Chapter 01

HLA-G and Endometrial Receptivity

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Abstract

The endometrium is a complex and dynamic tissue, which experiences physiological and cyclical changes each month, in response to ovarian hormones, cytokines and chemokines [1-3]. The embryo is capable of attach to the uterine endometrium during a short and self-limited period, in which the endometrial tissue acquires a functional condition that allows the interaction trophoblast-endometrium and therefore, it is receptive. The embryo enters the uterine cavity as an unhatched blastocyst and undergoes its final development through hatching to attachment to the uterine luminal epithelium within the environment of uterine fluid. The embryo first enters the uterine cavity as blastocyst and attached to the uterine epithelium [4] (Figure 1). Decidualization of endometrial stromal cells is mainly induced by ovarian steroids and progesterone-dependent decidualization is mediated in part by the second messenger cAMP [5], decidualization is taking place with the secretory transformation of the uterine glands, particularly of specialized uterine natural killer cells and vascular remodelling [6].

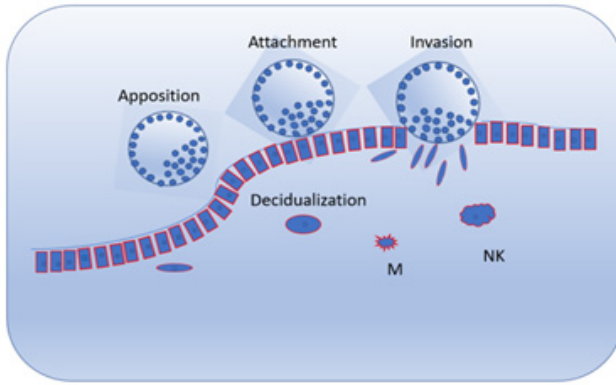

Figure 1: Human Blastocyst Implantation.
M-Macrophages; NK-Natural killer cells.

Endometrial receptivity (ER) is defined as a temporary unique factors sequence that make the endometrium receptive to the embryonic implantation [7]. This specific period is regulated by a combination of ovarian steroids hormones and genetic factors and is known as "window of implantation" (WOI). It takes place between 6 and 10 days after ovulation [8], and it remains receptive during a short period of time, about the 20-24th of a cycle of 28 days [9]. During this period the endometrium undergoes morphological, cytoskeletal, biochemical, and genetic changes to become functionally competent [10].

Embryo implantation is a process comprising several cellular, ultrastructural and molecular mechanisms initiated and mediated by the endometrium, the embryo and the interaction of both. In order that the embryonic implantation takes place, there is indispensable the concurrence of three fundamental elements: embryo quality, endometrial receptivity in WOI and embryo-endometrial interaction [11-13]. Figure.2 but timing endometrial receptivity is still a challenge.

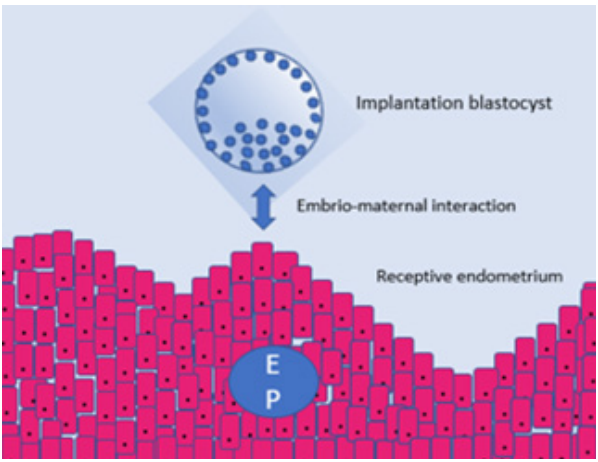

Figure 2: Embryo-Endometrial Interaction.

These processes are controlled by different factors, including ovarian steroids and its receptors, cytokines, growth factors, adhesion molecules, transcriptional factors and many others [14].

