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## Biomarkers Related to Endometrial Receptivity and Implantation

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### 1. Introduction

Implantation is a process requiring the delicate interaction between the embryo and a receptive endometrium. This intricate interaction requires a harmonized dialogue between embryonic and maternal tissues. [Aghajanova et al., 2008; Simon et al., 2000] The three stages of implantation are: apposition, adhesion, and invasion. Apposition describes trophoblast cells adhering to the receptive endometrial wall. Adhesion to the basal lamina and stromal extracellular matrix occurs in the presence of specific hormones, cytokines, and adhesion molecules. Once the blastocyst is anchored to the endometrial wall, it will become enclosed by an outer layer of syncytiotrophoblast, and an inner layer of cytotrophoblast. As the syncytiotrophoblast erodes the endometrium, the blastocyst will burrow into it and implantation will occur. [Ganong, 2005] During the last few years, research pursues enhancing both the quality of the embryo as well as understanding the highly dynamic tissue of the endometrial wall. Despite morphological and chromosomal criteria to improve the quality of transferred embryos, implantation rates remain at 25-35%. [Boomsma & Macklon, 2006]

The priming of the endometrium to optimize the window of implantation phase has been a subject of interest for decades, and much work has gone into understanding the preparation and capability of the endometrial wall to create a hospitable environment for the interaction with the blastocyst. While an embryo factor accounts for one third one implantation failure, lack of uterine receptivity explains approximately two thirds of implantation failures. [Achache, 2006; Ledee-Bataille et al., 2002] The actions of numerous cytokines, hormones, immunoglobulins, and other factors, are all orchestrated into preparing the endometrium for implantation. The morphological changes towards a receptive endometrium have been described as early as 1950 by Noyes, Hertig, and Rock [Strowitzki, 2006] and occur under the control of the sexual steroid hormones estrogen, and progesterone; with estrogen being the determinant hormone in the proliferative phase and progesterone being the determinant hormone in the secretory phase

During the luteal implantation phase; corresponding to cycle days 20-24, or seven to nine days after ovulation, the endometrium is receptive to the oncoming blastocyst. [Goiran &

Mignot, 1999] Essential expression of proteins, cytokines, and peptides can be detected at this time and serve as biomarkers for maximal endometrial receptivity. [Singh & Aplin, 2009; Lessey et al., 2002] The detection and investigation of biochemical markers during the implantation phase is an area of research receiving much interest and may serve to establish future treatments to help maximize the effectiveness of assisted reproductive techniques (ART) in the near future. According to Zhu, biomarkers are those that are present in the endometrium during the implantation phase, close to the implantation site, and disappear thereafter. [Cavagna & Mantese, 2003] This chapter will discuss biomarkers and their role in the attachment and invasion process during the implantation phase.

## **2. Biomarkers**

### **HLA-G**

Human leukocyte antigen G (HLA-G) is a major histocompatibility complex (MHC) class Ib gene thought to play an essential role in implantation by modulating cytokine secretion to maintain local immunotolerance and modulate cytokine secretion to control trophoblastic cell invasion. [Roussev & Coulam, 2007] At first, HLA-G was proposed as a protector against natural killer (NK)-cell-mediated cytotoxicity of target cells and to prevent allorecognition by maternal cytotoxic lymphocytes. Recently, it has been shown that these proteins regulate immune cells including T cells, NK cells, and antigen-presenting cells. [Fournel, 2000] Due to its essential role in the implantation process, recent attention has been focused on HLA-G and its diagnostic and therapeutic clinical applications. This has included the evaluation of couples with recurrent miscarriages and the mutation of the HLA-G gene. Serum sHLA-G levels during pregnancy may in the future become a diagnostic tool for evaluation of successful implantation but has yet to be established. [Roussev & Coulam, 2007]

