The Genetic and Biochemical Blueprint of Endometrial Receptivity: Past, Present, and Future Factors Involved in Embryo Implantation Success

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Abstract

In the field of assisted reproductive technology, endometrial receptivity is a crucial aspect that affects implantation rates in *in-vitro* fertilization procedures; in fact, impaired endometrial receptivity has been identified as the rate-limiting step for favorable pregnancy outcomes once factors regarding embryo quality have been optimized. The endometrium is a dynamic tissue that undergoes proliferative and secretory changes in each menstrual cycle, acquiring a short and transient period of embryo receptivity known as the Window of Implantation. Precise embryo-endometrial synchrony is necessary to achieve a successful pregnancy, and it involves complex and multifactorial processes related to morphological, biochemical, and genetic changes. On that behalf, defining the receptive window of each patient for personalized embryo transfer is a current goal. Here, we review different indicators of endometrial receptivity throughout the menstrual cycle, spotlighting the opening of the window of implantation: classical histological and biochemical markers, genetic factors, leading-edge transcriptomic signatures and miRNA profiles, and novel features such as the microbiome and secretome. Understanding the molecular mechanisms behind endometrial receptivity will facilitate the optimization and improvement of infertility treatments.

Keywords: endometrial receptivity, embryo implantation, menstrual cycle, window of implantation, decidualization

1. Introduction

The field of assisted reproductive technology (ART) has grown significantly in use and understanding over the past few decades, nevertheless, the rates of successful pregnancies in *in-vitro* fertilization (IVF) procedures are still relatively low. Impaired endometrial receptivity (ER) has been identified as the rate-limiting step for favorable pregnancy outcomes once all other factors, including the acquisition and selection of the best quality embryo(s), have been optimized. Correct and synchronized maturation of the endometrial tissue is essential for embryo
2. Factors involved in endometrial receptivity

2.1 Evaluation of endometrial morphology for receptivity assessment

Morphological changes during the endometrial cycle generate markers that have been used over decades to assess receptivity, such as histological evaluation of a biopsy and ultrasound examination of the endometrium. Endometrial biopsies are now
considered to provide little clinically relevant information [15, 16]. Additionally, the formation of pinopodes was thought to show potential as a clinical marker to assess ER [17]. However, the presence of pinopodes was demonstrated not only during the WOI but also in the post-receptive endometrium, precluding in this way its use as a marker of ER [17, 18]. On the other hand, ultrasound examination is a routinely used technique in IVF procedures [9, 19]. This non-invasive technique is based on the interpretation of a medical ultrasound of the endometrium. Various ultrasonographic parameters have been proposed as pregnancy predictors, such as endometrial thickness, volume, and blood flow patterns. The most commonly used is endometrial thickness [20, 21]. Due to differences in stimulation protocols, sonographic approaches, and difficulties in obtaining a standard sagittal view of the uterus, discrepancies in the cut-off value of endometrial thickness to achieve pregnancy arise [22]. Generally, it is considered that a minimum of 6–8 mm in endometrial thickness is necessary for a successful pregnancy [23–25]. Nevertheless, case reports have described pregnancy establishment despite an endometrial thickness of no more than 4 mm [26, 27]. Three-dimensional (3D) sonography assesses ER by considering endometrial thickness, volume, and angiogenic dynamics. The endometrial volume of fewer than 2 ml has been shown to decrease pregnancy rates significantly [19, 28, 29]. Another evaluated criterion is an endometrial pattern, which can be classified as triple-line, intermediate, or homogenous [30]. Among these, the triple-line pattern has been suggested to reflect ER [24, 31] broadly. Finally, the impact of ovarian stimulation on ER has yet to be determined. Abnormal hormone concentrations due to stimulation protocols during IVF might affect endometrial morphology and thereby ER [32]. Comprehensively, although morphological elements are important components of receptivity, there is still no consensus on the extent in which they can be used as WOI predictors.

2.2 Genetic factors involved in ER

Endometrial genetic abnormalities can lead to implantation failure due to dysregulation of critical processes such as trophoblast invasion and angiogenesis. Here, we discuss common genetic abnormalities that have been analyzed to determine their role in implantation failure (Table 1). Parental chromosomal abnormalities such as mutations and translocations should be considered relevant in the efficacy of improving reproductive outcome.

2.2.1 Angiogenetic factors: vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), TP53 tumor suppressor (TP53), murine double minute 2 (MDM2), herpes virus-associated ubiquitin-specific protease (HAUSP)

Successful pregnancy is dependent on adequate placental circulation and fetal vasculature. The development of a normal vascular network during implantation, embryo development, and placentation requires cooperation between different cell types and various growth factors. VEGF is a potent angiogenic factor that plays an essential role in embryo implantation/development. Four VEGF polymorphisms have been reported to affect VEGF activity and expression increasing aberrations in vascular formation and/or function. The polymorphism −1154G/A located in the promoter region has been associated with RPL [33], RSA [34, 35], and RIF [36, 37]. Moreover, a meta-analysis showed that genotypes −2578C/A, −634G/C, and 936C/T increase the risk of RSA as well [38]. Furthermore, eNOS, which is expressed in the terminal chorionic villous vessels, is important for vascular nitric oxide (NO) production to supply nutrients to the fetus. Only the eNOS Glu298Asp polymorphism has been shown to be significantly associated with RPL [39]. Additionally, successful trophoblast invasion requires the induction of paracrine apoptotic reactions to
| Gene | Polymorphism | rs code | Relevance | References |
|------|--------------|---------|-----------|------------|
| APOE |              |         | Heterozygous genotype is more frequent in women with RPL | [33] |
| eNOS | VNTR (B/A)   |         | Association to the risk of RPL | [77] |
|      | Glu298Asp    |         | No associated | [78] |
|      |              |         | Homozygote genotype T/T is associated with risk of IRM | [39] |
| ESR1 | IVS1-397T>C  | rs2234693 | Related to unknown thin endometrium in which P allele may be the risk and X allele its guard factor | [44] |
|      | IVS1-351A>G  | rs9340799 |         | |
| F2   | G20210A      | rs179963 | No association | [58, 60, 62, 63] |
|      |              |         | Heterozygous genotype is more frequent in women with RSA in the first trimester [57] and women with RPL [42] | |
| F5   | G1691A, Leiden | rs6025 | No association | [57, 58, 62, 63] |
|      |              |         | More frequent in women with RIF | [60] |
|      | H1299R (R2)  |         | No association | [58, 62, 63] |
|      | Y1702C       |         | No association | [58, 62, 63] |
| F8   | V34L         |         | More prevalent in women with RPL | [42, 58] |
|      |              |         | No association | [62, 63] |
| FGB  | G-455A       | rs1800790 | No association | [57, 58, 62, 63] |
| GPH1a| C1565T       |         | No association | [58] |
| HAUSP| rs1529916 G/A | rs1529916 | Allele A is associated with RIF | [40] |
| HPA1 | HPA1 a/b (L33P) |         | No association | [58, 62, 63] |
| LIF  | C715A        |         | No associated | [72] |
|      | G3400A       |         | More frequent in nulligravid women | [72, 73] |
|      | G3424A       |         | No associated | [72] |
|      | T1414G       | rs929271 | G/G genotype is associated with RIF | [40, 74] |
| MDM2 | T309G        | rs227944 | Allele G is associated with RIF | [40] |
| MTHFR| A1298C       | rs1801131 | No association | [58, 62–64] |
|      | C677T        | rs1801133 | More frequent in women with unexplained infertility [64], RPL [42, 58], and RSA [60] | [42, 58–60] |
|      |              |         | No association | [57, 58, 63] |
| MUC1 | VNTR         |         | Women with unexplained infertility might have susceptibility to implantation failure due to small MUC1 allele size | [67] |
|      |              |         | No association | [68, 69] |
| MUC4 | VNTR         |         | No association | [70] |
| PAI-1| 4G/5G        | rs1799889 | More prevalent in women with RIF [63] and RPL [42, 58] | [42, 58, 63] |
|      |              |         | No association | [62] |
secrete proteases capable of digesting the endometrial extracellular matrix (ECM) [36]. TP53 is a potent regulator of apoptosis, cell cycle, angiogenesis, and embryonic development. A TP53 polymorphism at codon 72, encoding either proline or arginine, was reported to alter the TP53 activity and affect human fertility [40]. The Arg72 variant has been shown to induce higher apoptotic activity than Pro72. Therefore, Pro72 variant might cause inadequate trophoblast invasion, increasing the risk of RPL [41] and RIF [37, 40]. In this manner, women with Pro/Pro genotype have a higher risk of RPL than women with the Arg/Arg or Arg/Pro genotypes. Following, MDM2 and HAUSP regulate TP53. MDM2 binds to TP53 to degrade it through poly-ubiquitination, blocking its ability to function as a transcription factor. The \( \text{MDM2} \) SNP309 is a functional SNP that increases MDM2 expression levels and attenuates TP53 pathway. HAUSP, on the other hand, acts as a specific deubiquitinase for TP53, the A allele has a significant association with infertility in young patients (<35 years) but not in the older patients, similarly to the \( \text{MDM2} \) SNP309 G allele. Those observations suggest that \( \text{MDM2} \) and \( \text{HAUSP} \) may be involved in the regulation of human fertility through the regulation of TP53 [40].

