Research Article

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Why Do Euploid Embryos Fail to Implant? The Role of CD138 and Chronic Endometritis

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Abstract

The objective of this prospective cohort study was to investigate the relationship between chronic endometritis (CE) and its treatment on pregnancy outcomes in patients undergoing frozen embryo transfers (FETs) from in vitro fertilization (IVF) with preimplantation genetic testing (PGT) who had previously failed ≥ 1 euploid FET. The diagnosis of CE was made using CD138 as a marker. This study occurred at a single, high volume academic-based fertility center. 305 patients were included with a history of ≥ 1 failed euploid transfers. 59 patients underwent endometrial biopsies (EMB) prior to subsequent FET (Tested), 246 patients did not undergo EMB prior to subsequent transfer (Untested). Patients in the Tested group had an EMB prior to subsequent FET, whereas the patients in the Untested group did not have an EMB prior to next FET. Patients tested and found positive for CE were treated with antibiotics and underwent test-of-cure (TOC) EMBs prior to next FET to assess for adequate treatment. Patients without CE on EMB proceeded to next FET without antibiotic treatment. Main outcome measures included positive Beta-hCG (β hCG) on cycle day 28, implantation rate (IR), and subsequent ongoing pregnancy/live birth rates (OPR/LBR). Our results showed that 51% of patients (30/59) were diagnosed with CE and treated with antibiotics (Tested, CE treated); negative TOC biopsies were obtained prior to next FET. 49% of patients (29/59) tested negative for CE (Tested, No CE) and were not treated prior to next FET. Tested patients without CE (CE treated and No CE) had significantly higher incidences of positive β hCG, IR and OPR/LBR than patients who were not tested. Those who were biopsied and treated for CE had significantly higher OPR/LBR compared to patients who were biopsied and negative for CE (80% vs. 55%). We conclude that CE is common, with a prevalence of 51% in patients who have failed euploid FETs. Testing for CE may provide a benefit to patients, assuring higher incidences for positive β hCG, implantation, and subsequent ongoing/live births. CE is a treatable condition warranting investigation in patients with poor pregnancy outcomes.

Keywords: Chronic endometritis, CD138, Syndecan-1, Failed euploid embryo transfer

Introduction

Why do patients fail to become pregnant after the transfer of chromosomally-normal embryos? Certainly undetected genetic variation in the embryo is one component; however, the endometrium represents a major contributing factor and an area of potential improvement for IVF pregnancy outcomes.

Chronic endometritis (CE), one such consideration, is a pathologic inflammation of the endometrium marked by the presence of plasma cells. These cells can be detected by traditional hematoxylin and eosin (H&E) staining and with the assistance of CD138 (or syndecan-1) immunohistochemistry staining of endometrial biopsy (EMB) samples. While the prevalence has been estimated between 0.8 to 19% in the general population, its true

prevalence is poorly understood as endometritis is not routinely screened for in all IVF or gynecologic settings, and most patients with the diagnosis are asymptomatic. However, it appears that certain higher risk populations like recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) groups may have a higher prevalence of CE, upwards of 42.9% and 57%, respectively [1-8].

As testing and subsequently treating CE with antibiotics are relatively simple interventions, the objective of this study was to evaluate the potential impact of these measures on subsequent IVF pregnancy outcomes in patients who had previously failed single, thawed, euploid embryo transfers (STEET).

Materials and Methods

Patient selection

Institutional Review Board approval (IRB #16-00154) was previously obtained by our single, high-volume

academic IVF center. Patients undergoing IVF with PGT who had ≥ 1 single euploid frozen embryo transfer (FET) from 2012-2017 were queried using Microsoft Access. As testing for CE became standard of care at our institution in 2016, patients in the Tested group were derived from failed single euploid FETs between 2016-2017; thus, all patients failing a euploid transfer were offered testing for CE after institutional practices had changed in 2016. Untested patients who failed ≥ 1 single euploid FET and proceeded to the next single euploid FET without undergoing EMB were used as the control group and were obtained from cycles between 2012-2017, and were mainly comprised of patients at a time when EMBs were not part of the standard evaluation for failed euploid transfers at our institution (Figure 1). Of note, patients who proceeded to FET without EMB after 2016 had declined this intervention at their personal preference.

