Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization

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Objective: To determine the role of endometrial sampling for identification and treatment of chronic endometritis (CE) in patients undergoing IVF-ET who repeatedly failed to conceive despite the transfer of good-quality embryos.
Design: Retrospective chart review.
Setting: University-based tertiary fertility center.
Patient(s): Thirty-three patients with recurrent implantation failure (RIF) who underwent endometrial sampling and subsequent ET were analyzed based on immunohistochemically confirmed CE: CE present on biopsy (group 1; n = 10) and CE absent on biopsy (group 2; n = 23). Patients with RIF undergoing IVF cycles during the same time period who did not have endometrial sampling were used as controls (group 3; n = 485).
Intervention(s): Endometrial sampling for CE and subsequent antibiotic treatment in affected patients followed by another IVF-ET cycle.
Result(s): Chronic endometritis was identified in 30.3% of patients with RIF. Group 1 had lower implantation rates (11.5%) in the IVF cycle following treatment than did group 2 and group 3 (32.7% and 20.3%, respectively). Clinical pregnancy and ongoing pregnancy rates were similar across groups.
Conclusion(s): Recurrent implantation failure warrants investigation of CE as a contributing factor. Women demonstrating CE on endometrial sampling have lower implantation rates in a subsequent IVF-ET cycle; however, there were no differences in subsequent clinical pregnancy or ongoing pregnancy rates after successful antibiotic treatment. (Fertil Steril® 2010;93:437–41. ©2010 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, chronic endometritis, recurrent implantation failure, endometrial biopsy

Implantation is a complex and multistep process, resulting in the blastocyst being embedded in the endometrial stroma, which is often viewed as the rate-limiting step for the success of IVF. It is well established that the success of embryo implantation depends on embryo quality, uterine integrity, and endometrial receptivity. Although embryo quality is the most consistent factor for predicting implantation and pregnancy rates in IVF-ET patients, this cannot be evaluated independently from uterine integrity or endometrial receptivity (1, 2). Identification and correction of benign uterine abnormalities such as polyps or submucosal fibroids is believed to increase pregnancy rates.

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The definition of recurrent implantation failure (RIF) remains controversial, generally being defined as failure to conceive following two or three embryo transfer cycles, or cumulative transfer of >10 good-quality embryos (3). Patients with RIF comprise a heterogeneous group that present with diverse clinical problems, and necessitates a thorough evaluation. In our program, evaluation of a couple with RIF includes assessment of maternal and paternal karyotypes, testing for antiphospholipid antibodies, and thorough assessment of the uterine cavity, including sampling of the endometrial lining.

Endometrial biopsy is one inexpensive and minimally invasive method to assess and study the physical and hormonal endometrial milieu. If abnormalities are identified and corrected, implantation rates could potentially be improved. Chronic endometritis (CE) is a persistent inflammation of the endometrial lining. Histologically, the diagnosis of CE is generally based on finding plasma cells infiltrates in endometrial biopsies (4). Chronic endometritis is thought to be related to infertility and spontaneous abortion, and it is usually asymptomatic and rarely suspected clinically (2, 5, 6). Additionally, correction of other structural uterine pathologies, such as endometrial polyps and myomas, has been shown to

improve clinical pregnancy and implantation rates following treatment (7).

In this study, we aimed to identify the incidence of CE in patients with RIF using a more specific immunohistochemistry evaluation of the endometrial samples. In addition, we evaluated the effects of antibiotic treatment of CE on subsequent IVF outcomes.

MATERIALS AND METHODS

Patient Selection

After obtaining institutional review board approval, 33 patients who underwent endometrial sampling for evaluation of RIF between 2001 and 2007 were identified. Recurrent implantation failure was defined as at least two cycles of IVF-ET in which at least one good-quality embryo was transferred in each cycle, without achieving a clinical pregnancy. Diagnosis of CE based on histologic examination of the endometrial sample was recorded, and antibiotic treatment regimens were reviewed. All patients treated for CE underwent a subsequent biopsy for a test of cure. Subsequent IVF treatment and cycle outcomes were analyzed. All patients undergoing biopsies had normal karyotypes, negative testing for antiphospholipid antibodies, and a normal uterine cavity assessed by hysteroscopy, sonohysterography, or hysterosalpingogram. All biopsied patients had the same number of embryos transferred pre- and post- biopsy. Group 1 ($n = 10$) included patients with diagnosis of CE identified by endometrial biopsy. Patients without endometritis identified in the endometrial samples comprised group 2 ($n = 23$). The control group (group 3; $n = 485$) included patients who failed at least two cycles of IVF-ET during the same time period, and did not have an endometrial biopsy for evaluation.