The detection of WOI in every patient, in a personalized way, would be essential and would allow to increase pregnancy rates in ART. Failure of the endometrium to achieve receptivity and the timing of the receptive period are now recognised as important issues in the success of IVF [4].

Markers of Endometrial Receptivity

In assisted reproductive technology (ART), it is very important to recognize this time before the embryo transfer. Sonographic exam can be performed, evaluation of endometrial blood flow or tri-dimensional features of the endometrium can assist to evaluate the receptivity. Knowledge of the length of human WOI has critical significance to all future studies identifying endometrial markers for ER [15]. It is necessary to examine other factors that might affect pregnancy rate. Though the study of ER is in his beginnings, it will improve our aptitude to diagnose and treat infertility [1]. Screening investigational methods ranging from immunohistochemistry to more complex techniques of chromatography such as matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) has been described, and proteins such as a3b5 integrins, leukemia inhibitor factor (LIF) and L-selectin, among others [16]. The detection of these markers is made in base of an endometrial biopsy, in a different cycle of the embryo transfer in ART.

ER in in vitro fertilization (IVF) cycles was assessed both on molecular level, as growth factors: leukemia-inhibitory factor (LIF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), granulocyte macrophage colony stimulating growth factor (GM-CSF) together with hemodynamic characteristics of uterine arteries. Sharfi, obtained an accuracy of 85.6% with LIF expression optic density in glandular and columnar endometrial epithelium, extracted in the assumed WOI period of the preceding IVF cycle; VEGF content in the cervical mucus on the transvaginal puncture day, systolic/ diastolic rate, resistance index of spiral arteries on the ovulation triggering day and cytokines [17].

It should be an important priority correlating biochemical markers and histochemical characteristics in WOI to determine favourable environment for implantation. There have been described the pinopodes, which are cytoplasmic prolongations as characteristic of ripeness endometrial that supports the implantation which his major expression has been demonstrated during WOI, nevertheless his value has not been established in IVF implantation failure.

Several biomarkers have been used to diagnosis ER, as histological, biochemical, and molecular markers.

Histological Markers

They are based on the morphologic changes of the endometrium during the menstrual cycle, described by Noyes on 1950. However, the use of Noyes criteria to predict the ER has been questioned in recent years [18].

Biochemical Markers

The molecules which have shown significant association with WOI are the integrins, leukemia inhibitory factor, homeobox A10, mucin 1, calcitonin, and cyclo-oxygenase 2 [19].

Molecular Markers

"Omics" technologies include genomics (the study of genomes and the complete collection of genes that they contain), epigenomics (study of epigenetic DNA modifications), transcriptomics (study of gene expression or transcriptomic profile), proteomics (the presence and quantification of proteins, the proteome), metabolomics or the composition and abundance of metabolites, the metabolome and lipidomics (collection of lipids, the lipidome, secretomics (secreted proteins, the secretome), interactomics (the interactome, or a 'systems biology' approach).

The endometrial markers with more possibility to use in clinical practice are:

Integrins

Some integrins, as α1β1, α4β1 y αvβ3, are regulated by hormonal changes showing an expression in certain moments [20], and a decrease of the integrins ß3 and a4ß1 in the endometrium they associate to implantation failure [21].

Antiadhesion Molecules

Mucin1 expression (MUC-1) increases between 2 and 3 days after the peak of luteinizing hormone (LH) [22] and it is one of the first molecules with which the blastocyst takes contact.

Chemokines

Endometrial chemokines are presents in blastocyst apposition phase.

Cytokines

Interleuquina 6 (IL-6) is present in the embryonic implantation, and increases during the luteal phase, especially in WOI. The inhibiting factor of the leukaemia (LIF) plays a basic role in endometrial receptivity regulation.

Growth Factor

Heparin Growth factor (HB-EGF) has maximum expression immediately before WOI.