| Gene | Polymorphism | rs code | Relevance | References |
|------|--------------|---------|-----------|------------|
| \( \text{PR} \) | H770H-C/T | | No association | [54] |
| G/T—Val660Leu | rs1042838 | | More prevalent in women with unexplained infertility | [65] |
| V660L | | | No association | [54] |
| \( \text{PT53} \) | Codon 72 Pro | rs1042522 | Homozygote genotype is associated with RPL [41, 75], IRM [76] and RIF [37, 40] | [37, 40, 41] |
| Codon 72 Arg | rs1042522 | Homozygote genotype is associated with RIF | [75] |
| \( \text{PTGS2} \) | G-765C | rs20417 | Association with implantation failure susceptibility | [55] |
| \( \text{TFF3} \) | rs225361 A/G | rs11701143 | Homozygous genotype is associated with less live births before their first spontaneous abortion | [61] |
| rs225361 A/G | rs11701143 | No association | [66] |
| rs11701143 T/C | rs225361 | Associated with idiopathic RSA | [61] |
| rs11701143 T/C | rs225361 | No association | [66] |
| rs225439 G/A | rs225439 | No association | [61, 66] |
| rs533093 C/T | rs533093 | No association | [61, 66] |
| rs77436142 G/C | rs77436142 | No association | [61, 66] |
| \( \text{VEGF} \) | G-1154A | rs1570360 | Homozygote A/A genotype associated with RSA [34, 35], RPL [33], and RIF [36, 37] | [33–37] |
| | | | No associated | [71] |
| C-2578A | rs699947 | No associated | [34] |
| G-634C | rs2010963 | No associated | [34] |
| C936T | rs3025039 | No associated | [34] |

Probably implicated mutations in implantation failure, studies are listed even when no association was found, rs code is mentioned whenever it is reported.

Table 1.
Genetic abnormalities involved in implantation failure.
2.2.2 Apolipoprotein E (APOE)

Due to the increase in total cholesterol levels during pregnancy, APOE plays a crucial role in lipid metabolism. APOE has three alleles in the long arm of chromosome 19 at position 13.2: ε2, ε3, and ε4. Individuals harboring the allele ε4 have higher cholesterol levels than the ones carrying the ε3/ε3 allele, whereas levels in those with the ε2 allele are lower [42].

2.2.3 Estrogen receptor α (ESR1)

ESR1 is a ligand-activated transcription factor essential for sexual development and reproductive function; its dysregulation leads to the development of various diseases such as cancer, cardiovascular disease, and inflammation, among others [43]. Due to alternative splicing of mRNA, it possesses three isoforms: ERαΔ3, ERα36, and ERα46 [43]; in a study performed by Yuan and Le [44], the polymorphisms rs2234693 and rs9340799 were related to the uncommonly thin endometrium.

2.2.4 Leukemia inhibitor factor (LIF)

LIF is an important implantation factor that promotes proliferation, invasion, and differentiation; its expression is regulated by the transcription factor tumor protein TP53 (TP53). Few studies have found a correlation between LIF gene polymorphism and reproductive capacity, Kang et al. demonstrated that SNP in the 3’UTR of the LIF (rs929271) gene is associated with infertility [40].

2.2.5 Mucin 1 (MUC1), Mucin 4 (MUC4)

MUC1 is an anti-adhesion molecule secreted by human endometrial epithelium, it has been suggested that its expression prevents the adherence of blastocyst to the endometrium. Interestingly, MUC1 must be locally removed in a paracrine fashion at the implantation site during the WOI to allow contact between the embryo and the endometrium, making it an important factor in determining ER [45–48]. MUC1 is a highly polymorphic gene that differs in the size of the region carrying the O-glycosylation sites: the variable number tandem repeat region (VNTR), which can go from 20 to 125 repeats [49]. Similarly, MUC4 is a greatly expressed mucin in endometrial epithelium [50], its gene is highly polymorphic and it contains a VNTR region that can go from 145 to 395 repeats [51]. Although its role in human infertility has not been fully explored, studies in other species have suggested that it plays a role in embryo implantation [52, 53].

2.2.6 Progesterone receptor (PR)

The PROGINS complex are three mutations in the PR gene that may be associated with unexplained infertility and implantation failure: a 306 bp insert in intron G of the dT2 allele in PR, the mutated alleleV660L, and guanine to thymine substitution in exon 4, resulting in a valine to leucine change in the hinge region of PR, and a cytosine to thymine substitution at exon 5 [54].

2.2.7 Prostaglandin-endoperoxide synthase 2 (PTGS2)

PTGS2 is a key enzyme involved in the conversion of arachidonic acid to prostaglandins (PGs). The −765G>C SNP mutation in the promoter region of PTGS2 upstream the transcriptional start site in the putative Sp1 site can cause alterations in Sp1 binding
[55]. Accordingly, the hypermethylation of the NF-IL6 site within the PTGS2 promoter results in elevated gene expression in eutopic endometrium in endometriosis [56].

2.2.8 Thrombolytic factors: coagulation factor II (F2), coagulation factor V (F5), coagulation factor XIII a chain (F13A1), methylenetetrahydrofolate reductase (MTHFR), plasminogen activator inhibitor-1 (PAI-1)

Thrombophilia, the predisposition for thrombosis, has been shown to be a risk factor for successful pregnancy due to impaired vascularization at the time of implantation. Therefore, the possible association between early pregnancy loss and polymorphisms at coagulation factors and thrombolytic genes responsible for inherited or acquired thrombophilia has been investigated. The coagulation factor II SNP G20210A in the 3′-untranslated region of F2 causes elevated prothrombin in plasma, leading to enhanced blood coagulation [57]. Furthermore, factor V Leiden mutation, G1691A, is a single nucleotide substitution in the F5 gene that results in reduced clearance of factor Va due to its blocked inactivation by activated protein C, increasing the risk of thrombosis [42, 57]. Also, factor XIII V34L polymorphism is a guanine to thymine substitution in exon 2 of F13A1 that leads to a valine for leucine change in residue 34; this SNP leads to reduced susceptibility to fibrinolysis and influences fibrin degradation [57]. Moreover, MTHFR is the rate-limiting enzyme in the methyl cycle. C677T polymorphism causes a substitution of valine for alanine, resulting in a thermolabile variant of the enzyme with reduced catalytic activity; combined with the SNP AC1298C, it is associated with hyper-homocysteinemia, a risk factor for venous and arterial thrombosis [42, 58–60]. Additionally, PAI-1 is a key regulatory element in the fibrinolysis cascade, it is believed to control proteolysis and remodeling of maternal tissue during trophoblast invasion. The 4G/5G polymorphism is located 657 bp upstream from the start site of transcription within the PAI-1 promoter and results in an allele with decreased transcriptional activity [42].

2.2.9 Trefoil factor 3 (TFF3)

TFF3 is a mucin-associated peptide co-expressed in mucus cells that acts as a mitogen to promote epithelial cell migrations and mediates epithelial repair after damage. The SNP rs11701143 is located in the promoter region of TFF3 within the regulatory region of the transcription binding site, whereas rs225361 is an intron variant located within a regulatory region. The exact function of both SNPs remains to be elucidated [61].

