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Research Article

The Use of Endometrial Culture For Targeted Treatment of Endometritis in Patients Experiencing Infertility and Recurrent Pregnancy Loss.

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Abstract

Aims: To evaluate whether endometrial culture in addition to endometrial biopsy reduces time to clear chronic endometritis (CE) and fewer endometrial biopsies in patients experiencing infertility and recurrent pregnancy loss (RPL).

Methods: This retrospective cohort study was performed at an academic tertiary care facility. We included patients (N=92) with endometritis (defined as either endometritis on pathologic evaluation or 5 plasma cells per high power field) who were evaluated in the reproductive infertility and endocrinology clinic for infertility or RPL from March 2018 to January 2022. In March 2021, the clinic implemented routine use of endometrial culture in addition to endometrial biopsy as part of the evaluation of infertility or RPL. We hypothesized that treatment of specific endometrial pathogens with reported sensitivities to antibiotics would result in a reduction in length of treatment to clear CE and fewer number of biopsies per patient. Patients evaluated prior to March 2021 with only endometrial biopsy (n=46) were compared to patients evaluated after March 2021 with endometrial culture in addition to endometrial biopsy (n=46). Patients who did not follow up to evaluate for clearance of endometritis were excluded from the study. Mean time to clearance and average number of biopsies were compared via student's t-test.

Results: The mean time needed to clear chronic endometritis in the endometrial biopsy only cohort was 73.1 days, while the average time needed in the endometrial culture plus endometrial biopsy cohort was 51.4 days (p=0.018). The average number of biopsies per patient in the endometrial biopsy only cohort was 2.89, while the average number of biopsies in the endometrial culture plus endometrial biopsy cohort was 1.98 (p=0.00001).

Conclusion: Endometrial culture in addition to endometrial biopsy leads to a statistically significant decreased time to treat patients with CE and significantly fewer endometrial biopsies required per patient. Endometrial culture is a diagnostic tool that could reduce time needed to treat CE by targeting specific pathogens. Futures studies should investigate if this simple diagnostic tool reduces time to pregnancy in patients with CE.

Keywords: Chronic Endometritis, Endometrial Culture, Infertility, Recurrent Pregnancy Loss and Endometrial Microbiome

1. Introduction

Chronic endometritis (CE) has been defined as plasmacyte infiltration of the endometrial stroma and is typically asymptomatic in nature. When symptoms of CE are present, they are often indistinguishable from other pathology leading to difficulty in diagnosis. The topic of CE is becoming increasingly more prevalent in the literature given its association with recurrent pregnancy loss (RPL), repeat implantation failure (RIF), and infertility. A retrospective cohort study amongst women with recurrent pregnancy loss demonstrated that CE was present in 57.8% of their cohort, and that one year following antibiotic use, women cured of CE had significantly higher successful pregnancies compared to women with persistent CE despite therapy

(78.4% vs 15.3%, respectively; p = .005) [1]. A meta-analysis of 12 studies estimated that the prevalence of CE in women with RPL was as high as 29.7% [2]. Chronic endometritis has been shown to be highly prevalent in patients with the diagnosis of unexplained infertility [3]. It has been proposed that impairment in endometrial receptivity caused by CE can thereby interfere with embryological implantation, leading to alterations in expression of genes involved in embryo implantation [4].

Chronic endometritis is traditionally diagnosed via one of three techniques: hysteroscopy, histology, or culture. Currently, no standardized diagnostic approach exists for the diagnosis of CE. Moreno, et al. has shown that when compared, histopathological evaluation usually underdiagnosed chronic endometritis while hysteroscopy tended to over diagnose [5]. It has been proposed that CE causes a structural transformation of the endometrium, evidenced by abnormalities typically seen on hysteroscopy. These abnormalities range from irregular contour of the endometrium causing distorted anatomy, stromal edema, hyperemia, or polyps. In addition to structural abnormalities, CE can also cause histological abnormalities.