Figure 1: Flow sheet of patients meeting inclusion criteria for study.

Patients in all categories were included if they had returned for subsequent FET following a failed cycle. Patients were excluded from Untested if they had undergone any uterine procedure (e.g. dilation and curettage, polypectomy) < 2 months before the subsequent transfer. Gestational carrier cycles were also excluded.

Endometrial biopsy

Endometrial samples were obtained using a standard pipelle (CooperSurgical, Inc.) in accordance with the FDA and manufacturer’s instructions for use, that is, to obtain an EMB in blind fashion. Samples were processed by the affiliated hospital’s Clinical Pathology Department or Enzo Clinical Labs, Inc. (Farmingdale, NY) as dictated by insurance coverage and physician preference. Each endometrial sample was preserved in 10% formalin; each sample was first analyzed to rule out incidental pathology such as malignancy, as is standard of care. If an unexpected outcome, such as hyperplasia or malignancy, was diagnosed, patients were notified and excluded from the study. Samples were further evaluated for the presence of plasma cells with standard H&E staining and immunohistochemical staining with CD138 (syndecan-1). Generally, samples were considered positive for CE if there was > 1 plasma cell present per high powered field. Timing of first and TOC endometrial biopsies occurred during luteal phase if possible and as evidenced by endometrial dating by the pathologist. This was not always possible due to expedited timing to next embryo transfer.

Antibiotic treatment

Patients who tested positive for CE were treated with antibiotic regimens at the discretion of the physician and included Fluroquinolone/Metronidazole for 14-21 days, Amoxicillin-Clavulanic acid/Doxycycline for 14-21 days. TOC biopsies were obtained to ensure adequacy of treatment prior to proceeding with next FET. Negative TOC biopsies were confirmed prior to subsequent transfer in all patients. If TOC biopsies were still positive, patients were treated with another antibiotic regimen, which consisted of another broad spectrum medication they had not taken previously, and underwent subsequent TOC to ensure adequacy of second regimen prior to next transfer.

Results

305 cycles met inclusion criteria (Figure 2).

Figure 2: Ongoing pregnancy/live birth rates for study and control subjects.

The demographics of such patients meeting inclusion criteria are included in Table 1. In general, most patients were Caucasian and had diagnoses of infertility at time of initial consultation. The two groups were similar in baseline characteristics.

59 patients had EMBs (Tested) prior to undergoing the subsequent FET and 246 patients proceeded directly to the next FET without having an EMB (Untested). 51% (30/59) of patients who underwent EMB tested positive for CE as evidenced by CD138 staining and were treated with antibiotics (Tested, CE Treated); negative TOC biopsies were obtained after completing antibiotics and prior to proceeding to the next FET. 11/30 (37%) patients required additional antibiotic treatment to adequately treat CE, which was confirmed with negative TOC biopsies following the second round of antibiotics. 49% (29/59) of patients tested negative for CE (Tested, No CE). Untested

patients were presumed to have an incidence of CE comparable to Tested patients.

Table 1: Demographics by group (tested or untested).