### **Pinopodes**

Pinopodes are organelles shown to be present on the endometrial wall during the implantation phase. They have been detected by electron microscopy and are specific markers for uterine receptivity. Progesterone dependent, pinopodes are present 20-21 days into the luteal cycle. [Cavagna & Mantese, 2003] Their function has not fully been established, but pinopodes are thought to play a role in protecting the blastocyst from being swept by the cilia on the endometrial wall promoting withdrawal of uterine fluid and facilitating molecular adhesion of the pinopodes with the blastocyst. The life span of fully developed pinopodes lasts no more than 48 hours suggesting a transient cell state. Following ovarian stimulation with clomiphene citrate and human chorionic gonadotropin (hCG), pinopodes formed a little earlier, on days 17 or 18 than in the natural state. [Cavagna & Mantese, 2003] It is thus possible that ovarian stimulation and early pinopode formation may have a role in shifting the window of receptivity resulting in asynchrony between the endometrium and blastocyst thereby negatively influencing implantation rates with IVF.

### **Integrins**

Integrins are a family of transmembrane glycoproteins, formed by the interaction of two different, non-covalently linked  $\alpha$  and  $\beta$  subunits. [Achache & Revel, 2006] They are adhesion molecules which participate in cell-adhesions and have also shown to play part in

adhesions between cells and extracellular components. [Ceydell, 2006] In addition, integrins participate in many physiologically important processes including embryological development, haemostasis, thrombosis, wound healing, immune and non-immune defense mechanisms and oncogenic transformation. Specifically, the  $\alpha v \beta 3$  integrin as well as its ligand osteopontin was positively detected by immunohistochemistry on the endometrial luminal epithelial surface, which first interacts with the trophoblast. [Achache & Revel, 2006; Apparao ET AL., 2001] The expression of the endometrial stromal integrins may be modulated by several factors and the expression of the  $\alpha v \beta 3$  integrin in the endometrial stroma was demonstrated to be stimulated by IL-  $\alpha$ , IL-  $\beta$  and TNF- $\alpha$ . [Ceydell, 2006] Integrins have been proposed as markers for endometrial receptivity, and the  $\alpha v \beta 3$  glycoprotein particularly has been directly associated with implantation.

### **L-selectin**

Selectins are lectin like proteins and include E-, L-, and P-selectins, all of which were originally thought to be expressed solely by hemangioblast descendants. P- selectins are expressed on the surface of platelets, E- selectins are expressed on activated endothelial cells, and L-selectins are expressed on lymphocytes. Glycoproteins carrying oligosaccharide formations including CD34, GlyCAM-1, PSGL-1, podocalyxin, and endoglycan, are recognized by the selectin molecules. [Foult et al., 2007] Selectins are responsible for the tether and roll mechanism on endothelial surfaces. Once leucocytes slow down and subsequently arrest, integrin activation triggers adhesion and transmigration through the vascular endothelium. [Torry et al., 2007] Recently, Genbacev et al. [Genbacev et al., 2003] have shown that hatched blastocysts expressed L-selectin and used this molecule to mediate its attachment to the luminal epithelial surface via MECA-79, its carbohydrate ligands, and related epitopes. [Foult et al., 2007] Also, Foult and Zdravkovic have shown that lack of expression of the L-selectin ligand MECA-79 in mid-luteal endometrial biopsies were indicative of low or no chance of pregnancy.

### **Heparin binding-epidermal growth factor**

Heparin binding-epidermal growth factor (HB-EGF) interacts with the EGF receptor and belongs to the epidermal growth factor family. It has been shown that HB-EGF expression is low during the proliferative endometrial phase, attaining its highest measure immediately prior to the implantation window, suggesting that it may have a role during the blastocyst implantation process. [Cavagna & Mantese, 2003; Lessey et al., 2002] It has been suggested that HB-EGF promotes implantation and trophoblast invasion through paracrine/autocrine signaling as cells penetrate the stroma. HB-EGF has also been shown to inhibit apoptosis and induces an invasive trophoblast phenotype. The co-existence of HB-EGF and pinopodes has been investigated with electron microscopy and immunochemistry, and shows that the expression of HB-EGF is highest when fully developed pinopodes are present, supporting the role of HB-EGF in the implantation process. [Cavagna & Mantese, 2003, Stavreus et al., 2001]

### **Chorionic gonadotropin and Notch 1**

Chorionic gonadotropin is one of the early embryonic secretions from the trophoblast cells of the pre-implantation embryo. This helps maintain the corpus luteum of pregnancy, and leads to the modifications in morphology and endometrial gene expression preparing for implantation.

