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Implantation of the Human Embryo

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1. Introduction

Implantation is the final frontier to embryogenesis and successful pregnancy. Over the past three decades, there have been tremendous advances in the understanding of human embryo development. Since the advent of *In Vitro Fertilization*, the embryo has been readily available to study outside the body. Indeed, the study has led to much advancement in embryonic stem cell derivation. Unfortunately, it is not so easy to evaluate the steps of implantation since the uterus cannot be accessed by most research tools. This has limited our understanding of early implantation. Both the physiological and pathological mechanisms of implantation occur largely unseen. The heterogeneity of these processes between species also limits our ability to develop appropriate animal models to study. In humans, there is a precise coordinated timeline in which pregnancy can occur in the uterus, the so called "window of implantation". However, in many cases implantation does not occur despite optimal timing and embryo quality. It is very frustrating to both a patient and her clinician to transfer a beautiful embryo into a prepared uterus only to have it fail to implant. This chapter will review the mechanisms of human embryo implantation and discuss some reasons why it fails to occur.

2. Phases of human embryo implantation

The human embryo enters the uterine cavity approximately 4 to 5 days post fertilization. After passing down the fallopian tube or an embryo transfer catheter, the embryo is moved within the uterine lumen by rhythmic myometrial contractions until it can physically attach itself to the endometrial epithelium. It hatches from the zona pellucida within 1 to 2 days after entering the cavity thereby exposing the trophoblastic cells of the trophectoderm to the uterine epithelium. Implantation occurs 6 or 7 days after fertilization. During implantation and placentation, a human embryo must attach itself to the uterus under conditions of shear stress. The embryo is rolling about within a mucus rich environment between the opposing surfaces of the endometrial walls of the uterus. This interactive process is a complex series of events that can be divided into three distinct steps: apposition, attachment and invasion (Norwitz et al., 2001).

2.1 Apposition

Once the human blastocyst hatches from the zona pellucida, the free-floating sphere of cells must orient itself as it approaches the endometrial surface and form an initial adhesion

(apposition) before it can firmly attach and begin the process of invasion. The apposition step is a transient and dynamic process whereby the embryo "tethers" itself to the endometrial surface. Uterine contractions and mucin secretion within the uterine cavity propel the blastocyst around the cavity. Despite these fluid dynamics which creates shear stress, the embryo is able to approach the wall, roll around to right itself so that the trophectoderm overlying the inner cell mass apposes to the endometrial surface. During this apposition phase, there is a dialogue between the floating blastocyst and endometrium using soluble mediators such as cytokines and chemokines acting in a bidirectional fashion to guide the blastocyst onto a 3-dimensional "docking" structure. The hormone-regulated pinopodes at the endometrial surface have been shown to mark the timing and appearance of optimal endometrial receptivity (Bentin-Ley et al., 2000).

Chemokines such as IL-8, RANTES, or MCP-1 are secreted locally by both the endometrium and blastocyst during the implantation window during the apposition phase. The L-selectin system, in particular, has been shown to be critically important during the blastocyst apposition phase (Genbacev et al., 2003). Selectins are lectin-like glycoproteins that include E-, L- and P-selectin, all of which were originally thought to be expressed exclusively by hemangioblast descendents. E-selectin is expressed on activated endothelial cells, P-selectin is expressed on the surfaces of activated platelets and endothelial cells and L-selectin is expressed on lymphocytes. Initial interest in the selectin system came because the implantation process bears some similarity to leukocyte transmigration across the blood vessel wall. Similar to a rolling blastocyst, the selectin adhesion systems allow leukocytes to tether and roll on the endothelial surface before invading into the interstitium. Leukocytes use specialized mechanisms, which involve the L-selectin adhesion system, to extravasate from flowing blood, under shear stress, into the endothelial wall. These specialized mechanisms in the vasculature enable cell adhesion to occur under shear flow. Interactions between leukocytes and endothelium are mediated by carbohydrate-binding proteins (selectins) that recognize specific oligosaccharide structures as their ligands. These interactions allow leukocytes to slow their passage on the endothelial surface (Alon & Feigelson, 2002). This step is followed by integrin activation which enable leukocytes to form a firm adhesion with the vascular endothelial surface and, subsequently, to transmigrate into tissues (Alon & Feigelson, 2002 and McEver, 2002). Selectins on the cell surface initiate tethering to their complementary ligands on specialized endothelial cells along the vascular wall until they become firmly attached (McEver, 2002). These lectin-like molecules recognize specific oligosaccharide structures carried on some glycoproteins including PSGL-1, CD34, GlyCAM-1, MAdCAM-1, podocalyxin and endoglycan. They are made up of at least 30% carbohydrates and bind to mucin oligosaccharide ligands on the endothelium. These types of interactions have rapid and reversible properties such that traveling leukocytes slow down, tether, release and roll on the epithelial cell surface until finally attaching at the site of extravasation. Once arrested, integrin activation triggers stable adhesion (shear-resistant) and subsequent transmigration through the vascular endothelium.

