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Endometrial biology during trophoblast invasion

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1. ABSTRACT

Attainment of successful implantation depends upon the synchronized changes in the endometrium before and after the arrival of blastocyst into the uterine cavity. The cues obtained from the receptive endometrium helps in proliferation and differentiation of the trophoblast cells. During the course of invasive differentiation, the trophoblast cells undergo several morphological, biochemical and molecular changes to gain the invasive capabilities. In turn, close apposition of the developing embryo brings out functional and morphological changes into the hormone primed receptive endometrium. Global gene expression profiling of the endometrium in response to the developing embryo or in response to the pregnancy hormone, human chorionic gonadotropin, in primate and human models, suggest that the endometrial-embryo cross-talk mainly influences three biological processes. Biological processes getting influenced by the blastocyst "signals" are associated with immunomodulation, biosensing and invasion. Pro- and anti-invasive paracrine factors expressed by different endometrial cell populations regulate the trophoblast invasion through activation of diverse signaling pathways. Identification of the gene signatures involved in embryo-endometrial dialogue would enhance our understanding about the pathologies like miscarriages and endometriosis.

2. INTRODUCTION

Success of embryo implantation depends upon the synchronized developmental events, which take place in the embryo as well as in the maternal endometrium, before and after they come in close contact during the "implantation window". In order to receive a developing embryo, the endometrium undergoes a series of morphological, biochemical and physiological changes collectively termed as decidualization. At the same time, the fertilized ovum undergoes several rounds of cell division to form the blastocyst during its journey from the fallopian tube to the uterine cavity. By the time the embryo arrives into the uterus, it develops to the blastocyst stage having the outer layer of trophoectodermal cells that contribute to form the placenta and the inner cell mass that forms the embryo proper. In the uterus, the trophoblast cells first make contact with the uterine epithelium for embryo apposition. Thereafter the embryo firmly gets implanted onto the endometrium, breeches the epithelial cell layer and invade into the maternal endometrium (which is now transformed into the decidua) to initiate pregnancy. Once in contact with the decidua, the cytotrophoblasts differentiate to form the invasive extravillous trophoblast (EVT) cells that invade into the deeper layers of the decidua until midgestation. The process of embryo implantation and trophoblast invasion are spatio-temporally

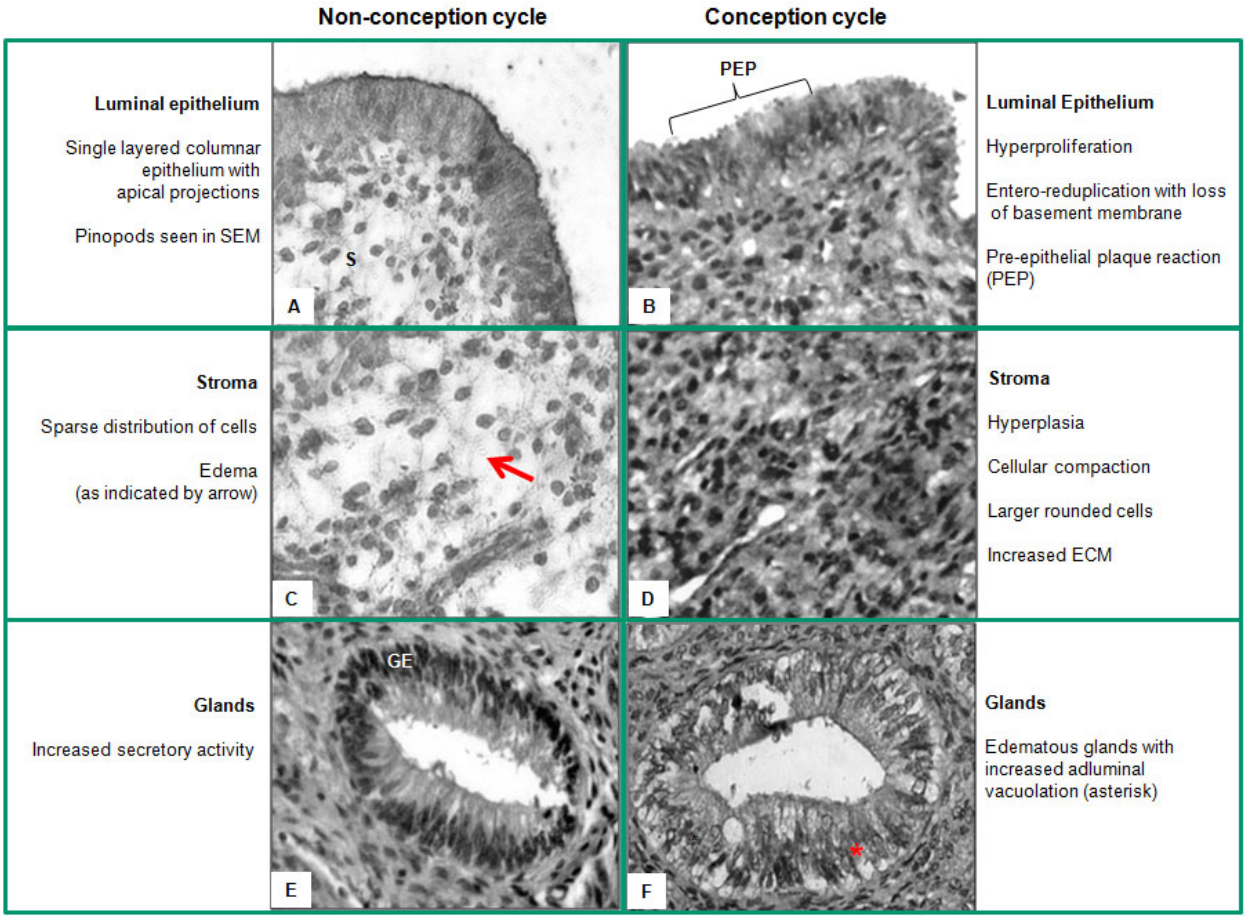

Figure 1. Morphological changes in the endometrium of the bonnet monkey (*Macaca radiata*) during early pregnancy: Endometrial biopsies were obtained from the bonnet monkey in the conception and non-conception cycles on day 6 post ovulation, as described previously (12) and evaluated for histological changes. Panels A, C and E are the representative photomicrographs from the non-conception cycle while, panels B, D and F are those from the conception cycle. Images in panel A, B, E and F were taken at 40X magnification of the objective while, panels C and D were digitally magnified.

controlled phenomena regulated by a feto-maternal cross-talk; if any of these processes go awry, it would lead to termination of the pregnancy or complications associated with the pregnancy. The focus of the review is to summarize, the cellular and molecular events associated with the embryo implantation and the invasion of trophoblastic cells into the endometrium. It will help in understanding the biological insinuation of the feto-maternal cross-talk and its implications in pregnancy related disorders. Considering the species specific differences in the mechanism of implantation and trophoblast invasion, herein we have restricted to the studies carried out in the human and non-human primate models, rather than being comprehensive.

3. MORPHOLOGICAL CHANGES IN THE ENDOMETRIUM DURING EARLY IMPLANTATION AND TROPHOBLAST INVASION

The dialogue that occurs between the pre-implantation embryo and the uterus is one of the true elegance (1). Presently, our knowledge about the molecules and the events associated with this process is partial. However, it is apparent that the initiation of pregnancy requires synchronised developmental changes in the endometrium as well as in the implanting blastocyst (1, 2). In primates, the uterus is refractory to the embryo implantation beyond a narrow “window of implantation” in the second half of the menstrual cycle. Approximately 8-10 days post ovulation; the uterus becomes "receptive" and enables the blastocyst to adhere to the luminal epithelium. The endowment of the "receptive window" is dependent on the ovarian steroids like estrogen and progesterone that prepare it for the establishment of pregnancy. Any disturbances at this stage leads to pregnancy failure. The receptive phase is characterized by the presence of columnar epithelium with microvilli (Figure 1), an increase in stromal cells proliferation, and the appearance of pinopode-like structures on the luminal epithelium (1). The morphological changes in the “receptive” endometrium are associated with the expression of a range of biochemical and molecular markers like transcription factors, integrins and their ligands, cytokines and growth factors (2). Efforts towards development of molecular signature of the “receptive window” using global gene profiling

technologies are ongoing and have been extensively reviewed (3, 4).

After fertilization, the developing zygote travels from the fallopian tube and reaches the uterine cavity. By this time, the embryo is at the blastocysts stage with well differentiated inner cell mass and the outer trophectoderm. The presence of a viable blastocyst induces a second phase of uterine receptivity with the help of blastocyst derived "signals". These signals are thought to superimpose with the already existing transforming signals present in the receptive endometrium and induce another round of differentiation that is distinct from those observed in a "receptive" stage endometrium (1). The morphological changes induced due to the presence of blastocyst derived signals are associated with alterations in the expression of receptivity related markers implying an existence of a blastocyst-uterine cross-talk at molecular level, even before any physical contact is established between them. These changes in the uterus are further modulated following the blastocyst attachment and implantation. A universal response at this stage is the significant increase in the permeability of the sub-epithelial capillaries surrounding the blastocyst resulting in decidualization (1). By definition, decidualization is morphological and functional remodeling of the endometrial stromal cells into highly specialized cells with a secretory function.

Thus, uterine receptivity and implantation can be categorized into three distinct phases. Phase I, regulated by estrogen and progesterone and is evident between days 8 to 10 post-ovulation of the normal menstrual cycle. Phase II is induced by the blastocyst 'signals' superimposed on the steroid primed receptive endometrium. This phase is associated with functional and morphological changes in the endometrium that are distinct from those observed at a comparable time of a non-pregnant cycle. Phase III is decidualization and endometrium remodeling following trophoblast invasion. Discussed below is a brief summary of the studies in the primates that demonstrate the modulation of the uterus by embryonic signals (Phase II and III).

