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Evaluation of the Endometrium in the XXI Century

Moreno Luna Estefanía

Moreno Luna Estefanía[1], 0009-0001-2931-1481; García de Miguel Laura[1].

ABSTRACT

The endometrium is a fundamental layer of the uterus that is relevant to female reproductive function. This specialized tissue lines the inner wall of the uterus and its condition and health are essential to the process of conception and subsequent embryo implantation.

Since the early days of reproductive medicine, the maternal endometrium has been considered a passive part of the reproductive process; a "good quality embryo" was all that mattered.

This fact requires revision since the efficacy of in vitro fertilization remains low despite considerable improvements in embryology and embryo transfer technologies in recent decades. We now know that human implantation is a very complex and multifactorial process. Successful implantation requires the presence of a healthy embryo, a receptive endometrium and a synchronized molecular dialogue between the two, as well as host immune tolerance/protection.

The study of the endometrium is one of the fundamental aspects to be taken into account in assisted reproduction treatments. It is a necessary practice that is routinely performed in assisted reproduction clinics, specifically in cases of implantation failure and repeated miscarriage.

KEYWORDS

Preimplantation genetic testing for aneuploidy, Recurrent implantation failure, Endometrial receptivity, Window of implantation, Embryo transfer.

ABBREVIATIONS

PRP	Platelet-rich plasma
RIF	Recurrent implantation failure
CE	Chronic endometritis
PGT-A	Preimplantation genetic testing for aneuploidy
BV	Bacterial vaginosis

MANUSCRIPT

Introduction

The endometrium is an essential layer of the uterus that is relevant to the female reproductive function. This specific tissue lines the inner wall of the uterus and its condition and health are essential to the process of conception and subsequent embryo implantation.

Nowadays, we know that human implantation is a very complex and multifactorial process.

[1] Clínica Tambre, Calle Tambre, 8. 28002 Madrid (Spain).

NOTE: The numbers following the affiliation markers are the author's ORCID iD.

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CORRESPONDENCE:

Moreno Luna Estefanía
emorenol@clinicatambre.com

Successful implantation requires the presence of a healthy embryo, a receptive endometrium and a synchronized molecular dialogue between the two, as well as the host immune tolerance /protection.

Implantation is initiated by adhesion of the blastocyst to the epithelial layer of the endometrium[1].

Preimplantation genetic testing for aneuploidy (PGT-A) reports the transfer of normal embryos based on chromosomal analysis[2] to avoid chromosomal aneuploidies, which can cause early pregnancy lost and implantation failure.

Despite of many publications on recurrent implantation failure (RIF)[3, 4], there is no universal accepted definition yet.

According to the study published by Pirtea in 2021, the incidence of RIF in women who have a normal uterus (anatomy) and undergo 3 consecutive transfers of single euploid embryos is less than 5%[5].

There are other publications that consider endometrial abnormalitiy the cause of implantation failure in assisted reproduction treatments. Ledee et al.[6] studied the endometrial immune function by measuring NK cells and other markers of inflammation in endometrial biopsies from the luteal phase. Excessive or insufficient immune responses, assessed by biopsies, are given as an explanation for implantation failure[7]. Unfortunately, there are no validated and available diagnostic tests to confirm immune-mediated implantation failure[8]. These investigators recommend different therapeutic options to treat the abnormal uterine immune response, from increasing the dosage of vaginal progesterone to intravenous intralipid administration and corticosteroid therapies. Therefore, the immune therapies are often initiated empirically without solid evidence of efficacy[8].

Abnormal results in the study of blood coagulation may also be a condition for implantation failure and pregnancy loss[9].

In the recent years, the timing of progesterone-induced endometrial changes has been assessed by gene expression panels in endometrial tissue[6,7,8] rather than histological changes. Gene assessments performed on luteal endometrial findings are reported as prereceptive, receptive, or postreceptive. Recommendations made by proponents of these tests are that adjustments need to be made in the timing of embryo transfers to achieve a synchronous window of implantation[10]. More recently, endometrial assessment strategies based solely on hormonal changes in the endometrium have been questioned, recognizing that the endometrium may simply be pathologic (or altered).

Refractory Endometrium

Another important factor to take into consideration is the endometrial thickness. It is a prognostic parameter for an embryo transfer. Refractory endometrium is considered endometrium of less than 7mm on the day of ovulation or on the day of human chorionic gonadotrophin (HCG) injection in fresh in vitro fertilization (IVF) cycles, or when progesterone starts in frozen and thawed embryo transfer cycles[11]. Endometrial thickness is directly correlated with levels of estrogens[12].

