INTRODUCTION

Human reproduction is an inefficient process. Epidemiological evidences suggest that only 30% of all conceptions get clinically recognized, and a large number of these are lost spontaneously. Furthermore, the success of assisted reproduction is low, as reasonably good quality embryos fail to implant and there is a high frequency of spontaneous abortions. These epidemiologic and clinical evidences indicate that uterine implantation governs reproductive success and maternal incompetence at the endometrial level can be a constraining factor. Thus, understanding the molecular events of embryo-maternal interaction is of interest to reproductive biologists, clinicians and couples affected by infertility. The understanding is also essential for designing rational management strategies.

Key words: Endometrium, Decidua, Implantation, Biosensor, Invasion, Trophoblast, Abortion.

*Corresponding Author: Deepak N. Modi, Molecular and Cellular Biology Laboratory, National Institute for Research in Reproductive Health, Parel, Mumbai, India.
Email: deepaknmodi@yahoo.com, modid@nirrh.res.in

Physiology of Embryo-Endometrial Cross Talk

Deepak N. Modi* and Pradeep Bhartiya

Implantation of the blastocyst stage embryo into the maternal endometrium is a critical determinant and a rate-limiting process for successful pregnancy. Embryo implantation requires synchronized changes in the endometrium before and after arrival of blastocyst into the uterine cavity. Extensive cross talks occur between the fetal and maternal compartments around the time of implantation which are reflected by morphologic, biochemical and molecular changes in the endometrial cells and the differentiating trophoblast cells. The embryo induced morphologic changes include occurrence of epithelial plaque reaction, stromal compaction and decidualization. Embryonic signals also alter the expression of a large number of transcription factors, growth factors and their receptors and integrins. Thus the embryo superimposes a unique signature on the receptive endometrium for successful implantation. Functionally, the embryo-endometrial cross talk is essential for endowing a “selector activity” to the receptive endometrium to ensure implantation of only a developmentally competent embryo. On selection, the decidua creates a conducive microenvironment for trophoblast invasion leading to placentation. Clinical evidences suggest that along with receptivity, a defective “selector” activity of the receptive uterus may be a cause of infertility and recurrent miscarriages. Defects in trophoblast invasion are associated with pregnancy complications like preeclampsia and intra-uterine growth retardation. It is envisaged that understanding of the embryo-endometrial dialogue leading to the “selector” activity, aids in development of appropriate therapeutic modalities for infertility related disorders and miscarriages. Conversely, it might also benefit the development of anti-implantation drugs for contraception.

for implantation failure and treatment of infertility.

Our current understanding of the process of embryo implantation and the determinants of successful pregnancy have mainly stemmed from animal models and in vitro studies using human tissues. Based on the data derived it is clear that endometrial receptivity and embryo implantation are complex processes involving a delicately poised balance of maternal hormones, endometrial factors and embryonic influences. The current review focuses on cellular and molecular events associated with endometrial receptivity and implantation to accomplish successful conception. The embryo-endometrial cross talk at the time of embryo apposition and implantation mainly in the primates will be discussed. The general understanding of the processes of endometrial receptivity and implantation has been a subject of recent reviews (Gellerson and Brosens, 2014; Ozturk and Demir, 2010; Young, 2013; Young and Lessey, 2010).

**Endometrial receptivity**

A mutual communication between the blastocyst and the uterus is indispensable for implantation. Akin to many developmental processes, it involves an elaborate sequence of genetic and cellular interactions, to be executed within an optimal temporal frame for successful pregnancy. In order to receive a developing embryo, the endometrium endures a series of morphological and physiological transformations. At the same time, the fertilized ovum undergoes several rounds of cell division and transforms into blastocyst. The blastocyst has an outermost layer of specialized cells, trophoblast cells, that surround the pluripotent inner cell mass. The trophoblast cells come in direct contact with the receptive endometrium establishing a firm attachment with the endometrial epithelial cells termed as apposition. Subsequently, the trophoblasts invade the endometrium and establish contact with the maternal circulation to form the placenta.

The endometrium is refractory to embryo implantation throughout the menstrual cycle except for a few days after ovulation. Approximately, on days 21–24 of the human menstrual cycle (8–10 days post ovulation), the uterus becomes "receptive", enabling blastocysts to adhere to the luminal epithelium. Termed as “window of receptivity”, the achievement of this stage is highly dependent on the ovarian steroids, estrogen and progesterone. The estrogen in the follicular phase leads to proliferation of endometrial epithelial cells, the progesterone surge that occurs in response to ovulation leads to differentiation of the estrogen primed endometrium to endow receptivity. Any disturbance in the levels of these hormones adversely affects endometrial physiology leading to failure of implantation. The endocrine regulation of the menstrual cycle and role of hormones in endowing receptivity
to the endometrium has been a subject of extensive studies (Jabbour et al., 2006; Young, 2013).