The notch family of receptors mediates a highly conserved pathway that regulated differentiation and pro-survival signals from humans to varied species of invertebrates. [Afshar et al., 2007; Paria et al., 2002] Notch proteins are ligand-dependant transmembrane receptors that transduce extracellular signals responsible for cell-fate and differentiation throughout development. Notch signaling often restricts the differentiation fates of a cell, directing it to a specific cell fate in cooperation with other signals, while at the same time inhibiting differentiation toward an alternate fate and promoting survival. Evidence indicates that Notch signaling regulates all three branches of the fate cell decision tree; differentiation, cell cycle progression and apoptotic cell death. Recently Afshar et al. have shown the co-expression of  $\alpha$ SMA and Notch 1, both arising from CG signaling, inhibits apoptosis of stromal cells during the establishment of pregnancy. Shedding of the uterine lining and the inability of the uterus to accept an embryo can be correlated with low expression of Notch 1. Survival of the uterine lining can be mediated by (h)CG supplementation or progesterone as they will induce the expression of Notch 1. [Afshar et al., 2007]

### **Mucins**

Mucins are glycoproteins high in molecular weight, which contain at least 50% of carbohydrate O-linked to a threonine/serine rich peptide core. [Gendler et al., 1990] MUC-1 is a large glycoprotein with a molecular weight >250 kDa. [Achache & Revel, 2006] When highly expressed on a cell surface, MUC-1 produces a steric hindrance phenomenon interfering with cellular adhesion. Cell-cell and cell-matrix adhesions are inhibited in direct correlation to the length of the MUC-1 ectodomain. [Hilkens et al, 1992] The apical surface of most epithelial cells is protected by a thick glycocalyx composed mostly of mucins that are believed to protect the cell surface from pathological processes. In the endometrium, MUC-1 is probably the first molecule the blastocyst encounters on the endometrial wall before implantation. This interaction would seem to indicate the blastocyst might be deterred from the endometrial wall until a proper location is encountered for implantation. In mice, rats, and pigs it has been shown that MUC-1 is down-regulated during the window of receptivity and thus optimizing the interaction between blastocyst and uterine wall. Paradoxically in humans, it has been shown that MUC-1 is up-regulated during the pre-implantation period. Therefore, it was suggested that humans must have a mechanism to induce inhibitory factors to down-regulate the MUC-1 barrier. High progesterone levels apparently reduce MUC-1 levels, thus unmasking intracellular adhesion molecules (CAM) on the surface of the endometrium and increasing uterine receptivity. [Bowen et al., 1996] Immunohistochemistry and scanning electron microscopy have shown that the MUC1 epitope corresponds only to ciliated cells. But the surface of non-ciliated cells such as pinopods has not been correlated to MUC-1. It has been suggested that pinopodes are important in providing a MUC-1 free area for blastocyst implantation. It seems that even though MUC-1 appears to have negative effects on implantation, its upregulation and extension beyond the glycocalyx covering the endometrium suggest it may have a temporary role in directing the embryo to effective implantation. [Achache & Revel, 2006]

### **Calcitonin**

Parafollicular cells of the thyroid release calcitonin in response to hypercalcemia to reduce calcium levels. Though its role remains to be determined, calcitonin is expressed in the human endometrium during the secretory phase with highest concentrations on luteal cycle days 19-21, coinciding with the implantation period. It has also been demonstrated

progesterone induces calcitonin gene expression in the endometrium. [Cavagna & Mantese, 2003; Kumar, 1998] By immunoreactivity for calcitonin mRNA, calcitonin seems to be absent during the proliferative and ovulatory phase. This finding may be another reason to suspect that calcitonin may be a marker for uterine receptivity. Calcitonin controls calcium homeostasis by binding to specific receptors identified as CR1a and CR1b. [Sexton et al., 1993; Wang, 1998] CR1a receptors have been found to be present in murine oocytes and zygotes in low concentrations, but significant increase of this receptor was found in embryos between the 8 cell and blastocyst stage. Wang et al. have also shown blastocysts differentiate in vitro at an accelerated rate when treated with 10 nM calcitonin for 30 minutes. [Cavagna & Mantese, 2003; Wang, 1998] Though this seems to demonstrate the role of calcitonin in embryonic development, further studies will need to be conducted to show the definitive role of calcitonin during implantation and development of the embryo.