This is a rare finding, present in only 2-3% of patients undergoing assisted reproductive technology. Multiple therapeutic approaches have been described with questionable results such as high doses of estrogens, different methods of estrogen administration (oral, vaginal, transdermal, intramuscular), adjuvant treatment with vasoactive agents such as aspirin, vitamin E, pentoxifylline, sildenafil citrate, granulocyte colony stimulating factor (G-CSF) IU infusion, stem cell therapy and platelet-rich plasma instillation.

Garcia-Velasco, showed that platelet-rich plasma and sildenafil citrate could have a beneficial effect in this type of patients[13].

Xin Li et al[14] described that the endometrial thickness in patients who received sildenafil citrate was significantly greater than in the control group (placebo or no treatment); the radial artery resistance index was significantly lower and the clinical and biochemical pregnancy rate were significantly higher in the sildenafil citrate group compared to the control group.

Uterine PRP is an experimental treatment consisting of a concentration of protein derived from blood, free of red blood cells, containing severe cytokines, as well as a large group of growth factors, such as interleukin 8 (IL-8), insulin-like growth factor I, II (IGF-I, II), vascular endothelial growth factor (VGEF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF) and connective tissue growth factor (CTGF); and it is supposed to increase endometrial growth and receptivity. The regulatory effect of PRP on the expression of growth factors and cytokines in the endometrium is due to its anti-inflammatory and pro-regenerative functions[12, 15,16]. Although PRP is widely used in other fields, its clinical efficacy in the field of obstetrics and gynecology is still very limited.

During the implantation window, around days 19-23 of each cycle, a molecular cascade leads to the creation of implantation and pregnancy proteins; cytokines, growth factors, prostaglandins and adhesion molecules are among these proteins, and their deficiency has been shown to be related to implantation

failure[17]. The hypothesis is that since PRP contains various growth factors and cytokines, it could stimulate proliferation and regeneration, enhance endometrial receptivity and improve implantation. Therefore, intrauterine infusion of PRP could have a positive effect on implantation and pregnancy[18].

Agarwal described the beneficial effect of hysteroscopic injection of PRP in the subendometrial region, showing an improvement in endometrial thickness and higher pregnancy rates in patients with previous thin endometrium[19].

Endometrial Microbiota in Human Reproduction and Chronic Endomeritis

The community of microorganisms (bacteria, fungi, archaea, viruses and parasites) that coexist with every human being inside or on the external surface of the body is called the "human microbiota and its genomic constitution is called the microbiome"[20].

The term microbiome "comprises the entire habitat, including the microorganisms, their genes and their environmental conditions"[21].

In the last few years, there is a great interest in the study of the endometrial microbiota and its impact on the success of embryo transfer.

The uterine microbiota of women of reproductive age is mainly composed of five types of "community states; four of them are lactic acid-producing Lactobacillus and the fifth are mainly aerobes and strict anaerobes[22].

Lactic acid production has been associated with contributing to the overall health of the vagina due to its direct and indirect effects on pathogens and host defense. Some bacterial species outside the Lactobacillus family can trigger immune responses and degrade the host mucosa, processes that increase susceptibility to infection and contribute to negative reproductive outcomes such as infertility and preterm delivery[23].

In general, the microbes that exist in the uterus present a mutualistic relationship with the host, that is beneficial for both, and they work as a first barrier of defense against the colonization of opportunistic pathogenic organisms.

As mentioned above the microorganisms found in the highest percentage in the vagina and endometrium belong to the Lactobacillus family, and these are: L.crispatus(CST-I), L.iners(cst-III), L.gasseri(CST-II) and L.jensenii(CST-V). These microbial communities are associated with healthy women of reproductive age and allow the production of large amounts of lactic acid achieving an acid pH < 4.5. Such an acidic environment is protective against infections or colonization of the endometrium by non-native pathogens and microbes[23].

The increased presence of Gardnerella, Atopobium, Mobiluncus, Prevotella and Clostridiales[24], means a decrease in Lactobacillus which is associated with clinical symptoms resulting in discharge, foul odor and irritation. Usually, this symptomatology translates into bacterial vaginosis (BV).

In general, the microbes that exist in the uterus present a mutualistic relationship with the host, that is, both benefit and function as a first barrier of defense against the colonization of opportunistic pathogenic organisms. As mentioned above the microorganisms found in the highest percentage in the vagina and endometrium belong to the Lactobacillus family, and these are: L.crispatus(CST-I), L.iners(cst-III), L.gasseri(CST-II) and L.jensenii(CST-V). These microbial communities are associated with healthy women of reproductive age and allow the production of large amounts of lactic acid achieving an acid pH < 4.5. Such an acidic environment is protective against infections or colonization of the endometrium by non-native pathogens and microbes[23]. The increased presence of Gardnerella, Atopobium, Mobiluncus, Prevotella and Clostridiales[24], means a decrease in Lactobacillus which is associated with clinical symptoms resulting in discharge, foul odor and irritation. Usually this symptomatology translates into bacterial vaginosis (BV).