Tissue samples of patients with CE often show increased endometrial superficial edematous changes, increased cell density, dissociated maturation between epithelium and stroma, and infiltration of plasma cells. The limitations of using histology for diagnosis of endometritis include subjectivity in pathology readings as well as its dependence on a focal area of endometrium and temporal conditions (phase of the menstrual cycle) during which the sample was collected. Although there is no accepted standard definition for the diagnosis of CE, the presence of plasma cells is the most specific and sensitive finding [6]. Recently, more than or equal to 5 plasma cell in a single high power field identified with CD138 staining has been proposed as a less subjective definition [7].

Cicinelli, et al. demonstrated an 81.3% cure rate following 3 cycles of antibiotic therapy in their cohort of patients with CE versus 6% cure rate in their untreated control group [8]. This study also added to the idea of an infectious nature behind CE. Standardized treatment for CE following diagnosis via hysteroscopy or biopsy varies based on institution. Yang, et al. conducted a prospective study investigating the effectiveness of treatment with doxycycline in patients with RIF. Their protocol involved using oral doxycycline 200 mg daily for 14 days. The success rate cited in their study was 92.3% (n = 108/117 subjects) [9]. A study by Johnston-MacAnanny, et al. used the same regimen when evaluating treatment efficacy in patients with CE and history of RIF, and 7 out of 10 patients receiving doxycycline cleared their CE [10]. The remaining 3 patients underwent treatment with ciprofloxacin and metronidazole which effectively eliminated their CE.

Increased interest in the role of the endometrium in terms of reproductive potential and reproductive success followed the discovery that the endometrium is not a sterile cavity. While the vagina is accepted as having a diverse microbiome with implications for health, the uterus was historically thought to be a sterile cavity [11]. Previously, bacteria in the uterus were thought to be associated only with a diagnosis of cervicitis, endometritis, and pelvic inflammatory disease [12]. More recently, studies have demonstrated the presence of bacteria in the uterus using endometrial culture in asymptomatic women [12-14]. This finding of bacteria on endometrial culture in the uteri of participants without clinical endometritis refuted the previous notion that the uterus is a sterile cavity [12-14].

Quantitative PCR has characterized the microbiome of the upper genital tract as being distinct from the lower genital tract and that low levels of bacteria within the uterus is not associated with inflammation [15]. Moreno and colleagues utilized this technique to analyze endometrial fluid as compared to vaginal aspirates from the same subjects and demonstrated that the endometrium harbors a complex microbiome that is not shared exclusively with the vagina using bacterial 16S RNA [16]. Advances in genomics and bioinformatics have defined microbial communities in various body organs by sequencing the unique bacterial 16S ribosomal RNA gene [17]. Further, the endometrial microbiome was characterized as lactobacillus dominant (LD) or non-lactobacillus dominant (NLD) communities [16].

The use of next generation sequencing technology has resulted in multiple studies demonstrating that the endometrium is often Lactobacillus predominant [18-20]. Tao, et al. analyzed bacteria present from embryo transfer catheter tips in IVF cycles and showed that Lactobacillus spp. were detected in all of their samples, with all samples having 70% or greater Lactobacillus abundance [19]. Non-lactobacillus dominant endometrial microbiota may impact implantation rates and miscarriage rates in patients undergoing in vitro fertilization (IVF) [16, 21]. Moreno, et al. evaluated the effect of the endometrial microbiome on reproduction in patients undergoing in-vitro fertilization treatments and found that NLD endometrium, compared to LD endometrium, was associated with adverse outcomes including lower implantation, pregnancy rates, on-going pregnancy rates, and increased miscarriage rates [16]. Another prospective cohort study of patients undergoing IVF demonstrated that LD endometrium was associated with increased live birth rates when compared to NLD endometrium. Additionally, in this cohort, NLD endometrium was associated with unsuccessful pregnancy or clinical miscarriage [22].

