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Results of *in vitro* fertilization after diagnosis and treatment of chronic endometritis

C.-C. VADUVA[1,2], M.S. SANDULESCU[1,2], M. TENOVICI[2,3], M.A. SIMINEL[4], M.B. NOVAC[5]

[1]Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Craiova, Romania
[2]HitMed Medical Center, Craiova, Romania
[3]Department of Pathology, Railroads Clinical Hospital, Craiova, Romania
[4]Department of Neonatology, University of Medicine and Pharmacy of Craiova, Craiova, Romania
[5]Department of Anesthesiology and Intensive Care, University of Medicine and Pharmacy of Craiova, Craiova, Romania

Abstract. – OBJECTIVE: A significant cause of infertility is the inability of the embryo to implant. Endometritis is one of the major causes affecting embryo implantation. The present study addressed the diagnosis and effects of chronic endometritis (CE) treatment on pregnancy rates after *in vitro* fertilization (IVF).

PATIENTS AND METHODS: We conducted this retrospective study on 578 infertile couples treated with IVF. In 446 couples, we performed a control hysteroscopy with biopsy before IVF. In addition, we examined the visual aspects of the hysteroscopy and the results of the endometrial biopsies, followed by antibiotic therapy if necessary. Finally, the results of IVF were compared.

RESULTS: Of the 446 cases studied, we diagnosed 192 (43%) with chronic endometritis, either by direct observation or based on the histopathological result. In addition, the cases diagnosed with CE we treated with a combination of antibiotics. The group diagnosed at CE and subsequently treated with antibiotic therapy had a significantly higher pregnancy rate after IVF (43.2%) than the group without treatment (27.3%).

CONCLUSIONS: Hysteroscopic examination of the uterine cavity was particularly important for the success of IVF. The initial CE diagnosis and treatment were an advantage for the cases in which we performed the IVF procedures.

Key Words:
Chronic endometritis, IVF, Hysteroscopy, Pregnancy, Antibiotic therapy.

Introduction

Currently, infertility affects 8-12% of couples[1]. Even if an *in vitro* fertilization (IVF) treatment is followed, the results do not exceed 30% per treatment cycle[2], and the results can be reduced even more over the age of 36 years. An important factor influencing fertility is implanting the embryo in the endometrium. A physiological inflammatory reaction in the endometrium accompanies the implantation of the embryo. It involves several local components, such as leukocytes, cytokines, immunoglobulins, and other factors that mediate the immune response and endometrial growth[3].

Understandably, the endometrium must have specific morphological and pathophysiological characteristics to support the implantation process. The endometrium must be of adequate thickness, have adequate hormonal impregnation, be in the luteal phase and, more precisely, be in the implantation window, and finally, not suffer from another condition[4]. Any endometrial pathology, such as that caused by a change in hormonal status or that caused by inflammation, can alter endometrial receptivity and lead to infertility.

Chronic endometritis (CE) is an inflammatory condition of the endometrium. It is characterized by a much higher percentage of plasma cells in the endometrium. It is a condition that involves a significant disturbance of the balance between microorganisms, normal flora of the endometrium cavity and the host's immune system[5]. This pathology can lead to infertility because this condition involves a much-increased lymphocyte infiltration and, consequently, an abnormal endometrial microenvironment for embryo implantation. It explains that CE prevalence among patients using an assisted reproduction technique is about 10%[6].

The conventional diagnostic technique of infertility, like ultrasound and hysterosalpingography, may omit minor intrauterine lesions. One of these abnormalities is CE[7]. Endometrial investi-

Corresponding Authors: Cristian-Constantin Vaduva, MD; e-mail: hitmed@gmail.com; Mirela Anisoara Siminel, MD; e-mail: mirelasiminel@gmail.com

gation can be best performed by direct visualization and histopathological analysis of biopsy specimens. Hysteroscopy is the only investigation that allows direct visualization of the uterine cavity. Hysteroscopy accompanied by endometrial biopsy is the best method to diagnose uterine pathology that interferes with embryo implantation[8].

The current study was necessary because of hysteroscopy's systematic investigation of female infertility. We reported the prevalence of CE in 12-46% of cases of infertile patients[9]. In most cases, CE is asymptomatic[9]. Therefore, the diagnosis is difficult, but its treatment may give positive results in infertility therapy[10].

Patients and Methods

The study is retrospective and involves four years: 2018 - 2021. The study included 578 couples with infertility investigated and treated by IVF procedure. Couples with male pathology were not admitted to the investigated group.