Bacterial vaginosis is usually caused by the presence of aerobes such as group B Streptococcus, Staphylococcus aureus, Escherichia coli and Enterococcus. These organisms produce an inflammatory response and destroy the acidic environment generated by the other community groups, meaning an increase in pH>4, which is associated with a diseased vagina.

Women with BV have an increased risk of adverse outcomes during childbirth, in addition to an increased likelihood of contracting sexually transmitted diseases such as HIV. It can also induce associated complications such as "chorioamnionitis, endometritis, salpingitis[24].

Currently for the study of human microbiota, 16S rRNA gene sequencing is used in the identification of complex microbial communities due to its feasibility to infer the representation of certain disease-causing microbial communities[20].

The 16S rRNA gene is a commonly used gene for the detection of microorganisms because it is present in the DNA of bacteria. It is transcribed and translated in the 16S rRNA, i.e. in the small subunit of the bacterial ribosome. It is a conserved sequence in all bacteria so

that there will be specific regions of the gene in all bacteria and other unique regions. The specific regions are used for the design of primers for subsequent PCR amplification of the gene. That is why the sequencing of the amplified 16S rRNA gene can serve as a marker to identify the bacteria in a given sample. For all these reasons, it is a very recurrent technique for the identification of the vaginal and endometrial microbiota in order to evaluate the presence of microorganisms that may be positively or negatively affecting the health of the female genital tract and to predict the success of IVF.

In the study by Chen et al, transcriptome and 16s rRNA sequencing technology was combined to analyze the interaction between endometrial microbial disorder caused by chronic endometritis and immune cells in the endometrium of patients with recurrent implantation failure. It was observed that the composition of endometrial microorganisms of patients with chronic endometritis and those without endometritis was significantly different. Phyllobacterium and Sphingomonas mainly regulated immune cells by interfering with the process of carbohydrate and/or fat metabolism in the endometrium[25].

We are currently having the question if it its necessary to perform a preliminary study of chronic endometritis (CE) in patients undergoing assisted reproduction treatments.

In most cases, chronic endometritis is caused by an alteration of the normal endometrial microbiome by bacterial pathogens. It is a possible origin of cronic endometritis the infection because antibiotic therapy normalizes the endometrium and improves clinical outcomes[26]. However, non-infectious forms of chronic endometritis may also exist. Clinically, it is silent or asymptomatic in most cases, which makes it difficult to diagnose. Symptoms are usually mild and nonspecific, with abnormal uterine bleeding, pelvic pain and dyspareunia.

Chronic endometritis is characterized by an alteration in the quantity and quality of leukocyte infiltration. Leukocytes are usually organized in nodular aggregates that infiltrate glands and vesicles, leading to structural alterations. In addition, abnormal leukocyte subpopulations (increased B cells and plasma cells, reduced NK cells cause alterations in the expression of inflammatory mediators and cytokines. The inflammatory milieu may alter the expression of genes involved in cell replication, the implantation process and also the immune tolerance of the endometrium to enhance embryo implantation[27]. Finally, an alteration of autophagy observed in chronic endometritis may affect endometrial cell function and impair endometrial decidualization[28].

Inflammatory mediators can alter uterine contractility during the mid-luteal phase, preventing fertilization and transuterine migration of the embryo before implantation[29].

Cicinelli's group considers a possible routine screening for chronic endometritis in all patients undergoing assisted reproductive technology or women with an adverse obstetric history (such as miscarriage) because of the deleterious effect of this pathology on reproductive outcomes[30].

However, the ASMR (American Society for Reproductive Medicine) does not recommend routine endometrial biopsy for screening for cronic endometritis prior to ART. Also, they say that there are not clear diagnostic criteria.

There are two methods to diagnose EC: hysteroscopic visualization of endometrial lesions with moderate specificity and sensitivity[31] and endometrial biopsy with histology.