### Prostaglandins

As implantation takes place, the blastocyst needs access and connection to the maternal vascular system. For this to occur there needs to be an increase of vascular permeability at the site of implantation. [Chakraborty et al., 1996] Prostaglandins (PGs) are known to possess vasoactive factors, play a definitive role in ovulation, fertilization, and labor, and recently have shown to be crucial during the implantation process. [Achache & Revel, 2006, Song et al., 2006]

Prostaglandins are eicosanoids consisting of four members, PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2</sub>α, and prostacyclin (PGI<sub>2</sub>). These are generated by the action of two enzymes, cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>), and cyclooxygenase (COX). Song et al. have demonstrated female mice lacking the cPLA<sub>2</sub> and COX enzymes are not able to produce PG, leading to significant implantation defects. cPLA<sub>2</sub> knockout mice also exhibited pregnancy failures and small litter size, secondary to delayed implantation. Exogenous administration of PG was able to restore embryo implantation at the correct time. It is not clear whether diminished expression of PG prevents human fertility because mice lacking PG will be fertile but present with fine tuning details. Thus it is postulated a similar process in humans leading to delayed implantation could lead to early pregnancy loss. Further investigation on the role of PGs at the time of human implantation and its possible role in late-pregnancy abnormalities needs to be further explored. [Achache & Revel, 2006, Song et al., 2006]

### HOX genes

Homeobox genes HOXA-10 and HOXA-11 have been linked with endometrial receptivity. Mutations in these genes have led to failure to achieve normal implantation in mice. [Cavagna & Mantese, 2003; Daftary & Taylor, 2001] Growth and development of the human endometrium have been linked with these genes, and shown to have significant up-regulation in the mid-secretory phase correlating with the implantation window. Female mice with homozygous mutations in the HOXA-10 or HOXA-11 have been shown to be infertile due to endometrial factors. [Satokata et al., 1995] According to Benson et al., the HOXA-10 gene may be important during morphogenesis for proper patterning of the reproductive tract and in adult endometrium for adequate implantation events.

In women with endometriosis, Taylor et al. observed HOX gene expression is altered resulting in endometrial molecular alterations resulting in decreased endometrial

receptivity. These observations further support the importance HOX gene expression may have during implantation process.

### **Angiogenesis**

Vascular development at the maternal fetal interface is an essential component for successful implantation and development. Trophoblasts, natural killer cells, and other cell types are responsible for this development. Trophoblasts are well known to produce angiogenic growth factors. [Cross et al., 2002; Torry et al., 2007] Ungranulated uterine natural killer cells (uNK) precursors are recruited to the endometrium during the transition of the endometrium to the secretory phase. Progesterone allows the development of the pre-uNK into large granulated uNK cells. These appear to be present during the implantation phase and have a role in releasing cytokines responsible for angiogenesis in early pregnancy, and development of spiral arteriole formation as the pregnancy progresses. [Leonard et al., 2006] In vitro models of mice have suggested that progesterone serves to up-regulate decidua IL-15, in turn serving as a main activator of uNK population. [Leede-Bataille et al., 2005]

Other cells such as B and T lymphocytes have also been implicated in angiogenesis during the early phases of pregnancy. B lymphocytes have been shown to express the c-Myc oncogene, which can induce angiogenesis by producing VEGF. [Ruddell et al., 2003] Vascular endothelial growth factor (VEGF) is a known angiogenic substance involved in the process of vascular proliferation. [Tammela et al., 2005] During the peri-implantation phases, certain VEGF receptors appear to be expressed and function to optimize blastocyst implantation by mediating vascular permeability. These are VEGFR-1, VEGFR-2, and NRP-1. [Halder et al., 2000; Torry et al., 2007] The function and expression of VEGF have shown to be pivotal for angiogenesis during the implantation process and early placental development. Disturbance of this process could lead to implantation failure and early pregnancy loss.