Several lines of evidence demonstrate that embryo derived factors directly or indirectly influence the endometrial receptivity and implantation in primates. The involvement of embryonic signals in the modulation of endometrial receptivity has been demonstrated *in vitro*. Remarkable modulation in the expression of chemokines, cytokines, growth factors and cell adhesion molecules have been observed in human endometrial epithelial cells co-cultured with the preimplantation stage blastocysts (5, 6). The technical challenges and ethical constraints associated with the collection of timed uterine biopsies in a conception cycle have been the major obstacles towards advancing our understanding of this phase in human beings. However, elegant *in vivo* studies have been published in this direction using the non-human primate model. The rhesus monkey has been utilized as a most common model to understand the mechanisms associated with implantation (7-11). Using preimplantation factor as a surrogate marker for the presence of conceptus in the uterine cavity, the morphological and biochemical profiling of the endometrium in a conception cycle have been studied in bonnet monkeys (12-17). In the baboons, the morphological and biochemical changes in response to chorionic gonadotropin (CG), when infused in a manner that mimics the transit of blastocyst have been reported (reviewed in 1 and 18).

3.1. Luminal and glandular epithelium

An early maternal response to pregnancy, prior to the implantation, is characterized by the hyperproliferative activity in the luminal epithelium (12). Epithelial cells in the focal areas undergo rapid proliferation with entero-reduplication of the nuclei without cytokinesis. There is associated loss of basement membrane along with the formation of large cellular clusters/ aggregates, which are known as acinar clusters. These focal changes are observed on day 10 post-implantation in the rhesus monkey and at day 15 in the baboons. These changes are collectively referred as epithelial plaque reaction. The epithelial plaque reaction is considered as a hallmark of pregnancy in the primate endometrium. In the fecund cycle of bonnet monkeys, on day 6 post-ovulation (just prior to implantation), similar focal hyperproliferative activity is observed in the luminal epithelium with large clumps of nuclei having poorly packed chromatin associated with the loss of basement membrane (Figure 1). However, these sites do not have the acinar clusters, a characteristic feature of an epithelial plaque. Since, other morphological features nearly resemble the epithelial plaque; the changes are possibly the events preceding an epithelial plaque reaction. Therefore, these changes are referred as 'pre-epithelial plaque' reaction (12).

In humans, at the ultra structural level, thinning of the basal lamina in the luminal epithelial cells has been reported in certain areas of the endometrium on days 18–41 after conception (19). In the bonnet monkey endometrium, thinning of the luminal epithelium associated with the thickening and diffusion of the apical and lateral gap junctions has been observed in the pre-implantation stage of the conception cycle (15). It is likely that the changes occurring in the gap junctions before embryo invasion paves the way for the implantation in the subsequent stages of the pregnancy. Intriguingly, along with the epithelial cells, granulocytic stromal cells are also observed in the luminal epithelium of the pregnant bonnet and rhesus monkeys prior to implantation (8, 15). It is plausible that thinning of the luminal epithelium, infiltration of the stromal cells and modulation of the tight junctions and/or remodeling of adherens junctions may contribute to the increased adhesiveness of the endometrial epithelial cells to the trophoblast cells.

The glandular epithelium of the fecund cycles also shows remarkable morphological differences as compared to non-fecund cycles. The pseudo-stratified appearance of the glandular cells is lost giving a slightly delayed maturational appearance in the conception cycles of the bonnet monkeys and humans (12, 15, 19). During the

conception cycle glandular epithelial cells show adluminal secretions (Figure 1). The uptake of glandular secretions from the endometrium by the trophoblast is an important pathway for the exchange of nutrients in the earliest stages of pregnancy, before the placenta is established. The increased vacuolation in a large percentage of luminal and glandular epithelial cells and presence of glands devoid of secretions suggests that the endometrium tends to accumulate material for the incoming blastocyst.

3.2. Endometrial stromal response to the embryo

The endometrial stroma in the functionalis zone undergoes hyperproliferation and compaction in response to the preimplantation embryonic signals (Figure 1) or in response to the treatment with hCG (7, 11, 12, 20). This compaction is most pronounced in the stroma underlying the luminal epithelium, where an epithelial plaque like reaction occurs (Figure 1). This compaction could be either due to the loss of edema or due to hyperplasia of the cells. However, dividing stromal cells could be seen in the endometrial bed of the pregnant bonnet monkeys (15), indicating that stromal compaction during early pregnancy is due to proliferation and not just due to the loss of edema.

Decidualization is the first functional change in the endometrium during pregnancy involving a series of morphological and molecular changes by which stromal fibroblasts differentiate into secretory decidual cells. In the humans, regardless of embryo implantation, stromal edema is observed on day 23 of the menstrual cycle. In next 2-3 days, there is beginning of predecidual reaction around the spiral arteries which spreads through the upper two-third of the endometrium. If implantation occurs, the predecidual reaction gets intensified and forms the decidua of pregnancy. In the non-human primates, decidualization is observed only in the conception cycle or following the treatment with hCG. Both, in the hCG treated baboons and in the pregnant bonnet monkeys, the stromal cells secrete insulin like growth factor binding protein-1 (IGFBP-1) and prolactin that are markers of a decidual response (11, 12, 21, 22). These biochemical changes in the stroma cells are associated with the cellular changes evident at the ultrastructural level. At this stage, the stroma cells become large and round with several projections containing actin filaments. There is also evidence of increased biosynthetic activity as revealed by the presence of many strands of endoplasmic reticulum, Golgi sacules and large secretory vesicles (15, 20). The decidual cells in the hCG treated baboons, also produce large amount of extracellular matrix, which is evident as the dense material surrounding the cells under the luminal epithelium (20). These observations suggest that in response to the embryo/embryonic factors, the endometrial stromal cells undergo decidualization.

3.3. Vascular changes

Enhanced microvasculature is the characteristic feature of a pregnant endometrium. In the bonnet monkeys, in response to the embryo, a large number of small blood vacuolation. These endometrial glands lack any kind of vessels appear in the stroma underlying the luminal epithelium, and the zona functionalis gets filled with several microcapillaries (12, 22). Increased vascularity and angiogenesis at the implantation site has also been reported in the rhesus monkeys (10). Expression of the endothelial cell markers such as vimentin and angiogenic factors such as placental growth factor (PGF) and vascular endothelial growth factor (VEGF) have also been found to be altered at the implantation site on day 12 post-ovulation (10). A similar increase in the number of small blood capillaries in the stroma of the endometrial functionalis have been demonstrated in the baboons infused with the physiological doses of hCG in the uterine lumen (20). These observations suggest that the maternal tissues initiate neovascularization in response to the embryo derived molecules such as hCG to influence the endometrium in a paracrine manner to commence an angiogenic reaction.

4. BIOCHEMICAL AND MOLECULAR CHANGES IN THE ENDOMETRIUM IN RESPONSE TO THE EMBRYONIC SIGNALS

Blastocyst implantation is dependent on the intrinsic embryonic program operating in conjunction with the extrinsic signals emanating from the female reproductive tract. Implanting blastocyst also regulate the endometrial changes during the conception cycle. The molecular dialogue that serves to synchronize the development in the embryonic and maternal tissues during the peri-implantation period requires highly orchestrated, progesterone-dependent changes in the endometrium to render it responsive to the embryonic signals. The cardinal factors of implantation process include growth factors, cytokines and their receptors, cell adhesion molecules and ligands, signal intermediates, proteases and transcription factors. Studies in the last decade using several *in vitro* and *in vivo* model systems have demonstrated that great deal of receptivity related molecules are further modulated by the embryo derived signals. The results from these studies have firmly established that the embryonic signals have profound impact to bring out biochemical changes in the maternal endometrium prior to and also during the implantation period to coordinate the cellular remodeling during early pregnancy. A synopsis of the molecules that are regulated by the embryonic signals during early pregnancy has been recently published and shall not be re-emphasized (16, 17). However, a list of such embryo regulated endometrial molecules is provided in Table 1 (22-45).

In an attempt to decipher the molecular mechanisms and the potential cross-talk between the various regulatory components within the endometrium, high throughput gene expression profiling technologies have been employed to gain a global eye view of the endometrial responses to the embryonic signals. Using the baboon model (46), microarray analysis on the whole endometrial sample identified a set of novel genes that are modulated by the embryo derived factor, hCG (47). CG

Table 1. Alterations in the expression of genes in the maternal compartment of non-human primates in response to embryonic signals