Morphologically, the receptive phase endometrium is characterized by presence of columnar epithelium with microvilli, an increase in stromal cell proliferation and appearance of pinopod-like structures on the luminal epithelium (Tu et al., 2014). The morphological features of the “receptive” endometrium are associated with expression of a range of biochemical and molecular markers, crucial for endowment of this phase of the uterus. Several markers like transcription factors, integrins and their ligands, cytokines and growth factors, have been associated with the receptive phase (Tu et al., 2014; Wang and Dey, 2006; Zhang et al., 2013). A molecular signature of the “receptive window” using global gene profiling technologies have been identified that can phenotype different phases of the menstrual cycle including the receptive stage to objectively classify the implantation window (Garrido-Gómez et al., 2013; Haouzi et al., 2012). These findings open the field for the diagnosis of the endometrial defects in assisted reproductive technology programs (Ruiz-Alonso et al., 2013).

**Embryo induced morphologic changes in the receptive endometrium**

In a conception cycle, the egg that has fertilized in the fallopian tube undergoes a series of cell divisions and reaches uterine lumen at the blastocyst stage. At this time the trophoblast cells are differentiated and the embryo is ready to hatch. From rodents to humans, this embryo induces a second round of differentiation both at the morphologic and biochemical level (Banerjee and Fazleabas, 2009). Distinct from the “receptive” stage endometrium, the embryo induced differentiation of the receptive endometrium is rather limited and largely derived from experimental studies in the non-human primates and endometrial biopsies obtained from conception cycles in humans. Three major non-human primate models to identify and dissect embryo induced morphological and physiological changes in the receptive stage endometrium are: 1) Timed hysterectomies and/or biopsies of the endometrium of baboons or rhesus macaques sequentially mated with males of proven fertility; 2) Endometria obtained from mated bonnet monkeys where the presence of the embryo has been verified using a preimplantation factor assay; and 3) Endometrial tissue obtained from baboons where human chorionic gonadotropin (hCG) has been infused in the uterine lumen in a manner that mimics the transit of blastocyst. The models have inherent advantages and disadvantages but are highly complementary and provide valuable information in terms of identification and deciphering the functional consequence of embryo induced changes in uterine receptivity.
**Epithelial changes:** The earliest endometrial response prior to implantation is characterized by an increased proliferative activity of the luminal and glandular epithelium. Distinct from the epithelial proliferation observed in the follicular phase, this proliferative activity in the pregnant uterus is restricted to focal areas. In the luminal epithelium, there are large clumps of nuclei with distinct entero-reduplication and poorly packed chromatin along with loss of basement membrane. These changes are termed as “epithelial plaque reaction” (Jones and Fazleabas, 2001; Rosario et al., 2005a). The formation of epithelial plaques is restricted to pregnant endometrium and reported in a variety of primate species including humans (Rossman, 1940). While consistently detected in the conception cycle, the epithelial plaque reaction is hormonally regulated and does not require presence of an embryo, as infusion of hCG directly in the uterus leads to formation of epithelial plaques similar to those observed in pregnant monkeys (Fazleabas et al., 1999; Jones and Fazleabas, 2001). The functional significance of epithelial plaques is not clear. It is speculated that the plaque may provide nutrition by means of intracellular glycogen (Enders et al., 1985; Rossman, 1940). The plaque response may stimulate precocious development of the maternal vasculature below the epithelium (Enders et al., 1985).

Beyond the plaque reaction, thinning of the basal lamina and thickening and diffusion of the apical and lateral gap junctions in luminal epithelial cells has been reported in pregnant human, bonnet and baboon endometrium (Demir et al., 2002; Rosario et al., 2008). Along with these changes, a few granulocytic stromal cells are also observed in the luminal epithelium of pregnant bonnet and rhesus monkeys prior to implantation (Ghosh et al., 1993; Rosario et al., 2008). It is conceivable that these changes occur to promote adhesiveness to the trophoblast cells at the time of apposition and invasion.