2.3 Immunological factors contributing to ER

The immune system plays a major role in the process of implantation and pregnancy maintenance [62]. During decidualization, endocrine processes transform uterine fibroblasts into cells that can produce hormones, growth factors, and matrix components to support embryo implantation [63, 64]. Furthermore, tolerance of the immune maternal system is required in pregnancy to avoid rejection of the semi-allograft or allograft embryo and for its successful implantation [65]. The decidua is a privileged site for immune tolerance; a large number of molecules and immune cell types participate in this process, leukocytes, macrophages, T lymphocytes, and dendritic cells comprise around 30 to 40% of the cells within the decidual stroma in early pregnancy. Among leukocytes, uterine natural killer (uNK) cells are activated and they significantly increase during decidualization (65–70%) [66, 67]. Increases in uNK cells denote three main functions in the endometrium: regulation of placental and trophoblast growth by cytokines [68, 69], local immunomodulation [70, 71], and
control of trophoblast invasion [69]. Furthermore, trophoblast cells play a major role in immune tolerance since these cells do not express major histocompatibility complex (MHC) class I (HLA-A and HLA-B) or class II molecules; ensuring that maternal T cells with αβ receptors cannot mount a classic cytotoxic attack against fetal paternal alloantigens. The trophoblast also protects itself by expressing Fas ligand (Fas L), which is important in the elimination of maternal reactive T cells by apoptosis induction [72–74]. Other important component of this process is T-regulatory (Treg) cells; these cells are essential for immunosuppression, prevention of autoimmunity, and maternal tolerance to the fetus [75–78]. Treg cells have been shown to be locally enriched in decidua during early normal pregnancy [79]. Furthermore, Forkhead box P3 (Foxp3) is a master regulator of Treg cell development, function, and differentiation [80]. Expression of FOXP3 was reduced approximately two-fold in endometrial biopsies of infertile women, implicating that the impaired differentiation of uterine T-cells into the Treg phenotype is a key determinant of fertility [81, 82]. On the other hand, helper T cells (CD4+) facilitate embryo implantation by regulating endometrial differentiation; they secrete interleukins and interferons that establish the implantation microenvironment. Successful pregnancy is dependent upon Th1/Th2 balance [83]. While Th1 cytokines are harmful for pregnancy, Th2 cytokines favor fetal growth and regulate uterine expression of fatty acid amide hydrolase (FAAH), LIF, and trophoblast release of human chorionic gonadotropin (hCG), which are known to play important roles during implantation [84–86]. Piccinni et al. demonstrated that T cells from decidua of women with a miscarriage show predominantly Th1-type cytokines with decreased Th2-type [84, 87, 88]. Finally, P4 and E2 mediate the downregulation of the maternal immune system [89]. P4 stimulates decidual proliferation; therefore, pregnancy results in an upregulation of P4 receptors on activated lymphocytes among placental cells and decidual CD56+ cells. In the presence of sufficient P4, these cells express progesterone-induced blocking factor (PIBF), a mediator that exerts substantial anti-abortive activities.

2.4 Biochemical markers involved in ER

We review molecular markers involved in the decidualization process that could be suitable as makers to assess ER.

2.4.1 Homeobox A10 (HOXA10)

HOXA10 is a transcription factor member of the homeobox family, known to be involved in the genetic control of embryonic development and in the regulation of the adult female reproductive tract [12, 90]. HOXA10 regulates downstream target genes that lead to endometrium development and receptivity acquisition [91], such as insulin-like growth factor binding protein 1 (IGFBP1) [12, 92, 93], genes of the Wnt pathway (reviewed by Sonderegger et al. [94], integrin β3 (ITGB3) [12, 90, 92], and empty spiracles homolog 2 (EMX2) [12, 95]. HOXA10 is regulated by P4 in a dose-dependent manner [12, 90, 91]. The expression of HOXA10 is low during the PP and it rapidly increases in the MSP [12, 90]. The diminished expression of HOXA10 in endometria of women with recurrent pregnancy loss (RPL) [96], adenomyosis [97], endometriosis [92], polycystic ovary syndrome (PCOS) [98], and idiopathic infertility [99] indicates that this gene could be essential for fertility [12].

2.4.2 Heparin-binding epidermal growth factor-like growth factor (HB-EGF)

HB-EGF is a member of the epidermal growth factor (EGF) family, it is expressed in the human uterus at the time of implantation and its expression is under steroidal hormone control [100–103]. The transmembrane form is associated
with cell adhesion and migration, it allows communication with the blastocyst by acting as a chemoattractant [101, 104]. HB-EGF expression is low in the PP and increases in the ESP immediately prior to the WOI, after which its levels decrease [101–103, 105]. Also, mRNA levels are low in pregnant endometrium and high in placental tissues at an early stage of development, suggesting that the HB-EGF ligand not only potentiates the health and survival of the peri-implantation embryo, but also induces the progression of its development [104]. HB-EGF stimulates epithelial expression of key endometrial proteins that are important biomarkers of the WOI, including LIF, HOXA10, and ITGB3 [100].

2.4.3 Leukemia inhibitory factor (LIF)

LIF is a member of a cytokine family with functional redundancy that includes interleukin 6 (IL6), oncostatin (OSM), ciliary neurotrophic factor (CNTF), and cardiotrophin 1 (CT1). They regulate proliferation, differentiation, and cell survival in different cellular systems [106]. LIF acts on cells by binding to the heterodimeric LIF receptor (LIFR), which consists of two transmembrane proteins, LIFR and glycoprotein 130 (gp130). LIFR activates several signaling pathways including the JAK/STAT, MAPK, and P13-kinase pathways, whereas gp130 participates in the activation of STAT1, STAT3, and STAT5B [107]. LIF induces the expression of cytokines and other regulatory molecules that could serve to regulate preimplantation development and embryo implantation [106–108]. LIF is one of the most important cytokines for receptivity during the WOI, the expression of LIF and LIFR reaches its highest level during the WOI in the MSP, LSP, and in early pregnancy in both surface and glandular epithelial cells under the influence of P4 [106, 107, 109, 110]. LIF can also be detected in decidual leukocytes, which are abundant at the implantation site; interestingly, LIF expression is low in women with unexplained infertility [106, 107, 111]. LIF also plays a crucial role in the regulation of fetal-maternal interactions during pregnancy, this cytokine mediates uterine receptivity through autocrine/paracrine interactions by binding to LIFR on the luminal epithelium to permit blastocyst attachment [106], but also regulates trophoblast function and vascular formation in the placenta [109].

2.4.4 Integrin β3 (ITGB3)

Integrins are ubiquitous cell adhesion molecules involved in maintaining normal tissue morphology and participate in cell–cell and cell-substrate interactions [100, 110, 112, 113]. In the human endometrium, integrins are involved in early embryo-endometrial interactions [90]. ITGB3 subunit is present after the ESP and its expression extend into the pregnancy [112, 114]. It has been reported that healthy fertile women show higher ITGB3 expression than patients with unexplained infertility [46, 96, 113–115]. Moreover, its dysregulation appears to characterize two distinct pathophysiological conditions that involve distinct mechanisms of defective ER: Type I and Type II. Type I defect is an out-of-phase endometrium with negative ITGB3 subunit expression, portrays a shifted WOI, and hormonal inadequacy or responsivenes is implicated, on the other hand, Type II defect is an “in-phase” endometrium with negative ITGB3 subunit expression and connotes the complete loss of the WOI. Furthermore, ITGB3 is expressed in EnSCs and endometrial glands with the highest levels in the MSP to LSP, suggesting a role in the regulation of endometrial function and implantation [115, 116]. Due to its temporal distribution and the effects of implantation when it is not present, ITGB3 is a useful molecular marker to assess ER. ITGB3 is regulated in the endometrium through a molecular mechanism via sex steroid signaling where HOXA10 acts as an intermediary [90, 96].
Other identified markers that are important for decidualization in the human endometrium include PR, particularly its encoded isoform progesterone receptor A (PR-A), homeobox A11 (HOXA11), PTGS2, MUC1, and interleukin 11 receptor (IL11R) [12].

2.5 Transcriptomic signature to determine the WOI

Microarray technology has been widely used to determine the transcriptomic profile of the endometrium by analyzing the expression of large batches of genes at different stages of the menstrual cycle. The most representative and commercially available test in this regard is perhaps the Endometrial Receptivity Array (ERA), developed in 2009 by Diaz-Gimeno et al., this test identifies the unique transcriptomic signature of the receptive endometrium by analyzing 238 differentially expressed genes, predicting the WOI for personalized embryo transfer (pET) [108]. Various research groups have analyzed changes in gene expression during the different phases of the endometrial cycle using microarray-based technologies [117–120], however, due to differences on results, unanimity about the main genes to be analyzed to determine the WOI has not been reached. Factors that contribute to the disagreement among studies results include differences on experimental design, utilized probes, sample acquisition day, sample size, collection method, and the application of distinct statistical analyses. Nevertheless, some genes have been reported to be expressed similarly in more than one work, here, we present a compilation of the expression profiles of those candidate genes in the human endometrium (Table 2).

2.6 miRNAs involved in ER

Micro-RNAs (miRNAs) are small, single-stranded, non-protein-coding RNA sequences of ~18–25 nucleotides in length that play an important post-transcriptional regulatory role in gene expression [121, 122] by targeting mRNAs for cleavage or transcriptional repression [123]. More than two decades have passed since the initial discovery of miRNAs in Caenorhabditis elegans by Lee et al. [124]; since then, great progress has been made in the understanding of miRNAs: what they are, how are synthesized, how regulate gene expression, and how they are involved in the formation and progression of pathological disorders. Extracellular miRNAs have been ubiquitously detected in body fluids [125]. Therefore, the presence and stability of miRNAs in biological fluids have advocated their potential as non-invasive biomarkers. Nevertheless, the identification of reliable miRNA biomarkers with reproducible profiles has been a challenge, and their diagnostic promise has remained a work in progress since they have still not entered the clinical field [126]. Nonetheless, given that miRNAs are differentially expressed in the endometrium across the menstrual cycle [127–131], several studies have been conducted to explore their role in ER [131–136]. Table 3 presents a summary of these studies.