At morphological level, there are parallels between leukocyte extravasation from the vasculature and attachment of embryo to the uterine wall since both types of adhesion occur under shear flow and are followed by integrin activation. Genbacev et al. (2003) have shown that the L-selectin adhesion system plays a crucial role during an initial step of blastocyst implantation. Hatched blastocyst expresses L-selectin and uses this molecule to mediate its

attachment to the luminal epithelial surface via its carbohydrate ligands, MECA-79 and related epitopes. Two different in vitro models illustrating L-selectin mediating adhesion were used to confirm the in-situ observations. In the first model, beads coated with synthetic L-selectin carbohydrate ligands were overlaid on placental chorionic villous explants under shear stress. It was shown the beads bound to cell column cytotrophoblasts (CTBs) on the explants and the binding was blocked by antibody to L-selectin. Additional immunolocalization experiment confirmed that the CTBs did express L-selectin. In the second model, isolated CTBs were overlaid on endometrial tissue sections under shear stress. It was shown that CTBs bound to epithelial surface of the endometrium tissues that were obtained during the luteal phase and did not bind to those that were obtained during the follicular phase. Again, the binding was blocked by anti-L-selectin antibody. Concurring with these observations, immunolocalization study of endometrial tissue demonstrated that the L-selectin carbohydrate ligand MECA-79 was upregulated from the day of ovulation to a peak 6 days post ovulation at the middle of the implantation window. It was negative throughout the follicular phase and remained negative if ovulation did not occur. Carson et al (2006) have shown that MUC-1, a transmembrane mucin glycoprotein expressed at the apical surface of the uterine epithelia, likely serves as a scaffold for these L- selectin carbohydrate ligands. Together, these studies suggests that the human embryo uses a mechanism well studied in leukocytes to mediate rolling and tethering onto the endometrial wall prior to firm adhesion when integrins begin their crucial role.

2.2 Embryo attachment

Shortly after the apposition step, integrin-dependent adhesion and attachment occurs. This receptor mediated event allows the blastocyst to attach firmly to the uterine wall and trophoblasts transmigrate across the luminal epithelium, burying the embryo beneath the uterine wall. Integrins are a family of cell adhesion molecules present on the plasma membrane as heterodimeric α and β glycoprotein subunits. These cell surface receptors on both the trophoblasts and the endometrium are involved at multiple functional levels during implantation. The trophoblast integrins have been shown to mediate cell-cell and cell-matrix interaction in trophoblast attachment, migration, differentiation and apoptosis. On the endometrial surface, most of the integrins serve housekeeping roles, but there are three heterodimers whose expression marks the boundaries of the implantation window (Lessey, 2002). The endometrial integrin α1β1 is expressed during the full span of the implantation window from ovulation to the late luteal phase. The expression of α4β1 begins with ovulation, but ceases as the window closes on cycle day 24. The endometrial integrin αvβ3 appears on the apical surface of the endometrial epithelium on cycle day 19 to 20, the opening of the implantation window and diminishes quickly through the next week. Its ligand, osteopontin is found concurrent with αvβ3 and might play a role in endometrial or embryo signaling, facilitating embryo attachment to the apical surface prior to invasion.

Absence of αvβ3 expression during the window of implantation has been reported with luteal phase deficiency and in some women with endometriosis, unexplained infertility and hydrosalpinges. This loss of normal integrin expression is thought to lead to implantation failure. Integrin knockout studies found that β1 null mice embryos develop normally to the blastocyst stage but fail to implant (Stephens et al., 1995). The human embryo regulates

these integrins in the human endometrium at the protein level in the apposition phase (Simon et al., 1997). Therefore, after the human blastocyst tethers to and breaks through the glycocalix barrier, it then induces by paracrine crosstalk a favorable epithelial integrin pattern for its firm attachment.

2.3 Trophoblast Invasion

The final phase of implantation is invasion which permits the creation of the hemochorial placenta. The term invasiveness implies the ability of cells to cross anatomic barriers. The first barrier is the layer of endometrial epithelial cells upon which the trophoblasts are attached. Immediately beneath the epithelial layer of cells is a specialized type of matrix called the basement membrane, a thin continuous layer composed mainly of type IV collagen. The basement membrane functions as an anchor for surface epithelium and a separating layer by ensheathing blood vessels, muscle cells and nervous tissue. Beyond the basement membrane lies a highly variable matrix, called the interstitial stroma which contains other tissue cells, vessels and lymphatic channels. Since the blastocyst is too large to squeeze through the epithelial layer, the attached trophoblasts use paracrine activity to induce an apoptotic reaction in the underlying endometrial epithelial cells (Galan et al., 2000).

To achieve successful invasion, trophoblasts then differentiate in a tightly regulated manner producing anchoring cytotrophoblasts and highly secretory synciotrophoblasts that degrades the extracellular matrix with several proteases. The expression of matrix metalloprotease – 9 coincides with the peak invasive potential of trophoblasts. By the 10th day after fertilization, the blastocyst is completely embedded in the stromal tissue of the endometrium with the epithelium regrown over to cover the site of implantation. Eventually, the cytotrophoblasts invade the entire endometrium, the uterine vasculature and into the inner third of the myometrium. The access to the uterine vessels creates the lacunar networks comprising the uteroplacental circulation which places the placental trophoblasts in direct contact with maternal blood. The placenta then serves its vital role as the transfer organ between mother and fetus for nutrients, gas and waste products, hormones and growth factors, among others.