**Stromal changes:** An almost universal reaction of the endometrial stromal cells in response to an embryo is decidualization. In its broadest sense, decidualization is defined as the postovulatory endometrial remodeling which includes secretory transformation of uterine stroma, influx of specialized uterine natural killer cells, and vascular remodeling. A more restricted definition of decidualization is an epithelioid transformation of the endometrial stromal cell with highly specialized and distinctive functions. Decidualization only occurs in species in which placentation involves breaching of the luminal epithelium by the trophoblasts. The extent of this differentiation process often correlates with the degree of trophoblast invasion (Dunn et al., 2003; Gellersen et al., 2007).

Morphologically, the elongated spindle like stromal cells of the secretory phase...
endometrium transform into cobblestone like enlarged decidual cells with multiple club shaped projections arising from the cell surface and contain abundant glycogen stores and lipid droplets (Welsh et al., 1985; Wynn et al., 1974). In humans, this transformation occurs even in absence of an embryo and is referred to as the pre-decidual response. In a conception cycle, under the continuous support of steroid hormones and blastocyst derived signals, decidualization of the entire endometrium is observed (Brosens et al., 2002; Gellersen and Brosens, 2014). The decidua forms a dense cellular matrix that allows coordinated trophoblast invasion while simultaneously protecting the conceptus from maternal and environmental insults (Kliman, 2000; Redhorse et al., 2004). In the non-human primates, decidualization is observed in conception cycle or on treatment with hCG (Jones and Fazleabas, 2001; Rosario et al., 2005a). These observations suggest that unlike in humans, embryo/embryonic factors are required for the endometrial stromal cells to undergo decidualization in monkeys.

Vascularization: A characteristic feature of the endometrium in a conception cycle is the enhanced microvasculature. In the pregnant bonnet monkeys, a large number of small blood vessels are detected in the stroma underlying luminal epithelium and the functionalis region of the endometrial bed (Rosario et al., 2005a). Increased vascularity and angiogenesis at the implantation site of rhesus monkeys has been reported (Sengupta and Ghosh, 2002). A similar increase in the number of small blood capillaries in the stroma of the endometrial functionalis have been demonstrated in baboons infused with physiological doses of hCG in the uterine lumen (Banerjee and Fazleabas, 2009; Jones and Fazleabas, 2001). These observations suggest that maternal tissues initiate neo-vascularization which may be required for immune cell differentiation and infiltration (See below).

Immune cell infiltration: The leukocyte population in the endometrium consists of T cells, macrophages and large granular lymphocytes. T cells and macrophages account for a substantial proportion of the leukocyte population in the human endometrium throughout the menstrual cycle (Jones et al., 1998; King, 2000). The largest leukocyte population in the human endometrium are the large granulated lymphocytes which express natural killer (NK) cell antigen CD56. The uterine NK (uNK) cell population is distinct from peripheral blood NK cells in phenotypic and molecular characteristics (Cooper et al., 2001; Fukui et al., 2011; King et al., 1991; Lysakova-Devine and O'Farrelly, 2014). Around the time of implantation, uNK cells comprise 70–80% of the leukocyte population in the endometrium and numbers increase if conception occurs (King, 2000; Kodama et al.,

It remains to be identified whether the increase in cell number is solely the result of in situ proliferation or homing from the peripheral circulation. In a conception cycle, the uNK cells differentiate into decidual NK (dNK) cells, functionally distinct from non-pregnant uterine counterparts (Kodama et al., 1998). The functional significance of uNK and dNK cells in the primate endometrium is largely speculative. Based on mouse studies and clinical observations, it appears that NK cells are crucial for pregnancy and failure of uNK transformation to dNK cells leads to pregnancy loss (Fukui et al., 2011; Gong et al., 2014; Quenby and Farquharson, 2006). Whether this transformation occurs exclusively in response to embryo derived signals or due to decidualization of the stromal cells, needs to be investigated.

Embryo induced molecular transformations in the receptive endometrium

The molecular dialogue between the embryo and endometrium involves a complex network of signaling molecules that mediate cell–cell or cell–extracellular matrix (ECM) interactions, and include factors such as cytokines, growth factors, cell-adhesion molecules and matrix metalloproteinases. There is some evidence indicating that the levels of steroid receptors, growth factors and cytokines are modulated in the endometrium during early pregnancy. The following section reviews the in situ molecular changes occurring in the primate endometrium in response to embryonic signals.