Table of Demographics		
	Tested	Not Tested
Number of Patients	59	246
Age	37.3 +/- 7.9	37.5 +/- 4.8
Race		
Caucasian	71.7% (43)	55.7% (137)
Asian	8.3% (5)	6.5% (16)
South Asian	1.7% (1)	1.6% (4)
Hispanic	1.7% (1)	0% (0)
African	3.3% (2)	2.0% (5)
Other/Not Reported	13.3% (7)	26.4% (65)
Gravidity/Parity		
Gravidity	0.72 +/- 1.0	0.80 +/- 1.2
Full Term	0.13 +/- 0.39	0.14 +/- 0.39
Premature	0.02 +/- 0.13	0.01 +/- 0.07
Sabs	0.56 +/- 0.82	0.67 +/- 1.02
LiveBorn	0.12 +/- 0.38	0.14 +/- 0.39
Diagnoses		
Infertility (nonspecific)	80.0% (48)	74.2% (161)
Tubal Factor	1.7% (1)	3.7% (8)
Oligomenorrhea	8.3% (5)	1.3% (3)
AMA/DOR/POI	5.0% (3)	7.8% (17)
PCOS	3.3% (2)	0.4% (1)
Male Factor	5.0% (3)	11.5% (25)
Recurrent Pregnancy Loss	6.7% (4)	7.3% (16)
Endometriosis	0% (0)	0.9% (2)
Same Sex Couple/Single Parent	5.0% (3)	3.6% (8)
PGT-M or PGT-SR	8.3% (5)	10.1% (22)
Other	1.7% (1)	2.8% (6)
Failed Outcome Type		
Negative Beta	0.83 +/- 0.62	0.63 +/- 0.48
Biochemical Pregnancy	0.34 +/- 0.54	0.28 +/- 0.45
SAb/Ectopic	0.08 +/- 0.28	0.09 +/- 0.29

Tested patients without CE (patients who were adequately treated for CE and patients who were negative for CE on initial biopsy) were analyzed together as an overall Tested group and also subcategorized by original CE diagnosis (if they had CE and were treated or

if they never had a diagnosis of CE). Tested patients (both CE treated and No CE groups) compared to Untested patients had significantly higher cycle day 28 β hCG (86% vs. 70%), IR (71% vs. 54%), and OPR/LBR (68% vs. 42%) with $p < 0.05$ for all categories (Table 2).

Table 2: Pregnancy outcomes according to testing and CE status.

Pregnancy Outcomes According to Testing and CE Status			
Outcome/Groups	Positive β hCG	IR	OPR/LBR
A) Untested	70% (171/246)	54% (132/246)	42% (104/246)
B) Tested	86% (51/59)	71% (42/59)	68% (40/59)
B1) CE Treated	93% (28/30)	80% (24/30)	80% (24/30)
B2) No CE	79% (23/29)	62% (18/29)	55% (16/29)
Significance	a,b	a,b	a,b,c

^a A vs B1 vs B2 $p < 0.05$, ^b A vs B $p < 0.05$, ^c B1 vs B2 $p < 0.05$

When analyzing the Tested group by initial EMB diagnosis, those who were CE Treated had significantly higher OPR/LBR compared to patients negative for CE and Untested (80% vs. 55% vs. 42% respectively, $p < 0.05$).

Discussion

The existence and ubiquity of chronic endometritis have been known and described since the early nineteenth century [9]. This inflammatory condition was diagnosed in much the same manner then as it is now, by the presence of plasma cells. The traditional method of diagnosis uses H&E staining to evaluate the endometrium primarily for the identification of plasma cells and also for features associated with CE like stromal spindling and edema depending on the expertise of the pathologist. However, this method has been associated with a false-negative rate of 16%, and thus, newer techniques have emerged to aid the histologic evaluation for plasma cells, like immunohistochemical staining with CD138, which has been shown in numerous studies to be more accurate and sensitive than H&E with less inter- and intra-observer variability bias [10,11-17]. CD138 staining has been utilized in multiple CE studies, including evaluation of patients with recurrent pregnancy loss [3],