Transcriptomics Biomarkers

The changes that occur during the menstrual cycle are ultimately the result of changes that occur at the level of gene transcription [23]. HOXA-10 and HOXA-11 genes, play an important role in endometrial receptivity and are overexpressed in WOI. Currently, transcriptom-

ics, based on microarray technology, is considered the most established technology available for evaluation of endometrial factor [10].

The Endometrial Receptivity Array (ERA) test is a microarray-based machine-learning predictive model used to diagnose human endometrial receptivity [24], leading personalised embryo transfer [25].

Genome-wide expression profiling of decidual responses to competent embryos showed that only 15 genes were responsive, whereas some 449 genes were dysregulated by poor quality embryos, that is, the decidua will determine if pregnancy take place after preimplantation events [4].

MiRNA

They are regulatory epigenetics of the gene expression. In WOI it is expressed mir-29a that induces the inhibition of the apoptosis [26].

Human leukocyte antigen

One factor is human leukocyte antigen (HLA), is part of the major histocompatibility complex (MHC) which is encoded by a series of grouped genes located on chromosome 6 and plays a role in control of adaptive immunity, particularly T-cell-mediated immunity towards pathogens. During the normal pregnancy the maternal immune system undergoes changes that lead to foetal tolerance. The maternal immune system is a critical component of implantation process and any evidences suggest that HLA-G may play a role in protecting the foetus from the maternal immune response [27]. The immune tolerance of pregnancy is a paradox as the mother's immune system does not reject a foetus even being a partially foreign tissue, or even being from an oocyte donor. The non-classical major histocompatibility complex (MHC) molecule HLA-G is essential for this immune tolerance induction in pregnancy.

Soluble human leukocyte antigen G (sHLA-G) is present in many body fluids and may confer immune tolerance to embryo. HLA-G expression has been detected in early preimplantation embryos and it is postulated that a relationship between embryonic expression of this factor and successful pregnancy may exist [28,29]. More recent studies indicate a relationship between sHLA-G secretion, embryo quality and pregnancy rate [30,31].

HLA-G protein is expressed as a membrane bound exhibiting a very limited tissue distribution as extravillous cytotrophoblast cells in the placenta, maternal spiral arteries, endothelial cells of foetal vessels in the chorionic villi, amnion cells, thymus, and interferon-ɣ - stimulated blood monocytes [32].

There are four membrane-bound HLA isoforms with a transmembrane region and an intra-cytoplasmic tail, and three secreted isoforms HLA-G5, HLA-G6 and HLA-G7.

Because trophoblast forms the physical interface between foetus and mother, HLA-G might play a role in maternal immunological accommodation of the semi-allogeneic foetus, Apps conclude that the evidence for trophoblast HLA-G stimulating leukocyte immunoglobulin-like receptor B1 receptors on decidual leukocytes is compelling. These findings suggest how a foetal molecule might influence the local maternal immune response. As HLA-G+ trophoblast cells infiltrate the uterine mucosa, they might deliver a pregnancy specific signal to the local maternal leukocytes and modify their function to accommodate the foetus-placental unit [33]. It is assumed the importance of HLA in the immune response and in the modulation of maternal-foetal immune relationship during pregnancy.

There is an immunomodulation of cytokines secretion that is believed that creates a chemical dialogue between embryo and maternal immune tolerance.

Soluble HLA-G suppresses the functional activity of Natural Killer (NK) cells and inhibits NK cell-mediated cytotoxicity [34] that

suggests is important to induce immunotolerance, control trophoblast invasion and contribute to vascular remodelling of spiral arteries to allow implantation and pregnancy maintenance. All this point to the fundamental role that sHLA-G expression of the invasive cytotrophoblasts has in creating a tolerogenic condition at the foetus-maternal interface.