Category	Factor	Epithelium (luminal or glandular)	Stroma/Decidua	Model	Reference
Transcription factors	HOXA10	++	++	Bonnet monkey	(22)
	HOXA11	Unknown	Unknown	Bonnet monkey	(22)
	Estrogen receptor (ER) alpha	++	++	Bonnet monkey	(15)
	ER	Down	Down	Baboon	(23)
	Prolactin receptor (PR)	++ ++	++ No change	Baboon, Rhesus monkey	(23) (24,25)
	C/EBP beta	+	+++	Baboon	(26)
Cytokines	TNF alpha	++	No change	Bonnet monkey	(14)
	TNF receptor 1 and receptor 2	++	No change	Bonnet monkey	(14)
	IL6	++	++	Bonnet monkey, Rhesus monkey	(13,27)
	IL1 alpha	++	++	Rhesus monkey	(27)
	IL1 beta	++	++	Rhesus monkey	(27)
	IL11	+ +	++ ++	Rhesus monkey, Cynomologus monkey	(28)
	IL11 receptor alpha	+ +	++ ++	Rhesus monkey, Cynomologus monkey	(28)
	LIF	++ No change	++ No change	Rhesus monkey, Bonnet monkey	(27) (13)
	TGF beta	++	No change	Bonnet monkey	(13)
	TGF alpha	++	+	Baboon	(29)
	TGF beta receptor	++	No change	Bonnet monkey	(13)
Hormones and growth factors	IGFBP1	++	?	Bonnet monkey, Rhesus monkey, Baboon	(22) (30) (31)
	Prolactin	++ +	No change ++	Bonnet monkey Baboon	(22) (32)
	Prolactin receptor	+	++	Baboon	(32)
	Glycodelin	++ ++	Not detected	Bonnet monkey Baboon	(13) (33)
	VEGF	++	++	Rhesus monkey	(34)
	Placental growth factor (PIGF)	++	++	Rhesus monkey	(34)
	IGF1	++	+	Rhesus monkey	(35)
	IGF1 Receptor	Not detected	++	Baboon	(36)
	IGFII	+	++	Rhesus monkey	(35)
	EGF	++	+	Baboon	(29)
	EGFR	++	+	Baboon	(29)
	Cyclooxygenase 1 (COX1)	++ Down	++ Down	Bonnet monkey Baboon	(15) (37)
	Cyclooxygenase 2 (COX2)	++ ++	++ ++	Bonnet monkey Baboon	(17) (37)
Signaling molecules	Protein Kinase A regulatory subunit 1A	++	++	Bonnet monkey	(17)
	Protein Kinase A Catalytic subunit A	++	++	Bonnet monkey	(17)
	Nitric oxide synthase (NOS)	++	++	Rhesus monkey	(38)
	Switch-associated protein 70 (SWAP70)	++	++	Rhesus monkey	(39)
Cytoskeletal and adhesion	Beta actin	++	++	Bonnet monkey	(15)
	Alpha smooth muscle actin	Not detected Not detected	++ Not detected (Increased in blood vessels)	Baboon Bonnet monkey	(40) (12)
	Smooth muscle myosin II	Variable	++	Baboon	(40)
	Mucin 1	Down	Not detected	Baboon	(41)
	Integrin alpha1	Down	++	Baboon	(42)
	Integrin alpha3	++	++	Baboon	(42)
	Integrin alpha 4	Down	++	Baboon	(42)
	Integrin alphaV beta3	++	++	Baboon	(42)
Proteases	MMP2	Not detected	++	Rhesus monkey	(43,44)
	MMP9	++	++	Rhesus monkey	(43)
	MMP14	Not detected	++	Rhesus monkey	(43)
	TIMP1	Not detected	++	Rhesus monkey	(43,44)
	TIMP2	Not detected	++	Rhesus monkey	(43)
	TIMP3	Not detected	Not detected	Rhesus monkey	(43,44)
	Ubiqutin	Not detected	++	Baboon	(45)
	Ubiquitin cross reactive proteins	Not detected	++	Baboon	(45)

'+' or '++' indicate weak or strong induction in the cells as compared to non pregnant receptive stage endometrium. 'Down' is downregulated compared to the non pregnant receptive stage endometrium '?' is for those whose cellular localization is unknown Models: Rhesus monkey (Macaca mulatta), Baboon (Papio anubis), Cynomologus monkey (Macaca fascicularis), Bonnet monkey (Macaca radiata). Data derived from rhesus monkey, baboon and cynomologus monkey are of endometrial/decidua studied at post implantation stages (day 10-20). Bonnet monkey data is obtained at preimplantation on day 6 post-ovulation and presence of an embryo was verified by a bioassay.

Table 2. List of differentially expressed genes in stromal cells in response to paracrine signals from the trophoblast cells identified by microarray analysis

Category	Upregualted	Downregulated
Immunomodulatory	*CXCL1, CXCL6, CXCL2 CXCR4, CCL8, CCL2 (MCP1), CCL8 (MCP2), IL-6, IL-8, IL-1BR1, IL-15, IL-7R, IL-2RA, , IL-13A2, IL-15R, TNFAIP -2, -3,-6, TNFAIP interacting protein, IFNGR1, IFN-alpha- IFI-30, -35, -44,-T4*	*TGFB1, CD24*
Proteolysis	*MMP-1, -3, -10, -12, -14, ADAMTS3, ADAMTS7, uPA*	*MMP11, Calpain, Cystatin*
Transcription	*CEBPD, GCM1, MYC, JUN, GATA3, PTX3*	*DACH1, TOX, ETV1*
Cell signaling	*CGA, SNX10, PBEF1, ADORA2B, PDE3B*	*PI3K p85a, CD24, PDGFD*
Ion binding /transport	*Metallothionein (MT)-1F, -1E, -1F, -1H, -1L, -1M, -1X, -2A, Stanniocalcin*	
Growth regulators	*GAS1, GOS, IP-15*	*IGFBP5, IGF-1, FGF-1, TRH degrading enzyme*
Cell cycle and apoptosis	*IER, S100A3*	*CCNE2*
Development	*DKK1*	*WNT, FZD2, CHODL, MPPED2, DSCR1L1*
Angiogenesis	*S100P*	*Angiopoietin 1*
Cell adhesion and motility		*Trophinin, ANK3, ADRA2A, ITGA6, Fibronectin 1alpha-smooth muscle actin*

Adapted from the microarray analysis reported previously (56,57)

induces the expression of leukemia inhibitory factor (LIF), the complement component C3, superoxide dismutase-2 (SOD-2), the matrix metalloproteinase 7 (MMP7) and glycodelin. On the other hand, it decreases the expression of the regulator of the Wnt-signaling pathway and the soluble frizzled receptor protein 4. LIF plays a crucial role in implantation and trophoblast invasion by regulating the adhesion and promoting the invasion of EVTs (48, 49). The CG mediated regulation of C3, glycodelin and SOD2 expression suggests that it may have a role in the regulation of the function of immune system and protection against oxidative damage during pregnancy. Metalloproteinases help in remodeling of the ECM of the endometrium during normal cycle as well as during the implantation (50). Upregulation of the expression of endometrial MMP7 under the influence of CG in both the baboons and the humans would possibly help in promoting the trophoblast invasion (47, 51, 52).

Once the physical contact of the embryonic cells and the maternal epithelial cells is established, the trophoblast cells breach the epithelial layer and enter into the decidua. As the invading trophoblasts journeys through the maternal decidua, it is predominantly encountered by the decidualized endometrial stromal fibroblasts, which are in close proximity to the invading trophoblasts. These trophoblast cells *in situ* would presumably further rework on the decidual cells to gain access to the various components of the maternal compartment and establish placentation. Despite the technological advancements, we have little understanding about the *in vivo* interaction between the maternal endometrium and the invading trophoblasts. *In vitro* experiments have been performed in this direction to provide mechanical and biochemical insights on the process of trophoblast-endometrium interaction (53-55).

Two microarray studies conducted in this direction have attempted to delineate the molecular phenotype of the endometrial cells in response to the trophoblast signals. In one study, Hess and his group focused on the paracrine modulation in the stromal cells by studying the changes in the global gene expression profile of the endometrial stromal cells after treatment with the trophoblast cell conditioned medium (56). The study revealed that the decidualized human endometrial stromal cells respond to the secreted products from the human EVTs by markedly upregulating the expression of various cytokines, chemokines, angiogenic factors and downregulating the expression of genes which are responsible for stromal cell mitosis and other processes. In another study, Popovici and his colleagues established an *in vitro* co-culture system of endometrial stromal cells with the first-trimester trophoblast explants and the effect of trophoblast on the endometrial stromal cells during implantation was analyzed by comprehensive gene expression profiling (57). Table 2 gives a comprehensive list of differentially expressed genes identified by these studies. It is evident that the trophoblast cells alter the expression profile of genes that belong to various classes and have diverse functions in the regulation of cellular physiology. Functionally, it appears that the presence of trophoblast cells modulates immunomodulatory, proliferative and cell death associated processes in the stromal/decidual cells. At the same time, they direct the decidual cells to activate the expression of proteases and inhibit the expression of cell adhesion molecules perhaps to facilitate its invasion. Intriguingly, there is altered expression of the molecules related to the immune function, adhesion and angiogenesis in the endometrium of fecund cycles of the bonnet and rhesus monkeys and CG treated baboons (10, 12, 14, 17, 46, 47). These observations imply that the embryonic signals modulate the maternal

compartment and alter their functionality to support the initiation of pregnancy.

5. PURPOSEFUL CONNOTATION OF THE EMBRYO-ENDOMETRIAL CROSS-TALK

There are ample clinical evidences which suggest that the endometrial refractoriness during the putative "window of implantation" is a cause of subfertility and failure of *In vitro* Fertilization (IVF) (58, 59). The histological features and the "molecular fingerprints" of the receptive and the conception cycle of the endometrium can be exploited for the diagnostic and the therapeutic applications. In the biological perspective, three major functions of the embryo-endometrial cross-talk have emerged which include i) immunomodulation, ii) biosensing and iii) invasion. Summarized below are evidences that support the above notion.

