Implantation

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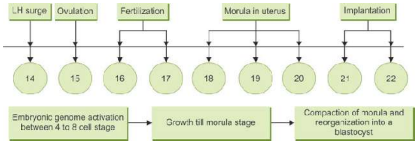
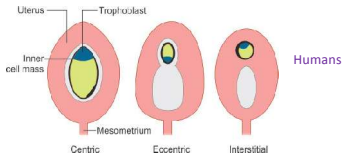
Implantation

Implantation is the process by which the blastocyst comes into intimate physical and physiological contact with the uterine endometrium.



Types of implantation

Based on the different types of blastocyst uterine cell interactions, implantation has been classified into three broad categories: (1) centric, (2) eccentric, and (3) interstitial



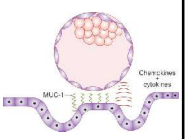
Morphological steps of implantation

The process of implantation classified is into three stage

1. Apposition and rolling
2. Adhesion (attachment)
3. Penetration (invasion)

Apposition

Stage	physiology	molecules
Apposition (unstable adhesion of blastocyst to endometrial surface)	• Once embryo reaches uterus via tapis roulant of Mucin (MUC) flow, which is continuous toward cervix • Apposition is progressively increasing intimacy of contact between trophoblast and uterine epithelium Characterized by: • Enlargement of embryo • Edema of endometrium • Decrease in uterine fluid volume • Obliteration of uterine lumen by closer apposition of apical end of endometrial and trophoblastic cells • Appearance of pinopodes	1. MUC-1 2. Chemokines – Alpha (CXC chemokines) – Beta (CC chemokines



Molecules involved in Apposition

Mucin – 1

It is present on endometrium .First molecule that blastocyst encounters on the endometrialwall before implantation Expression is highest in luteal phase and implantation period· Cell-cell and cell-matrix adhesions are inhibited . It repels and guide the blastocyst to find the correct place for implantation There is a local loss of MUC-1 at the site of embryo attachment and in the immediate vicinity, whereas its expression is increased at a distance farther away from the implantation site
TNF-alpha (proinflammatory cytokine) and sheddases bring proteolysis of MUC-1 at the site of implantation .

In women with recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL), studies have shown reduced midsecretory phase levels of MUC-1 and its epitopes

Molecules involved in Apposition

Chemokines

Chemokines are a family of small polypeptides (70–80 amino acids) specialized in the attraction of specific leukocytes

Alpha chemokines : IL 8 activator of neutrophils and T lymphocytes
Beta chemokines : MCP 1 (momocyte chemo attractant protein)activator of monocytes , macrophages, T lymphocytes ,basophils , mastcells, NK cells.
RANTES (regulated upon activation normal T cells expression and secretion) is a chemoattractant of monocytes , eosinophils , basophils .

IL-8, MCP-1 were mainly expressed in glandular and luminal epithelium and endothelial cells; RANTES was localized mainly in the stromal and perivascular cells of blood vessels .

human embryos expresses some of these chemokine receptors,

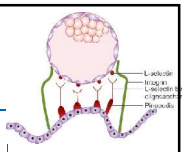
Chemokines act on a range of leukocyte subsets, which in turn release proteases and mediators, which facilitate embryo apposition and invasion

Molecules involved in Apposition

ENDOMETRIUM	EMBRYO
MUC -1	-
MCP-1 Beta chemokine	CCR2B receptor
RANTES Beta chemokines	CCR5 receptor

Rolling

Stage	Physiology	Molecules
Rolling over	Blastocyst enters the uterus and role over endometrium freely	L-selectin



Molecules involved Rolling

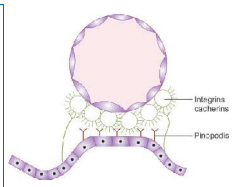
L-selectin
It is expressed by the hatched embryo

L- Selectin oligosaccharide based ligand MECA-79, is upregulated during the window of implantation (WOI)
Their interaction facilitates the apposition of blastocyst to the endometrial epithelium

