>P value&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(I-a) CE+ if ≥1 cell/10HPF (I-b) CE+ if ≥1.95 cells/10HPF</td>
<td>(II-a) CE+ if ≥1 cell/section (II-b) CE+ if ≥2.95 cells/section</td>
<td>(III-b) CE+ if ≥5.15 cells/0.1 mm&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fertile (n=40)</td>
<td>17.5%</td>
<td>30.0%</td>
<td>5%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Reproductive Failure (n=180)</td>
<td>28.9%</td>
<td>46.1%</td>
<td>22.2%</td>
<td>10.0%</td>
</tr>
<tr>
<td>RM (n=93)</td>
<td>19.4%</td>
<td>38.7%</td>
<td>17.2%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RIF (n=39)</td>
<td>23.1%</td>
<td>51.3%</td>
<td>20.5%</td>
<td>7.7%</td>
</tr>
<tr>
<td>Infertility (n=48)</td>
<td>37.5%</td>
<td>56.3%</td>
<td>33.3%</td>
<td>10.4%</td>
</tr>
</tbody>
</table>

*Note:* RM = recurrent miscarriage. RIF = recurrent implantation failure.

Chi-Square test was used to compare the difference between subgroups.

a, an arbitrary criteria used in literature.
b, criteria based on reference range (95<sup>th</sup> percentile) derived from fertile subjects.

Three types of comparison are made:

- Using the same quantification method, the prevalence rate based on two different diagnostic criteria, a and b, are compared (I-a vs I-b; II-a vs II-b); and
- Using the same diagnostic criteria based on reference range derived from fertile subjects, i.e. b, the prevalence rate determined by three different quantification methods (I-b, II-b, III-b) are compared; and
- Using the same quantification method and diagnostic criteria, the prevalence rate between fertile subjects and women with reproductive failure and subgroups are compared.

<sup>a</sup> criteria based on reference range (95<sup>th</sup> percentile) derived from fertile subjects.

<sup>b</sup> criteria based on reference range (95<sup>th</sup> percentile) derived from fertile subjects.
**Supplementary Table 1** A comparison of demographics between different groups of subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fertile (n=40)</th>
<th>RM (n=93)</th>
<th>RIF (n=39)</th>
<th>Infertility (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>29.6 ± 3.4</td>
<td>35.0 ± 3.4</td>
<td>36.1 ± 2.7</td>
<td>35.7 ± 2.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.6 ± 2.0</td>
<td>22.1 ± 2.9</td>
<td>21.1 ± 2.4</td>
<td>21.7 ± 3.4</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1 - 3)</td>
<td>3 (3 - 7)</td>
<td>0 (0 - 3)</td>
<td>1 (0 - 6)</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (1 - 3)</td>
<td>0 (0 - 1)</td>
<td>0 (0 - 1)</td>
<td>0 (0 - 2)</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>0 (0 - 1)</td>
<td>3 (3 - 6)</td>
<td>0 (0 - 1)</td>
<td>0 (0 - 2)</td>
</tr>
</tbody>
</table>

*Note: RM = Recurrent miscarriage. RIF = Recurrent implantation failure. BMI = Body mass index. ANOVA analysis was used to compare the difference between subgroups.*

*mean ± SD

°median (range)

°P<0.01: Fertile vs RM; Fertile vs RIF; Fertile vs Infertility.
**Supplementary Table 2** A comparison of the intra-observer variability (intraclass correlation coefficient, Observer A) among three quantification methods of CD138+ cell (I) CD138+ cell count per 10 randomly chosen HPF; (II) CD138+ cell count per whole section; (III) CD138+ cell count per unit area.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>(I) CD138+ cell count per 10 randomly chosen HPF</th>
<th>(II) CD138+ cell count per whole section</th>
<th>(III) CD138+ cell count per unit area</th>
<th>Specimen area (mm²)</th>
<th>1st Observation</th>
<th>2nd Observation</th>
<th>1st Observation</th>
<th>2nd Observation</th>
</tr>
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<tbody>
<tr>
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<td>3</td>
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</tbody>
</table>

Intra-CC: 0.46 (95% CI: 0.06 to 0.74) (0.90: 0.76 to 0.96) (0.97: 0.92 to 0.99) (0.84: 0.64 to 0.93)

**Note:** HPF = high power field, \( \times 400 \) magnification. Intra-CC = Intraclass Correlation Coefficient. 95% CI = 95% Confidence Interval.
Supplementary Table 3 A comparison of the inter-observer variability (intraclass correlation coefficient, Observer A and B) among three quantification methods of CD138+ cell (I) CD138+ cell count per 10 randomly chosen HPF; (II) CD138+ cell count per whole section; (III) CD138+ cell count per unit area.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>(I) CD138+ cell count per 10 randomly chosen HPF</th>
<th>(II) CD138+ cell count per whole section</th>
<th>Specimen area (mm²)</th>
<th>(III) CD138+ cell count per unit area</th>
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<tbody>
<tr>
<td></td>
<td>Observer A</td>
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</tbody>
</table>

Intra-CC 0.39 0.88 0.96 0.83
(95% CI) (-0.06 to 0.70) (0.72 to 0.95) (0.90 to 0.98) (0.62 to 0.93)

Note: HPF = high power field, ×400 magnification; Intra-CC = Intraclass Correlation Coefficient. 95% CI = 95% Confidence Interval.