It is now well established that that the female reproductive tract has a normal or typical flora - namely, the *Lactobacillus* species. Major pathogens associated with the diagnosis of endometritis are *Enterobacteriaceae, Gardnerella, Streptococcus, Staphylococcus, Escherichia coli, Proteus species, Klebsiella pneumoniae, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida, Corynebacterium, Mycoplasma* and *Ureaplasma* species [23, 24]. We hypothesized that standardized implementation of

endometrial culture leads not only to more targeted treatment of specific endometritis pathogens, but also to more expedited care in patients undergoing fertility treatment. Currently, no standardized method for diagnosing and treating CE exists. Endometrial culture may be an effective, objective, and reliable method for diagnosing CE or an adjunct to histology or hysteroscopy.

We proposed a retrospective review comparing the number of endometrial biopsies required to clear chronic endometritis compared with the number of procedures required after implementing routine endometrial culture at the time of endometrial biopsy as the standard of care in our clinic.

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2. Materials and Methods

This was a retrospective cohort study conducted via chart review of patients with the diagnosis code of female infertility or RPL who were seen in our institution's reproductive endocrinology and infertility clinic from March 2018 to January 2022. This original research was approved by our institution's review board prior to its initiation. In March 2018, our clinic began collecting routine endometrial biopsy on patients undergoing evaluation for infertility or RPL to evaluate for presence of CE. Endometrial biopsy was collected during the patient's follicular phase (aim of cycle day 7-10).

If the patient had a positive result for CE, defined at our institution as positive CD138 staining with five or more plasma cells per high power field on histology, they were treated empirically with course of doxycycline. A repeat endometrial biopsy was then performed on cycle day 7-10 after completion of antibiotics to determine if the CE had been cleared. If the patient was determined to still have CE, they were treated with a longer course of doxycycline versus an alternative antibiotic based on the provider's discretion or patient preference (Figure 1).

Figure 1: Initial Protocol for Follow Up.

In March 2021, our clinic began implementing routine use of endometrial culture in addition to endometrial biopsy as part of the evaluation of infertility and RPL. Endometrial culture was added in addition to histology due to the thought that identifying specific pathogens may lead to targeted treatment of the source of endometritis. Specimens were sent to our institution's pathology lab where they underwent aerobic culture. During patient encounters following implementation of endometrial culture, endometrial biopsy and culture were collected at the same time. For culture, the cervix was prepped with saline and an endometrial biopsy pipelle (Cooper Surgical Endometrial Suction Curette - CE 2797) was used to collect an endometrial tissue sample. A small portion of that sample was placed in a container with sterile saline, to be used for endometrial culture.

The remaining sample was placed in formalin, to be used for endometrial biopsy. Both specimens were then sent to our institution's laboratory for evaluation. A report was then provided, demonstrating the pathogenic flora and sensitivities. Antibiotics based on these sensitivities were then prescribed. The patient would then present on cycle day 7-10 of their next menstrual cycle for repeat sampling to determine clearance of pathogenic flora, see Figure 2 for the modified protocol.

Figure 2: Modified Protocol For Follow Up.

All extracted patient data used for this retrospective cohort study was de-identified by our statistician prior to analysis. Patients who had undergone endometrial biopsy from March 2018 to March 2021 were subdivided from patients who had undergone endometrial biopsy with histology and endometrial culture from March 2021 to January 2022. From these two subsets, patients with a result of CE on histology were selected. Patients who did not follow up for a repeat biopsy and/or culture to determine clearance of endometritis were excluded from the study. Mean time to clearance of CE and average number of biopsies were compared via student's t-test.

3. Results

Data from patients seen in our REI clinic from March 2018 to January 2022 was collected and de-identified. Patients with the ICD code for infertility or RPL were included for an N = 410. These patients were further queried for a pathology report indicating endometritis, defined at our institution as endometritis on histological evaluation or greater than equal to 5 plasma cells in a high-power field. The patients were then subdivided into two groups, group 1 being patients evaluated prior to March 2021 with only endometrial biopsy (n=46) and group 2 being patients evaluated after March 2021 with endometrial culture in addition to endometrial histology (n=46).