2.7 The endometrial secretome as a potential tool to ascertain ER

The aim to develop alternative non-invasive strategies to provide accurate receptivity assessment has drawn assiduity to the endometrial secretome, which is based in the identification of factors secreted by cells or tissues at a particular time in either physiological states or pathological conditions [137], including proteins, lipids, and metabolites. Therefore, the analysis of differentially present molecules in the uterine cavity at different time points of the menstrual cycle could potentially help to identify the WOI and to diagnose uterine pathologies. Sample collection of endometrial fluid (EF) collection in the peri-implantation period is an easy procedure performed with minimally invasive tools that could easily be implemented
| Gene                              | Functional category                              | Expression profile | References          |
|----------------------------------|--------------------------------------------------|--------------------|---------------------|
| Annexin 4 [ANX4]                | Apoptosis                                        | PP to MSP          | [73, 86, 91–95]     |
|                                  |                                                  | ESP to MSP         |                     |
|                                  |                                                  | MSP to LSP         |                     |
| Apolipoprotein D [APOD]          | Cholesterol transport and trafficking            | PP to ESP          | [85, 92, 96]        |
|                                  |                                                  | ESP to MSP         |                     |
| Claudin 4 [CLDN4]                | Cell adhesion                                    | ESP to MSP         | [73, 85, 93, 94, 96, 97] |
| Decay-accelerated factor [DAF]   | Immune modulators/cytokines                      | ESP to MSP         | [85, 93, 94, 98]    |
| Dickkopf-1 [Dkk1]                | Regulation of WNT signaling pathway              | PP to ESP          | [73, 85, 92, 94, 96, 97, 99] |
|                                  |                                                  | ESP to MSP         |                     |
| Endothelin 3 [EDN3]             | Vasoactive substances                            | ESP to MSP         | [73, 92, 93, 100]   |
| Growth arrest and DNA-damage-inducible protein [GADD45] | Cell cycle                                      | PP to MSP          | [73, 92–94, 97, 101] |
|                                  |                                                  | ESP to MSP         |                     |
|                                  |                                                  | MSP to LSP         |                     |
| Glutathione peroxidase 3 [GPX3] | Response to stress and oxidoreductase activity   | ESP to MSP         | [73, 86, 96]        |
| Homeobox A10 [HOXA10]           | Transcription factor                             | ESP to MSP         | [55, 64, 94, 102, 103] |
|                                  |                                                  | MSP to LSP         |                     |
| Inhibitor of DNA binding 4, dominant negative helix-loop helix protein [ID4] | Anatomical structure development               | ESP to MSP         | [73, 92–94, 104]    |
| Insulin-like growth factor binding protein 1 [IGFBI-1] | Anatomical structure development               | ESP to MSP         | [73, 94]            |
| IL15 precursor [IL15]           | Immune response                                  | ESP to MSP         | [73, 85, 92–94, 96] |
| Mitogen-activated protein kinase kinase 5 [MAPKKK5] | Signal transduction                             | PP to ESP          | [92, 101]           |
| Matrix metalloproteinase 26 [DAMMP26] | Tissue remodeling and blastocyst invasion    | MP to LPP          | [87, 94]            |
|                                  |                                                  | PP to ESP          | [73]                |
|                                  |                                                  | MSP to LSP         |                     |
| Msh homeobox homologs 1,2 [MSX1] | Anatomical structure development                | ESP to MSP         | [73, 88, 91, 93, 96] |
| Natural cytotoxicity-triggering receptor 3 [NCR3] | Immune response                                 | ESP to MSP         | [86, 87]            |
|                                  |                                                  | MSP to LSP         |                     |
|                                  |                                                  | LSP to MP          |                     |
|                                  |                                                  | MP to PP           |                     |
| Olfactomedin 1 [OLF1]           | Anatomical structure development                 | PP to ESP          | [73, 88, 92, 93]    |
|                                  |                                                  | ESP to MSP         |                     |
| Sex-determining region Y-box 4 [SOX4] | Apoptotic pathways                              | PP to ESP          | [87, 88]            |
|                                  |                                                  | MSP to LSP         |                     |
|                                  |                                                  | MP to LPP          |                     |
| Osteopontin [SPP1]              | Cell adhesion                                    | PP to SP           | [73, 85, 91–93, 96, 97, 101] |
|                                  |                                                  | ESP to MSP         |                     |
| Tissue inhibitor metalloproteinase 3 [TIMP-3] | Degradation of the extracellular matrix         | PP to ESP          | [73, 94, 95]        |
|                                  |                                                  | ESP to MSP         |                     |
|                                  |                                                  | MSP to LSP         |                     |

Genes expressed in human endometrium and its expression profiles at the different phases of the endometrial cycle. A compilation of the genes exhibiting the same expression profile in more than one work is presented, regardless of the differences among studies.

Table 2.
Expression profiles of genes involved in ER.
### Reference and relevance

**[132]** Shows distinct miRNA profiles in LPP and MSP

| Samples: Endometrial biopsies LPP [n = 4] and MSP [n = 4] | Results: 49 differentially expressed miRNAs: 12 ↓ in the MSP [miR-214, 503, 134, 450, 382, 376A, 369-5p, 222, 370, 542-3p, 105, and 127] and 12 ↓ in LPP [miR-210, 193a-3p, 345, 29b, 29c, 30b, 204, 203, 582-5p, 30d, 200c, and 31] | Predicted: Cell cycle pathways in the LPP. Wnt signaling pathway in the PP. **Validated:** Decreased transcripts of predicted targets [cyclins, CDKs, and E2F3] |

**[134]** Evaluates differentially expressed miRNAs in RIF-IVF patients

| Samples: Secretory endometrium; 12 fertile women versus 11 women with RIF | Results: 13 differentially expressed miRNAs: 10 ↓ [miR-23b, 145, 99a, 99b, 652, 139-5p, 195, 342-3p, 150, and 374b] and 3 ↓ [miR-32, 628-5p, and 874] | Predicted: Wnt signaling, adherents junctions, p53 signaling, cell adhesion, and cell cycle pathways. **Validated:** Decreased transcripts of N-cadherin, H2AFX, NTN4, and SFRP4. Wnt and cell cycle pathways ↓ in RIF-IVF |

**[154]** Makes comparison between natural vs. stimulated cycles. Suggests that ovarian stimulation may shift the WOI

| Samples: Infertile women. Receptive [LH + 7, n = 5] vs. prereceptive [LH + 2, n = 5] in natural cycles. Receptive [hCG + 7, n = 5] vs. prereceptive [hCG + 4, n = 5] in stimulated IVF cycles | Results: 20 differentially expressed miRNAs in natural cycles: 8 ↑ [miR-30d, 30b, 30a, 31, 21, 193a-5p, 193a3p, 203] and 12 ↓ [miR-33a, 452, 125b, 455-3p, 455-5p, 483-5p, 143, 100, 504, 424, 424, 503]. 22 differentially expressed miRNAs in stimulated cycles: 19 ↑ [miR-187, 708, 433, 320a, 320b, 34c-5p, 320c, 320d, 485-5p, 574-5p, 375, 23b, 423-5p, 193b, 34b, 503, 424, 455-5p, 483-5p] and 3 ↓ [miR-886-5p, let-7f, let-7a] | Predicted: Cell cycle, transport, cell adhesion, cell death, and metabolism |

**[136]** Provides miRNA signature of EnSCs during decidualization in vitro

| Samples: Endometrial samples on oocyte retrieval day from healthy ovum donors [n = 50]. EnSCs [n = 20] isolated and cultured | Results: 43 differentially expressed miRNAs: 26 ↑ [miR-95, 888, 936, 1185, 518f, 548 k, 593, 486-5p, 29c, 449b, 300, 371-5p, 1224-3p, 891a, 365, 541, 409-5p, 33b, 154, 376a, 133a, 218–2, 22, 614, 369-3p, 185] and 17 ↓ [miR-146a, 155, 181b, 181a, 135b, 181d, 200c, 141, 182, 429, 483-3p, 200a, 96, 183, 9, 30a, 126] | Predicted: Growth factors, interleukins, ECM remodeling enzymes.  • Top pathways by ↑ miRNAs: axon guidance, adherent junction, actin cytoskeleton regulation, ErbB [EGFR] signaling, and renal cell carcinoma.  • Top pathways by ↑ miRNAs: actin cytoskeleton regulation, adherent junction, axon guidance, Wnt signaling, and MAPK |