unexplained infertility [14], and recurrent implantation failure [5,7] which overall suggest that these high risk groups may have a higher prevalence of CE than the general population. It must be noted that CD138 may diagnose CE more frequently than traditional staining techniques, and thus it may be associated with a higher false positive rate due to its ability not only to stain plasma cells, but also endometrial glands and stroma [9,10]. Even if an immunohistochemical stain is used and adequately interpreted, the question still stands – how many plasma cells are needed to consider a patient positive for CE? Thus, CE is a challenging diagnosis for both clinicians and pathologists. Adding to the confusion, each study of CE differs in its diagnostic criteria for CE and the patient populations studied. Further, some argue that the diagnosis of CE should be excluded in the context of structural pathology like leiomyomas and polyps that are associated with increased plasma cells [18]. We acknowledge this as a limitation in our study, as we did not exclude patients with fibroids and did not pursue workup to evaluate for endometrial polyps. Most would agree that there is an infectious component to the etiology of CE; to this end, we evaluated each patient for gonorrhea and chlamydia as per usual routine and none were positive for these at the time of treatment. However, without diagnostic consensus, we find ourselves in a similar situation to our counterparts from the early 1900s.

Amidst this confusion, there is data to suggest that CE plays a role in infertility [2]. The current study employed two institutions that are well-versed in CD138 staining of endometrial biopsies and plasma cell identification. Further, by including only cycles derived from STEET, we controlled for a potential embryo factor with the best testing currently available, which is PGT using the NGS platform. This is the first study to our knowledge to evaluate the effect of CE using CD138 as a marker on IVF pregnancy outcomes that were solely obtained by using genetically tested, euploid embryos and to follow up subsequent pregnancy outcomes in such cycles post-biopsy and/or treatment. However, we cannot rule out a small chance of errors in PGT, undetected genetic variation/mosaicism, or damage to embryos from manipulation, no matter how much the technology has improved. Additionally, while the majority of patients did not have a presenting diagnosis of RPL, we must note a limitation that this group of patients constitutes a separate reproductive category than those failing only one prior transfer and were not isolated from analysis. Another topic of debate would be what constitutes RPL in the literature, as well, as we were only considering

patients with failed transfers of euploid embryos for this study.

The treatment of CE is unfortunately not much clearer. Traditionally, cultures have not been performed to test for a causal organism – one might speculate that such cultures would have to be obtained from the endometrium and might be fraught with vaginal contamination. One study by our institution aimed to answer this question and is being prepared for publication, and more studies are needed to answer this question. Thus, the gold standard of treatment for CE is broad spectrum antibiotics. First-line treatment is cited as either ofloxacin 400mg twice daily or levofloxacin 500mg daily, along with metronidazole 500mg twice daily for 14 days. Alternative regimens have included a 14-day course of doxycycline 100mg twice daily. From the limited studies that exist, the former regimen has a 94% positive TOC rate while the latter has a 70% TOC rate [2,5].

It is striking to compare the OP/LB rates obtained when patients were tested and treated for CE compared to patients who proceeded to FET without a biopsy (OPR/LBR 80% vs. 42%, respectively, $p < 0.05$). As the Cochrane review on intentional endometrial injury (i.e., endometrial “scratch”) procedures indicates, the biopsy itself may promote beneficial inflammation and implantation in a select group of patients; this procedure is essentially an endometrial biopsy without histologic characterization of the endometrial tissue. To account for this, we obtained a clean control group, without endometrial biopsies or recent uterine procedures prior to FET, in order to evaluate the potential effect of the biopsy itself on pregnancy outcomes. In doing so, we found significantly higher rates of all primary outcomes - positive β hCG, IR and OPR/LBR - in patients who underwent endometrial biopsies (Tested) versus those who did not (Untested, see table 2); this suggests an improvement in outcomes due to diagnosis and treatment of CE and perhaps a small benefit of the endometrial biopsy itself. However, it must be mentioned that not all studies show a benefit to the endometrial scratch and we were unable to definitively rule out endometrial pathology like polyps or submucous myomas in our control group [13]. Delving further into the Tested group, patients who were diagnosed with CE and treated had better pregnancy outcomes compared to the Tested, No CE group which may highlight error in EMB sampling or analysis, as a diagnosis may have been missed in some patients or overestimated in others. Alternatively the increase may be due to the added benefit of the endometrial scratch as others have published,