Biomarkers in Endometrial Fluid

Embryo implantation depends not only on the endometrial receptivity, but also on the uterine environment. The endometrial fluid content selective transudation from the blood, carriage from the Fallopian tubes and likely also the peritoneal cavity and, importantly, secretions from the endometrial glands [4], with ions, amino acids, carbohydrates, lipids and proteins, including hormones, cytokines, enzymes, grow factors, etc. [35]. Meng report the application of a new methodology that allows to measure biomarkers of endometrial development within uterine secretions, using non-invasive testing, instead of a traditional endometrial biopsy [36]

The advantage is the possibility to obtain the diagnosis of biomarkers with aspiration of the endometrial fluid in the same embryo transfer cycle, with minimal invasion.

Proteins

Li found a total of 31 identified proteins that could be classified into the following functional categories of the correlation with implantation process: cell migration or assimilation (five proteins), enzymic activity (nine proteins), signal transduction and gene regulation (nine proteins), immunoregulation (four proteins), vascularization (two proteins), and blood clotting or fibrinolysis system (two proteins) and suggests that the proteomic analysis might serve as a tool for predicting the endometrial remodelling from the pre-receptivity to receptivity phase in humans. These results may allow further understanding of the biological mechanisms underlying human endometrial receptivity [37].

Table 1: Proteins Regulated in Endometrium from LH D7 versus LH D2 (37).

Protein description	Function
Membrane-associated P receptor component 1	Membrane proteins
Coagulation factor XIII A chain	Miscellaneous
Vimentin	Structural factors
Tubulin beta-2C chain	Structural factors
FK506-binding protein 4	Intracellular/signalling factors
Transaldolase	Enzyme
Prolyl 4-hydroxylase alpha-2 subunit	Enzyme
Protein DJ-1	Miscellaneous
Reticulo calbin-1	Ion binding proteins
Heat shock protein HSP 90-beta	Membrane proteins
DNA mismatch repair protein Msh2	Miscellaneous
Alcohol dehydrogenase (NADP+)	Enzyme
Rab GDP dissociation inhibitor beta	Intracellular/signalling factors
Elongation factor 2	Intracellular/signalling factors
Ezrin	Intracellular/signalling factors
Putative ATP-dependent Clp protease proteolytic subunit	Enzyme
ETHE1 protein	Miscellaneous
Triosephosphate isomerase	Enzyme
Thioredoxin domain-containing protein 4 (precursor)	Miscellaneous
Cathepsin B	Enzyme
Enoyl-CoA hydratase	Enzyme
Gelsolin	Structural factors
Protein-glutamine gamma-glutamy	Enzyme
Annexin A4	Ion binding protein
Bystin	Cell adhension molecules
Collagen alpha-2(VI) chain	Structural factors
Acyl-CoA dehydrogenase, short/ branched chain specific	Enzyme
Peroxiredoxin-1	Miscellaneous
Annexin A2	Ion binding protein
Fibrinogen beta chain	Coagulation factor
Superoxide dismutase (Mn)	Enzyme

Recently, S100A10, that concerns migration, decidualization and apoptosis, some major biological functions involved in the implantation process, might play a key role during implantation, would be a candidate biomarker for predicting implantation failure, particularly due to inadequate endometrial receptivity [38].

Nucleolar channel systems (NCSs) can be detected in exfoliated endometrial epithelial cells (EECs) of uterine secretions. This detection may represent the development of less invasive methods for assessment of endometrial receptivity based on endometrial secretions, and that identifies only maximal endometrial receptivity [36].

Lipids

Braga detected in his study that seven lipids were strongly represented in the pregnancy group, and four lipids in the no pregnancy group, and he found that Ceramide, strongly represented in non-receptive endometria may point to a possible temporal displacement on the window of implantation [39].

On the other hand, Vilella showed a PGE2 and PGF2α profile in the human EF, which can be used to detect the WOI in natural, IVF, and ovum recipient cycles, which is nullified with the insertion of an IUD (ie, in refractive endometrium). This evidence suggests that PGE2 and PGF2α concentrations in the human EF can be used as no invasive biomarkers to personalize ART treatments [40].

Conclusions

At present, numerous investigations have been carried out to detect an endometrial marker more specific to detect the WOI. This will be essential to increase pregnancy rates in ART cycles.

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