### **Insulin like growth factor-II (IGF-II)**

Insulin-like growth factors along with their binding proteins are thought to be responsible for differentiation, endometrial growth, angiogenesis, and apoptosis. [Cavagna & Mantese, 2003] IGF-II in particular is a known mediator of trophoblast function and is required for suitable placental growth and transport function. [Herr et al., 2003] Trophoblasts have been shown to express IGF-II while vessels near the implantation site have similarly been shown to express IGF-II receptors indicating IGF-II may directly act as an angiogenic growth factor. [Torry et al., 2007] In mice, IGF-II has demonstrated its vessel proliferation potential by inducing angiogenic growth factors such as VEGF and proliferin. Insulin like growth factors are regulated by insulin-like growth factor binding proteins (IGFBP). [Cavagna & Mantese, 2003] Licht et al. have shown that secretion of IGFBP-1 by the endometrium occurs approximately 10 days after the LH surge, correlating with the implantation window. [Licht et al., 2002] With IGFBP-1 being the predominant regulatory factor for IGF-II, it may play an important role in endometrial receptivity and implantation.

### **Leukemia Inhibitory Factor (LIF)**

In 1992, Hilton demonstrated LIF to be a haemopoietic factor by its capability to stimulate macrophage differentiation of the mouse myeloid leukemia cell line. [Achache & Revel,

2006; Hilton, 1992] Proliferation, cell survival, and differentiation, are some of the autocrine and paracrine effects of LIF, and have led researchers into investigating its function in blastocyst development and implantation. A study by Stewart [Stewart, 1994] showed that female mice expressing homozygous LIF gene deficiency displayed failed embryo implantation. Further evidence of the importance of LIF was observed as LIF supplementation rescued embryo implantation in the previously affected mice. LIF expression was observed to reach maximum concentrations in the mid- to late-secretory phase. Endometrial biopsies have shown LIF mRNA expression on days 18 to 28 of the menstrual cycle with maximum expression on day 20. [Charnock-Jones et al., 1994] Infertile patients and those with repeated implantation failures have been shown to have abnormal levels of LIF supporting the role of LIF as a fundamental element in the implantation process. [Achache & Revel, 2006] Preclinical and clinical trials have investigated the effects of recombinant human LIF (r-hLIF) in improving endometrial receptivity. [Brinsden et al., 2003] In light of the importance of LIF in the implantation process, r-hLIF could be an important tool in the near future to optimize endometrial receptivity.

### **Serum-and Glucocorticoid-Regulated Kinase 1 (SGK1)**

Recently, Feroze-Zaidi et al. demonstrated that women with unexplained fertility or recurrent implantation failure after IVF showed an abnormal expression of the SGK1 gene in the luminal epithelial cells during the midsecretory receptive phase corresponding with the implantation window. [Fakhera et al., 2007] Regulation of epithelial Na<sup>+</sup> channels (ENaCs) is known to be controlled by SGK1. Uterine fluid homeostasis could thus be directly influenced by SGK1 leading to decreased uterine receptivity and disruption of successful implantation. Differentiating human endometrial stromal cells (HESCs) also activate SGK1, which stimulates the expression of prolactin (PRL), a most important decidual marker gene. [Brosens & Gellersen, 2003, 2006]

FOXO proteins are known to be able to regulate genes involved in proapoptotic properties, and also genes involved in differentiation, cell cycle arrest, DNA repair, and oxidative defenses. [Fakhera et al., 2007; Sunter et al., 2003] Phosphorylation of transcription factors regulating expression of FOXO proteins, are targeted by kinases including SGK1 which serve to inactivate such proteins. [Brunet et al., 2001; Rena et al., 2003] Increased activity of SGK1 in the midsecretory phase may disrupt implantation by disrupting normal activity of ENaC-mediated Na<sup>+</sup> and water transport or by interrupting focal apoptosis.