Improper invasion can lead to compromised placental development and complications of pregnancy. Where there is excessive invasion, a placenta may attach onto the myometrium (accreta), into the myometrium (increta) and completely through the myometrium (percreta). Each of these is associated with higher risks of hemorrhage, operative delivery and hysterectomy. If invasion is too shallow, it may lead to intrauterine growth restriction and/or preeclampsia (Zhou et al., 1997).

3. Optimizing embryonic implantation

For couples who suffer from infertility, assisted reproductive techniques have proven to be the most effective in overcoming the majority of barriers. The effectiveness of in vitro fertilization (IVF) is achieved by the ability to bypass certain barriers such as low sperm counts or tubal dysfunction and to choose the most competent embryo to be placed in the uterine cavity at the best time. The factors that limit IVF success can be generally be distilled down to the health of the embryo versus the health of the endometrium, or in essence an evaluation of the seed versus the soil.

3.1 Embryo (seed)

In the past decade, there have been tremendous strides into the study of embryonic competence, or the assessment of what constitutes the embryo with the highest potential to implant and create a baby. Historically, the most common means of assessment is based on morphological observation of an embryo's development in optimal culture conditions. Although this has steadily improved the ability to select the embryos with the highest reproductive potential, thereby increasing pregnancy rates while decreasing multiple gestations, the vast majority of embryos fail to implant. These failures and frustrations have led to the expansion of tools and means to better evaluate the embryo. Metabolomics and proteomics are fields of study into the culture conditions of the embryos looking for soluble markers of improved embryo health. Unfortunately, assessment by morphology and/or culture conditions are not enough since aneuploidy contributes to the majority of unhealthy embryos. Genomics has much promise since chromosomal abnormalities represent a substantial prevalence of reproductive waste (Hassold, 2007). Clinically, this is demonstrated by a woman's age with reduced fecundity, greater miscarriage rates and higher numbers of births with aneuploidy. Indeed, in clinical practice, the age of the oocyte is the single most important variable influencing the outcome of assisted reproduction.

This has led to preimplantation genetic diagnosis (PGD) or assessment to determine the chromosomal constitution before embryo transfer. Initially, polar body biopsy and blastomere biopsies from day 3 embryos were assessed with fluorescent-in-situ hybridization (FISH) for evaluation of a select number of chromosomes. A full discussion of PGD assessment is outside the scope of this chapter but briefly, because of the limitations of FISH, the assay techniques and timing of biopsy have continued to evolve into the development of comprehensive chromosomal screening platforms (Schoolcraft et al., 2011). Several advancements have improved the ability to correctly determine the reproductive potential of a single blastocyst. Combining the technique of trophectoderm biopsy, vitrification and 23 chromosome assessment by single-nucleotide polymorphism (SNP)-based microarray screening has shown promising data at markedly improving the implantation rate to over 60% and reducing the miscarriage rate. This represents an improvement in implantation rates simply by selecting the most reproductively competent seed. However, it is sobering to note that an implantation rate as high as 60% still means that 40% of the most highly selected embryos with a normal chromosome complement still fail to implant. Hence, the health of the seed is not the only component. Undoubtedly, further answers will come from the endometrial side of the equation.

3.2 Endometrium (soil)

Prior to implantation the endometrium undergoes extensive hormonal preparation to produce an epithelium capable of implantation. The development of the receptive endometrium is dependent on estrogen inducing rapid proliferation of the tissue followed by progesterone inducing a secretory pattern. The importance of these steroids cannot be understated as pregnancy cannot occur without them. Their effect is to organize the endometrium or "soil" appropriately so that local cytokines and chemokines can direct the activities in implantation. If the soil is ill prepared by improper hormonal signaling, the following events are much less likely to occur. When optimally primed, the endometrium becomes the most receptive. During the menstrual cycle between cycle days 20-24, or 6 – 10 days post ovulation, the endometrium

becomes receptive to implantation during a well defined span of time, the so called "window of implantation". Outside this window, the endometrium is refractory to implantation. It is as if there are resistant factors that inhibit implantation. In fact, an embryo has a better chance of implanting in a fallopian tube or on the peritoneal surface then it does on the unreceptive endometrium. The acquisition of receptivity is a regulated process. Certain disorders that can lead to infertility may be either the absence of receptive molecules or the presence of resistant molecules that inhibit implantation. It has been suggested that MUC1 may act as an anti-adhesion molecule during embryo attachment in the mouse (Surveyor et al., 1995). One theory proposed is that MUC1 acts like bare branches on a Christmas tree that are resistant until adorned with ornaments that serve as ligands to embryonic receptors (see fig. 1). Since MUC1 acts as a scaffold to ligands of L-selectin, it is possible that a disruption of this glycoprotein complex may lead to failed implantation.

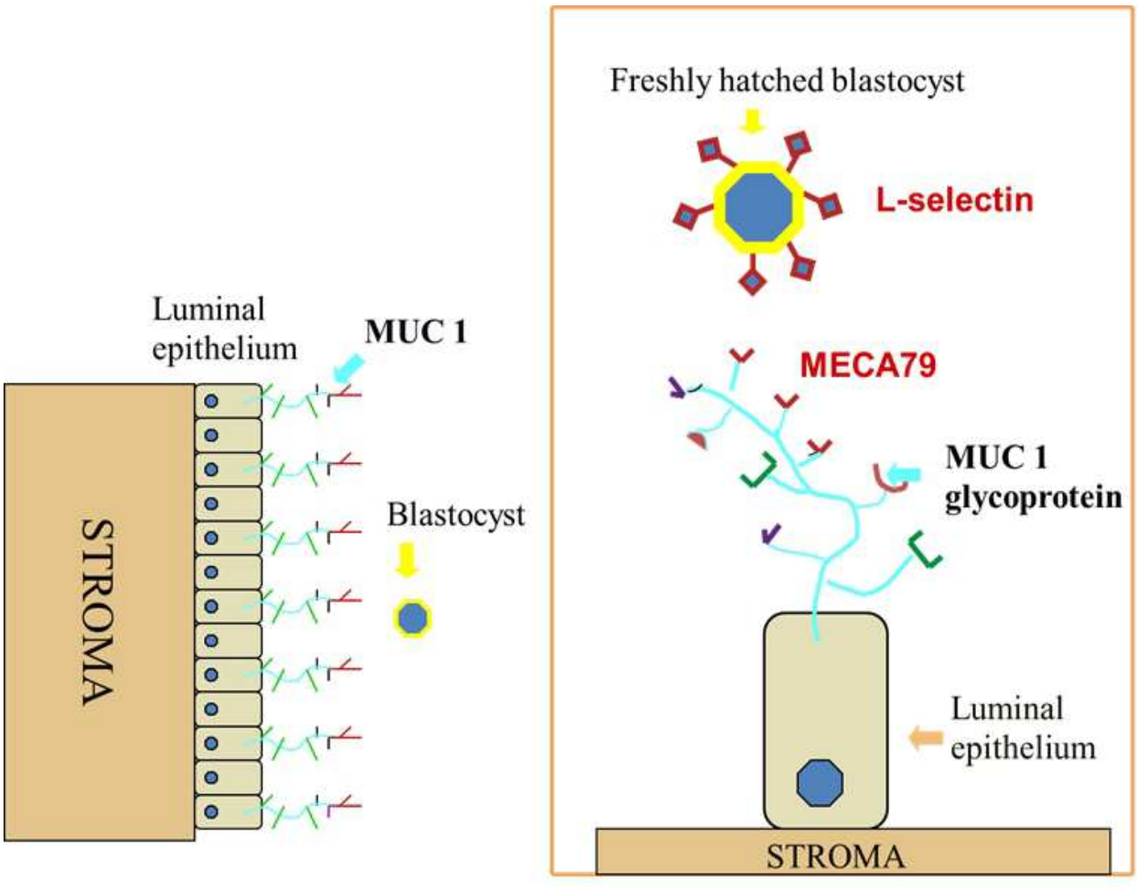


Fig. 1. Model of initial apposition of hatched blastocyst to uterine luminal epithelium

Unlike soil which takes a relatively passive role to the active functions of a seed, the endometrium must engage equally to become receptive and permit the peaceful invasion of a foreign group of cells. The critical component creating this receptivity is the dialogue that occurs between the endometrium and the attaching blastocyst. The endometrium prepares its "landing zones" at the same time a hatching blastocyst prepares it's "tethering tentacles" as it moves about the lumen. As the two appose one another the receptive endometrium

actively participates in the implantation process through paracrine bidirectional cross-talk. Using the analogy above, the approaching embryo instructs the tree branches (i.e. endometrium) to self decorate with appropriate ornaments or ligands for tethering by the L-selectin molecules expressed on the exposed trophectoderm. One could hypothesize that infertile patients who suffer from repeated implantation failure would either lack or have diminished ligands on their endometrium during the window of implantation.

4. Study into the failure of implantation

Given the many and varied causes of infertility and early pregnancy loss, we hypothesized that defects in the selectin adhesion system could account for a portion of unexplained reproductive failures. In practice, there are subgroups of women undergoing in vitro fertilization who repeatedly fail to implant despite the transfer of top quality embryos. Our goal was to assess whether the absence of the L-selectin ligand MECA-79 on the endometrium occurs more frequently in patients with repeated implantation failure (RIF), and if the lack of the endometrial L-selectin ligand correlates with unsuccessful implantation.

4.1 Study design

We compared the presence of endometrial L-selectin ligand in a fertile population to a subgroup of patients with a history of repeated implantation failures. The subjects underwent endometrial biopsies during the mid luteal phase (e.g. implantation window) of an ovulation induction cycle. Those patients with implantation failure continued their pursuits of pregnancy through further treatments. The outcome of each patient was followed and tabulated according to whether they did or did not have the L-selectin ligand recognized by immunostaining with the antibody MECA-79.