**[133]** Provides miRNA signature of fertile human endometria: receptive vs. prereceptive

| Samples: Receptive MSP [LH + 7, n = 4] vs. prereceptive ESP [LH + 2, n = 5] biopsies from 9 healthy fertile women | Results: 4 significantly expressed different miRNAs in receptive samples: 2 ↑ [hsa-miR-30b and 30d] and 2 ↓ [hsa-miR-494 and 923] Suggests 12 genes that could serve as a new panel for ER: CAST, CFT, DPYSL2, FLJ, FGFR2, LIF, MTF1, NPAS2, P4HA2, PPARGC1A, TACC2, RAB40B | Predicted: Transcription, cell proliferation, and apoptosis. Involvement in pathways such as axon guidance, Wnt/β-catenin, ERK/MAPK, TGF-β, p53, and leukocyte extravasation. They identified SEPT7, CRM1, SLC4A1, HES1, FXR2, and TNF444B as genes that interact with genes MIR30B and MIR30D |
Proteomics of EF has already rendered valuable information regarding ER; Casado-Vela et al. identified 803 proteins in EF aspirates using three different proteomic strategies [138]. Additionally, Boomsma et al. [139] analyzed endometrial secretions prior to embryo transfer from 210 women undergoing IVF to determine differences in cytokine profiling at the time of implantation, finding a negative and a positive association of monocyte chemo-attractant protein-1 (MCP-1) and IFN-γ-inducible 10 kDa protein (IP-10) levels and implantation, respectively. Lipidomics, on the other hand, seems to have rendered slight information on receptivity [140], nevertheless, a study performed by Berlanga et al. [141] and followed by Vilella et al. [142, 143] carried out lipidomic analyses of EF from patients at different stages of their menstrual cycle, they determined a significant increase in Prostaglandin E2 (PGE2) and Prostaglandin F2α (PGF2α) between days 19 and 21, coincident with the WOI. In a recent study performed by Durairaj, Aberkane et al. [144], the contribution of EnSCs to failed implantation was examined by analyzing the secretome profile of EnSCs cultures in-vitro. From there, they encountered that secretome profiles of pregnant women are less divergent in implantation-positive cultures particularly in Day 0 (undifferentiated cells), suggesting that endometrial defects linked to reproductive failure could be more prominent in the PP, a phase that is commonly thought to be not relevant for ER studies. This research group also demonstrated that the secretome of undifferentiated EnSCs compromises blastocyst development. Finally, they determined that a deficiency of endometrial mesenchymal stem-like cells (MSCs) could lead to aberrant EnSC function and implantation failure. Overall, this study remarks the importance of progenitor cell populations in the endometrium that supports the acquisition of receptivity and

| Reference and relevance | Methodology | Results | Targets |
|-------------------------|-------------|---------|---------|
| [130] Differential miRNAs across cycle. Release of miRNAs into the EF and its uptake by the embryo | **Samples**: EF [n = 20] at different phases of the menstrual cycle of healthy women. Timing of sample collection: EPP, LPP, ESP, WOI, and LSP | Compared with the WOI, 9 differentially expressed miRNAs were identified in the EPP, 8 in the LPP, 6 in the ESP and 4 in the LSP. MiR-30d was the most differentially secreted maternal miRNA in the EF during the WOI | **Predicted**: Cell cycle and endocrine processes |
| [135] Shows a significant different expression of miRNAs in the WOI of RIF patients that may contribute to impaired ER | **Samples**: Endometrial biopsies from the WOI [5–7 days after ovulation]: 7 from RIF group and 5 from control group [infertile patients that delivered after one transfer attempt] | With a 2-fold threshold: 105 miRNAs were differentially expressed: 93 ↑ and 12 ↓. After raw signal value correction, 15 were found to be significantly different. 10 ↑ [hsa-miR-374a-5p, 145-5p, 30b-5p, 196b-5p, 199a-5p, 199b-5p, 449a, 424-5p, 125b-5p, 21-5p] and 5 ↓ [hsa-miR-1207-5p, 4306, 572, 5739, 6088] | **Predicted**: TAM analysis: miR-30 family, human embryonic stem cell regulation, epithelial-mesenchymal transition, and miRNA tumor suppressors. Network regulatory analysis: 176 miRNA-miRNA interactions. The top core miRNA were ABP1, AQP3, ASS1, and TIMP3, the top core miRNAs were has-miR-4668-5p, 429, and 5088 |

Studies conducted to analyze miRNA expression profiles during the menstrual cycle as potential biomarkers of the WOI. A summary of the differentially expressed miRNAs and predicted targets found in recent studies is presented. Its relevance regarding the role of miRNAs is also addressed.

Table 3. Studies of miRNA-profiling during the WOI.
raises the prospect of screening the endometrium before the initiation of an ART procedure.

2.8 The microbiome as a novel aspect of ER

Historically, the uterus was assumed to be free of bacteria as the fetal environment was considered to be physiologically sterile [145], this notion implies that the neonate’s microbiome is acquired only during and after birth. Although recent research still supports this conception [146], others have characterized upper genital tract microbiota [147–150], suggesting that the endometrial and vaginal microbiota not be identical [151]. What is more, the study by Moreno et al. [147, 152], which defined the microbiota in the EF as Lactobacillus-dominated (LD) or non-Lactobacillus-dominated (NLD), suggested that the presence of an NLD microbiota in a receptive endometrium was associated with a significant decrease in implantation, pregnancy, ongoing pregnancy, and live birth rates. Nevertheless, they acknowledge that in the absence of pathological signs, an NLD microbiota could be considered normal since Lactobacillus-deficient communities have been identified in the genital tract of otherwise healthy asymptomatic women [153]. This conception sets the stage for further research of the human microbiome, expands the possibilities to assess individualized receptivity based on the endometrial microbiome, and opens the door to explore targeted therapies for an altered endometrial microbial habitat.

3. Remarks

This review encloses different aspects of ER, spotlighting the opening of the WOI. Altogether, this compilation could aid in the development of new clinical practices that define an individual’s receptive window for pET to improve ART results ultimately.

Nowadays, ER is the rate-limiting step in successful ART procedures that end up in pregnancy and child delivery. The endometrial tissue is a ponderous element in fertility; it constitutes the soil in which a viable embryo will implant to achieve progeny. The attainment of ER involves an extensive assortment of genetic and biochemical mechanisms that must integrate in a parallel manner. Understanding of this process as an entity is still insufficient; nevertheless, the surge of new technologies is contributing in the deciphering of receptivity mechanisms and in search of novel biomarkers that could serve to detect the WOI. Notably, although most studies focus on individual genetic mutations, a more comprehensive view of the parental genetics is needed to determine whether an endometrium is adequate for embryo transfer before the initiation of an ART procedure. Due to ambiguous or non-conclusive results in search of genetic predispositions of endometrial-associated infertility, it would be controversial to provide genetic counseling currently. Perhaps, in the future, massive sequencing could help to provide insights into the importance of single and multiple genetic mutations to establish a receptive or non-receptive profile. If assertive, this profile could be applied as an endometrial pre-implantation parental test to improve the rates of healthy pregnancies and live births. With this in mind, in our opinion, the best short-term approaches towards detecting or improving ER are the transcriptome, microbiome, and miRNA signatures, all achievable using the power of NGS. This review encloses different aspects of ER, spotlighting the opening of the WOI. Altogether, this compilation could aid in the development of new clinical practices that define an individual’s receptive window for pET to improve ART results ultimately.
| Abbreviation | Full Form |
|--------------|-----------|
| 3D           | three-dimensional |
| ApoE         | apolipoprotein E |
| ART          | assisted reproductive tech |
| CNTF         | ciliary neurotrophic factor |
| CT1          | cardiotrophin 1 |
| E2           | estrogen |
| ECM          | extracellular matrix |
| EF           | endometrial fluid |
| EGF          | epithelial growth factor |
| EMX2         | empty spiracles homolog 2 |
| eNOS         | endometrial nitric-oxide synthase |
| EnSCs        | endometrial stromal cells |
| EPP          | early-proliferative phase |
| ER           | endometrial receptivity |
| ERA          | endometrial receptivity array |
| ESP          | early-secretory phase |
| ESR1         | estrogen receptor 1 |
| F13A1        | coagulation factor XIII A chain |
| F2           | coagulation factor II |
| F5           | coagulation factor V |
| F8           | coagulation factor 8 |
| FAAH         | fatty acid amide hydrolase |
| Fas L        | Fas ligand |
| FGB          | β fibrinogen |
| Foxp3        | Forkhead box P3 |
| gp130        | glycoprotein 130 |
| GPIIIa        | glycoprotein IIIa |
| HAUSP       | herpesvirus-associated ubiquitin-specific protease |
| HB-EGF      | heparin-binding epithelial growth factor-like growth factor |
| hCG         | human chorionic gonadotropin |
| HOXA10       | homeobox A10 |
| HOXA11       | homeobox A11 |
| HPA1         | human platelet alloantigens 1 |
| HSCORE       | histological score |
| IGFBP1       | insulin-like growth factor binding protein 1 |
| IL11R        | interleukin 11 receptor |
| IL6          | interleukin 6 |
| IP-10        | IFN-γ-inducible 10 kDa protein |
| ITGB3        | integrin β3 |
| IVF          | in-vitro fertilization |
| LD           | lactobacillus-dominated |
| LH           | luteinizing hormone |
| LIF          | leukemia inhibitory factor |
| LIFR         | leukemia inhibitory factor receptor |
| LPP          | late-proliferative phase |
| LSP          | late-secretory phase |
| MCP-1        | monocyte chemo-attractant protein-1 |
| MDM2         | murine double minute 2 |
| MHC          | major histocompatibility complex |
| MiRNA        | microRNA |
| MP           | menstrual phase |
| Abbreviation | Full Form |
|--------------|-----------|
| MSCs         | mesenchymal stem-like cells |
| MSP          | mid-secretory phase |
| MTHFR        | methylenetetrahydrofolate reductase |
| MUC1         | Mucin 1 |
| MUC4         | Mucin 4 |
| NGS          | next generation sequencing |
| NLD          | non-lactobacillus-dominated |
| OSM          | oncostatin |
| P4           | progesterone |
| TP53         | tumor protein 53 |
| PAI-1        | plasminogen activator-inhibitor-1 |
| PCOS         | polycystic ovarian syndrome |
| PCR          | polymerase chain reaction |
| pET          | personalized embryo transfer |
| PGE2         | prostaglandin E2 |
| PGF2α        | prostaglandin F2α |
| PGs          | prostaglandins |
| PIBF         | progesterone-induced blocking factor |
| PP           | proliferative phase |
| PR           | progesterone receptor |
| PR-A         | progesterone receptor A |
| RIF          | repeated implantation failure |
| RPL          | recurrent pregnancy loss |
| RSA          | recurrent spontaneous abortion |
| SNP          | single-nucleotide polymorphism |
| SP           | secretory phase |
| TAM          | tool annotations human miRNAs |
| TFF3         | trefoil factor 3 |
| Treg         | T-regulatory cells |
| uNK          | uterine natural killer |
| VEGF         | vascular endothelial growth factor |
| VNTR         | variable number tandem repeats |
| WOI          | window of implantation |
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References