**Estrogen receptors (ER) and Progesterone receptors (PR)**

Sex steroids exert their effects through their receptors, estrogen receptor (ER) and progesterone receptor (PR). As compared to non-conception cycle, both ER and PR expression is higher in the conception cycle around implantation in bonnets, baboons and rhesus (Ghosh and Sengupta, 1988, Rosario et al., 2008). Post apposition ER expression is lost in the epithelium and stroma but retained in the wall of spiral arteries, blood vessels, and myometrial smooth muscle cells (Hild-Petito et al., 1992; Perrot-Applanat et al., 1994).

While PR is most abundantly expressed in the uterine glands and stroma in the receptive phase, expression of PR is down-regulated in the glands but present in the stroma surrounding the glands and spiral arteries, wall of spiral arteries, blood vessels, and smooth muscle cells of the myometrium (Ghosh and Sengupta, 1988; Hild-Petito et al., 1992; Perrot-Applanat et al., 1994).

**Homeobox genes HOXA10 and HOXA11**

HOX genes are essential for endometrial growth, differentiation and receptivity by mediating some functions of progesterone. Both HOXA10 and HOXA11 are expressed in human endometrial epithelial and stromal cells, and their expression is significantly
higher in mid- and late-secretory phases, coinciding with time of embryo implantation and high levels of estrogen and progesterone (Daftary and Taylor, 2006; Godbole et al., 2007; Modi and Taylor, 1998; Xu et al., 2014).

Unlike steroid receptors, the expression of HOXA10 is induced in the endometria of bonnet monkeys in the conception cycle. Abundant expression of HOXA10 protein is detectable in stromal and glandular cells of the pregnant bonnet monkeys (Godbole et al., 2007). Interestingly, treatment of endometrial cells with spent blastocyst culture medium and/or hCG resulted in increased transcription of HOXA10 (Blitek et al., 2011; Fogle et al., 2010; Sakkas et al., 2003). However, unlike the glands and the stroma, in luminal epithelium of the conception cycle, HOXA10 expression is reduced and expression is virtually absent in the pre-epithelial plaques (Godbole et al., 2007; Modi and Godbole, 2009). These observations are surprising, as in the mouse, suppression of HOXA10 in epithelial cells leads to inhibition of embryo implantation; overexpression leads to increase in litter size in mouse (Bagot et al., 2000). While this might reflect the fundamental differences in the mechanisms associated with implantation in rodents and primates, observations in the monkey indicates that products of HOXA10-modulated transcriptome in luminal epithelium may be inhibitory for implantation, and hence may be down regulated by embryonic stimuli. As transcription factors, HOX genes regulate other downstream target genes leading to proper development of endometrium and receptivity to implantation. A number of molecular and morphological markers specific to the implantation window are regulated by HOX genes, including pinopodes, β3 integrin and insulin-like growth factor-binding protein-1 (Daftary and Taylor, 2006; Modi and Godbole, 2009). All the HOX targets are also modulated in the endometria in response to the embryo (Nimbkar-Joshi et al., 2009).

**Cytokines and growth factors**

Leukemia inhibitory factor (LIF), Interleukin-6 and -11 (IL-6 and IL-11) are members of a single family of cytokines that share the signal transducer receptor unit gp130 in target cells to elicit biologic effects. All these three cytokines play key roles in implantation. First identified in the mouse where targeted disruption of the LIF gene showed implantation failure (Stewart et al., 1992), reduced LIF expression/secretion has been reported in infertile women with defects in implantation (Mikolajczyk et al., 2006; Tawfeek et al., 2012). While LIF seems to be a critical requirement for implantation, the expression is not modulated by embryonic signals as the levels do not alter in the implantation phase endometria of bonnet monkeys in conception as compared to non-conception cycle (Rosario et al., 2005b). However, in rhesus the expression of LIF and
its receptors are increased in the endometria of pregnant monkeys as compared to non-pregnant controls (Sengupta et al., 2003). LIF is crucial for implantation in primates as administration of an antagonist for LIF receptor or antibody against LIF directly in to the uterine cavity of monkeys and mice, results in failure of pregnancy (Sengupta et al., 2006; Terakawa et al., 2011; White et al., 2007). The results indicate that LIF is essential in the process of blastocyst implantation. LIF is also a promotor of trophoblast invasion (Suman et al., 2013a; 2013b).