In most cases, we included hysteroscopy in the investigation scheme. However, we did not perform this procedure in patients with contraindications (patients with untreated vaginal infections, vaginal bleeding, cardiovascular disease or neoplasms, etc.) or who refused this examination despite the risks and disadvantages explained. We performed hysteroscopies postmenstrual. Usually, in cycles in which contraceptives were administered with the role of endometrial decidualization, which allows a better view of it.

Hysteroscopies were performed under short-term intravenous anesthesia with Propofol (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). Hysteroscopies were made with a Bettocchi endoscope (Karl Storz SE & Co. KG, Tuttlingen, Germany) with an outer diameter of 5 mm, provided with a channel for biopsy forceps. The solution for uterine distension was saline at a maximum pressure of 100 mmHg. We methodically investigated the endocervical canal, uterine cavity, endometrium, and tubal orifices. In all cases, one or more endometrial biopsies were taken during the endoscopic procedure using grasping forceps (Karl Storz SE & Co. KG, Tuttlingen, Germany). We took the endometrial tissue pieces and sent them to the pathological anatomy laboratory. Here they were analyzed by an pathologist specialized in examining endometrial pathology. The tissue was fixed in a 10% formalin solution for the microscopic analysis and processed according to the paraffin inclusion technique. The blocks were cut to a thickness of 4 μm using the HMB 350 microtome (Fisher Scientific UK, Loughborough, UK) equipped with a STS water-based transfer system (Fisher Scientific UK, Loughborough, UK). We used the Hematoxylin-Eosin (HE), Periodic Acid–Schiff (PAS) Hematoxylin and Masson's trichrome staining as described in a previous article[11]. We monitored the presence of endometritis lesions according to the Kurman criteria: the presence or absence of inflammatory cells such as neutrophils, lymphocytes, or plasma cells[12].

In all cases, we established a histopathological or hysteroscopic CE diagnosis. We followed by repeated antibiotic treatment for two weeks after a month's interval. Both partners of the couple we treated simultaneously. A combination of quinolones and nitroimidazole was administered orally twice daily. All 578 couples underwent the IVF procedure. We used the same stimulation protocol described in a previous article[13]. The ovarian stimulation was done with the antagonist protocol using ampoules of 75 IU of FSH + LH (Menopur, Ferring GmbH, Kiel, Germany) and ampoules of Ganirelix (Orgalutran, N.V. Organon, AB Oss, Netherlands). Ovulation was triggered with 250 μg r-hCG (Ovitrelle, Merck Europe B.V, Amsterdam, Nederlands). In all cases of IVF, we used the ICSI technique.

Statistical Analysis

We analyzed the pregnancy rate achieved. We compared the data from the group of patients who were diagnosed and treated at CE with those in the group that did not receive treatment. We checked the continuous data of the two groups and the normality of their distribution with a Kolmogorov-Smirnov test, which showed that the distribution was normal. To determine whether the difference was statistically significant between the results of the two groups of patients, the Chi-squared test, Fisher's exact test, was used. $p < 0.05$ was considered statistically significant. The software StatsDirect 3 (Version 2.8.0, StatsDirect Ltd, Cambridge, UK) was used for power calculation and data analysis.

Results

Most of the 446 couples investigated by hysteroscopy had a history of pelvic inflammation - 312 couples (69.9%).

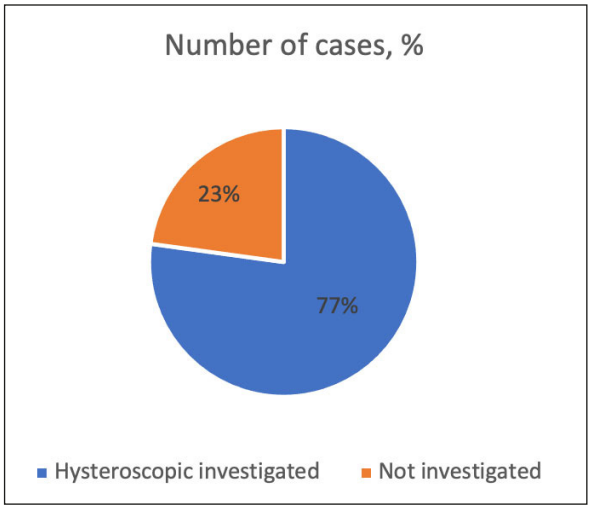

Figure 1. Hysteroscopic investigation.