but as previously stated, this is controversial in the field [12,13]. Perhaps there is a benefit to administering the broad spectrum antibiotics for a pathogen for which we are not routinely testing; however, this is not to suggest that all patients desiring pregnancy should be routinely administered antibiotics. Finally, in the group of patients who did not have an endometrial biopsy after failing a euploid transfer (Untested), it can be assumed by the data in the study that 51% would have tested positive. Thus, of the 246 that had another transfer, 125 of them had CD138 and presumably failed to have an ongoing pregnancy due to that fact. By inference then, 104 out of 246 (42%) that had an ongoing pregnancy could have been 104 out of 121 (246 minus 125) and the ongoing rate would have been 86% which is comparable to the CE treated group.

Studies of CE may be improved by sampling technique and careful avoidance of sampling the lower uterine segment and cervix, as these areas can lead to false positive results [9]. This may be overcome through hysteroscopy, as standard endometrial biopsies as performed in this study are blind procedures. We acknowledge that our study was vulnerable to this potential confounder, in addition to other limitations including using multiple pathologists for CE evaluation, varying doses and antibiotic regimens, and the small number of patients who were biopsied. However, the effectiveness of the different medications was tested by obtaining negative TOC biopsies prior to undergoing next FET, although one cannot rule out that CE may have been underdiagnosed as the entire uterine cavity was not sampled by pipelle or surveyed by hysteroscopy. While an increased sample size is always desirable, our Tested population is on par with or above the average number of patients studied in the CE literature. Moreover, this is one of the first studies to focus on the endometrial factor by using only PGT normal embryos and using a large control group to evaluate for the potential effect of the biopsy procedure.

Conclusions

Chronic endometritis using CD138 as a marker of plasma cells is common with a prevalence of 51% in patients who have failed euploid FETs. Testing for CE appears to provide a benefit to the patients we studied, assuring higher incidences for positive β hCG, implantation, and ongoing and live births. It is unclear whether the benefit was derived from assurance of no CE or partially from the effect of the endometrial scratch during EMBs; this determination will require further investigation with a

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larger sample size, although we suspect the former. CE is a treatable condition warranting investigation in patients with poor pregnancy outcomes in the context of PGT-normal embryo transfers. It may represent a piece of the puzzle as to why patients fail euploid transfers.

Declarations

Ethics approval and consent to participate

Institution Review Board approval was obtained (IRB #16-00154). Patients who were included in the prospective study were consented and given the opportunity to discuss all questions and concerns.

Consent for publication

IRB approval and patient consent obtained.

Availability of data and material

Available to patients upon request.

Competing interests

None

Funding

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Authors' contributions

Study initiated by Drs. Grifo, Keefe and Masbou. Drs. Fino and Hodes-Wertz contributed patients to this study. Dr. Blakemore provided logistical support and helped greatly with clinical assistance.

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References

1. Polisseni F, Bambirra EA, Camargos AF. Detection of chronic endometritis by diagnostic hysteroscopy in asymptomatic infertile patients. *Gynecol Obstet Invest.* 2003;55(4):205-210. Doi: <https://doi.org/10.1159/000072075>
2. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. *Fertil Steril.* 2014;101(4):1026-1030. Doi: <https://doi.org/10.1016/j.fertnstert.2013.12.031>
3. Kitaya K, Tada Y, Hayashi T, et al. Comprehensive