### **Interleukin-6 (IL-6)**

IL-6 is a cytokine classically known to induce immunoglobulin production in activated B cells, but also found to display a wide variety of functions outside the B-lymphocyte system. IL-6 expression in the human endometrium has been detected with the highest levels corresponding to the luteal phase. [Achache & Revel, 2006] mRNA expression of IL-6 steadily increases during the mid- to late-secretory phase and then decreases again in the late-secretory phase. During the crucial window of implantation, immunoreactivity for IL-6 becomes markedly detectable. The epithelial and glandular cells are the areas where the protein is mostly pronounced, compared to the stroma. During the window of implantation, receptors for IL-6 can be found not only in the endometrium, but are also expressed in the blastocyst, suggesting the paracrine/autocrine role of IL-6 during the peri-implantation period. Experiments performed using mice with disrupted IL-6 genes have shown despite

implantation, the growth and development of the blastocyst becomes compromised. [Achache & Revel, 2006; Salamonsen et al., 2000] This suggests that even though IL-6 may not be an essential element for implantation, the lack of its presence could still explain infertility in some cases. Recent findings of patients with recurrent abortions have shown that IL-6 endometrial m-RNA is suppressed in the mid-secretory phase, thus supporting the role and importance of IL-6 in infertility.

### **Interleukin-1 (IL-1)**

IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1 receptor antagonist (IL-1ra) are all included in the family of IL-1, and serve as pivotal mediators of the immunologic and inflammatory response. In past experiments with mice, knockout mice for IL-1 were still able to proceed with implantation, but of interest, mice who received intraperitoneal injections of the IL-1ra displayed blastocysts unable to implant on the endometrial wall. Simon attributed this to the down regulation of crucial integrins at the luminal epithelial surfaces by the IL-1ra. In humans, it has been observed that administration of IL-1 causes an increase of  $\beta$ 3 expression in the culture media of EECs thereby optimizing blastocyst implantation. [Achache & Revel, 2006] Leptin has also been shown to increase integrin  $\beta$ 3 expression. Interestingly enough, IL-1 $\beta$  acts in stimulating leptin secretion and up-regulating its Ob-R receptor in EECs. IL-1 Rtl mRNA and protein have shown to be present in maximal levels during the luteal phase in the human epithelial endometrium, and expression of the IL-1 antagonist has been shown to be reduced during the period of the implantation window. This finding suggests that suppression of the IL-1 antagonist during this crucial period of implantation maximizes successful implantation. [Boucher et al., 2001]

In women with endometriosis, the levels of IL-1ra and IL-1 $\alpha$  were found to be markedly increased when compared to control groups in the PF and serum, and may serve as an explanation of the pathogenesis and infertility in such patients. [Kondera-Anasz et al. 2005]

### **Leptin**

Acting both at the endocrine and paracrine level, leptin has been associated with regulation of body weight and reproductive function. [Cervero et al., 2004] Leptin is the product of the OB gene. Studies with rodents have determined this ligand-receptor system to be necessary for implantation. Receptors associated with leptin include total leptin receptor (OB-RT), the long form (OB-RL), and HuB219.1 and HuB219.3 short isoforms found in the endometrium. Studies with mice expressing ob/ob mutations resulted in phenotypically obese and sterile mice. Exogenous leptin treatment was able to restore sterility in these mice, but food restriction was not, implicating leptin as a requirement for normal reproductive functioning. [Cervero et al., 2004] Additionally, leptin has also been shown to increase integrin  $\beta$ 3 expression, an important ligand protein essential for endometrial receptivity and implantation. The leptin receptors OB-RT, OB-RL, HuB219.1, and HuB219.3 have all demonstrated maximal expression in the late luteal phase. [Achache & Revel, 2006]

### **Cadherins**

Cadherins are responsible for calcium-dependent cell-to-cell adhesion mechanisms and belong to a group of glycoproteins divided into N-, P-, and E-cadherins, all displaying specific functions and tissue distributions. Of all the cadherins, E-cadherin is the most studied pertaining to implantation, is ubiquitous, and is believed to be responsible for

maintainance of adherens junctions in epithelial cells. [Singh & Aplin, 2009] E-cadherin suppression is responsible for cell-cell adhesion dysfunction. Riethmacher et al. demonstrated that targeted mutation of the E-cadherin gene resulted in defective pre-implantation development in mice.