The results demonstrated that the mean time needed to clear CE in the endometrial biopsy cohort was 73.1 days, while the mean time needed to clear CE in the endometrial histology plus culture cohort was 51.4 days (p = 0.018). In addition to this, the mean number of biopsies needed for clearance of CE in the endometrial biopsy alone group was 2.89, while the mean number of biopsies in the histology and culture group was 1.98 (p = 0.00001). (Tables 1, 2).

Table 1: Mean Time to Clear Chronic Endometritis.

Intervention	Time Frame	Mean ± SD (Days)	Median (days)	P- Value
Endometrial biopsy only	3/2018- 3/2021	73.1 ± 43	63.0	P = 0.018
Endometrial biopsy + Culture	4/2021-1/2022	51.4 ± 36	35.7	

Table 2: Mean Number of Biopsies Required to Clear Chronic Endometritis.

Intervention	Time Frame	Mean ± SD (# biopsies)	Median (# biopsies)	P- Value
Endometrial biopsy only	3/2018- 3/2021	2.89 ± 1.4	2	P = 0.00001
Endometrial biopsy + Culture	4/2021-1/2022	1.98 ± 0.9	2	

In addition to this, the pathogenic results from endometrial culture data were analyzed and *Streptococcus agalactiae* (group B) was the most commonly identified pathogen followed by *Enterococcus faecalis, Gardnerella vaginalis, Escherichia coli,* and *Staphylococcus aureus* (Table 3). A total of 10 patients were lost to follow up and were removed from the final data set (n = 10, ~9.8%).

Table 3: Most Common Pathogens Identified in Patients With Chronic Endometritis.

Bacteria	Number of Patients (%)
Streptococcus agalactiae (group B)	15 (18)
Enterococcus faecalis	13 (16)
Gardnerella Vaginalis	6(7)
Escherichia Coli	5(6)
Staphylococcus aureus	3(4)

4. Discussion

Our study provides an applicable option for the clinical management of CE. Chronic endometritis remains difficult to diagnose and is often overlooked due to its insidious symptoms. Multiple studies have shown that CE is infectious in nature, stemming from alteration of normal endometrial microbiota. The infectious origin of CE is further inferred by normalization of endometrial flora following the use of antibiotics.

Chronic endometritis can be associated with both gram-positive and gram-negative bacteria, which is supported by the pathogen prevalence reported in this study (Table 3). The wide variety of pathogens represented begs the question of whether the traditional empiric therapy often used is more likely to fail when compared with targeted therapy. Our study supports that when therapy is tailored to a specific pathogen, patients experience a statistically significant decreased time to clear CE and significantly decreased number of biopsies.

Limitations of the study include lack of sufficient data to assess pregnancy outcomes following implementation of endometrial culture, lack of anaerobic or Ureaplasma/ Mycoplasma cultures, size of the patient population, and

number of patients lost to follow up which were removed from the final data set. In addition, the culture could be cervical due to contamination of the catheter as it passed through to the uterus. Future studies with a larger patient population could evaluate culture and successful pregnancy outcome.

5. Conclusion

Chronic endometritis is thought to cause a hostile intrauterine environment that can lead to impairment of implantation and unsuccessful pregnancy outcomes. Endometrial culture is a simple and minimally invasive diagnostic tool that can be implemented in the clinical setting to investigate for diagnosis of CE in patients with infertility, RIF and RPL. In addition to this, endometrial culture is the only method of diagnosis that allows objective diagnosis without the subjectivity associated with histology and hysteroscopy. With CE being closely linked to RPL, RIF and unexplained infertility, the question remains as to whether diagnosis and treatment of CE prior to pursuing infertility treatment should be pursued to optimize patients' chance for successful pregnancy outcomes.

In addition, endometrial culture has the added value of targeting specific pathogens within the endometrial microbiota that may be the source of CE. Further studies should investigate if using endometrial culture reduces time to achieve pregnancy in patients with CE. There is also a need for randomized control trials to determine whether treatment of CE affects pregnancy outcomes. Future studies should include a standardized sample collection protocol as well as well-defined criteria for histologic diagnosis of CE. With further studies of this topic, there is potential for improved spontaneous pregnancy rates and live birth rates in patients with CE who are diagnosed and treated.

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