The following graph shows the proportion of hysteroscopic investigated cases (Figure 1).

Hysteroscopically, an impairment of tubal permeability, at least unilateral, was detected in 319 cases (71.5%).

We noted and analyzed all atypical hysteroscopic visual aspects. However, most were macroscopic endometrial inflammatory aspects (Figure 2).

On hysteroscopic examination, CE had multiple appearances. We discovered these lesions in 232 cases (52%). Many of them appeared simultaneously.

More common CE was hypervascularity, congestion, mosaic, and red-dotted plaques with vascular dystrophy. The vascular network appears accentuated, especially at the peri-glandular region: vascular dystrophy, focal or diffuse peri-glandular hyperemia. The presence of bright red endometrial areas with central white dots focally or diffusely distributed on the endometrium surface creates a "strawberry-like" appearance. These areas bleed easily in direct contact. Localized or diffuse endometrial hypertrophy is also joint. Diffuse or localized polyposis was another specific sign of CE we discovered on hysteroscopy. We frequently encountered the presence of micropolyps, which were easily detected because they floated in the distension medium (Figure 3).

Histopathological examination on harvested endometrial biopsy pieces revealed aspects of CE in 192 cases (43%). The histopathological diagnosis of inflammation was made at the level of biopsies performed: focal or diffuse hyperemia, stromal oedema, thickening of the endometrium and the presence of neutrophils, lymphocytes, or plasma cells in the examined tissue stroma (Figure 4).

In all cases where we have made a histopathological or hysteroscopic diagnosis CE, we have recommended repeated antibiotic treatment.

Finally, we compared the results obtained after the IVF procedure. In the group investigated hysteroscopically, we established a diagnosis of endometritis, followed by antibiotic treatment.

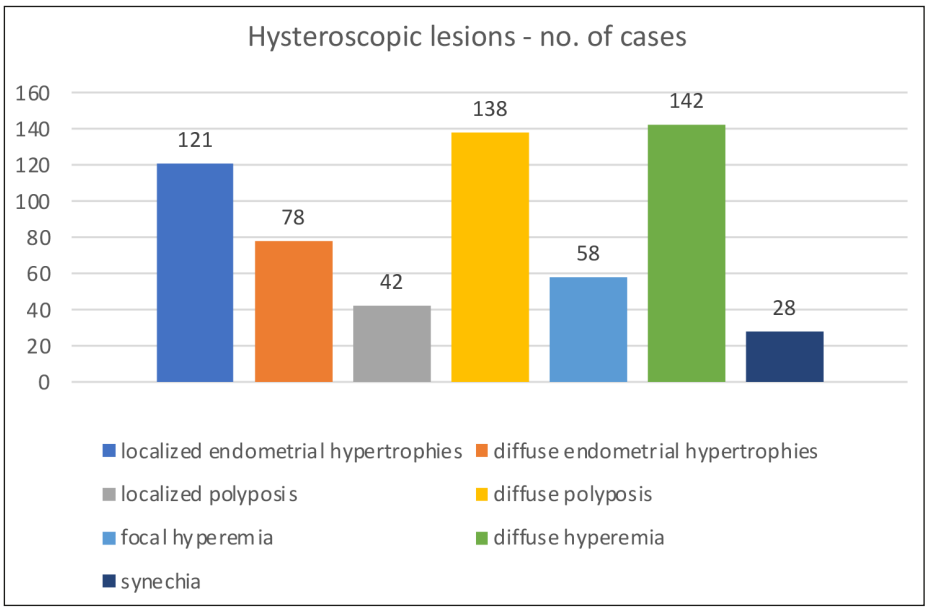

Figure 2. Endometrial lesions observed at hysteroscopy.