5.1. Uterine immunomodulatory factors are regulated by the embryonic signals

Microarray analysis of the decidual cells co-cultured with the trophoblast cells or the CG treated baboon endometrium have identified genes having immunomodulatory activity. CG treatment to the baboon endometrium increases the expression of glycodelin and C3 (47). The co-culture of stromal cells with the trophoblast cells showed altered expression of a large number of inflammatory cytokines, chemokines, NFkB (Nuclear Factor Kappa B) and many more genes involved in the regulation of immune function and the modulation of inflammatory reaction (Table 2). The increase in the expression of inflammatory cytokines is not surprising as these are modulated by the presence of embryo in the endometrium of the pregnant bonnet monkeys (Table 2). There is a pre-requisite of an inflammatory environment to support the implantation of the blastocyst into the endometrium. These observations prompt us to hypothesize that the initial requirement of the inflammation milieu is facilitated by the factors secreted by the embryos. The embryo/trophoblast derived signals seems to direct the endometrial cells to produce inflammatory cytokines and chemokines, which in turn would stimulate the endometrial immune cells like-T cells and natural killer (NK) cells to facilitate the pregnancy. Indeed, the conditioned medium from the decidual cultures is reported to influence the uterine NK (uNK) cell proliferation and their functioning (60).

5.2. Embryonic signals direct the endometrial cells to dictate their selection (biosensor activity)

In an attempt to identify factors involved in the embryo-endometrial cross-talk, supernatants were collected form the decidual cells co-cultured with the blastocysts and assayed for the expression of a range of the putative implantation associated cytokines, growth factors and chemokines (61). The results demonstrated that the developing human embryos had no detectable effects on the decidual secretions. However, an unusual response was noticed upon co-culture of the morphologically arresting blastocyst with the decidual cells. Decidual cells co-cultured with arrested embryos responded by downregulating the expression of the key receptivity and immune regulators like IL-1b, -6, -10, -17, -18, eotaxin and heparin-binding EGF-like growth factor. This effect was selective as the presence or absence of a developmentally compromised embryo had no effect on the expression of IL-12, -15, TNF-alpha, monocyte chemotactic protein-1 or chemokine (C–X–C motif) ligand 10 production (61, 62). This differential sensitivity of the decidual cells towards the normal and abnormal embryos was not observed with the non-decidualized stromal cells (61), suggesting that the decidual but not the stromal cells have the ability to sense the embryo quality (62). An altered expression of some of these factors has also been observed *in vivo* in the endometrium during the fecund cycles or in response to the CG (13, 14, 17, 47). These observations suggest that the decidua not only senses the signals derived from the embryo and responds to create a pro-implantation condition, but also sense the quality of the embryonic signals and even terminate the "window of endometrial receptivity" to enable the mother to dispose off the compromised embryos. This observation adds up another dimension to the potential of decidualizing endometrial stromal cells as the sensors of the embryo quality during the implantation process.

5.3. Embryonic signals generate a microenvironment within the uterus to support the trophoblast invasion

Trophoblast cells require a complex array of molecules that permit their controlled migration/invasion into the decidua. While, several of these are already expressed by the endometrium during the "window of receptivity", many others are induced upon receiving the embryonic signals. The hypothesis that the change in the gene expression profile in the human endometrium by the embryo derived signals promote the trophoblast invasion got the experimental proof, when co-culture of trophoblast and endometrial decidual cells or treatment of trophoblast cells with decidual cell culture medium led to an increase in the trophoblast invasion (63, 64). We have shown that decidual cell secretome enhances the invasion of trophoblast cells through altered expression of the MMPs and tissue inhibitor of metalloproteinases (TIMPs) (64). In addition to these, there could be involvement of other proteases like uPA and their inhibitors in the regulation of trophoblast invasion (65). In a Matrigel based co-culture system that allows cell-cell contact, an enhanced invasive potential of the decidualized cells was observed in the presence of trophoblast cells. The enhanced invasiveness was associated with an increase in the expression of MMPs by the decidual cells (63). These observations suggests that under the influence of the products derived from the embryo (in this case, the trophoblast cells), the decidual cells are not only highly migratory, but also highly invasive to actively support the movement of the trophoblast cells into the decidual layer by encircling them (63). Thus, the trophoblast cells seem to direct the maternal endometrium towards a pro-invasive milieu.

Figure 2 summarizes the morphological events in the endometrium that are primed by the embryo and the current understanding of the functional connotation. The embryonic signals, pre/post- implantation, affect all the

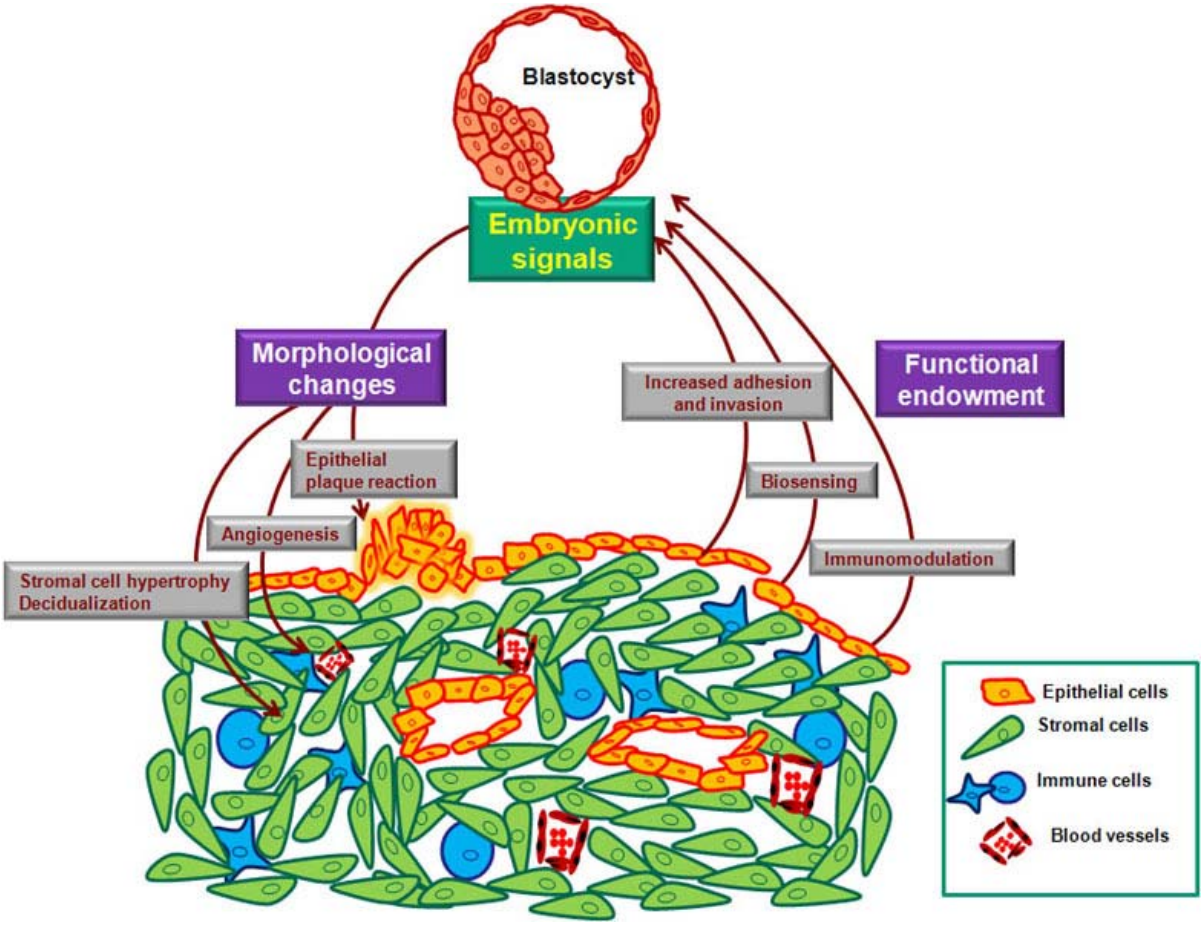

Figure 2. Embryonic signal induced morphological changes and functional endowments achieved by the endometrium at implantation: Embryonic signals pre/post-implantation induces the endometrial epithelial and stromal cell hypertrophy, decidualization and increased angiogenic activity. At the same time, the endometrium gains its ability to sense a good vs. poor quality embryo, allowing it to adhere and eventually invade the endometrium. It also creates an inflammatory milieu that not only supports invasion but, dictate the decidual immune cells to evade rejection.

compartments of the endometrium and alter the expression of a plethora of biomolecules. These alterations bring about immunomodualtory changes and endow the endometrium with biosensing, adhesive and invasive capabilities. The endometrium would sense a normal embryo and allow it to adhere to the endometrial epithelium. The embryo would further prime the decidual immune cells, so that it can evade the maternal immune responses. At the same time, the endometrial cells are stimulated to secrete multitude of molecules having pro-invasive potential, thereby promoting the invasion of trophoblast cells of the adhered blastocysts.

From above discussion, it is perceptible that the trophoblast cells are self facilitatory that amend the maternal compartment not only to suite its survival but also its functionality. All these events ultimately pave the way for an embryo friendly milieu. However, this is not a one way process where the endometrium takes dictation from the blastocyst and modulates itself to support pregnancy. It is rather a two way mutually acceptable dialogue where the endometrial cells also dictates terms to the incoming embryo, mainly the trophoblast cells. The EVT cells are intrinsically highly invasive in nature and shares expression of several molecular and functional signatures of tumor cells. Trophoblasts, like tumor cells can voraciously proliferate and invade *in vitro* extracellular matrix as well as under *in vivo* situation at extra uterine sites. However, in the decidua their invasion is highly restricted both in time and space. The differentiated EVTs just post-implantation are allowed to rapidly proliferate and invade through the decidua funtionalis (upper 2/3rd of the decidua), reach the maternal spiral arteries and establish placentation. The trophoblast cells are however, permitted to continue migration and invasion only upto the superficial layer of the myometrium (decidua basalis) and their invasion is completely stopped by mid gestation. The decidual restraint of trophoblast invasion plays a major role in spatio-temporal regulation of trophoblast invasion, which is missing in extrauterine pregnancies and malignancies.