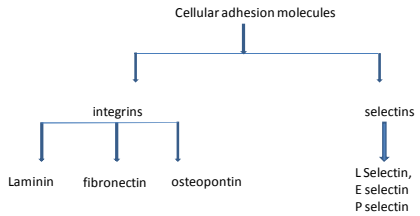
ENDOMETRUIM	EMBRYO
L-Selectin oligosaccharide based ligand MECA-79	L-selectin

Adhesion

Stage	physiology	Molecules
Adhesion	• Direct contact occurs between the maternal endometrial epithelium cell (EEC) and the embryonic trophectoderm (TE) • The first sign of attachment reaction occurs on day 20–21 in humans, and coincides with a localized increase in the stromal vascular permeability at the site of blastocyst attachment • Endometrium and embryo now express extracellular matrix (ECM) component which helps to mediate adhesion through Cell adhesion molecules.	Cellular adhesion molecules • Integrins • Fibronectin • laminin • Cadherins • Immunoglobulin



Molecules involved in Adhesion



Molecules involved in adhesion

Cellular adhesion molecules

Family of transmembrane glycoproteins. They establish a firmer adhesion after the appositioning process is initiated by selectins . They are expressed both by the endometrium and blastocyst,

Integrins

Alpha 5 beta 3 integrin is first integrin that interacts with trophoblas. Its expression in the endometrial stroma has been shown to be stimulated by IL-α, IL-β, and tumor necrosis factor-α (TNF-α) .
Integrins have been proposed as markers for endometrial receptivity, particularly the α5β3 glycoprotein.

laminin

They are ECM proteins abundantly secreted by decidualized endometrialstroma, and are under progesterone control.
Laminin facilitates trophoblast invasion, probably via lowering of insulin-like growth factor binding protein-1 (IGFBP-1) and prolactin, which are the two major secretory proteins of decidualized stromal cells

Fibronectin

Fibronectin interacts with integrins expressed by trophoblast, and this integration inhibits trophoblast invasiveness

Molecules involved in Adhesion

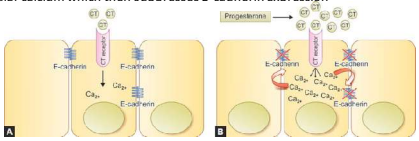
Immunoglobulin

Intercellular adhesion molecule-1 (ICAM1) or CD54 is a transmembrane glycoprotein ,which is constitutively expressed on the epithelial cells of endomerium.

Decreased expression in women with implantation failures

Cadherins

Cadherins are a group of glycoproteins responsible for calcium -dependent cell to cell adhesion through homotypic binding. In the luteal phase, E-cadherin may be downregulated to enable epithelial cells dissociation and blastocyst invasion. Interestingly, calcitonin (CT) expression in the embryo is induced by progesterone leading to increased intracellular calcium which then suppresses E-cadherin expression

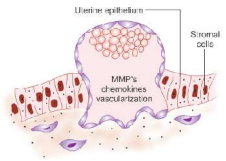


Molecules involved in Adhesion

EMBRYO	ENDOMETRIUM
Integrins	Integrins (α5β3)
Calcitonin receptors	Cadherins (down regulation)
	ICAM1
	Fibronectin Laminin

Invasion

Invasion	Finally, in the invasion phase, the embryonic trophoblast breaches the basement membrane and invades the endometrial stroma up to the uterine vessels . Three types of interaction occur between implanting trophoblast and uterine epithelium: 1. Trophectoderm cell from the blastocyst migrate between the epithelial cells, displacing them and penetrating as far as the basement membrane[21] 2. Epithelial cells lift off the basement membranes, an action that allows trophoblast to insinuate underneath the epithelium 3. Fusion of trophoblast with uterine epithelial cells	• Chemokines • Matrix metalloproteinase-2 (MMP-2)/MMP-9 • Vascular endothelial growth factor (VEGF) • Collagenases • Plasminogen activators • Selectins • Integrins



Window of implantation

The endometrium is normally receptive to embryo only in the window of implantation

It is restricted to time frame in the mid luteal phase

Opens on day 19 or 20 of menstrual cycle or days 4 or 5 after progesterone presence

Days 7 – 10 after LH surge

In ART cycle WOI can be induced by supplying E2 and P4 to syncronise with embryo transfer

Remains open for an extended period at lower estrogen levels but rapidly closes at higher levels due to abbarent expression of implantation related genes.