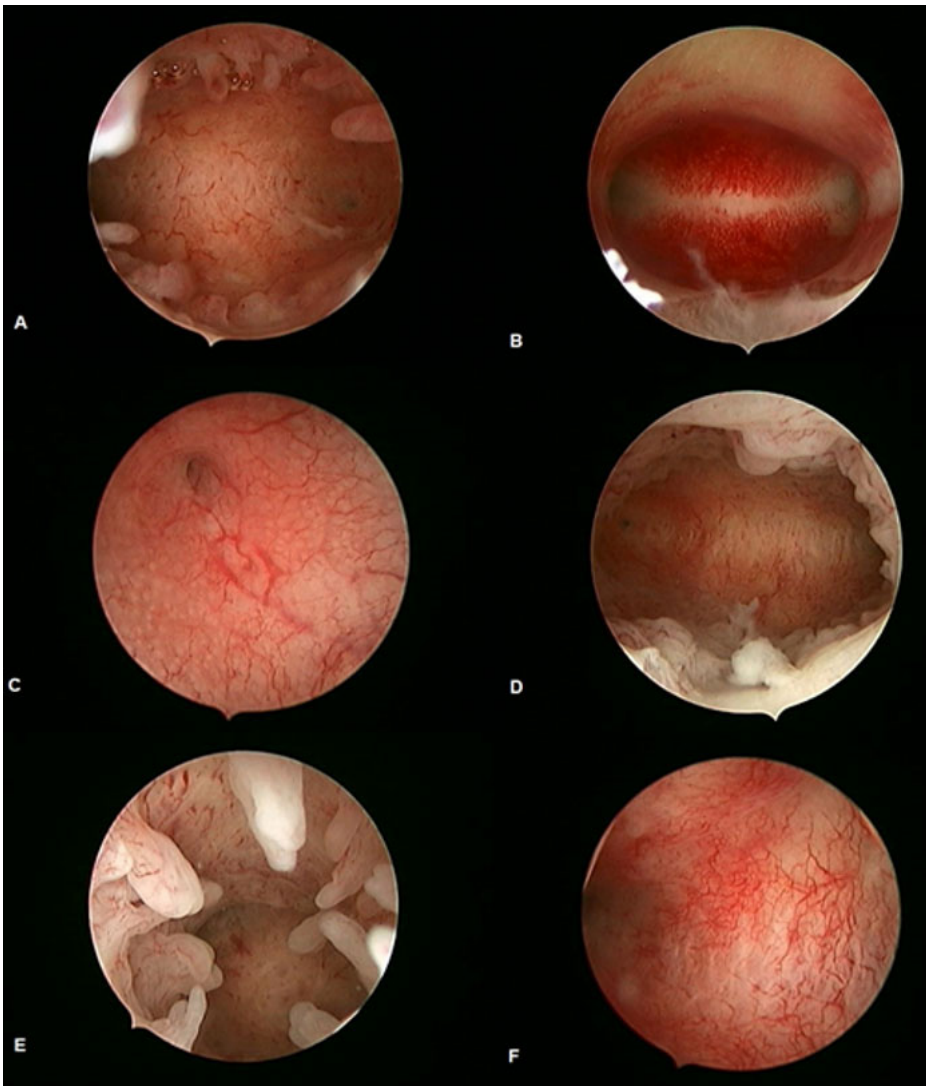

Figure 3. Chronic Endometritis – Hysteroscopic aspects. **A**, Micropolyps and microvascularization. **B**, Congestive endometrium. **C**, Bright red areas of endometrium with white central dots (strawberry aspect). **D**, Sessile micropolyps, non-homogenous endometrial thickening. **E**, Peduncolatous and vascularized polyps. **F**, Vascular dystrophy, focal or diffuse periglandular hyperhemia..

As a result, we obtained pregnancies in 64 cases, a percentage of 43.2%. In the group that was investigated hysteroscopically and did not receive antibiotic treatment, we obtained pregnancies in 12 cases, a percentage of 27.3%.

We wanted to determine if the difference was statistically significant. Therefore, we applied the Chi-squared test and Fisher's exact test, and the results were statistically significant ($p = 0.0405$).

Discussion

This is a historical cohort study on the effect of antibiotic treatment on patients diagnosed hysteroscopic and histopathologic with CE and undergoing a human-assisted reproductive procedure.

Endometritis is defined as an inflammation of the endometrium, which can be caused by many factors. It can manifest in acute or chronic forms depending on the causative factor or the symptoms presented by the patient[14].

The acute form is very rarely diagnosed and, in many cases, is a short-term transitional phase to the chronic form. It is usually caused by persistent placental or abortive residues or can result from an untreated pelvic inflammatory disease or another inflammatory pathology in the urogenital tract[14,15]. Numerous studies[15,17,18] have shown that prolonged menstrual bleeding, previous abortions, uterine curettage, tubal pathology or a history of bacterial vaginal infections are risk factors for this pathology.