4.2 Patient selection

Control subjects were healthy young women with proven fertility who were serving as anonymous ovum donors. Twenty RIF patients were recruited at the Nevada Center for Reproductive Medicine. Approval for this research was obtained from the Renown Medical Center Institutional Review Board of the Committee for Human Rights in Research. Patients were recruited if they had at least two unsuccessful in-vitro fertilization cycles due to implantation failure. The diagnosis of implantation failure was made if no implantation occurred despite the transfer of top-quality embryos into a normal appearing endometrial cavity. The embryo morphology was scored according to criteria by Veeck (Veeck, 1999). Only patients who had 6-8 cell stage embryos, grade 1 and 2 transferred were included. The uterine cavity was assessed pre-cycle by sonohysterography and in cycle by transvaginal sonographic evaluation of the endometrial thickness and appearance. Only patients with an endometrial thickness greater than 7 mm and a trilaminar appearance were included.

4.3 Endometrial biopsy

Informed consent was obtained from all patients using an IRB protocol that was approved by the Renown Medical Center Committee on Human Research. The phase of menstrual cycle was confirmed by either a controlled stimulation cycle or a natural cycle monitoring. The

stimulation comprised 14 days of estradiol (estrace 2 mg orally bid), followed by micronized progesterone (prometrium 200 mg orally bid) plus estradiol (estrace 2 mg orally qd). In the natural cycles ovulation was confirmed by both a late follicular ultrasound and LH monitoring. An endometrial biopsy was obtained on day 6 of progesterone or the 7th day post LH surge by advancing a Unimar pipelle to the fundus, creating negative pressure and pulling the catheter out in a spiral fashion. The tissue was immediately fixed in 3% paraformaldehyde for 24 hrs. It was rinsed three times in PBS, infiltrated with 5 to 15% sucrose followed by OCT compound and frozen in liquid nitrogen. The tissue was sectioned (5 μm) using a cryomicrotome (Leica Microsystems, Bannockvurn, IL) for immunolocalization.

4.4 Endometrial dating

The morphology of the endometrial biopsies was scored according the dating criteria of Noyes by an experienced histologist (Noyes, 1950). The histologist was blinded to the patient history, including the cycle day of endometrial biopsy.

4.5 Immunohistochemistry

Rat monoclonal antibodies that recognize L-selectin ligand, MECA-79, were from BD Biosciences, San Jose, CA. The MECA-79 antibody recognizes a high-affinity L-selectin ligand carbohydrate epitope containing SO3 → 6GlcNAc (Yeh, 2001). The primary antibody was added at a concentration of 5 μg/μl. After the specimens were incubated at 4 ^{o}C overnight, they were washed three times in PBS and incubated with goat FITC-conjugated anti-rat IgM (Jackson ImmunoResearch Laboratories). As controls, an irrelevant rat IgM antibody (anti-KLH, eBioscience) or PBS was substituted for primary antibody. Staining was evaluated by using a Zeiss Axiophot fluorescence microscope.

4.6 Results

First we tested the expression of MECA-79 in the endometrial biopsies from control group that consisted of 20 healthy women who were proven to be fertile. All biopsy specimens had morphological characteristics of mid-luteal phase endometrium. The immunostaining with MECA-79 antibody revealed that this L-selectin ligand was present in all samples from the control group and that positive immunostaining was associated with the surface of the luminal and glandular epithelium.

Interestingly, one control patient who initially stained negative for MECA-79 was found to be out of phase during a natural cycle. Later we discovered she had not ovulated. She was placed on a controlled stimulation cycle and subsequently stained positive at the mid-luteal phase. The group of RIF patients was very heterogeneous. The immunostaining with MECA-79 was positive in 15 out of 20 specimens (i.e., in 75% of examined biopsies – see Figure 2). Of these 15 positive specimens, four exhibited weak or patchy staining and eleven were normal. All five MECA-79 negative samples were associated with severe uterine anomalies, including congenital anomalies (i.e. unicornuate uterus), asherman's syndrome, adenomyosis and multiple myomectomies. None of these patients became pregnant after an average of five separate attempts of embryo transfer (see Table 1). Out of 15 patients with MECA-79 positive biopsies, ten became pregnant and 5 quit treatments after an average of one more transfer following the biopsy.

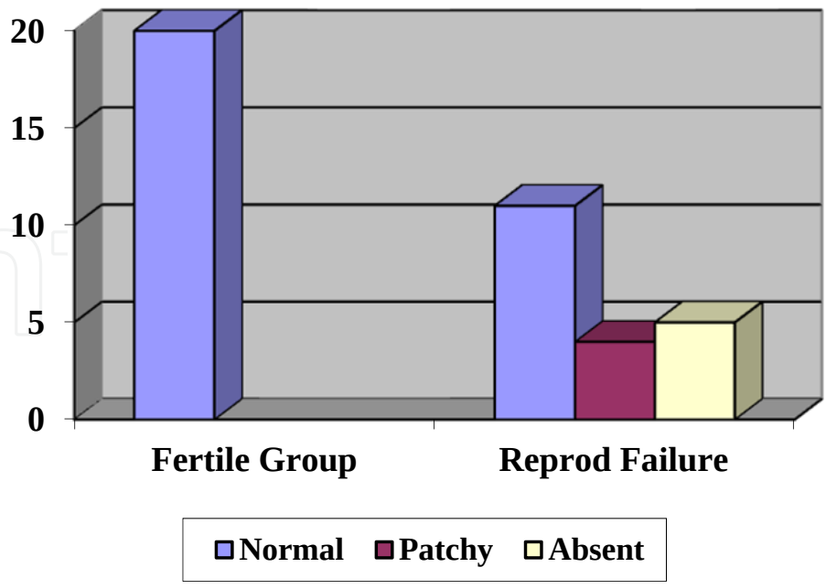


Fig. 2. Presence of L-selectin ligand MECA-79 immunostaining among patients

Of those that continued, the majority became pregnant over an average of two more transfers (see Table 2). Those patients that were positive for the ligand, but did not conceive stopped their treatments an average of 1.5 cycles sooner than those that did get pregnant (2.8 vs. 4.3 cycles respectively). There were no complications with the pregnancies to date.

L-Selectin Ligand (N)	Age (SD)	Uterine History	IVF History (no. of cycles)	Implantation Rate (pregnancies / no. embryos)
Absent (5)	39.0 (1.67)	Unicornuate (2) Asherman's syndrome Adenomyosis Many myomectomies	25/5 (5)	0/72
Patchy (4)	34.7 (4.43)	Normal (1) Hydrosalpingectomy(2) Postpartum curettage	17/4 (4.25)	3/47 (15.7%)
Normal (11)	40.1 (6.73)	Normal (6) Myomectomy (2) Mural myomas (2) Uterine septoplasty	40/11 (3.6)	11/76 (14.5%)

Table 1. Summary of Patients with Repeated Implantation Failure

The results of this pilot study have some important implications. First, the lack of expression of L-selectin ligand MECA-79 in the mid-luteal endometrial biopsy specimen in this group of patients was indicative of a very low or no chance of pregnancy. The predictive value is 100% with a sensitivity of 50% and specificity of 100% (see Table 3). Undoubtedly, the sensitivity would improve if the patients that quit treatments had persisted and become

pregnant. Second, patients with a positive MECA-79, that had failed two good prognosis cycles, had about 32% chance per cycle to achieve pregnancy subsequently. Third, in 8 patients with normal uterus and positive immunostaining with MECA-79 the probability of pregnancy was higher than 85% cumulatively, or 53.8% (7/13) per cycle. The implantation rate of those that became pregnant, however, was only 12% overall which is less than one third the rate seen for all patients at the treatment center. Regardless of the presence of the L-selectin ligand, these patients clearly represent a sub-fertile group.

Patients	**L-selectin ligand**	**Implantation**	**Normal Uterus**	**Implantation Rate (%)**	**Mean Number of Cycles**
5	Absent	None	0/5	0/72 embryos	5
10	Present	10	7/10	14/116 (12.1%) --after endometrial biopsy 14/57(25%)	4.3
5	Present	None	1/5	0/37	2.8

Table 2. L-selectin ligand and outcome in RIF patients

L-Selectin Ligand	Not Pregnant	Pregnant	Positive Predictive Value	Negative Predictive Value	# subsequent cycles	Pregnancy per cycle (%)
Absent	5	0	100%		15	0/15
Present	5	30		86%	31	10/31 (32.2)

Table 3. Predictive value of L-selectin ligand absence to the lack of a subsequent pregnancy (screening test performed after at least two failed cycles)

4.7 Discussion

The apical surface of the endometrium contains key elements for the initiation of molecular interactions to capture the human blastocyst. The endometrium becomes hormonally primed through the menstrual cycle to create a period of optimal receptivity to successful embryonic implantation. This "window of implantation" occurs between days 20 to 24. Outside this window, the endometrium is resistant to embryo attachment (Navot, 1991). Lai, et. al described the expression of L-selectin ligand throughout the natural menstrual cycle (Lai, 2005) and controlled ovarian stimulation cycle (Lai, 2006). Its expression increased from the periovulatory interval to the mid-secretory phase. Peak immunostaining for L-selectin ligand was seen at the early to midsecretory interval on the luminal surface which coincides with the window of implantation. Interestly, a reduction in expression was seen in subjects who received ovulation induction medication. Vlahos, et. al found that progesterone supplementation enhances L-selectin ligand expression in the luteal phase following controlled ovarian stimulation (Vlahos, 2006). Preliminary work by Khan et al (Khan, 2005) found that N-acetylglucosamine-6-O-sulfotransferase (GlcNAc-6-OST), the gene responsible for high affinity L-selectin ligand epitope production, is regulated by estrogen and

progesterone. Estrogen up regulates the gene, while progesterone amplifies this action. Progesterone alone however will suppress GlcNAc -6-OST expression, presumably rendering the endometrium non-receptive.

In our study group, those patients who lacked the ligand all had high risk histories for uterine defects. Iatrogenic causes include curettage and myomectomy while natural states like a congenital anomaly can also give rise to endometrial defects. Clearly, not all patients with such histories fail to achieve pregnancy, but perhaps the L-selectin ligand MECA-79 may act as a marker for the extent of injury or anomaly prior to attempts at pregnancy in high risk groups.

There could be other disease states that affect the expression of L-selectin ligand. Lessey et al described aberrant integrin expression in the endometrium of women with endometriosis (Lessey, 1994). Similarly, Kao et al found the gene, GlcNAc -6-OST, is down-regulated in patients with endometriosis (Kao, 2003). Mak et al found androgens suppress the gene expression which may play a role in poorer reproductive outcomes among patients with polycystic ovarian syndrome (Mak, 2005).

Shamonki et al correlated L-selectin ligand expression with the pregnancy rate in subsequent donor egg cycles (Shamonki, 2006). They demonstrated significantly higher immunohistochemical reactivity for the L-selectin ligand at the apex of endometrial surface epithelium obtained during mock cycle from donor egg recipients who subsequently conceived compared to those who did not. They scored the intensity of staining and correlated it to pregnancy rate, rather than the presence or absence as we have done. The study further supports our finding that L-selectin plays a role in implantation not only by its presence but also by its degree.

We demonstrated that the absence of L-selectin ligand in patients with multiple failed implantation cycles will continue to fail further attempts at implantation. Those who have failed but test positive for the L-selectin ligand, have a very good prognosis on subsequent trials of implantation despite having other unknown contributors to their subfertility.

5. Conclusion

Implantation of the human embryo is complex interaction between the endometrium and the mobile blastocyst. It must occur within a relatively narrow time frame under conditions of a primed receptive surface epithelium and a morphologically changing trophectoderm. Mechanically, the movement of the blastocyst must be arrested in order for attachment and then invasion can occur. The L-selectin ligand adhesion system is becoming more convincingly believed to play a major role in mediating initial embryonic apposition. By loose tethering, the blastocyst is able to attach despite the shear forces within the uterine lumen and orient itself for stable attachment. Once anchored, a cascade of events unfolds allowing the embryo to burrow into the endometrial wall and establish a hemochorial placenta.

Dysfunction in either the blastocyst or the endometrium can limit the implantation efficiency. Many studies into embryo reproductive competence have demonstrated that improved selection of the embryo can drastically improve the implantation rate. While the endometrial side of the equation is more challenging to study, we are uncovering areas

where proper preparation and determination of a receptive endometrium is improving outcomes. At each phase along the implantation process, certain disease states have been shown to disrupt the delicate dialogue required for implantation. Regarding the apposition step, the L-selectin ligand may be used as a marker for implantation efficiency. Clinically, in high risk groups, one could biopsy a patient and potentially prevent many futile attempts of costly treatments if the ligand is absent. This would provide the patient with important information as to why she is unable to become pregnant and open options such as gestational surrogacy to help her to become a mother. Beyond apposition, defects that disrupt integrin expression have been shown in disease states such as endometriosis and hydrosalpinges. Treatment of these defects have improved outcomes. Finally, the invasion of cytotrophoblasts to the proper depth of the uterus is critical in determining the outcome of pregnancy. Excessive invasion leads to placenta accreta, while inadequate invasion has been implicated in the pathophysiology of preeclampsia, the leading cause of maternal death in the industrialized world.

Normal implantation is crucial for successful pregnancy. For the infertile couple and their treating physician, a better understanding of the processes of implantation will enable better diagnosis and treatments to overcome the reasons why they cannot have a healthy child. Together, by selecting of the healthiest embryo and establishing the most receptive endometrium we can increase implantation efficiency. Future research into markers of endometrial receptivity will allow clinicians to define the optimal environment in which to transfer the best embryos, thereby improving pregnancy rates and decreasing complications such as multiple pregnancies, miscarriages and ultimately conditions that compromise the healthy intrauterine development of baby.

6. References

Alon R., Feigelson S. (2002). From Rolling to Arrest on Blood Vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at sub-second contacts. *Semin Immunol,* Vol. 14, pp 93-104

Bentin-Ley U., Sjogren A., Nilsson L., Hamberger L., Larsen J.F., Horn T. (2000). Relevance of endometrial pinopodes for human blastocyst implantation. *Hum Reprod Suppl*., Vol. 6, pp. 67-73

Carson D.D., Julian J., Lessey B., Prakobphol A., Fisher S. (2006). MUC1 is a Scaffold for Selectin Ligands in the Human Uterus. *Frontiers in Bioscience,* Vol. 11, pp. 2903-2909

Galan A., Herrer R., Remohi J., Pellicer A., Simon C. (2000). Embryonic regulation of endometrial epithelial apoptosis during human implantation. *Hum Reprod*., Vol. 15(suppl. 6), pp. 74-80

Genbacev O., Prakobphol A., Foulk R., Krtolica A., Ilic D., Singer M., Yang Z.Q., Kiessling L., Rosen S., Fisher S. (2003). Trophoblast L-Selectin-Mediated Adhesion at the Maternal-Fetal Interface. *Science,* Vol.299, pp. 405-408

Hassold T., Hall H., Hunt P. (2007). The origin of human aneuploidy: where we have been, where we are going. *Hum Mol Genet*, Vol. 16, pp. R203-208

Kao L.C., Germeyer A., Tulac S. (2003). Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology*, Vol. 144, pp. 2870-2881

Khan S., Pisarska D., Kao L.C. (2005). Hormonal Regulation of N-acetylglucosamine-6-O-sulfotransferase (GlcNAc-6-OST), Expression in a Human Endometrial Cell Model. *Abs presented at conjoint meeting ASRM/CFAS* Sept.2005, Vol. 85:suppl 1:S434

Lai T.H., Shih I.M., Vlahos N., Ho C.L., Wallach E., Zhao Y. (2005). Differential Expression of L-selectin ligand in the Endometrum During the Menstrual Cycle. *Fertil Steril*, Vol. 83, pp. 1297-1302

Lai T.H., Zhao Y., Shih I.M., Ho C.L., Bankowski B., Vlahos N. (2006). Expression of L-selectin ligands in human endometrium during the implantation window after controlled ovarian stimulation for oocyte donation. *Fertil Steril* Vol. 85(3), pp. 761-763

Lessey B., Castlebaum A., Sawin S. (1994). Aberrant integrin expression in the endometrium of women with endometriosis. *J Clin Endocrinol Metab*, Vol. 79, pp. 643-649.

Lessey B.A. (2002). Adhesion molecules and implantation. *J Reprod Immunol* Vol. 55, pp. 101-112

Mak W., Khan S., Pisarska D. (2005). Androgen Suppression of a target human implantation gene N-acetylglucosamine-6-O-sulfotransferase (GlcNAc-6-OST) in a human endometrial cell model. *Abs presented at conjoint meeting ASRM/CFAS* Sept.2005, Vol 85:suppl 1:S10

McEver R.P. (2002). Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol*, Vol. 14, pp. 581

Navot D., Scott R.T., Droesch K. (1991). The Window of Embryo Transfer and the Efficiency of Human Conception in vitro. *Fertil Steril*, Vol. 55, pp.114-118

Norwitz E., Schust D., Fisher S. (2001). Implantation and the survival of early pregnancy. *NEJM,* Vol. 342,19, pp 1400-1408

Noyes W., Hertig A.I., Rock J. (1950). Dating the endometrial biopsy. *Fertil Steril* Vol. 1, pp. 3-25.

Prakobphol A., Thomsson K., Hansson G., Rosen S., Singer M., Phillips N., Medzihradszky K., Burlingame A., Leffler H., Fisher S. (1998). Human low-molecular-weight salivary mucin expresses the sialyl lewis - X determinant and has L-selectin ligand activity. *Biochemistry* Vol. 37, pp. 4916–4927

Prakobphol A., Boren T., Ma W., Zhixiang P., Fisher S. (2005). Highly glycosylated human salivary molecules present oligosaccharides that mediate adhesion of leukocytes and Helicobacter pylori. *Biochemistry* Vol. 44, pp. 2216–2224

Rosen S. (2004). Ligands for L-selectin: homing, inflammation, and beyond. *Annul. Rev. Immunol*. Vol. 22, pp. 129–156

Schoolcraft W., Treff N., Stevens J., Ferry K., Katz-Jaffe M., Scott R. (2011). Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism micro-array-based comprehensive chromosomal screening in infertile patients. *Fertil Steril*, Vol96(3), pp. 638-642

Shamonki M.I., Kligman I., Shamonki J.M. (2006). Immunohistochemical expression of endometrial L-selectin ligand is higher in donor egg recipients with embryonic implantation. *Fertil Steril* Vol.86(5), pp. 1365-1375

Simon C., Gimeno M.J., Mercader A., O'Connor J.E., Remohi J., Polan M.L., Pellicer A. (1997). Embryonic regulation of integrins beta 3, alpha 4, and alpha 1 in human endometrial epithelial cells in vitro. *J Clin Endocrinol Metab,* Vol. 82, pp. 2607-2616

Stephens L.E., Sutherland A.E., Klimanskaya I.V., Andreiux A., Meneses J., Pedersen R.A., Damsky C.H. (1995). Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality. *Genes Dev,* Vol. 9, pp. 1883-1895

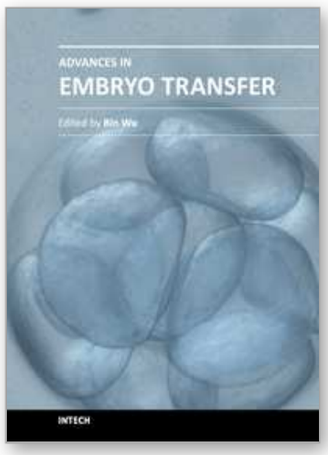
Surveyor G.A., Gendler S., Pemberton L., Das S., Chakraborty I., Pimental R. (1995). Expression and steroid hormonal control of muc-1 in the mouse uterus. *Endocrinology*, Vol. 136, pp. 3639-3647

Veeck L.L. (1999). *An atlas of human gametes and conceptuses*. New York, Parthenon, pp. 46-51

Vlahos N.F., Lipari C.W., Bankowski B. (2006). Effect of luteal-phase support on endometrial L-selectin ligand expression after recombinant follicle-stimulating hormone and ganirelix acetate for in Vitro Fertilization. *Clin Endocrine Met*, Vol. 91(10), pp. 4043-4049

Yeh, J.C., Hiraoka N. (2001). Novel Sulfated Lymphocyte Homing Receptors and Their Control by a Corel Extension Beta 1,3-N,acetylglucosaminyltransferase. *Cell* Vol.105, pp. 957-969

Zhou Y., Damsky C., Fisher S. (1997). Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype: one cause of defective endovascular invasion in this syndrome? *J Clin Invest*. Vol.99, pp. 2152-2164



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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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