In response to the decidual signals, the trophoblast cells actively divide, differentiate, migrate and invade through various layers of the maternal tissue to establish placentation. Of these processes, the events of invasion has achieved greatest attention as it is a prime process required to be performed by the trophoblasts to

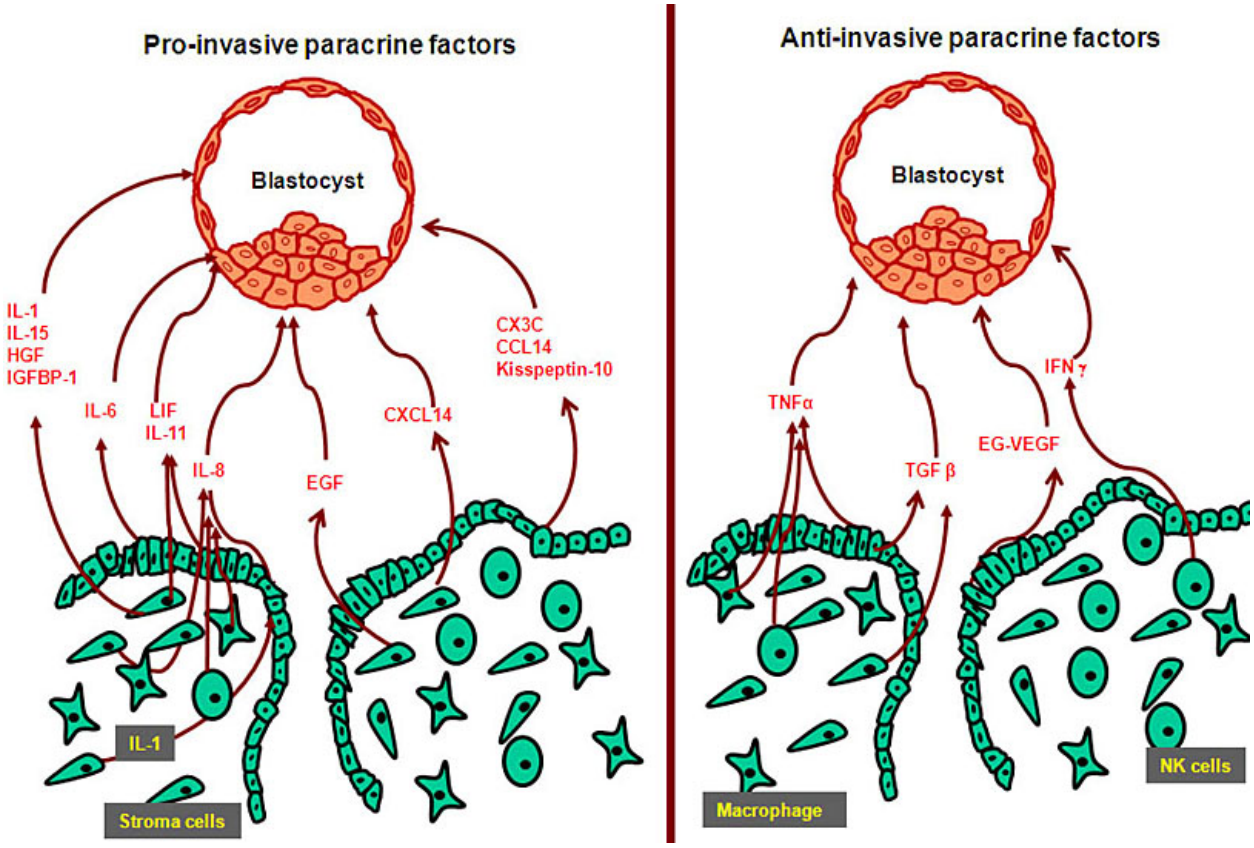

Figure 3. Paracrine factors in the regulation of trophoblast invasion: The cross-talk between endometrium and blastocyst is vital for implantation leading to successful conception. Endometrium has several cell populations like stromal cells, uNK cells, macrophages, which contributes to secrete several growth factors and cytokines to modulate the invasion of trophoblast cells. The pro-invasive paracrine factors secreted by endometrium include IL-6, IL-11, LIF, IL-1, IL-8, IL-15, EGF, HGF, IGFBP-1 etc. The paracrine factors produced by the endometrium that negatively regulate trophoblast invasion include TGF-beta, TNF-alpha, IFN-gamma, Kisspeptin etc.

establish correct placentation. Understanding the process of invasion has clinical relevance as flaws in the process of invasion causes a number of pregnancy complications. In the next section, we review the endometrial signals modulating the trophoblast invasion and the molecular mechanisms operative within the trophoblast cells in response to the extrinsic stimuli during this process.

6. PARACRINE REGULATION OF THE TROPHOBLAST INVASION BY THE ENDOMETRIAL FACTORS

Various cell types such as endometrial epithelial cells, stromal cells, uNK cells, decidual macrophages etc present in the receptive endometrium produce a variety of factors which act in a paracrine fashion to regulate the invasion of trophoblast cells. We and others have shown that secretions from the decidual cells alter the expression of invasion related molecules like MMPs and integrins in the trophoblast cells and also influence its invasion (64 and references therein). These results imply that the decidua must secrete molecules that are sensed by the trophoblast cells and they amend their own physiology depending on the cues they get from the maternal cells. Figure 3 shows some of the important cytokines, chemokines and growth factors which are known to be produced by the decidua and shown to either promote or inhibit the invasion of trophoblast cells.

IL-6, IL-11 and LIF are the major cytokines of IL-6 family, which are mainly expressed by the endometrial cells and play potential role in the regulation of the embryo implantation (66). IL-6 is expressed by the endometrial glands during the proliferative phase of the menstrual cycle, which increases by 5 to 10 fold during the mid-secretory phase (67). The expression of IL-11 and LIF has also been observed by the endometrial glandular and luminal epithelium as well as by the stromal cells during the secretory phase of the menstrual cycle (68). Interestingly, the expression of LIF, IL-6 and IL-11 increases during the decidualization of human endometrial stroma cells and it is further modulated by the presence of embryo in the endometrium (13, 17, 69, Table 1). The expression of IL-6 group of cytokines during the secretory phase of the menstrual cycle i. e. the putative "window of implantation" and their modulation by the embryo suggests their critical role in the regulation of endometrial and trophoblastic functions. Indeed, IL-6 increases the invasion

of trophoblastic HTR-8/SVneo cells (derived from the human first trimester placenta explants cultures, immortalized by SV40 large T antigen) by altering the expression of adhesion molecules like integrin alpha5, integrin alpha1 and integrin beta1 (70). In trophoblastic cells, IL-6 also increases the enzymatic activity of MMP2 and MMP9 without increasing their immunoreactivity (71). LIF promotes the adhesion of the EVTs through change in the integrin expression. However, it increases the invasiveness of JEG-3 choriocarcinoma cells through down regulation of the tissue inhibitor of metalloproteinase 1 (TIMP1) expression (72). In contrast to IL-6 and LIF, IL-11 has dual role in the regulation of trophoblast invasion, as it increases the invasion/migration of trophoblastic JEG-3/AC1-M88 (primary extravillous trophoblast cell-JEG-3 choriocarcinoma cell hybrid) cells, while decreases the invasiveness of HTR-8/SVneo cells (73, 74). A reduced expression of these three cytokines in the endometrium has been found to be associated with the recurrent abortions and pregnancy failure (75).

Epidermal growth factor (EGF) is expressed by both the proliferative and secretory phase endometrium, but its expression is relatively higher in the decidual cells. It increases the invasion of trophoblastic cells by increasing the expression of MMP9 and TIMP1 (76). Hepatocyte growth factor (HGF) is largely expressed by the endometrial stromal cells while, its cognate receptor (c-met) is largely expressed on the trophoblast cells making it a potent growth factor, which can act in a paracrine way to regulate the trophoblast biology (77). It increases the invasiveness of trophoblastic cells by up-regulating the expression of H2.0-like homeobox (HLX) gene (78). HLX is expressed in the proliferating and migrating, but not in the invading human trophoblast cells and its expression is significantly low in the cases of human fetal growth restriction (78). A decrease in the HGF expression correlates with the progression of pre-eclampsia in the pregnant women (75). Insulin like growth factor binding protein-1 (IGFBP-1) is produced by the decidualized endometrial cells and increases the invasion of the trophoblastic cells by increasing their gelatinolytic activity (79). Endometrial endocrine gland-derived vascular endothelial growth factor (EG-VEGF) is highly expressed in early pregnancy but, falls after 11 weeks. Expression of EG-VEGF is induced in the endometrium of the rhesus monkeys in the conception cycle (10). EG-VEGF inhibits the EVT migration, invasion and tube-like organization with a decrease in the MMP2 and MMP9 production. EG-VEGF levels have been found to be elevated in the pre-eclampsia patients making it a novel paracrine regulator of the trophoblast invasion (80). TGF-beta is expressed by the endometrial epithelial, stromal as well as decidual cells (13). Its expression increases in the glandular cells during early pregnancy (13). TGF-beta also decreases the invasion of trophoblastic cells by up-regulating the expression of TIMP1 and 2, plasminogen activator inhibitor-1 (PAI-1) and -2 (PAI-2) (81). In addition to this, TGF-beta1 up-regulates the expression of molecules associated with cell to cell adhesion (ezrin and E-cadherin) in trophoblast cells leading to a reduction in invasion (82). The role of TGF superfamily in the regulation of placental function has been reviewed in detail elsewhere (83). In an *in vitro* model of the trophoblast invasion where, first trimester human chorionic villous explants were co-cultured with the primary human endometrial fibroblast, addition of TGF-beta inhibited the invasion of extravillous trophoblast cell into the endometrial cell layer (84).