During the luteal phase, E-cadherin mRNA levels are significantly elevated and regulation seems to be mainly controlled by intracellular calcium levels. E-cadherin cytoskeletal organization and disassembly at the adherens junction are mediated by rising levels of calcium which work by acting on signaling pathways. In vitro studies have shown that calcitonin produces a transient rise in intracellular calcium levels, suppressing E-cadherin at cellular contact sites. These experiments were performed by Li et al. on cultured Ishikawa cells.

Calcitonin appears to be an important regulator of implantation. Progesterone acts to increase calcitonin levels, which in-turn acts to increase intracellular calcium thus regulating E-cadherin expression. E-cadherin then seems to serve two main functions of uterine receptivity: adhesiveness in the preliminary phases; and inactivation by the actions of progesterone and calcitonin in the secretory phase to allow epithelial cell disassociation and implantation. [Achache & Revel, 2006]

### **Cyclin E and p27**

Cyclins are known to control mitotic phase progression in cells. The G1 to S phase transition is controlled by the rate limiting step of Cyclin E, whereas prevention of the cell cycle progression is controlled by the p27 cyclin-dependent kinase inhibitor. [Kliman et al., 2006] While the plausible role of cyclin E involves proliferation, p27 is mostly responsible for differentiation. [Dubowy et al., 2003] Consistent with these actions, estrogen has positive regulatory effects on Cyclin E and progesterone seems to induce a dominant p27 state. Cyclin E activity is present in the cytoplasm of epithelial cells whereas p27 activity is exclusively active in the nucleus. While present in the early phases of the menstrual cycle, Cyclin E reactivity seems to rapidly decrease after cycle day 19; this could be explained by its subsequent movement towards the nucleus where it binds to p27 thereby becoming inactivated.

The Endometrial Function Test (EFT) is a means to assess Cyclin E by immunohistochemically staining endometrial biopsies using antibodies against Cyclin E; and also as a means to identify an abnormally developing endometrium. [Dubowy et al., 2003] EFT showing a persistence of Cyclin E was associated with glandular developmental arrest (GDA), and observed in women with infertility. The overexpression of Cyclin E seemed to indicate that cells were arrested at an earlier phase of the menstrual cycle, possibly due to a premature expression of p27. [Kliman et al., 2006] The development of the EFT associated with cyclin markers and their correlation to estrogen and progesterone could serve as an important tool in the near future to assess endometrial receptivity and the effects of exogenous hormone administration in infertile patients. [Kliman et al., 2006]

### **Colony Stimulating Factor-1 (CSF-1)**

CSF-1 is a haemopoietic growth factor inducing proliferation and differentiation of cells belonging to the mononuclear phagocytic lineage. Pollard et al. have demonstrated that

op/op mice with mutations in CSF gene displayed multiple skeletal defects and decreased implantation rates. Other studies have shown CSF-1 to also be an important factor when it comes to ovulation. Op/op mice compared to wild type mice showed significant lower follicular development and ovulation rates. It has been shown that women with lower preconceptional CSF-1 levels are more prone to recurrent abortions compared to women with higher preconceptional CSF-1 levels. [Cavagna & Mantese, 2003]

## 2. Clinical implications

Continuing investigation into understanding and exploring new markers of endometrial receptivity remain a high priority in reproductive endocrinology. Recent studies performed by Haouzi and associates have found new genes expressed during the implantation window by the human endometrium. [Haouzi et al., 2009] This information along with knowledge of previously discussed biomarkers can lead investigators to a more thorough approach when performing endometrial biopsies during a natural cycle especially in patients who have had unsuccessful IVF cycles. The goal of such investigation is to better understand the requirements of a hospitable environment for blastocyst implantation. Such knowledge may decrease unsuccessful implantation and facilitate a single embryo transfer in a well known receptive environment during an IVF cycle.