In Vitro Fertilization Protocols

All the women in the study underwent one of two controlled ovulation hyperstimulation protocols—the long luteal phase GnRH agonist protocol or the GnRH antagonist protocol—as determined by their primary physician depending on patient age, antral follicle count, and serum FSH levels. Patients who used the long GnRH agonist protocol started 0.5 mg of leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL) in the midluteal phase of the preceding cycle. A transvaginal ultrasound and serum E_2 were then performed after the onset of menses to confirm pituitary suppression, as shown by the absence of follicular activity and a serum E_2 level of <75 pg/mL. Once pituitary suppression was achieved, controlled ovarian stimulation was initiated as described below, and the dose of leuprolide was then reduced to 0.25 mg daily and continued until the day of trigger of oocyte maturation. Patients who used the GnRH antagonist protocol were evaluated on day 2 of their menses, and gonadotropins were commenced if the ovaries were quiescent on ultrasound. Ganirelix acetate (Ganirelix, Organon Pharmaceuticals, Roseland, NJ) was initiated once the leading follicle was ≥ 14 mm or $E_2 \geq 350$ pg/mL, and continued daily until the day of trigger. All the patients used either recombinant FSH (Gonal F; Serono, Inc., Rockland, MA) alone or in combination with purified urinary hMG (Repronex; Ferring Pharmaceuticals, Inc., Suffern, NY) for ovarian stimulation. In both groups, 5,000 to 10,000 IU of human chorionic gonadotropin (hCG) were administered subcutaneously when at least two follicles reached ≥ 17 mm in diameter, followed 35 hours later by ultrasound-guided transvaginal oocyte retrieval. All patients received luteal phase intramuscular (intramuscular) progesterone (P) 50 mg daily until a viable fetus was seen on ultrasound. Serum P levels were assessed at the time of hCG test, 11 days after embryo transfer, to ensure serum levels ≥ 20 ng/mL. If serum hCG was detected, hCG and P4 levels were repeated 48 hours after initial evaluation. Clinical pregnancy was defined as ultrasound detection of an intrauterine gestational sac with a fetal pole and cardiac activity between 6 and 7 weeks gestational age. Ongoing pregnancies were those clinical pregnancies that exceeded 12 weeks estimated gestational age.

Endometrial Sampling

Endometrial sampling was performed using either a Pipelle biopsy catheter (Sepal Reproductive Devices, Boston, MA) or by sharp curettage following hysteroscopy, after confirmation of a negative pregnancy test.

Immunohistochemistry

Chronic endometritis was defined as the presence of plasma cells in the endometrial stroma. Endometrial biopsies were initially diagnosed as positive or negative for CE by hematoxylin and eosin staining. Although not done routinely, for the purposes of our study and to confirm the diagnosis, all biopsies were subsequently immunohistochemically stained with an antibody specific to plasma cells, CD138. Slides were cut from original paraffin embedded biopsies and prepared for immunohistochemical staining by dewaxing and antigen retrieval. Prepared slides were then incubated with the mouse monoclonal CD138 antibody (Biocare Medical, Concord, CA) at 1:100 dilution for 1 hour. Dako Real Envision Detection System was then used (Dako, Carpinteria, CA) in which a labeled polymer horseradish peroxidase antimouse antibody is applied and incubated for 30 minutes. Slides were then washed and incubated with diaminobenzidine. The biopsies were considered negative for CE if less than one plasma cell per high-power field was seen. A repeat biopsy showing normal endometrium was required before initiation of further IVF treatment. The test of cure biopsy was similarly stained and examined. All biopsies were evaluated and stained by the same pathologist at the University of Connecticut (M.S.).

Antibiotic Treatment

Patients identified with CE were treated with a 14-day course of oral doxycycline 100 mg twice a day. Persistent positive biopsies were retreated with a combination of ciprofloxacin