IL-1 is expressed by the decidualized stroma cells but not by the endometrial epithelial cells (79). It increases the invasion of trophoblastic cells by increasing the expression of MMP2, MMP9, membrane type-matrix metalloproteinase-1 (MT-MMP-1), -2 (MT-MMP-2) and urokinase-type plasminogen activator (uPA) (79). In addition to altering the expression of proteases, IL-1beta also promotes the secretion of leptin- a known factor to promote the trophoblast invasion (85). IL-1beta increases the trophoblastic cell to matrix interaction and reduces the cell to cell adhesion by decreasing the expression of ezrin and E-cadherin expression (82). With respect to the expression of proteases, their inhibitors and the adhesion molecules, IL-1beta and TGF-beta1 acts antagonistic to each other to regulate the trophoblast invasion. IL-1 stimulates the production of IL-8 by the endometrial epithelial cells, which subsequently increases the migration of villous Cytotrophoblast cells (86). Depletion of IL-8 from the culture supernatant of the IL-1 stimulated endometrial epithelial cells showed decrease in the invasion of cytotrophoblast cells. In the human uterus, IL-8 is expressed by the endometrial and decidualized epithelial and stromal cells. It is also expressed by the decidual NK cells, macrophages and CD8+ T lymphocytes (87, 88). IL-8 increases the invasion of trophoblastic cells by up-regulating the expression of MMP2 and MMP9 (87).

IL-15- an IL-2 family of cytokine, is expressed by the proliferative and secretory phase endometrium as well as by the first trimester decidua (89). The expression of IL-15 is markedly increased by the stromal cells during decidualization (69). In addition to this, macrophages are also an important contributor of IL-15 in the uterine milieu (89). IL-15 increases the invasion of JEG-3 cells by upregulating the MMP1 expression (90). Kisspeptin (KiSS)- a neuronal decapeptide, is central to the regulation of gonadotropin secretion. It is expressed during first trimester by the endometrial as well as by the syncytiotrophoblast cells (91, 92). It inhibits the trophoblast invasion through binding to the G protein-coupled receptor Kisspeptin-1 receptor (KiSS-1R) with an increase in the intracellular Ca^{+2} levels (91, 92).

Tumor necrosis factor-alpha (TNF-alpha) is expressed by the endometrial cells, decidual macrophages and NK cells (79). Its expression increases in the endometrium during the early pregnancy (14, 17). Reportedly, TNF-alpha inhibits the trophoblastic HTR-8/SVneo cell invasion by up-regulating the expression of PAI-1 (93). In the uterus, uNK cells are the major source of interferon-gamma (IFN-gamma) and inhibit the invasion of EVT through increase in apoptosis as well as reduction in the expression of MMP2 (94). IFN-gamma decreases the invasion of EVT by decreasing the expression of insulin like growth factor receptor-II (95). Further, co-stimulation of EVT with both TNF-alpha and IFN-gamma also

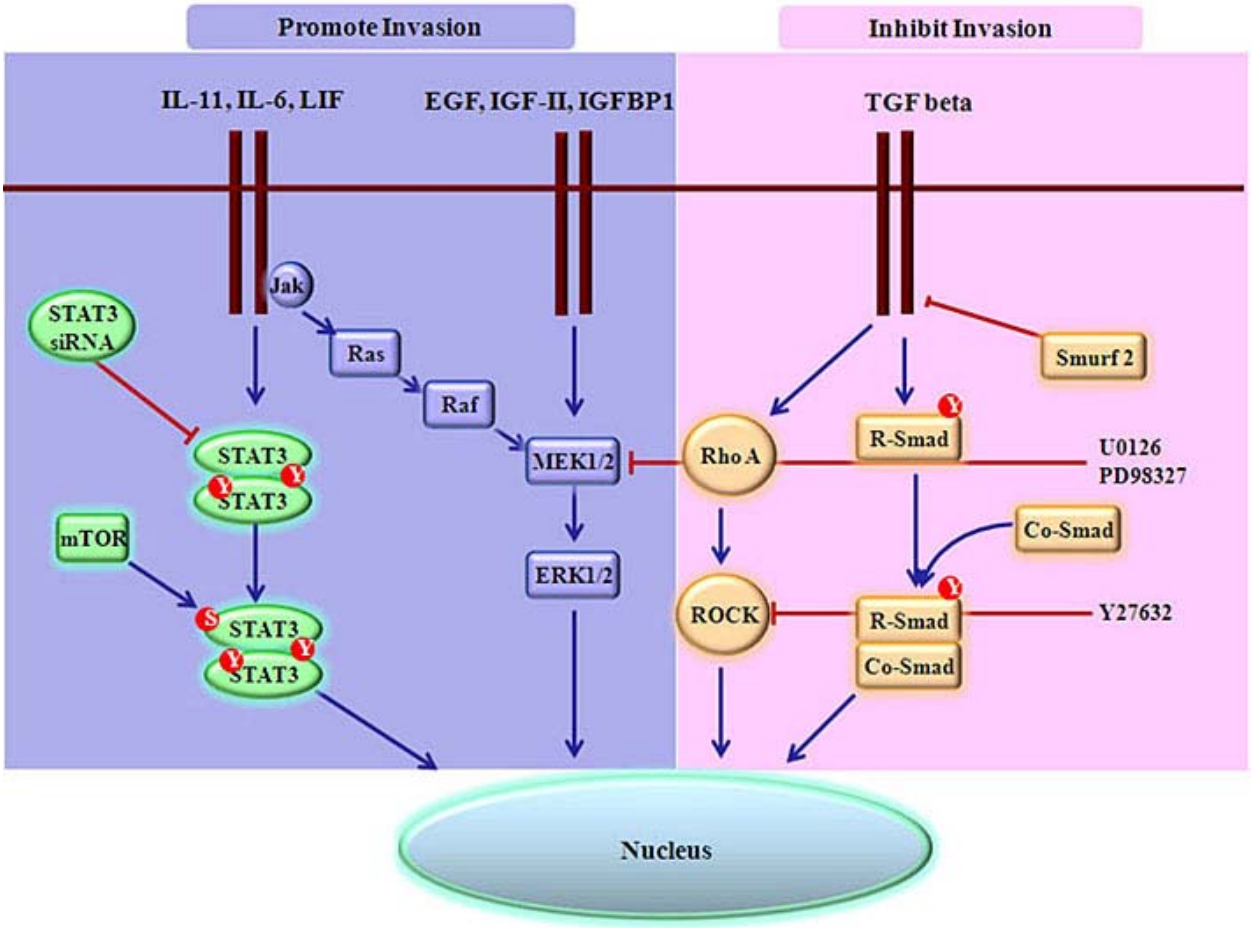

Figure 4. Downstream signaling pathways activated by the paracrine factors in trophoblast cells: Endometrium derived paracrine factors predominantly activate Jak-STAT, MAPK and TGF-beta mediated signaling pathways to influence the invasion of trophoblastic cells. Jak-STAT pathway is activated by IL-6 group of cytokines and inhibition of STAT3 expression by siRNA inhibits the IL-11 and LIF mediated invasion in trophoblastic cells. Similarly, ERK1/2 dependent MAPK pathway is activated by EGF, IGF-II, IGFBP etc and to a certain extent by LIF and IL-11. However, the role of ERK1/2 activation by IL-11 and LIF during invasion of trophoblast cells has not been elucidated. In contrast to these, TGF-beta dependent signaling pathway inhibits the invasion of trophoblast cells through activation of Smad and Rho A dependent signaling mechanism. Red ball with 'Y': phosphorylation at tyrosine residue while, red ball with 'S': phosphorylation at serine residue. U0126 is the inhibitor for both MEK1 and MEK2 while PD98327 is the inhibitor of MEK1. Y27632 is a selective inhibitor of the ROCK.

inhibited their invasiveness with a decrease in the expression of MMP2 and increase in the expression of uPA (96). Uterine NK cells are the major immune cell population in the decidua. Decidual NK cell have high secretory activity and secrete cytokines like TNF-alpha, TGF-beta1 and IFN-gamma. Hence, it was hypothesized that the culture supernatant obtained from uNK cell culture would inhibit the trophoblast invasion. However, in contrary to that, culture supernatant of the uNK cells obtained from 12 to 14 week gestational age increased the EVT invasion by up-regulating the expression of MMP9 (97). These results suggest that the effect of a combination of cytokine/growth factor may lead to the expression of distinct set of genes as compared to when they act independently.

Chemokine (C-X-C motif) ligand 14 (CXCL14) is a small cytokine of CXC chemokine family. It is expressed by both the decidualized stromal cells as well as the villous trophoblasts. It inhibits the invasion of trophoblastic cells by suppressing their gelatinase activity (98). Chemokines C-X3-C motif ligand 1 (CX3CL1) and C-C motif ligand 14 (CCL14) are abundantly expressed in the endometrial vasculature, epithelial and decidual cells while, their receptors (CX3CR1 and CCR1) are present on the invading human trophoblasts. CX3CL1 and CCL14 promote the trophoblast migration by mediating changes in the expression of adhesion molecules and extracellular matrix proteins like α-catenin, extracellular matrix protein 1, osteopontin, MMP12 and integrin beta5 (99, 100).