- endometrial immunoglobulin subclass analysis in infertile women suffering from repeated implantation failure with or without chronic endometritis. *Am J Reprod Immunol*. 2014;72(4):386-391. Doi: <https://doi.org/10.1111/aji.12277>
4. Zolghadri J, Momtahan M, Aminian K, et al. The value of hysteroscopy in diagnosis of chronic endometritis in patients with unexplained recurrent spontaneous abortion. *Eur J Obstet Gynecol Reprod Biol*. 2011;155(2):217-220. Doi: <https://doi.org/10.1016/j.ejogrb.2010.12.010>
 5. Johnston-MacAnanny EB, Hartnett J, Engmann LL, et al. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. *Fertil Steril*. 2010;93(2):437-441. Doi: <https://doi.org/10.1016/j.fertnstert.2008.12.131>
 6. Romero R, Espinoza J, Mazor M. Can endometrial infection/inflammation explain implantation failure, spontaneous abortion, and preterm birth after in vitro fertilization? *Fertil Steril*. 2004;82(4):799-804. Doi: <https://doi.org/10.1016/j.fertnstert.2004.05.076>
 7. Cicinelli E, Matteo M, Tinelli R, et al. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci*. 2014;21(5):640-647. Doi: <https://doi.org/10.1177/1933719113508817>
 8. McQueen DB, Perfetto CO, Hazard FK, et al. Pregnancy outcomes in women with chronic endometritis and recurrent pregnancy loss. *Fertil Steril*. 2015;104(4):927-931. Doi: <https://doi.org/10.1016/j.fertnstert.2015.06.044>
 9. Groth J, McQueen D, Stephenson M. Inflammation of the endometrium in reproduction. In: Simón C, Giudice L, eds. *The Endometrial Factor: A Reproductive Precision Medicine Approach*. New York: CRC Press; 2017:193-203. Doi: <https://doi.org/10.1201/9781315151472-13>
 10. Liu Y, Chen X, Huang J, et al. Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure. *Fertil Steril*. 2018;109(5):832-839. Doi: <https://doi.org/10.1016/j.fertnstert.2018.01.022>
 11. Smith M, Hagerty KA, Skipper B, et al. Chronic endometritis: A combined histopathologic and clinical review of cases from 2002 to 2007. *Int J Gynecol Pathol*. 2010;29(1):44-50. Doi: <https://doi.org/10.1097/PGP.0b013e3181ae81bb>
 12. Pye C, Chatters R, Cohen J, et al. Induced endometrial trauma (Endometrial scratch) in the mid-luteal menstrual cycle phase preceding first cycle IVF/ICSI versus usual IVF/ICSI therapy: Study protocol for a randomized controlled trial. *BMJ Open*. 2018;8(5):e020755. Doi: <https://doi.org/10.1136/bmjopen-2017-020755>
 13. Lensen S, Osavlyuk D, Armstrong S, et al. A randomized trial of endometrial scratching before in vitro fertilization. *N Engl J Med*. 2019;380(4):325-334. Doi: <https://doi.org/10.1056/NEJMoa1808737>
 14. Kasius JC, Fatemi HM, Bourgain C, et al. The impact of chronic endometritis on reproductive outcome. *Fertil Steril*. 2011;96(6):1451-1456. Doi: <https://doi.org/10.1016/j.fertnstert.2011.09.039>
 15. Bayer-Garner IB, Korourian S. Plasma cells in chronic endometritis are easily identified when stained with syndecan-1. *Mod Pathol*. 2001;14(9):877-879. Doi: <https://doi.org/10.1038/modpathol.3880405>
 16. Bayer-Garner IB, Nickell JA, Korourian S. Routine syndecan-1 immunohistochemistry aids in the diagnosis of chronic endometritis. *Arch Pathol Lab Med*. 2004;128(9):1000-1003. Doi: [https://doi.org/10.1043/1543-2165\(2004\)128<1000:RSIAIT>2.0.CO;2](https://doi.org/10.1043/1543-2165(2004)128<1000:RSIAIT>2.0.CO;2)
 17. Kitaya K, Yasuo T. Inter-observer and intra-observer variability in immunohistochemical detection of endometrial stromal plasmacytes in chronic endometritis. *Exp Ther Med*. 2013;5(2):485-488. Doi: <https://doi.org/10.3892/etm.2012.824>
 18. Kurman RJ, Ellenson LH, Ronnett BM, eds. *Blaustein's pathology of the female genital tract*. 6th ed. New York: Springer, US; 2011. <https://www.springer.com/gp/book/9783319463339>.

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