IL-6 and IL-11 are pleiotropic cytokines required for implantation. IL-11 expression is increased during decidualization (Godbole and Modi, 2010), recombinant IL-6 and IL-11 promote decidualization of human endometrial cells in vitro (Dimitriadis et al., 2005; Menkhorst et al., 2010). IL-6 and IL-11 are also promoters of trophoblast invasion (Champion et al., 2012; Modi et al., 2011; Suman et al., 2009; 2013). IL-11 and the receptor IL-11Rα are detected in the decidua mainly at implantation sites in cynomolgus and rhesus monkeys (Champion et al., 2012; Dimitriadis et al., 2005). It is also detected in the vascular endothelial cells and epithelial plaques. Likewise, the expression of IL-6 is significantly higher in endometria of animals in the conception cycles as compared to non-conception of rhesus and bonnet monkeys (Rosario et al., 2005b; Sengupta et al., 2003).

Several growth factors like Tumor Growth Factor (TGF) beta, Epidermal Growth Factor (EGF) and Tumor Necrosis Factor (TNF) alpha are pro-inflammatory cytokines that have emerged to be critical mediators for implantation owing to their direct effects on immune cells (Dimitriadis et al., 2005; Omwandho et al., 2010). In the window of implantation, a significant increase in endometrial TGF beta and its receptor occurs in the glandular epithelium of animals in the conception cycles as compared to non-conception cycles (Rosario et al., 2005b; Sachdeva et al., 2001). TNF alpha and its receptor population increases in the endometria of animals in the conception cycles as compared to non-conception cycles (Nimbkar-Joshi et al., 2009; Rosario et al., 2005c). EGF and its receptors are detected in both the glands and stromal compartments of the receptive phase endometrium; expression is increased mainly in the stromal cells of the pregnant animals (Slowey et al., 1994). An increase in endometrial LIF, EGF, TGF and TNF by endometrial cells in presence of embryonic stimuli prior to apposition suggests induction of an inflammatory like condition in the implantation phase endometrium, which may be a requirement for initiation of pregnancy; the increase in expression in stromal compartment implies involvement with decidualization.
**Integrin and their ligands**

Integrins are heterodimeric glycoproteins which undergo dynamic temporal and spatial changes in their distribution in the endometrium during the menstrual cycle in women (Lessey and Arnold, 1998; Reddy and Mangale, 2003). Likewise the ECM ligands for these receptors are likely to play a role in the establishment of a receptive endometrium. The integrins and their cognate ligands show dynamic changes in levels of expression and polarization during early pregnancy. In the baboon, the collagen receptor alpha1beta1 and fibronectin receptor alpha4beta1 expressed in glandular epithelium during window of receptivity are lost with the establishment of pregnancy. The vitronectin receptor, alpha4beta3 is expressed in the glandular epithelium in pregnant animals. The osteopontin receptor, alphavbeta3, is expressed in both glandular epithelium, and decidualizing stromal cells of pregnant animals (Fazleabas et al., 1997; Mangale and Reddy, 2007). In the mouse decidua, interactions between integrin alphav beta3 and vitronectin is required to maintain a balance between cell proliferation and apoptosis, along with modulation of inflammatory responses (Mangale et al., 2008).

Recently the dynamics of integrin expression mainly alphavbeta3 in the uterine epithelium has been detailed in early pregnancy using the bonnet model. The results revealed that expression of alpha v increases in luminal epithelial cells of pregnant animals, show a shift in localization at the site of attachment (Nimbkar-Joshi et al., 2012). At the non-attachment pole, the alpha(v) integrin is mainly in the basal zone of the luminal epithelial cells. However, at the attachment pole, alphav is redistributed and also detected in the apical pole. The differential subcellular distribution of integrin is directed by embryonic stimuli as treatment of epithelial cultures with conditioned medium of human embryos obtained at IVF leads to increased distribution of alphav on the apical membrane (Nimbkar-Joshi et al., 2012). These observations imply that embryonic stimuli not only directs cellular reprogramming by changing gene expression, but also controls intracellular protein trafficking leading to preferential sorting of proteins.

From the above studies it is clear that embryo induces distinct changes in the receptive stage endometrium and affects almost all the compartments in preparation of pregnancy. These changes seem to be induced in response to secretions by the embryonic cells and are highly localized in nature. A summary of the morphological and molecular changes that occur in the receptive endometrium in presence of an embryo are shown in Fig 1.
From the discussion above it is clear that remarkable changes occur in the molecular profile of endometrium at the time of apposition and implantation, distinct from those during the window of receptivity. The changes seem to be induced in response to secretions by the embryonic cells and are localized in nature. However, the functional connotations of such observations remain far from clear. This is mainly due to our inability to perform genetic manipulations in the endometria of primates. Nevertheless, in recent years, elegant in vitro models have been designed to decipher functional consequences of embryo induced changes in endometrial cells (Weimar et al., 2012). While it would be beyond the scope of this article to review these studies, the data derived from these studies, combined with changes seen in vivo, it appears that the embryo signals the endometrial bed prior to implantation making it competent for embryo quality control and trophoblast invasion.