## 3. Future applications

With recent significant attention given to endometrial receptivity, it is with no surprise that new methods of investigating the endometrial factor are under investigation and may soon become routine when exploring causes for infertility. Recently performed studies have now started to analyze endometrial secretions prior to embryo transfers in IVF and IUI patients. [Boomsma et al., 2009] Recent research conducted by Boomsma and associates evaluated secretions of different cytokines including interleukins, tumor necrosis factor- $\alpha$ , macrophage migration inhibitory factor, eotaxin, monocyte chemotactic protein-1, and heparin-binding epidermal growth factor. [Boomsma et al., 2009] Such novel modalities may soon elucidate new therapies and treatments of defective endometrial receptivity.

## 4. Conclusion

With the precisely timed roles of different cytokines, hormones, and immune regulatory mechanisms, implantation is an intricate process requiring the collaboration of synchronized timed events and chemical interactions. As previously discussed, the “window of implantation” corresponds to a short period of time between days 20 and 24 of the menstrual cycle when the endometrium becomes receptive to the oncoming blastocyst. During the first part of the menstrual cycle, estrogen is present as the predominant hormone causing endometrial cell proliferation. Progesterone secreted by luteinized follicles after ovulation in the latter phase of the menstrual cycle serves to induce cell differentiation.

Approximately five-six days after ovulation, the blastocyst will enter the uterine cavity in search of a well prepared endometrium for implantation. Biomarkers such as the ones previously discussed are vital to ensure this process is successful. Selectins and mucins play a role in leading the blastocyst to a receptive endometrium, while integrins and

cadherins serve as adhesion molecules for nidation. This fine orchestration of biomarkers and timed events has lead scientists toward improved understanding of the endometrium and its role during implantation. During the last decades, many advances have been made to improve ovulation and the quality of embryos. While remarkable advances such as IVF and other ART have been achieved, scientists are starting to realize the importance of a “fertile ground” at the embryo-uterus interface. Current research leading to the better understanding of biomarkers and endometrial receptivity may lead to optimization of embryo implantation in the future. Screening for receptivity markers and treating patients accordingly may allow for increasing use of a single embryo transfer with IVF leading to fewer complications encountered from multiple gestations. Patients will in tandem benefit by avoiding high costs of recurrent ART treatments and emotional despair from failed procedures. Some physicians are already taking proactive approaches in assessing endometrial receptivity by assessing biomarkers such as integrins, cyclin E, p27, and recently, even genes from endometrial biopsies. Such screenings may be standard in the near future and may lead to favorable treatments with subsequent higher rates of successful implantation.

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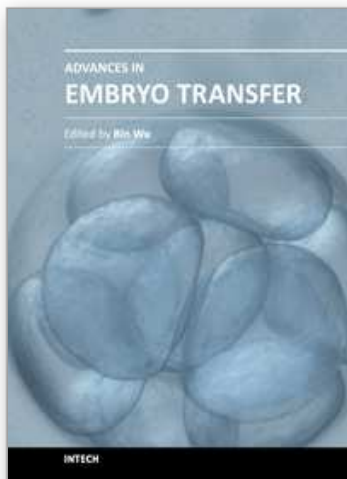
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Embryo transfer has become one of the prominent high businesses worldwide. This book updates and reviews some new developed theories and technologies in the human embryo transfer and mainly focus on discussing some encountered problems during embryo transfer, which gives some examples how to improve pregnancy rate by innovated techniques so that readers, especially embryologists and physicians for human IVF programs, may acquire some new and usable information as well as some key practice techniques. Major contents include the optimal stimulation scheme for ovaries, advance in insemination technology, improved embryo transfer technology and endometrial receptivity and embryo implantation mechanism. Thus, this book will greatly add new information for readers to improve human embryo transfer pregnancy rate.

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