Mediators of Implantation

1. Ovarian hormones
   - Estrogen
   - Progesterone
   - Relaxin.
2. Endometrium
   - *Cytokines*: LIF, CSF, and IL -1
   - *Growth factors*: Insulin-like growth factor-1 (IGF-1), HB-EGF, transforming growth factor -beta (TGF-β) (inhibins and activins), and vascular endothelial growth factor (VEGF)
   - *Genes*: *HOXA10* and *HOXA11*, *BMP2*, and *WNT4*
   - *Proteins*: Glycodelin, matrix metalloproteinase-2 (MMP-2), and MMP-9, signal transducer and activator of transcription 3 (STAT3) protein.
3. Embryo
   - IL-1
   - VEGF
   - IGF-2
   - Chorionic gonadotropin (CG).
4. Peripheral
   - Cyclooxygenase-2 (COX-2) signaling.
5. Endocrine
   - Corticotropin releasing hormone (CRH)
   - Leptin

Ovarian harmones

➢ Estrogen :

it initiate a cascade of paracrine and autocrine signal transduction which, via cell adhesion processes, will lead to attachment and the subsequent invasion of the embryo into the endometrium.

- Upregulation of P4 receptors
- Also upregulates VEGF, IGF-1, L-selectin, and HB-EGF.

➢ Progesterone:

- Morphological changes in the endometrium
- Upregulates pinopode formation
- Upregulates CSF, LIF, IL -1, prostaglandin (PG), VEGF, glycodelin, fibronectin, Mucin-1 (MUC-1), and L-selectin
- Downregulation of beta3 integrin
- Downregulation of estrogen receptors

➢ Relaxin

it is ovarian peptide harmone

Increases the production of glycodelin and VEGF secretion

There is cycle dependent concentration of relaxin in the serum with a peak at the WOI

Endometrium

Cytokines

Leukemia inhibiting factor: LIF, a member of IL-6 type family, behaves as a pleotropic glycoprotein. LIF seems to be regulated by Progesterone and some locally produced factors. LIF acts on cells by interacting with LIF receptor (LIFR), which exists as LIFR and gp130 and is expressed on TE and luminal and glandular epithelium. In humans, LIF acts on cytotrophoblast (CTB), causing them to differentiate into anchoring phenotype, which is achieved by increased synthesis of fibronectin and decreased production of hCG protein, while inducing secretion of oncofetal fibronectin.

Colony-stimulating factor is a hematopoietic growth factor inducing proliferation and differentiation of cells belonging to mononuclear phagocytic
It has a trophic effect on trophoblast cells.
Responsible for decidual function and placental growth.

Interleukin-1 and others : IL 1 alpha , IL 1 beta , IL 1 receptor antagonist and signal transduction receptor comprise the IL 1 family
IL-1, a known product of monocytes and macrophages fine tunes cell proliferation and differentiation and is present in both endometrium and embryo. It is under control of progesterone . Only the type 1 receptor is functional in human endometrium .
IL 1 and its receptor are localised on human oocyte and embryo.
IL 1 beta produced from embryo acts on IL-1RT-1 (receptor type-1) on endometrium and upregulates integrin (alpha 5 beta 3) cascade, plays a role in adhesion.