Decidua as a “selector” for embryo quality control
The exceptional rate of early pregnancy loss may be due to the high prevalence of
chromosomal abnormalities in the embryo. Genetic analysis of blastomeres taken from good quality embryos obtained at in vitro fertilization (IVF) showed that around 70% harbor complex chromosomal abnormalities (Chow et al., 2014; Mertzanidou et al., 2013). Such observations raise the question of how to safeguard the mother against prolonged investment in potentially developmentally abnormal embryos. One school of thought believes that abnormal embryos by themselves are incompetent towards implantation resulting in pregnancy failure. In recent years however, experimental evidence indicate that spontaneous decidualization of endometrium coupled to menstruation is a judicious strategy to meet the challenge. The decidua may play a key role in discriminating normal and abnormal blastocysts to allow pregnancy.

Evidences to support this hypothesis were obtained from co-culture of decidual cells with morphologically normal and abnormal human embryos obtained at IVF. While morphologically normal embryos had no major effects on production of a selected set of cytokines; media derived from decidual cells co-cultured with morphologically arrested or abnormal blastocysts led to down-regulation of IL-1b, -6, -10, -17, -18, eotaxin and heparin-binding EGF-like growth factor (Teklenburg et al., 2010a). Such down-regulation is associated with closure of endometrial competence for implantation and menstruation (Evans and Salamonsen, 2014). Microarray analysis of decidual cells challenged with conditioned medium from good and poor quality embryos, identified 449 decidual genes deregulated in response to medium conditioned by poor-quality embryos (Brosens et al., 2014). One of the down regulated genes in response to signals in conditioned media derived from poor quality embryos was HSPA8. The protein functions in protein assembly and folding, clatherin-mediated endocytosis, assembly of multiprotein complexes, transport of nascent polypeptides, and regulation of protein folding (Stritcher et al., 2013). The observation suggests that soluble signal from developmentally impaired human embryos induce endoplasmic reticulum (ER) stress response in decidualizing cells. An in vivo proof for the in vitro observations came from studies in uteri of mice flushed with conditioned culture medium of developmentally competent and incompetent embryos. Analysis of the uterine transcriptome revealed that medium derived from competent embryos evoked a supportive intrauterine environment, whereas medium derived from poor quality embryos led to ER stress (Brosens et al., 2014). Thus, it implies that the endometrium not only senses signals derived from the embryo and responds to create a pro-implantation condition, but is also capable of terminating the window of endometrial receptivity to enable the mother to dispose of compromised embryos. The observation adds another dimension to the potential of
decidualizing endometrial stromal cells as sensors of embryo quality during implantation.

Thus, we propose a dual-phase response of the endometrium. The steroid primed receptive phase endometrium responds to the incoming embryo creating an obligatory environment for implantation. At the same time the decidua gains a 'selector' activity to recognize developmental competence of the implanting embryo. Based on the blastocyst competence as judged by the decidua, either pregnancy is continued or the maternal response is aborted and culminates in menstruation.

**Regulation of trophoblast invasion**

Once the endometrium encounters a developmentally competent blastocyst and decides to continue with pregnancy, the embryo apposes and trophoblast cells begin to breach the luminal epithelium and invade into the maternal decidua to establish placentation. Trophoblast cells are inherently invasive and can invade any tissue. However, in the pregnant endometrium the invasion is highly controlled. It is believed that the decidualized stromal cells secrete a complex array of molecules that permit the controlled migration/invasion. While several of the molecules are already expressed by the receptive endometrium, others are induced post decidualization and receiving of the embryonic signals. Co-culture of trophoblast and decidual cells or spent medium increases trophoblast invasion (Godbole *et al*., 2011; Menkhorst *et al*., 2012). We have demonstrated that decidual cell secretome enhances invasion of trophoblast cells through altered expression of matrix metalloproteases (MMPs) and tissue inhibitors of matrix metalloproteases (TIMPs) (Godbole *et al*., 2011). Conversely, in response to trophoblasts, the decidual cells also gain a migratory and invasive phenotype (Gellersen *et al*., 2010; Weimar *et al*., 2013). Thus, decidualization and embryo driven changes in the uterine cells creates a microenvironment favorable for implantation and placentation.