Endometrial biopsy is considered the gold standard for the diagnosis of EC, based on the identification and counting of plasma cells in the endometrial stroma[32]. For this purpose, IHC staining with CD138 is routinely used, which allows a simple and reliable identification of plasma cells (PC) in endometrial tissues, thus gaining popularity over the classical hematoxylin-eosin analysis. However, there is no clear evidence for the number of plasma cells required for the diagnosis of CD; we note that it varies among investigators. Some studies use 1 to 5 PC per HPF, while others use 1 PC per 10 HPF, and still others use different criteria[33]. These levels were arbitrarily selected without having a clear reference population. The dilemma of the Plasma cells originates from the fact that PCs are not evenly distributed throughout the endometrial stroma and may even be concentrated in patches or scattered throughout the stroma.

In Pirtea's work, they describe that the clinical impact of CE is of short duration and usually resolves without treatment or is very infrequent[5], so they do not support the idea of the systematic study of CE in patients prior to ART.

On the other hand, patients diagnosed with endometriosis have a higher incidence of chronic endometritis. This does not mean that it has an impact on the final results of assisted reproduction treatments and, in particular, on live birth rates, since deferred transfer protocols are currently preferred in endometriosis[30].

Another factor to be taken into account is uterine disorders. Uterine pathology is a risk factor for the development of chronic endometritis[34]. Endometrial polyps and uterine synechiae are the two pathologies

most significantly associated with CE. The diagnosis of CE is less frequent in patients with a septate uterus. Hysteroscopic surgery can cure most cases of CE without antibiotherapy simply by surgically correcting the pathology, regardless of the type of intrauterine abnormalities. This type of surgery should be indicated before the administration of antibiotics[34].

Endometrial Receptivity Test

Methods to explore endometrial receptivity have been carried out by genomic studies. This is the case of the endometrial receptivity microarray (ERA) developed in 2008 and whose objective is to "define the transcriptomic of the endometrial receptivity"[35]. For this purpose, a cohort study was performed, including three groups of subjects: one to select the genes to be included in the array, another for endometrial dating and a last one with pathological endometrial samples to train the predictor. In total, 95 patients were included in the this study. For the selection of genes that were related to endometrial receptivity, expression profiles of the entire human genome were analyzed, focusing on the receptive and pre-receptive endometrium. The inclusion criteria for these were those showing a fold change > 3 and a p value < 0.05. Agilent technology was used to fabricate the microarray and it underwent a training process to train the predictor and locate the receptivity genes. The results showed that there were 238 genes that were differentially expressed in the transition between the pre-receptive and the receptive phase. The objective was to demonstrate the importance of studying the state of the endometrium in reproductive medicine during the WOI (window of implantation).

In 2018, another study was published. The objective was to demonstrate the existence of another test called ER Map/ ER Grade that could predict endometrial receptivity status by RT-qPCR using a new panel of genes involved in endometrial proliferation and maternal immune response associated with embryo implantation. For this purpose, a cohort of 216 patients with endometrial samples including fertile women and patients who had undergone fertility treatments was analyzed for the expression of 184 genes involved in endometrial receptivity. The results in this case showed that 85 of the 184 genes had significant differential expression and showed that these genes were associated with aspects such as cell division and proliferation, vascular proliferation, embryo implantation. In addition, 40 of these genes allowed the classification of the endometrium according to its state of receptivity into pre-receptive, receptive and post-receptive. The conclusions of this new study were similar to the one published in 2008 and establish that identifying the optimal time to perform embryo transfer is essential to increase the success rate in assisted reproduction[36]. According to Simón et al. personalized embryo transfers statistically significantly improved pregnancy, implantation and cumulative live birth rates[37]. In general, 62.5% of the population have receptive implantation windows with 5 days of progesterone impregnation[37].

However, and despite what has been published, the efficiency of this type of test is increasingly controversial and there are multiple publications that affirm that personalized transfer according to the ERA protocol does not provide any benefit in patients undergoing euploid embryo transfer[38,40].

In patients who did obtain an euploid blastocyst as an outcome after IVF treatment, the use of receptivity testing to guide the timing of frozen embryo transfer compared to the standard timing of transfer did not significantly improve the live birth rate[39,40].

The evidence does not support the routine use of endometrial receptivity tests to guide the timing of embryo transfer during in vitro fertilization[40].

CONCLUSIONS

The study of the endometrium is one of the fundamental aspects to be considered in assisted reproduction treatments. It is a necessary practice that is routinely performed in assisted reproduction clinics, specifically in cases of implantation failure and repeated miscarriage.

The impact and management of thin endometrium and RIF are a common challenge for patients undergoing assisted reproduction. Both are an infrequent but challenging occurrence in assisted reproduction.

Currently, there is minimal evidence to support any specific protocols or adjuvants to significantly improve pregnancy outcomes in patients with thin endometrium.

PGT-A may be beneficial for patients with recurrent implantation failure. However, endometrial receptivity test does not appear to be clinically useful for patients with RIF.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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