Growth factors

HB-EGF Heparin binding epidermal growth factor	• Maximal expression during late secretory phase, at the sites of active blastocyst displaying ErbB4 • This induction is followed by expression of betacellulin, epiregulin, neuregulin-1, and COX2 at the time of attachment
IGF Insulin like growth factor	• Insulin like growth factor system comprises of IGF -1 and IGF-2 • All IGF participate in regulation of cellular growth and differentiation and have metabolic, antiapoptotic, and angiogenic effects, via increase in VEGF • In cycling human endometrium, IGF expression is restricted to stromal cells • IGF-2 dominates in secretory endometrium, expressed by trophoblast, while IGFBP mainly by decidual cells • IGFBP-1 regulates the invading trophoblast, by modulating MMP-2 and MMP-9 levels • IGFBP 3 makes more IGF 2 available in extra embryonic matrix as it has low affinity to IGF.

TGF-beta	• Inhibin and activin belong to TGF-beta subfamily • Inhibin A and activin A levels increase during WOI • Modulates maternal immune-tolerance during implantation • TGF-beta signaling associated with onset of uterine receptivity and embryo-attachment reaction, whereas it diminishes when trophoblast invasion starts • Serum concentrations in the first trimester of pregnancy can possibly discriminate between a viable pregnancy and an abortion. This is mainly due to rescue of corpus luteal function by inhibin A
VEGF	VEGF is a major modulator of vascular growth and remodeling and it increases vascular permeability in the endometrium • It increases vascular permeability in endometrium at implantation site, making it receptive to implantation

Genes :

➢ *HOXA10* and *HOXA11 Genes*
They are Transcription factors and regulators of embryonic morthogenesis and differentiation
HOXA 10 and HOXA 11 are upregeulated in window of implantation also responsible for upregulation of IGF binding protein 1 , pinopodes , beta 3 integrin.
Reduced expression in hydrosalpinx , PCOS .

➢ *WNT4 gene :* once WTN protein binds to its ligands, WNT gene is activated and leads to transcriptin of target genes by either beta catenin signaling or beta catenin independent pathways . For embryo implantation beta catenin signaling path way is used.
It plays a role in
- Cell-cell adhesion in apposition phase
- Morula to blastocyst transition
- Embryo spacing via induction of evenly spaced bands in smooth muscle of uterus
- Decidualization of uterus

➢ BMP 2 Gene : is a major player in decidualization .

Proteins

Glycodelin: Glycodelin-A, also known as PP14 (placental protein 14) or progesterone-associated endometrial protein (PEP), is the most abundantly secreted and consistently upregulated glycoprotein in late secretory endometrium and gestational decidua. Glycodelin has immunosuppressive role, suppresses the activity of natural killer (NK) cells, and protect the embryo from maternal immune rejection.
Progesterone, hCG, and relaxin seems to be the regulatory pathways for glycodelin production.

Matrix metalloproteinase-2 and -9: Tissue remodeling and angiogenesis are hallmark events during implantation and decidualization. MMP and tissue inhibitors of metalloproteinase (TIMP) are thought to be key mediators for matrix degradation during implantation. There is evidence that a balance between a select set of MMP and TIMP is important for implantation.
Progesterone, growth factors, and cytokines including the EGF and TGF -β family members and LIF have been shown to modulate MMP and TIMP.

Janus kinase-signal transducers and activators of transcription (STAT 3): In human endometrial stromal cells, STAT3 protein production is regulated by progesterone, LIF, and IL -11. it is proposed for trophoblast invasiveness .

Embryo

Interleukin-1β is the major form produced by the preimplantation embryo. Acts on IL-1 receptor on maternal side and causes endometrial transformation via integrin cascade, which are known to initiate NK cell differentiation
Interleukin-1 regulates the expression of several molecules in the endometrium, including IL-6, IL-8, LIF, CSF, tumor necrosis factor (TNF) alpha, COX-2, prostaglandin E2 (PGE2), PGF2 alpha, MMP-1 and -9, and TIMP-1 and -3

Vascular Endothelial Growth Factor
In the developing embryo, release of IL-1β upregulates VEGF production from embryo. Both VEGF and its functional receptor are expressed by the trophoblast, most notably by the invasive first trimester extravillous cytotrophoblast, suggesting that VEGF participates in regulating the proliferation, invasion, and metabolic activity of the trophoblast in an autocrine fashion.
The VEGF-A is the dominant subtype in the endometrium and VEGF-R1 and VEGF-R2 have been described as main subtypes involved in implantation.

Insulin-like Growth Factor-2

- Insulin-like growth factor-2 dominates in secretory endometrium, released by trophoblast, while IGFBP-1 is secreted by decidua.
- All IGF participate in regulation of cellular growth and differentiation and have metabolic, antiapoptotic and angiogenic effects, via increase in VEGF.
- Within the extraembryonic matrix (EEM), IGFBP-3 binds IGF, but IGFBP-3 has a low affinity towards IGF, so making it available around EEM to provide a free supply for embryo.

Chorionic Gonadotropin

Embryonic action via hCG on the endometrium is through two effects:

1. An indirect, endocrine effect via rescue of the corpus luteum and subsequent progesterone release
2. A direct, paracrine effect on the endometrium

Progesterone controls endometrial function by increasing LIF secretion, mediated via IL-4 which originates from T cells

Peripheral

Cyclooxygenase-2 Signaling

Progesterone influences the PG level in the human menstrual cycle. PGE2 and PGF2 alpha peak in midluteal phase, where WOI is situated, helps in implantation by increasing vascular permeability and bringing decidualization of endometrium.

In early pregnancy, two processes concerning PG take place

1. The decidual PG levels drop remarkably low as compared to nonpregnant levels.
2. Increased local production of PG at the implantation site.

The implanting embryo seems to be capable of triggering this mechanism as well, possibly by its own PG production.

PGE2, PGF2alpha ,PGI2 increases vascular permiability and edema at implantation site and promotes implantation

Endocrine Signals

Corticotropin-releasing Hormone

Corticotropin-releasing hormone has been localized in endometrial glands and decidual stroma as well as trophoblast.
Progesterone upregulates CRH production, which causes decidualization.
Also upregulates IL-1, IL-6, and PGE2.
Corticotropin-releasing hormone participates in local inflammatory phenomena, which takes place at the implantation site, rendering the endometrial surface adhesive for the implanting blastocyst
, CRH induces the synthesis of proapoptotic Fas ligand (FasL) on human invasive extravillous trophoblast and maternal decidual cells. Therefore, it potentiates the ability of these cells to induce apoptosis of surrounding activated maternal T cells bearing the Fas receptor (FasR) on their surface and facilitating trophoblast invasion.

Leptin

Leptin is a product of the *OB* gene. Its receptors are found in the endometrium with maximal expression in the late luteal phase. Additionally, leptin has also been shown to increase integrin β3 expression, which is essential for endometrial receptivity and implantation.

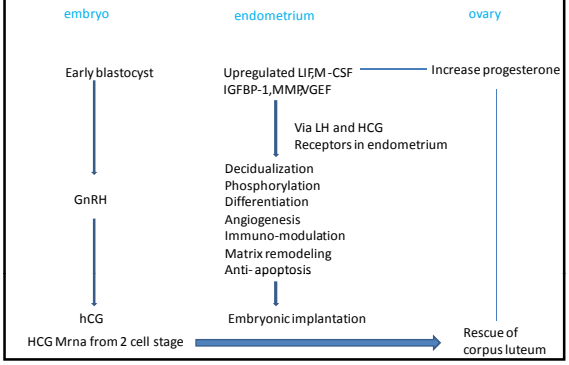
EMBRYO–MATERNAL CROSS DIALOGUE

The EEM and zona pellucida represents the interface between the mother and embryo. The start of full embryo–maternal signaling can be expected to occur at around day 6 following the luteinizing hormone (LH) peak and have to pass this matrix to reach their destination, since the human embryo does not hatch till day 6. Hence, EEM of preimplantation embryos acts a mailbox, through which signals traverse from embryo to mother or vice versa

Only a few embryonic signals, which are clearly directed toward the mother during the preimplantation period are known; the most well-known is hCG.

hCG mRNA is detectable as early as in the 2-cell stage and detectable concentrations of hCG are already produced before implantation. The critical days of the establishment of pregnancy are days 6–10 after the LH peak, which relates to the time of increasing hCG plasma concentrations.

Role of HCG in embryo maternal signaling



IMMUNE ACCEPTANCE OF PREGNANCY

How Pregnancy Affects Immune System ?

Peripheral Immune System

Increase in the peripheral white blood cell (WBC) count is the first recognized change to occur in the peripheral maternal immune system during pregnancy. The following changes are noted in:

T Cells:

This shift away from type 1 cytokine to type 2 cytokine production which is beneficial for pregnancy,since type 1 cytokines [e.g. interferon gamma (IFNγ) and TNF-α) are harmful for pregnancy because they inhibit embryonic and fetal development

Immune response

shift from a cellular to a humoral immune response during pregnancy.

Peripheral Natural Killer Cells:

The number of peripheral NK cells is decreased in pregnant women as compared with nonpregnant women.

Compliment : No activation of compliment

TH1 response	TH2 response
Pro-inflammatory cytokines IL-1,IL2,IL6,IL12,IL15 IL18,INFgama,TNFalpha	Anti inflammatory cytokines IL4,IL5,IL10,ILL13 GM-CSF
Endometrial invasion Wound like healing process Recruit immune cells to decidua uNK cells and APC(macrophages and dendritic cells) Osteoponitin	Tissue remodelling and angiogenesis Tolerance towards semi allograft fetus

Immune System in the Decidua

The decidual cells may play an important role in acceptance of the fetus and the control of trophoblast invasion. Hence, the decidua contains a diverse population of cells, including decidualized stroma cells, lymphocytes, uNK cells, monocytes, and epithelial cells . the proportion of leucocytes in the decidua are cycle dependent, from less than 10% in early proliferative phase to 20% in the late secretory, to more than 40% in early pregnancy. This is mainly due to increase in uNK cells, which comprise 60% of LKs.

Uterine NK cells : Uterine NK cells are different to mature circulating NK cells, yet phenotypically resemble the smaller unique NK cell subset, which is CD56/CD16/CD3 and has low direct cytotoxicity . Proliferation of uNK cells is through the production of IL-15 by placental macrophages. Although the uNK cells are present in the decidua in large amounts, they do not attack the semi-allogeneic nonvillous cytotrophoblast. This is due to the fact that uNK cells express inhibitory receptors . These receptors bind to the MHC 1 b on trophoblast, so ob binding to these MHC I antigens, the inhibitory receptors inhibit the lytic activity of the uNK cells.

Decidual T Lymphocytes : Similar to the peripheral blood, the most accepted theory is the dominance of Th2 response over Th1 promote allograft tolerance, and may improve fetal survival.

These $CD56^+$ NK cells and $CD8^+$ T cells accumulate at the decidua parietalis and trophoblast invasion front and facilitate deep invasion of cytotrophoblasts into the myometrial segments thereby promoting spiral artery remodeling and angiogenesis.

MECHANISMS AT THE TROPHOBLAST TO ESCAPE IMMUNE ATTACK

MHC

Nonvillous cytotrophoblast cells express MHC Ib molecules not MHC 1a so they cannot be recognised as forigen by maternal T cells but they are at increased risk of lysis by uterine NK cells . But as MHC Class 1 B bind to inhibitory receptors on uterine NK cells the lytic activity of NK cells is inhibited .

Haptoglobulin and uretero globulin , prostaglandins E act as immunomodulators.

T cell activity down regulation

Fas ligand system induced apoptosis of T cells .

Increased FasR expression leads to increased susceptibility of the T cells for induction of apoptosis, called "activation induced cell death (AICD)". Apoptosis may be induced either by FasL expressing LKs or by FasL positive invading trophoblast cells

Induction of anergy : due to missing of costimulation signal CD 86.