[1] Lessey BA. The role of the endometrium during embryo implantation. Human Reproduction. 2000;15(Suppl 6):39-50

[2] Diedrich K et al. The role of the endometrium and embryo in human implantation. Human Reproduction Update. 2007;13(4):365-377

[3] Strowitzki T et al. The human endometrium as a fertility-determining factor. Human Reproduction Update. 2006;12(5):617-630

[4] Teh WT, McBain J, Rogers P. What is the contribution of embryo-endometrial asynchrony to implantation failure? Journal of Assisted Reproduction and Genetics. 2016;33(11):1419-1430

[5] Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Human Reproduction Update. 2006;12(6):731-746

[6] Reed BG, Carr BR. The normal menstrual cycle and the control of ovulation. In: De Groot LJ et al., editors. South Dartmouth (MA): Endotext; 2000

[7] Cha J, Sun X, Dey SK. Mechanisms of implantation: Strategies for successful pregnancy. Nature Medicine. 2012;18(12):1754-1767

[8] Mahajan N. Endometrial receptivity array: Clinical application. Journal of Human Reproductive Sciences. 2015;8(3):121-129

[9] Heger A, Sator M, Pietrowski D. Endometrial receptivity and its predictive value for IVF/ICSI-outcome. Geburtshilfe und Frauenheilkunde. 2012;72(8):710-715

[10] Beier HM, Beier-Hellwig K. Molecular and cellular aspects of endometrial receptivity.

[11] Lockwood CJ et al. The role of decidualization in regulating endometrial hemostasis during the menstrual cycle, gestation, and in pathological states. Seminars in Thrombosis and Hemostasis. 2007;33(1):111-117

[12] Lu Z, Hardt J, Kim JJ. Global analysis of genes regulated by HOXA10 in decidualization reveals a role in cell proliferation. Molecular Human Reproduction. 2008;14(6):357-366

[13] Jabbour HN et al. Endocrine regulation of menstruation. Endocrine Reviews. 2006;27(1):17-46

[14] Fox C et al. Local and systemic factors and implantation: What is the evidence? Fertility and Sterility. 2016;105(4):873-884

[15] Murray MJ et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. Fertility and Sterility. 2004;81(5):1333-1343

[16] Coutifaris C et al. Histological dating of timed endometrial biopsy tissue is not related to fertility status. Fertility and Sterility. 2004;82(5):1264-1272

[17] Nikas G. Endometrial receptivity: Changes in cell-surface morphology. Seminars in Reproductive Medicine. 2000;18(3):229-235

[18] Quinn CE, Casper RF. Pinopodes: A questionable role in endometrial receptivity. Human Reproduction Update. 2009;15(2):229-236

[19] Zhang T et al. The role of three-dimensional power Doppler ultrasound parameters measured on hCG day in the prediction of pregnancy during in vitro
fertilization treatment. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2016;203:66-71

[20] Abdalla HI et al. Endometrial thickness: A predictor of implantation in ovum recipients? Human Reproduction. 1994;9(2):363-365

[21] Yuval Y et al. The relationships between endometrial thickness, and blood flow and pregnancy rates in in-vitro fertilization. Human Reproduction. 1999;14(4):1067-1071

[22] Schild RL et al. Endometrial receptivity in an in vitro fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. Fertility and Sterility. 2001;75(2):361-366

[23] Noyes N et al. Factors useful in predicting the success of oocyte donation: A 3-year retrospective analysis. Fertility and Sterility. 2001;76(1):92-97

[24] Friedler S et al. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: A critical review. Human Reproduction Update. 1996;2(4):323-335

[25] Khalifa E et al. Sonographic appearance of the endometrium: The predictive value for the outcome of in-vitro fertilization in stimulated cycles. Human Reproduction. 1992;7(5):677-680

[26] Sundström P. Establishment of a successful pregnancy following in-vitro fertilization with an endometrial thickness of no more than 4 mm. Human Reproduction (Oxford, England). 1998;13(6):1550-1552

[27] Remohi J et al. Endometrial thickness and serum oestradiol concentrations as predictors of outcome in oocyte donation. Human Reproduction (Oxford, England). 1997;12(10):2271-2276

[28] Mercé LT. Ultrasound markers of implantation. The Ultrasound Review of Obstetrics and Gynecology. 2002;2(2):110-123

[29] Raga F et al. Assessment of endometrial volume by three-dimensional ultrasound prior to embryo transfer: Clues to endometrial receptivity. Human Reproduction. 1999;14(11):2851-2854

[30] Zhao J, Zhang Q, Li Y. The effect of endometrial thickness and pattern measured by ultrasonography on pregnancy outcomes during IVF-ET cycles. Reproductive Biology and Endocrinology. 2012;10:100

[31] Zhao J et al. Endometrial pattern, thickness and growth in predicting pregnancy outcome following 3319 IVF cycle. Reproductive Biomedicine Online. 2014;29(3):291-298

[32] Fatemi HM, Popovic-Todorovic B. Implantation in assisted reproduction: A look at endometrial receptivity. Reproductive Biomedicine Online. 2013;27(5):530-538

[33] Coulam CB, Jeyendran RS. Vascular endothelial growth factor gene polymorphisms and recurrent pregnancy loss. American Journal of Reproductive Immunology. 2008;59(4):301-305

[34] Papazoglou D et al. Vascular endothelial growth factor gene polymorphisms and idiopathic recurrent pregnancy loss. Fertility and Sterility. 2005;83(4):959-963

[35] Lee HH et al. Association study of vascular endothelial growth factor polymorphisms with the risk of recurrent spontaneous abortion. Fertility and Sterility. 2010;93(4):1244-1247
[36] Goodman C, Jeyendran RS, Coulam CB. Vascular endothelial growth factor gene polymorphism and implantation failure. Reproductive Biomedicine Online. 2008;16(5):720-723

[37] Goodman C, Jeyendran RS, Coulam CB. P53 tumor suppressor factor, plasminogen activator inhibitor, and vascular endothelial growth factor gene polymorphisms and recurrent implantation failure. Fertility and Sterility. 2009;92(2):494-498

[38] Xu X et al. Association of VEGF genetic polymorphisms with recurrent spontaneous abortion risk: A systematic review and meta-analysis. PLoS One. 2015;10(4):e0123696

[39] Su MT, Lin SH, Chen YC. Genetic association studies of angiogenesis- and vasoconstriction-related genes in women with recurrent pregnancy loss: A systematic review and meta-analysis. Human Reproduction Update. 2011;17(6):803-812

[40] Kang HJ et al. Single-nucleotide polymorphisms in the p53 pathway regulate fertility in humans. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(24):9761-9766

[41] Coulam CB, Kay C, Jeyendran RS. Role of p53 codon 72 polymorphism in recurrent pregnancy loss. Reproductive Biomedicine Online. 2006;12(3):378-382

[42] Yenicesu GI et al. A prospective case-control study analyzes 12 thrombophilic gene mutations in Turkish couples with recurrent pregnancy loss. American Journal of Reproductive Immunology. 2010;63(2):126-136

[43] Jia M, Dahlman-Wright K, Gustafsson JA. Estrogen receptor alpha and beta in health and disease. Best Practice & Research. Clinical Endocrinology & Metabolism. 2015;29(4):557-568

[44] Yuan R, Le AW. A study on the estrogen receptor alpha gene polymorphism and its expression in thin endometrium of unknown etiology. Gynecologic and Obstetric Investigation. 2012;74(1):13-20

[45] Hey NA et al. The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase. The Journal of Clinical Endocrinology and Metabolism. 1994;78(2):337-342

[46] Xu B et al. Pinopodes, leukemia inhibitory factor, integrin-beta3, and mucin-1 expression in the peri-implantation endometrium of women with unexplained recurrent pregnancy loss. Fertility and Sterility. 2012;98(2):389-395

[47] Meseguer M et al. Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. Biology of Reproduction. 2001;64(2):590-601

[48] Brayman M, Thathiah A, Carson DD. MUC1: A multifunctional cell surface component of reproductive tissue epithelia. Reproductive Biology and Endocrinology. 2004;2:4

[49] Carson DD, DeSouza MM, Regisford EG. Mucin and proteoglycan functions in embryo implantation. BioEssays. 1998;20(7):577-583

[50] Audie JP et al. Mucin gene expression in the human endocervix. Human Reproduction. 1995;10(1):98-102

[51] Nollet S et al. Human mucin gene MUC4: Organization of its 5'-region and polymorphism of its central tandem repeat array. The Biochemical Journal. 1998;332(Pt 3):739-748

[52] Ferrell AD et al. Sialomucin complex (Muc4) expression in porcine endometrium during the oestrous cycle
and early pregnancy. Reproduction in Domestic Animals. 2003;38(1):63-65

[53] Carraway KL, Idris N. Regulation of sialomucin complex/Muc4 in the female rat reproductive tract. Biochemical Society Transactions. 2001;29(Pt 2):162-166

[54] Coulam CB, Jeyendran R, Roussev R. Association of progesterone receptor polymorphisms with recurrent implantation failure after in vitro fertilization and embryo transfer. Journal of Assisted Reproduction and Genetics. 2008;25(4):119-122

[55] Salazar LA et al. Association of −765G>C polymorphism of the COX-2 gene with recurrent embryo implantation failure in Southern Chilean women. Clinica Chimica Acta. 2010;411(21-22):1822-1824

[56] Wang D et al. DNA hypomethylation of the COX-2 gene promoter is associated with up-regulation of its mRNA expression in eutopic endometrium of endometriosis. European Journal of Medical Research. 2012;17:12

[57] Pihusch R et al. Thrombophilic gene mutations and recurrent spontaneous abortion: Prothrombin mutation increases the risk in the first trimester. American Journal of Reproductive Immunology. 2001;46(2):124-131

[58] Goodman CS et al. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? American Journal of Reproductive Immunology. 2006;56(4):230-236

[59] Vacquier VD. Oogenesis: Developmental biology. Science. 1985;229(4718):1078-1079

[60] Qublan HS et al. Acquired and inherited thrombophilia: Implication in recurrent IVF and embryo transfer failure. Human Reproduction. 2006;21(10):2694-2698

[61] Haroun S et al. Association between trefoil factor 3 gene variants and idiopathic recurrent spontaneous abortion. Reproductive Biomedicine Online. 2014;29(6):737-744

[62] Singh M, Chaudhry P, Asselin E. Bridging endometrial receptivity and implantation: Network of hormones, cytokines, and growth factors. The Journal of Endocrinology. 2011;210(1):5-14

[63] Croy BA et al. Decidual natural killer cells: Key regulators of placental development (a review). Journal of Reproductive Immunology. 2002;57(1-2):151-168

[64] Abrahamsohn PA, Zorn DH. Redirecting reproductive immunology research toward pregnancy as a period of temporary immune tolerance. Journal of Assisted Reproduction and Genetics. 2017;34(4):425-430

[65] Gleicher N, Kushnir VA, Barad DH. Redirecting reproductive immunology research toward pregnancy as a period of temporary immune tolerance. Journal of Assisted Reproduction and Genetics. 2017;34(4):425-430

[66] Lash GE, Robson SC, Bulmer JN. Review: Functional role of uterine natural killer (uNK) cells in human early pregnancy decidua. Placenta. 2010;31(Suppl):S87-S92

[67] Bulmer JN, Williams PJ, Lash GE. Immune cells in the placental bed. The International Journal of Developmental Biology. 2010;54(2-3):281-294

[68] King A, Loke YW. Effect of IFN-gamma and IFN-alpha on killing of human trophoblast by decidual LAK cells. Journal of Reproductive Immunology. 1993;23(1):51-62

[69] Dosiou C, Giudice LC. Natural killer cells in pregnancy and recurrent pregnancy loss: Endocrine and immunologic perspectives. Endocrine Reviews. 2005;26(1):44-62
[70] Koopman LA et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. The Journal of Experimental Medicine. 2003;198(8):1201-1212

[71] Rachmilewitz J et al. Negative regulation of T cell activation by placental protein 14 is mediated by the tyrosine phosphatase receptor CD45. The Journal of Biological Chemistry. 2003;278(16):14059-14065

[72] Poole JA, Claman HN. Immunology of pregnancy. Clinical Reviews in Allergy & Immunology. 2004;26(3):161-170

[73] Runci R et al. Apoptosis and Fas expression in human fetal Membranes1. The Journal of Clinical Endocrinology & Metabolism. 1998;83(2):660-666

[74] Aschkenazi S et al. Differential regulation and function of the Fas/Fas ligand system in human trophoblast Cells1. Biology of Reproduction. 2002;66(6):1853-1861

[75] Saito S, Sasaki Y, Sakai M. CD4(+) CD25high regulatory T cells in human pregnancy. Journal of Reproductive Immunology. 2005;65(2):111-120

[76] La Rocca C et al. The immunology of pregnancy: Regulatory T cells control maternal immune tolerance toward the fetus. Immunology Letters. 2014;162 (1 Pt A):41-48

[77] Guerin LR, Prins JR, Robertson SA. Regulatory T-cells and immune tolerance in pregnancy: A new target for infertility treatment? Human Reproduction Update. 2009;15(5):517-535

[78] Heitmann RJ et al. Maternal T regulatory cell depletion impairs embryo implantation which can be corrected with adoptive T regulatory cell transfer. Reproductive Sciences. 2017;24(7):1014-1024

[79] Dimova T et al. Maternal Foxp3 expressing CD4+ CD25+ and CD4+ CD25-regulatory T-cell populations are enriched in human early normal pregnancy decidua: A phenotypic study of paired decidual and peripheral blood samples. American Journal of Reproductive Immunology. 2011;66(Suppl 1):44-56

[80] Haiqi H, Yong Z, Yi L. Transcriptional regulation of Foxp3 in regulatory T cells. Immunobiology. 2011;216(6):678-685

[81] Jasper MJ, Tremellen KP, Robertson SA. Primary unexplained infertility is associated with reduced expression of the T-regulatory cell transcription factor Foxp3 in endometrial tissue. Molecular Human Reproduction. 2006;12(5):301-308

[82] Betz AG. Tolerating pregnancy. Nature. 2012;490:47

[83] Weetman AP. The immunology of pregnancy. Thyroid. 1999;9(7):643-646

[84] Clark DA, Croitoru K. TH1/TH2,3 imbalance due to cytokine-producing NK, gammadelta T and NK-gammadelta T cells in murine pregnancy decidua in success or failure of pregnancy. American Journal of Reproductive Immunology. 2001;45(5):257-265

[85] Saito S. Cytokine network at the feto-maternal interface. Journal of Reproductive Immunology. 2000;47(2):87-103

[86] Szekeres-Bartho J. Immunological relationship between the mother and the fetus. International Reviews of Immunology. 2002;21(6):471-495

[87] Piccinni MP, Romagnani S. Regulation of fetal allograft survival by a hormone-controlled Th1- and Th2-type cytokines. Immunologic Research. 1996;15(2):141-150

[88] Piccinni MP et al. Role of hormone-controlled Th1- and Th2-type cytokines
in successful pregnancy. Journal of Neuroimmunology. 2000;109(1):30-33

[89] Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1Th2 balance. Journal of Reproductive Immunology. 1996;31(1):81-95

[90] Daftary GS et al. Direct regulation of beta3-integrin subunit gene expression by HOXA10 in endometrial cells. Molecular Endocrinology. 2002;16(3):571-579

[91] Taylor HS et al. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. The Journal of Clinical Investigation. 1998;101(7):1379-1384

[92] Kim JJ et al. Altered expression of HOXA10 in endometriosis: Potential role in decidualization. Molecular Human Reproduction. 2007;13(5):323-332

[93] Ruiz-Alonso M, Blesa D, Simon C. The genomics of the human endometrium. Biochimica et Biophysica Acta. 2012;1822(12):1931-1942

[94] Sonderegger S, Pollheimer J, Knofler M. Wnt signalling in implantation, decidualisation and placental differentiation--review. Placenta. 2010;31(10):839-847

[95] Troy PJ et al. Transcriptional repression of peri-implantation EMX2 expression in mammalian reproduction by HOXA10. Molecular and Cellular Biology. 2003;23(1):1-13

[96] Germeyer A et al. Endometrial beta3 integrin profile reflects endometrial receptivity defects in women with unexplained recurrent pregnancy loss. Reproductive Biology and Endocrinology. 2014;12:53

[97] Fischer CP, Kayisili U, Taylor HS. HOXA10 expression is decreased in endometrium of women with adenomyosis. Fertility and Sterility. 2011;95(3):1133-1136

[98] Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. The Journal of Clinical Endocrinology and Metabolism. 2003;88(1):238-243

[99] Szczepanska M et al. Expression of HOXA-10 and HOXA-11 in the endometria of women with idiopathic infertility. Folia Histochemica et Cytobiologica. 2011;49(1):111-118

[100] Lessey BA et al. Regulated expression of heparin-binding EGF-like growth factor (HB-EGF) in the human endometrium: A potential paracrine role during implantation. Molecular Reproduction and Development. 2002;62(4):446-455

[101] Stavreus-Evers A et al. Co-existence of heparin-binding epidermal growth factor-like growth factor and pinopodes in human endometrium at the time of implantation. Molecular Human Reproduction. 2002;8(8):765-769

[102] Yoo HJ, Barlow DH, Mardon HJ. Temporal and spatial regulation of expression of heparin-binding epidermal growth factor-like growth factor in the human endometrium: A possible role in blastocyst implantation. Developmental Genetics. 1997;21(1):102-108

[103] Birdsall MA et al. Expression of heparin-binding epidermal growth factor messenger RNA in the human endometrium. Molecular Human Reproduction. 1996;2(1):31-34

[104] Chobotova K et al. Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. Mechanisms of Development. 2002;119(2):137-144
[105] Leach RE et al. Multiple roles for heparin-binding epidermal growth factor-like growth factor are suggested by its cell-specific expression during the human endometrial cycle and early placentation. The Journal of Clinical Endocrinology and Metabolism. 1999;84(9):3355-3363

[106] Cullinan EB et al. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(7):3115-3120

[107] Aghajanova L et al. Coexpression of pinopodes and leukemia inhibitory factor, as well as its receptor, in human endometrium. Fertility and Sterility. 2003;79(Suppl 1):808-814

[108] Diaz-Gimeno P et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertility and Sterility. 2011;95(1):50-60 60e1-15

[109] Guzeloglu-Kayisli O, Kayisli UA, Taylor HS. The role of growth factors and cytokines during implantation: Endocrine and paracrine interactions. Seminars in Reproductive Medicine. 2009;27(1):62-79

[110] Lessey BA et al. Distribution of integrin cell adhesion molecules in endometrial cancer. The American Journal of Pathology. 1995;146(3):717-726

[111] Steck T et al. Leukaemia inhibitory factor (LIF) gene mutations in women with unexplained infertility and recurrent failure of implantation after IVF and embryo transfer. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2004;112(1):69-73

[112] Lessey BA et al. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. The Journal of Clinical Investigation. 1992;90(1):188-195

[113] Lessey BA et al. Further characterization of endometrial integrins during the menstrual cycle and in pregnancy. Fertility and Sterility. 1994;62(3):497-506

[114] Lessey BA et al. Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertility and Sterility. 1995;63(3):535-542

[115] Dorostghoal M et al. Endometrial expression of beta3 integrin, calcitonin and plexin-B1 in the window of implantation in women with unexplained infertility. International Journal of Reproductive BioMedicine (Yazd, Iran). 2017;15(1):33-40

[116] von Wolff M et al. Endometrial osteopontin, a ligand of beta3-integrin, is maximally expressed around the time of the "implantation window". Fertility and Sterility. 2001;76(4):775-781

[117] Carson DD et al. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. Molecular Human Reproduction. 2002;8(9):871-879

[118] Ponnampalam AP et al. Molecular classification of human endometrial cycle stages by transcriptional profiling. Molecular Human Reproduction. 2004;10(12):879-893

[119] Punyadeera C et al. Oestrogen-modulated gene expression in the human endometrium. Cellular and Molecular Life Sciences. 2005;62(2):239-250
[120] Talbi S et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. Endocrinology. 2006;147(3):1097-1121

[121] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-297

[122] Shukla GC, Singh J, Barik S. MicroRNAs: Processing, maturation, target recognition and regulatory functions. Molecular and Cellular Pharmacology. 2011;3(3):83-92

[123] Lim LP et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature. 2005;433(7027):769-773

[124] Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843-854

[125] Weber JA et al. The microRNA spectrum in 12 body fluids. Clinical Chemistry. 2010;56(11):1733-1741

[126] Pogribny IP. MicroRNAs as biomarkers for clinical studies. Experimental Biology and Medicine (Maywood, NJ). 2018;243(3):283-290

[127] Pan Q, Chegini N. MicroRNA signature and regulatory functions in the endometrium during normal and disease states. Seminars in Reproductive Medicine. 2008;26(6):479-493

[128] Hull ML, Nisenblat V. Tissue and circulating microRNA influence reproductive function in endometrial disease. Reproductive Biomedicine Online. 2013;27(5):515-529

[129] Katzorke N et al. Diagnosis of endometrial-factor infertility: Current approaches and new avenues for research. Geburtshilfe und Frauenheilkunde. 2016;76(6):699-703

[130] Vilella F et al. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome. Development. 2015;142(18):3210-3221

[131] Cuman C et al. Human blastocyst secreted microRNA regulate endometrial epithelial cell adhesion. eBioMedicine. 2015;2(10):1528-1535

[132] Kuokkanen S et al. Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. Biology of Reproduction. 2010;82(4):791-801

[133] Altmae S et al. MicroRNAs miR-30b, miR-30d, and miR-494 regulate human endometrial receptivity. Reproductive Sciences. 2013;20(3):308-317

[134] Revel A et al. MicroRNAs are associated with human embryo implantation defects. Human Reproduction. 2011;26(10):2830-2840

[135] Shi C et al. Endometrial MicroRNA signature during the window of implantation changed in patients with repeated implantation failure. Chinese Medical Journal. 2017;130(5):566-573

[136] Estella C et al. miRNA signature and dicer requirement during human endometrial stromal decidualization in vitro. PLoS One. 2012;7(7):e41080

[137] Zullo J et al. The cell secretome, a mediator of cell-to-cell communication. Prostaglandins & Other Lipid Mediators. 2015;120:17-20

[138] Casado-Vela J et al. Comprehensive proteomic analysis
of human endometrial fluid aspirate. Journal of Proteome Research. 2009;8(10):4622-4632

[139] Boomsma CM et al. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Human Reproduction. 2009;24(6):1427-1435

[140] Demiral I et al. Genomic, proteomic and lipidomic evaluation of endometrial receptivity. Turkish Journal of Obstetrics and Gynecology. 2015;12(4):237-243

[141] Berlanga O et al. How endometrial secretomics can help in predicting implantation. Placenta. 2011;32(Suppl 3):S271-S275

[142] Vilella F, Ramirez LB, Simon C. Lipidomics as an emerging tool to predict endometrial receptivity. Fertility and Sterility. 2013;99(4):1100-1106

[143] Vilella F et al. PGE2 and PGF2alpha concentrations in human endometrial fluid as biomarkers for embryonic implantation. The Journal of Clinical Endocrinology and Metabolism. 2013;98(10):4123-4132

[144] Peter Durairaj RR et al. Deregulation of the endometrial stromal cell secretome precedes embryo implantation failure. Molecular Human Reproduction. 2017;23(8):582

[145] Ansbacher R, Boyson WA, Morris JA. Sterility of the uterine cavity. American Journal of Obstetrics and Gynecology. 1967;99(3):394-396

[146] Perez-Munoz ME et al. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. Microbiome. 2017;5(1):48

[147] Moreno I et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. American Journal of Obstetrics and Gynecology. 2016;215(6):684-703

[148] Aagaard K et al. The placenta harbors a unique microbiome. Science Translational Medicine. 2014;6(237):237ra65

[149] Verstraelen H et al. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. Peer Journal. 2016;4:e1602

[150] Franasiak JM et al. Endometrial microbiome at the time of embryo transfer: Next-generation sequencing of the 16S ribosomal subunit. Journal of Assisted Reproduction and Genetics. 2016;33(1):129-136

[151] Mitchell CM et al. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. American Journal of Obstetrics and Gynecology. 2015;212(5):611.e1-611.e9

[152] Moreno I, Franasiak JM. Endometrial microbiota-new player in town. Fertility and Sterility. 2017;108(1):32-39

[153] Ravel J et al. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(Suppl 1):4680-4687

[154] Sha AG, Liu JL, Jiang XM, Ren JZ, Ma CH, Lei W, Su RW, Yang ZM. Genome-wide identification of micro-ribonucleic acids associated with human endometrial receptivity in natural and stimulated cycles by deep sequencing. Fertil Steril. 2011;96(1):150-155 e155