As mentioned above, chronic inflammation can follow the acute stage or appear as chronic

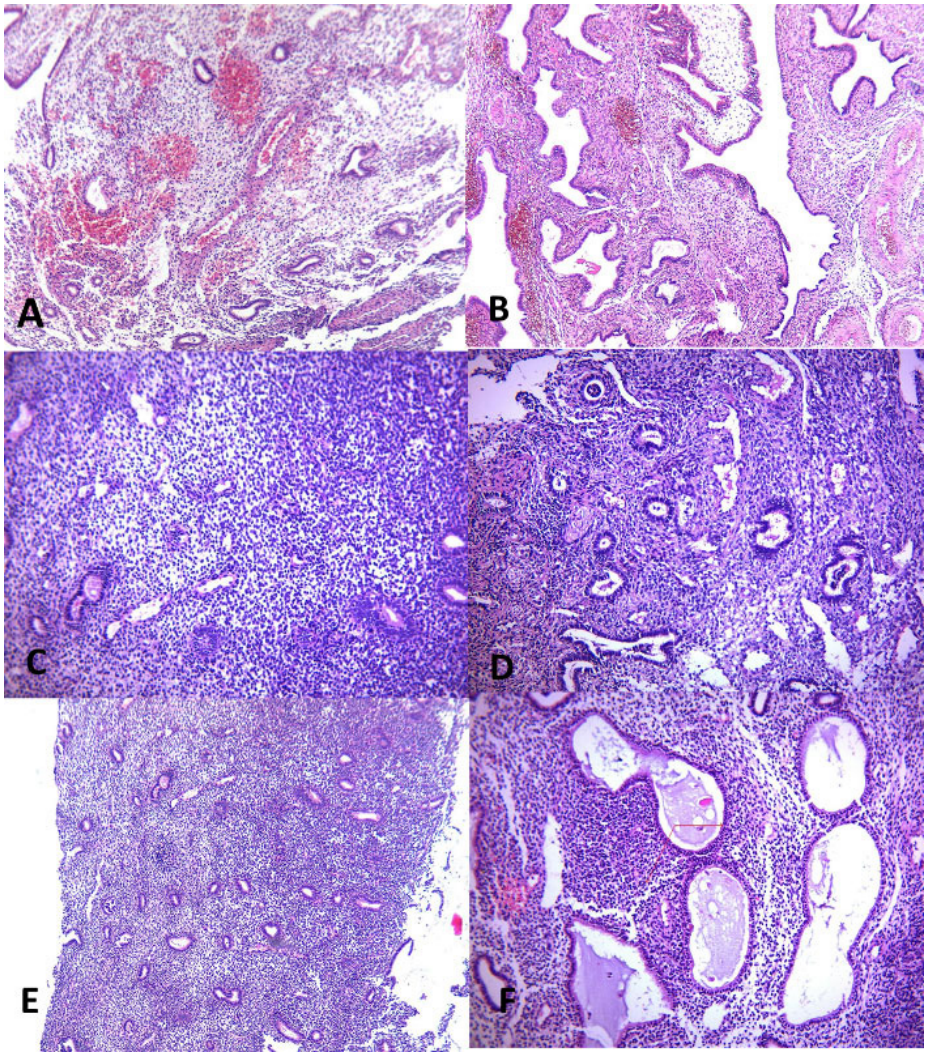

Figure 4. Endometrial biopsy (HE). **A**, CE with polypoid appearance and rich hemorrhagic infiltrate in the stroma (x20). **B**, CE with micropolyps (x20). **C**, CE with periglandular lymphoplasmic infiltrate (x40). **D**, CE with stromal hyaline areas (x20). **E**, Lymphocytic CE with a tendency to form germinal centers (x20). **F**, CE with simple hyperplasia without atypical and rich chronic inflammatory periglandular lymphocytic infiltrate (x20).

inflammation "*ab initio*". This chronic form can result from multiple factors: inflammation, genetics, and immunity system. Recent research[16] demonstrates the remarkable therapeutic effects of stem cells in infertility related to thyroid autoimmunity, one of the women's most prevalent autoimmune disorders. However, the most common form of CE is the inflammatory one. CE has a microbiological origin or a mechanical-chemical origin. Foreign bodies generally cause CE of chemical-mechanical origin in the genitals, such as cervical weighing or intrauterine devices.

Our study shows a high number of CE in patients with infertility who are to undergo the IVF procedure (43%). Similar studies[17-20] confirm this observation (10.8-53.6%). Therefore, it was expected to be found in couples with a long history of infertility who underwent numerous infertility treatments and eventually reached the IVF procedure. However, we noticed that most of these had a history of pelvic inflammation (69.9%). Nevertheless, this pathology had an insidious or absent symptomatology, further delaying the decision to treat infertility.