The above studies demonstrate that various endometrial derived factors *in vitro* activate or suppress the invasion of trophoblast cells by altering the expression of invasion related genes. At present, the precise concentrations of these factors *in vivo,* at the site of implantation, are not known; the cumulative effects of these effectors on the process of invasion need to be investigated. However, culture supernatants from early decidualized endometrial stromal cells stimulate invasion (64 and references therein) while those derived from the first trimester decidual cells inhibit invasion (Graham and Lala from 64). This suggests that the decidual secretome may dynamically alter during various stages of gestation. It is likely that at the early implantation stages, decidual secretome is programmed to stimulate the trophoblast invasion whereas, at later stages the secretome may contain higher level of anti-invasive factors to limit trophoblast invasion.

From above discussions, it is evident that the process of invasion is a two way dialogue between the embryo and the endometrium where both the compartments respond to signals from each other and appropriately modulate their physiology to ultimately facilitate pregnancy. At the cellular level, the paracrine factors secreted by the endometrial cells bind to the cognate receptors onto the trophoblast cells and induce various signaling mechanisms to further regulate the production of a variety of factors involved in the regulation of invasion of trophoblast cells. The next section summarizes the signaling mechanisms that operate within the trophoblast cells in response to the various extrinsic factors in regulating their invasion.

7. MOLECULAR MECHANISMS OF THE TROPHOBLAST INVASION

In the trophoblast cells, activation of diverse signaling pathways through paracrine mediators alters the expression of effector molecules. A final outcome of these signaling events decides whether there will be an increase or a decrease in the invasion. From several experimental observations, it appears that the way in which these activatory and inhibitory signaling pathways are controlled make the trophoblastic cells different from several malignant carcinomas, where there is no check on invasion. As discussed above, there are several extrinsic factors that control the process of invasion. So, it is logical that there must be existence of a multitude of signaling pathways within the trophoblastic cells to respond to these stimuli. Not surprisingly, the expression of most members of diverse signaling pathways have been detected in the trophoblastic cells and their mechanism of operation in context of invasion has been a subject of several reviews. In this section, rather than being comprehensive, we have chosen to review three key signaling pathways, which get activated by the decidua derived paracrine mediators and affect the process of trophoblast invasion. The picture that has emerged from these studies is schematically shown in Figure 4 (75, 101-104) and discussed below.

7.1. Janus kinase (Jak)-signal transducer and activator of transcription (STAT) pathway

IL-6 group of cytokines transduce its signal by binding to the cytokine specific as well as the group specific gp130 co-receptor. Cytokine binding to its receptor leads to oligomerization of the gp130 core, bringing the intracellular Jaks in close proximity to cross-phosphorylate each other. Activated Jaks in turn phosphorylate the cytoplasmic tail of the gp130 co-receptor, which then acts as docking site for proteins having SH-2 domain [STAT, Ras, Phosphoinositide 3-kinase (PI3K) and Phospholipase C-delta (PLC-delta)] (75). Cytoplasmic STATs get phosphorylated by activated Jaks through phosphorylation at STAT3 Tyr705 residue. In addition to this, STAT3 also gets phosphorylated at Ser727 through several other kinases, like mammalian target of rapamycin (mTOR) (105). Activated STAT3 forms homo-/hetero- dimers leading to their translocation into the nucleus, where they act as a transcription factor to regulate gene expression of several genes (75).

Amongst the IL-6 family of cytokines, LIF, IL-6 and IL-11 are known to activate trophoblast invasion and all the three utilize the Jak-STAT pathway. In JEG-3 cells, LIF increases the activation of STAT3 through phosphorylation of Tyr705. The involvement of STAT3 in LIF mediated invasion is evident from the observations where silencing of STAT3 expression by siRNA led to a significant decrease in the invasion, irrespective of the addition of LIF (75). IL-6 increases the invasion of JEG-3 and HTR-8/SVneo trophoblastic cells and silencing of its expression in JEG-3 cells led to a decrease in invasion due to decrease in endogenous level of IL-6 (70, 106). In HTR-8/SVneo cells, IL-6 increases the phosphorylation of STAT3 Tyr705. Whether, STAT3 silencing leads to decrease in IL-6 mediated invasiveness of trophoblast cells is not known. In JEG-3 choriocarcinoma cells, IL-11 increases their invasiveness through activation and expression of STAT3 (74). Ablation of STAT3 expression by siRNA decreases the invasiveness of these cells (74). This effect of IL-11 requires gp130 as silencing the expression of gp130 in JEG-3 cells abrogated the IL-11 mediated increase in invasiveness while, IL-6R silencing had no bearing on the regulation of IL-11 mediated increase in the invasion (74). IL-11 also increases the migration of EVT cells and a hybrid cell line, AC1-M88 through STAT3 activation (107). However, STAT3 does not appear to be an exclusive pathway that IL-11 may utilize to stimulate invasion as addition of excess IL-11 to JEG-3 cells partially rescued the inhibitory effect of STAT3 silencing (74). In contrast to these observations, in HTR-8/SVneo and EVT cells, IL-11 decreases the invasion through activation of STAT3 (73). At present the reason of such conflicting effects of IL-11 in trophoblast invasion through STAT3 are unclear. It definitely demands a careful analysis of the differences in the basic cellular signaling machinery of JEG-3 and HTR-8/SVneo cells.

7.2. Extracellular signal-regulated kinase 1/2 (ERK1/2) mediated signaling pathway

Activation of ERK1/2 has been found to be associated with several malignancies and it also controls diverse cellular processes like proliferation and apoptosis in various cell types. These observations raised the possibility of its involvement in trophoblast invasion. Activated ERK1/2 is detectable 1n the placental villi till 12th week of

gestation, suggesting a role in the placental development during the first trimester of pregnancy (108).

Among the several stimulators, IGF-II, IGFBP-1 and EGF utilize ERK mediated signaling pathway to regulate the trophoblast invasion. The increase in migration of HTR-8/SVneo cells under the influence of IGF-II and IGFBP-1 is associated with the activation of ERK1/2 (109, 110). IGFBP-1 binds to the integrin alpha5beta1 through its Arg-Gly-Asp (RGD) domain and activation of ERK1/2 takes place through activation of focal adhesion kinase (FAK). Involvement of ERK1/2 in the process of invasion is evident from the findings that increase in invasion of trophoblastic cells under the influence of IGF-II and IGFBP-1 can be inhibited by using MEK-1 inhibitor, which abrogates the activation of ERK1/2 downstream to this kinase (109, 110).

In HTR-8/SVneo cells, ERK1/2 pathway is activated by EGF, which upon treatment with MEK1/2 inhibitor abrogates the EGF dependent ERK1/2 activation. EGF mediated increase in the expression of MMP2, MMP9 and TIMP1 in HTR-8/SVneo cells not only require the activation of ERK1/2 but, also of PI3K (111-113). In JAR choriocarcinoma cell line and first trimester trophoblast cells, EGF via ERK1/2 stimulates the production of MMP2 through increase in the binding activity and the expression of phophorylated p53, activator protein-2alpha (AP-2alpha) and -2gamma (AP-2gamma), CCAAT enhancer-binding protein-epsilon (C/EBP-epsilon) and -lambda (C/EBP-lambda) to their responding sequences, found in the MMP2 promoter (114).

7.3. TGF mediated signaling pathway

TGF is an important regulator of the trophoblastic cell functions (103, 115, 116). Members of the TGF-beta superfamily transduce its signal by binding to several isoforms of Type I and II Ser/Thr-receptor tyrosine kinases also known as activin receptor-like kinases (ALKs). Each TGF-beta member binds to the specific combination of type I and II receptor, leading to their autophosphorylation and further activation of several downstream signaling pathways. Amongst these pathways, Smad mediated signaling pathway is well characterized (103). The R-Smads (TGF-beta-Responsive Smads); Smad2 and Smad3 are cytoplasmic proteins which gets activated at the cell membrane by the TGF-beta receptors and translocate to the nucleus upon binding with common-mediator Smad (coSmad). TGF-beta signaling can also activate ERK, Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38MAPK) and Rho GTPases. JEG-3 cells express Smad2, 3, 4, and 7 isoforms. Analysis of the transcriptional activity of TGF-beta in JEG-3 cells using two TGF-beta responsive reporter constructs, p3TP-Lux and pAR3-Lux revealed that Smad2, 3 and 4 enhance, while Smad7 inhibits the TGF-beta1 mediated gene expression (117).

In JEG-3 cells, TGF-beta1 regulates the transcriptional activity of aromatase gene through activation and binding of Smad2 to the promoter sequence and inhibits the estradiol production (118). An important regulator for the TGF-beta signaling pathway is Smad ubiquitination regulatory factor 2 (Smurf2). Smurf2 is homologous to E6-AP carboxy terminus (HECT) type E3 ubiquitin ligase, which targets the TGF-beta receptors and various Smads for the proteasome-mediated degradation. In trophoblast cells, silencing the expression of Smurf2 by siRNA led to an increase in the expression of TGF-beta type I receptor without any change in Smad2 expression, which is also considered as a potential target of Smurf2. Over expression of Smurf2 in HTR-8/SVneo cells resulted in the decrease in TGF-beta type I receptor and an increase in the cellular migration and invasion (119). TGF-beta also activates the RhoA/Rho-associated kinase (ROCK) pathway to regulate the migration of cells. Inhibition of ROCK with their chemical inhibitor Y27632 abolished the TGF-beta mediated inhibition of the EVT migration on the endometrial cell layer (84). These observations suggest a role of ROCK in the TGF-beta-dependent control of the trophoblast migration.

In summary, trophoblast cells respond to different stimuli by activation and inhibition of diverse signaling pathways depending upon the kind of the extracellular signals it experiences. It will be of interest to determine how these different signaling cascades cross-talk and direct the transcriptional machinery to control the trophoblast invasion. Unlike tumor cells, as trophoblast cell invasion is highly controlled in space and time, it is tempting to hypothesize that in responses to the maternal stimuli, the invasion promoting and inhibiting signaling cascades (for e.g. Jak-STAT/ERK vs. Smad) in the trophoblast cells may constantly compete with each other and the outcome of this would be sensed by the maternal system to decide the production of the different pro- and anti-invasive molecules, which ultimately regulate the trophoblast invasion.

8. CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

Endometrial receptivity has been considered as the major rate limiting step and a bottleneck for successful mammalian reproduction. Beyond ovarian hormones, it is now evident that the embryonic signals further prime the uterus resulting in advanced level of molecular, biochemical and morphological modifications. Embryo implantation is the result of a well-orchestrated sequence of events including cellular adhesion, invasion and immune regulation under the influence of the ovarian hormones, which is further modulated by the embryonic signals. It is proposed that embryo implantation is a well-orchestrated drama, in which the ovarian hormones and the embryonic signals direct the play. Some gene products are main characters with roles at several different stages, some act as a chorus, in a combinatorial fashion with others, whereas a few play a critical role in one scene and then disappear. The mechanism presumably evolved to be delicately poised to respond firstly to the hormonal trigger to achieve receptivity and then to amplify this decision under the embryonic signals to permit trophoblast invasion. So, it seems that embryo implantation is a system full of back-ups and functional redundancy.

In the last decade, the use of genomics and proteomics approaches have greatly enhanced our knowledge on the process of endometrial receptivity and implantation and also led to identification of aberrations in gene expression in menstrual disorders. Several researchers have applied such global gene profiling approaches to understand the primate endometrial function and identify the genes that may be crucial in preparation of the endometrium for the process of implantation. Data is also accumulating on the gene signatures of several endometrial pathologies such as endometriosis. These studies provide greater insight about the various genetic and molecular pathways associated with the pathology and the cellular and phenotypic changes associated with them.

Studies so far have mainly focused on the molecular mechanisms of receptivity during physiological and pathological situations. However, now it appears that beyond the receptivity state, the endometrial response under the influence of embryonic signals may be the basis of a number of underlying fertility associated disorders. Current literature does indicate that defective embryo-endometrial cross-talk may be the basis of two important fertility related problems *i. e.* 1) miscarriages, and 2) endometriosis.

8.1. Miscarriages

As described above that embryo-endometrial cross-talk enables the biosensing ability to the decidual cells to segregate the normal and the abnormal embryos and allowing only the normal embryos to implant (62). In recurrent miscarriages, it has been reported that the endometrial stromal cells of women with pregnancy losses respond differently as compared to the controls and there is a failure of endometrial stromal cells to express an appropriate decidual phenotype. This deregulated maternal response leads to prolonged endometrial receptivity phase compromising the biosensing ability of the stromal cells and permits the implantation of the defective embryos (62). Such pregnancies at later stages would get rejected and presented clinically as a miscarriage. Such lack of embryo selection would provide the basis to explain the chromosomal and non-chromosomal pregnancy failure and the possible association between miscarriage and late obstetrical complications in subsequent pregnancy.

8.2. Endometriosis

Endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity. This is one of the most common reproductive disorders in women which are associated with infertility. Patients with endometriosis show reduced rate of follicular growth, reduced functional capacity of the preovulatory follicle, reduced fertilization rates, abnormal preimplantation embryonic development and altered early luteal function (120). However, beyond these studies that investigated the outcome of IVF treatment for patients with endometriosis have concluded that the success rates for women with endometriosis were significantly lower compared with women without the disease (121, 122). The poor success rate is attributed to the reduced implantation rates in patients with endometriosis. Indeed, the eutopic endometria of women with endometriosis or in those of baboons with experimentally induced endometriosis, there is aberrant expression of several genes including those required for receptivity (123, 124). While, these studies have contributed immensely to our understanding towards the pathophysiology of endometriosis, it is unclear of how this might cause infertility. From all these studies, it appears that the eutopic endometrium in cases of endometriosis might have a defective embryo-endometrial cross-talk.

HOXA10 is a key molecule that is required for the endometrial receptivity and its expression is induced further by the embryonic stimuli during decidualization (22, 69). We and others have shown that HOXA10 regulates the expression of key decidualization related markers like IGFBP1 and prolactin (69, 125, 126). In women with endometriosis and in baboons with induced endometriosis, the expression of HOXA10 is dramatically curtailed (127-131). Since HOXA10 is one of the important responders of the embryonic signals in the decidual cells, loss in its expression would result in a failure of the receptivity and decidual functioning. Interestingly, the decidual response gets reportedly altered in the eutopic pregnancies associated with the complications of endometriosis (132-134). This is associated with altered signaling events culminating the IGFBP-1 production (127, 129, 134, 135).

Beyond decidualization, during endometriosis, the embryo-endometrial cross-talk is also defective in eutopic endometrium. In baboons, several genes whose expression is modulated by CG in the normal receptive endometrium, is blunted in endometria of animals with experimentally induced endometriosis (136). Some of the genes regulated by CG show attenuated responses while others respond in the opposite manner to that observed in normal animals. These include the immunomoduators (C3, glycodelin, SOD, CXCL14, COX1 and 2, IL1R2), proteases (MMP7, SERPINA3, ADAMTS8, MME), signaling molecules (PKCB1, ARHGAP12 and 20), cytoskeletal and adhesion related molecules (N-cadherin, collagens: COL16A1, COL24A1, COL7A1, ITGA8, TUBG2) and several transport molecules. These altered responses would logically prevent the acquisition of the full endometrial molecular repertoire necessary for implantation. These observations imply that reduced implantation rates in women with endometriosis may not be just due to altered physiology of the receptive eutopic endometria but, is also due to disturbed embryo-endometrial cross-talk. It is tempting to propose that the infertility observed in women with endometriosis is multifaceted that involve deregulation of the several pathways, including decidualization that are required by the blastocyst for implantation. In a long run this information will be of vital importance in devising management strategies for this enigmatic disease.

Beyond infertility and abortions, alteration in feto-maternal cross talk may be also associated with pregnancy associated with complications. From the trophoblast side, defective trophoblast invasion has been associated with pregnancy induced hypertension and intra

uterine growth restriction (137-139). Presently, it is unknown if the flaw in placentation is due to an intrinsic trophoblastic cell defect or a failure of the embryo endometrial-cross talk. There have been evidences regarding defective decidual functions in cases with intrauterine growth restriction (140-142). If true, it would be alluring to believe that a defective endometrial-embryo cross-talk may not only have implications in implantation and early pregnancy pathologies but, may be of significance even in late obstetric complications.

Finally, and potentially far-reaching, the embryo-endometrial cross-talk paradigm surmise that the early pregnancy associated complications can be averted by targeting the endometrial decidual response prior to the pregnancy or immediately after implantation. This hypothesis is clinically supported by the observations that hormones such as progesterone, weak androgens like dehydroepiandrosterone, glucocorticoids, heparin etc that directly modulate the decidual process prevent impending miscarriages (143-145). Future research, therefore, must be directed towards deciphering the functional characteristics of the endometrial receptivity and the embryo-endometrial cross-talk. The knowledge acquired from such scientific endevours, is envisaged not only to assist in the development of specific therapeutics for infertility disorders but may also lead to the development of new and improved methods for the endometrium based contraception.

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Abbreviations: hCG: human chorionic gonadotropin; STAT: signal transducer and activator of transcription; EGF: epidermal growth factor; TGF-beta: transforming growth factor-beta; TNF-alpha: tumor necrosis factor-alpha; ADAMTS: disintegrin and metalloproteinase with thrombospondin motifs**;** CEBPD: CCAAT/enhancer binding protein delta; ANK3: ankyrin-3; FZD2: frizzled-2; IFNGR1: interferon gamma receptor 1; CHODL: Chondrolectin; MPPED2: metallophosphoesterase domain containing 2; DSCR1L1: Down syndrome critical region gene 1-like 1; CCNE2: cyclin E2; IER: immediate early response; S100A3: S100 calcium binding protein A3; ADRA2A: adrenergic receptor Alpha-2A; S100P: S100 calcium binding protein P; DKK1: Dickkopf-related protein 1; GAS: growth arrest-specific; IP15: interferon-inducible-protein-15; CGA: chicken glycoprotein hormones, alpha polypeptide; SNX10: sorting nexin 10; PBEF1: pre-B cell colony-enhancing factor; ADORA2B: adenosine A2b receptor; PDE3B: phosphodiesterase 3B**;** DACH1: Dachshund homolog 1; ETV1: ets variant 1; PTX3: pentraxin 3; TOX: Thymus high mobility group box protein; GATA3: GATA binding protein 3; GCM1: glial cell missing 1; PDGFD: platelet derived growth factor D; CD24: cluster of differentiation 24; IFI: interferon gamma inducible, ITGA6: integrin alpha 6; ITGA8: integrin alpha 8; CXCL: chemokine CXC ligand; CCL: chemokine CC ligand; MCP: monocyte chemotactic protein; CXCR1: CXC chemokine receptor 1; MMP: matrixmetalloproteinase; IGF-1: insulin-like growth factor-1; FGF-1: fibroblast growth factor-1; SERPINA3: serpin peptidase inhibitor, clade A; ADAMTS8: a disintegrin and metalloproteinase with thrombospondin motifs 8; MME: membrane metallo-endopeptidase; PKCB1: protein kinase C beta 1; ARHGAP: Rho GTPase activating protein; COX: cyclooxygenase; IL1R2: lnterleukin 1 receptor, type II

Key Words: Endometrial Receptivity, Endometrium-Embryo Cross-Talk, Trophoblast Invasion, Implantation, Review

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