Numerous growth factors that regulate the proliferation and invasion of trophoblast cells have been identified at the fetal-maternal interface. The various factors secreted by the decidual cells and/or the associated cell types and their influence on trophoblast invasion has been recently reviewed (Knöfler, 2010; Modi *et al*., 2012). Amongst the various factors, IL-6, LIF and IL-11 are abundantly produced by the endometrial stromal and decidual cells, and play a key role in trophoblast invasion (Fitzgerald *et al*., 2008; Modi *et al*., 2012; Suman *et al*., 2013a; Suman and Gupta, 2014). IL-6 and LIF stimulates invasion of primary trophoblast and JEG-3 choriocarcinoma cells via the STAT3 signaling pathway (Jovanović and Vićovac, 2009; Suman and Gupta, 2014). The role of IL-11 in trophoblast invasion is less clear as it inhibits the invasion of primary
trophoblast and HTR-8/SVneo cells, but increases invasion of the choriocarcinoma JEG-3 cells (Suman et al., 2009; 2012; 2013b). The discrepancy may originate from differences in the transcription factor content of the two cell lines. However, the data suggests that locally produced IL-6, LIF and IL-11 act to finely tune invasion. While the cumulative effects of various factors and their roles under in vivo conditions need investigations, the observations together suggest that decidualization driven transformation of endometrial stromal cells creates a uterine microenvironment that controls trophoblast invasion.

Clinical Repercussions of the Embryo-Endometrial Cross-Talk
Endometrial receptivity is a major rate limiting step and bottleneck for the success of assisted reproductive technologies. The discovery that embryonic signals potentiate the already primed uterus has opened several avenues for understanding of the process of implantation and initiation of pregnancy. Given the experimental evidence demonstrating the embryo-endometrial cross talk plays a key role in endowing receptivity as well as selectivity to the endometrium, a logical consequence of a reduced ability to recognize embryonic signals is implantation failure and/or miscarriage. Suboptimal response to signals of high quality embryos will result in a suboptimal environment for subsequent development and placenta formation, a major cause of pregnancy related complications like fetal growth restriction and gestational hypertension leading to preeclampsia.

In converse, impaired endometrial selectivity can result in superfertility. The hypothesis stems from the observations that women with recurrent miscarriages are highly fecund and time to pregnancy is reduced in those women with a history of five or more miscarriages (Teklenburg et al., 2010b). Since developmentally incompetent blastocyst implant (due to failure of selectivity), these would lead to late first trimester abortions. Thus lack the “selector” activity in the endometrium may be a causative factor towards compromised pregnancy. Indeed, a loss of selection sensing has been observed in endometrial stromal cells derived from women experiencing recurrent miscarriages (Salker et al., 2010). Furthermore, when flushed through the mouse uterus, secreted factors from decidualizing cultures of stromal cells derived from patients with recurrent miscarriages prolonged the window of receptivity and also increased the incidence of pathological implantation sites, immune defects and fetal demise (Brosens et al., 2014). Additionally, endometrial stromal cells from patients with recurrent miscarriages show altered responses to hCG, and failure to discriminate between high and low quality human embryos (Salker et al., 2010; Weimar et al., 2012). Thus, the “selector” activity of
the decidua may be a key to successful pregnancy and defects in the process may cause recurrent miscarriages.