CE is a pathology that is difficult to identify. It cannot be detected by clinical or vaginal ultrasound examinations, which are performed on an outpatient basis. CE can only be suspected in rare cases where complications occur: synechiae, hydrometry or pyometry[20]. In this context, hysteroscopy has long been proven one of the best techniques for identifying CE[21].

In recent years, the interest in studying CE has dramatically increased because it has been associated with impaired fertility. After all, endometrial inflammation appears to alter the physiological mechanisms of oocyte fertilization, prevent embryo implantation or may induce early abortion[22,23].

Some authors[3,24,25] consider that CE would not affect the results of IVI/ICSI therapy. Other authors[26,28,31] argue that inflammatory aspects of the endometrium observed hysteroscopically correlate with IVF failure, and there is a clear link between endometrial congestion and positive sperm culture. We found a 52% frequency of endometrial congestion. Micropolyps mainly manifest chronic CE; they are 3.2 times more common in infertile patients who have been investigated hysteroscopically[14]. The same conclusion was reached by Feghali et al[27] who found signs of CE in 45% of the IVF cases examined hysteroscopically. We found the presence of CE in 43% of the cases studied. Repeated pregnancy loss was also associated with chronic endometritis[28].

The leading cause of CE is an infection of the endometrium caused by microorganisms that frequently populate the lower genital tract. Endometrial cultures are positive in about 60% of cases. The most common incriminating agents were pyogenic pathogens (70% of cases), such as streptococci, staphylococci, enterococci, and Escherichia coli. However, we can also find cases of bacteria such as *Chlamydia trachomatis* (10%), Mycoplasma and Ureaplasma (about 20%)[18]. Although it has a low incidence, tuberculous endometritis is also worth mentioning. In many cases, due to the polymorphism encountered, the etiology is polymicrobial[23].

In specialized literature[29], there are data regarding CE therapy. In this regard, injecting embryo culture supernatant into the endometrial cavity was not beneficial.

Usually, treatment of CE is based on the administration of broad-spectrum antibiotics performed according to the antibiogram, when it can be performed, or empirically. The most prescribed treatment is doxycycline or cephalosporins, macrolides or quinolones. The partner should undergo the same treatment[30]. Thus, doxycycline administered in doses of 100 mg repeated at 12 hours for 14 days had a cure rate in 60% of cases[31].

We observed that prior therapy of CE has a positive effect in terms of the percentage of pregnancies obtained through IVF/ICSI.

For this reason, we recommended a previous antibiotic treatment in all cases where we found endometrial inflammatory aspects by direct observation and histological examination. Our study used a combination of antibiotics from the quinolone group and the imidazole group. Moreover, this is because germs cause most upper genital tract infections: Ureaplasma, Mycoplasma, and Chlamydia but also other non-specific anaerobic germs that come by ascending them from the vagina. From the point of view of pregnancy rate obtained by IVF, the difference was statistically significant between the two studied groups, with and without antibiotic treatment ($p < 0.05$).

Other authors[23,32] have observed that the antibiotic treatment offers optimal results, and the hysteroscopic and biopsy examination greatly helps.

Furthermore, it should finally be emphasized that treating chronic endometritis can increase the number of embryo transfers performed on frozen cycles. Embryo transfer with frozen embryos, especially in the case of patients initially diagnosed with chronic endometritis, should not be discouraged because, as demonstrated, this type of embryo transfer is not followed by poorer results compared to embryo transfer with fresh embryos[33-35].

Conclusions

The appearance of CE is mainly due to the local action of the germs in the genital tract. Therefore, diagnosis of CE by hysteroscopy and treatment with antibiotics gives the possibility of obtaining superior results following the IVF procedure. However, further studies are needed to determine whether and which interventions – hysteroscopy, biopsy, treatment, or a combination of these – can most influence the results achieved by IVF.

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Conflicts of Interest
The authors declare no conflicts of interest.

Informed Consent
Informed consent was obtained from all the subjects involved in the study.

Availability of Data and Materials
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' Contributions

CCV and MT (Conceptualization, data curation, investigation, formal analysis, methodology, and writing of the original draft); MSS and MAS (Conceptualization, Methodology, Writing, review and editing); CCV and MBN (supervision, validation, project administration). All the authors have read and agreed to the published version of the manuscript.

ORCID ID

Cristian C. Vaduva: 0000-0001-7526-1756

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