Once the embryo has implanted, the trophoblast cells invade to establish placentation. Multiple stages of placentation could be compromised that can lead to diseases. Pre-eclampsia affecting 3–5% of pregnancies, which is characterized by gestational hypertension and severe proteinuria, and is a major cause of fetal and maternal deaths. While pre-eclampsia is detected later in gestation (20 weeks onwards), its pathogenesis is established early in gestation where trophoblast invasion is defective. It has been shown that in women with preeclampsia shallow placental invasion and inadequate plugging of the spiral artery affects blood supply into the intervillous space and alters the consistency of the blood flow. This can lead to fluctuations in the supply of oxygen to the placenta (Ji et al., 2013; Saito and Nakashima, 2014), triggering a maternal response by increasing blood pressure and compromising fetal development (Furuya et al., 2008). Another disorder caused by defects in trophoblast invasion is intrauterine growth restriction (IUGR). IUGR arises as a result of inadequate blood supply and/or inadequate transport of nutrients across the placenta to the fetus, resulting in a range of mechanisms including reduced uteroplacental blood flow, compromised feto-placental angiogenesis and subsequent villous development (Gourvas et al., 2012). It is believed that 'poor placentation' along with hypoxic micromilieu of fetoplacental site, shear stress of uteroplacental blood flow, and aberrantly secreted proinflammatory substances into maternal circulation, synergistically contribute to progression of preeclampsia and IUGR (Furuya et al., 2008; Gourvas et al., 2012; Ji et al., 2013; Saito and Nakashima, 2014). Since trophoblast invasion and placentation are dependent on proper decidualization, defective embryo-endometrial cross talk can lead to improper decidual response thereby causing poor trophoblast invasion, shallow placentation and hence preeclampsia. Preliminary evidence suggests that decidualization defects might exist in decidua of women with pregnancies complicated with preeclampsia (Saito and Nakashima, 2014).

Thus, there is a need for better understanding of the basic processes of placentation and mechanisms that go awry in women with preeclampsia and IUGR for effective therapeutic approaches to these common disorders.

**CONCLUSIONS**

The embryo-endometrial cross talk has evolved as a delicately poised mechanism to respond initially to the hormonal trigger to achieve receptivity and then to amplify the decision under embryonic signals to permit pregnancy. During this critical period, the
receptive endometrium gains the ability for selectivity, where response of the luminal epithelium serves to transduce and amplify signals coming from competent embryos renders the underlying decidual layer more receptive to invasion (Fig. 1). This permits embryo apposition followed by trophoblast invasion to establish placentation (Fig. 2). In the event the endometrium experiences presence of a poor-quality embryo, the supportive network is not activated, but the decidua mount a stress response, leading to withdrawal of receptivity resulting in menstruation and failure of pregnancy (Fig. 2). Such a selector mechanism of the decidua is highly desirous to avoid investing energy in pregnancy with abnormal fetuses which may not survive till term or have compromised survival ability. Once the receptive and the selective competence of the endometrium are ensured and the right blastocyst implants, the decidua creates a local microenvironment that is conducive for trophoblast invasion and placentation (Fig. 2).

The significance of such bimodal and biphasic endometrial response to the implanting embryo is potentially far reaching. To date, treatment of recurrent miscarriages and implantation failure are inefficacious and highly empirical. The recent understanding of the dual processes has revealed that recurrent implantation failure may be caused by defects in endometrial embryo cross talk. It will be necessary to unravel the molecular processes that control the timely transition of the receptive uterus to a selective decidua which

Figure 2: Biosensor activity of the implantation stage endometrium. A) In presence of a normal good quality embryo the endometrium undergoes extensive biochemical and molecular transformation allowing the apposition of the embryo to the luminal epithelium. Consequently, the secretory factors from the decidua promote invasion of trophoblast cells allowing placentation. B) In presence of a poor quality embryo the endometrium responds by activating a strong inflammatory cascade thereby triggering closure of receptivity leading to menstruation.

subsequently permits trophoblast invasion to establish placentation. Once elucidated, effective approaches to modulate implantation and treat pregnancy complications will be feasible proposition. The knowledge acquired from such studies, is envisaged to assist in the development of specific therapeutics for infertility disorders, and may also lead to the development of new and improved methods for endometrium based contraception.

ACKNOWLEDGEMENTS

The authors acknowledge Indian Council of Medical Research (ICMR), New Delhi, for financial assistance.

CONFLICT OF INTEREST

The authors claim no conflict of interest.

REFERENCES


Kodama T, Hara T, Okamoto E, Kusunoki Y, Ohama K. Characteristic changes of large


Sengupta J, Dhawan L, Ghosh D. Immunohistochemical localization of leukemia inhibitory factor, interleukins 1 and 6 at the primary implantation site in the rhesus...


Suman P, Shembekar N, Gupta SK. Leukemia inhibitory factor increases the invasiveness of trophoblastic cells through integrated increase in the expression of adhesion molecules and pappalysin 1 with a concomitant decrease in the expression of tissue inhibitor of matrix metalloproteinases. *Fertil Steril* 2013b;99: 533–542.


Terakawa J, Wakitani S, Sugiyama M, Inoue N, Ohmori Y, Kiso Y. Embryo implantation is blocked by intraperitoneal injection with anti-LIF antibody in mice. *J Reprod Dev* 2011;57:


