

REVIEW

Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors

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Abstract

The prerequisite of successful implantation depends on achieving the appropriate embryo development to the blastocyst stage and at the same time the development of an endometrium that is receptive to the embryo. Implantation is a very intricate process, which is controlled by a number of complex molecules like hormones, cytokines, and growth factors and their cross talk. A network of these molecules plays a crucial role in preparing receptive endometrium and blastocyst. Furthermore, timely regulation of the expression of embryonic and maternal endometrial growth factors and cytokines plays a major role in determining the fate of embryo. Most of the existing data comes from animal studies

due to ethical issues. In this study, we comprehend the data from both animal models and humans for better understanding of implantation and positive outcomes of pregnancy. The purpose of this review is to describe the potential roles of embryonic and uterine factors in implantation process such as prostaglandins, cyclooxygenases, leukemia inhibitory factor, interleukin (IL) 6, IL11, transforming growth factor- β , IGF, activins, NODAL, epidermal growth factor (EGF), and heparin binding-EGF. Understanding the function of these players will help us to address the reasons of implantation failure and infertility.

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Introduction

It is generally accepted that successful implantation depends on the quality of blastocyst, a receptive endometrium and the synchronization between the developmental stages of the embryo itself. The key to this process is the dynamic and precisely controlled molecular and cellular events that drive implantation and establishment of pregnancy. This dynamic process involves coordinated effects of autocrine, paracrine, and endocrine factors. The cross talk between trophoblastic cells and receptive endometrium during the time of blastocyst adhesion and invasion is poorly understood in humans. Because it is not possible to study the implantation process in women *in vivo* due to ethical and technical issues, most of the existing data has been derived from animal studies. Animal models have provided valuable insights into the molecular mechanisms that occur during embryo implantation (Wang *et al.* 1998). Despite extensive research in this field, the majority of pregnancy losses occur before or during implantation. Every ninth couple in Europe and the USA is affected by implantation disorders and pregnancy wastage (Krusell *et al.* 2003). The complex process of embryo

implantation requires a plethora of locally acting molecules that are involved in this early embryo–uterine interaction.

In this study, we review the data on the functional role of major hormones, cytokines, and growth factors during implantation process. Due to space limitation and previously published reviews, we have discussed research carried out over the last two decades. Given the broad array of these molecules, special attention is given here to factors that are released at the implantation site, and particularly on hormones, growth factors, and cytokines, which are likely to play an important role in regulating trophoblast differentiation and invasion.

Process of implantation

The first step in implantation is the initiation of dialogue between the free-floating blastocyst and the receptive endometrium, which is mediated by locally acting hormones and growth factors (Tabibzadeh & Babaknia 1995). The next step is apposition, where the trophoblast cells adhere to the receptive luminal epithelium of the endometrium by

establishing contact with the micro protrusions present on the surface of uterine epithelium known as pinopodes (Lopata *et al.* 2002). Consequently, a stable adhesion of blastocyst to the endometrial basal lamina and stromal extracellular matrix (ECM) occurs. A stronger attachment is achieved through local paracrine signaling between the embryo and the endometrium. The first sign of the attachment reaction occurs on the evening of day 4 in mice/rats, or days 20–21 in humans, and it coincides with a localized increase in the stromal vascular permeability at the site of blastocyst attachment (Sharkey & Smith 2003). The last step of implantation is the invasion process, which involves penetration of the embryo through the luminal epithelium into the stroma, thereby establishing a vascular relationship with the mother. This activity is mainly controlled by trophoblast cells. However, the decidua also limits the extent of invasion (Norwitz *et al.* 2001). In response to invasion and the presence of constant progesterone stimulation, the endometrial stromal cells and endometrial ECM undergo decidualization. During decidualization, endometrial tissue remodels, which includes secretory transformation of the uterine glands, influx of specialized uterine natural killer (NK) cells, and vascular remodeling (Gellersen *et al.* 2007). The decidua impedes the movement of invasive trophoblasts both by forming a physical barrier to cell penetration and by generating a local cytokine milieu that promotes trophoblast attachment rather than invasion (Graham & Lala 1992, Clark 1993). The timely completion of attachment and decidualization is essential for the viability of the pregnancy.

The success of implantation depends on achieving the appropriate embryo development to the blastocyst stage and at the same time the development of an endometrium that is receptive to the embryo. The phenomenon of endometrial receptivity was first established in the rat and later validated in other species (Psychoyos 1986). Endometrium is known to become receptive only for short periods in rodents and humans as well. Beyond this period of receptivity, the embryo is unable to successfully establish contact with refractive endometrium. Therefore, timely arrival of embryo in a receptive endometrium is very much crucial for successful implantation (Ma *et al.* 2003). This time period is called the 'window of implantation', during which the uterine environment is conducive to blastocyst implantation. In addition to the physical interaction of the embryonic tissue with the uterine cells, this process is undoubtedly influenced by maternal steroid hormones, growth factors, and cytokines in a paracrine manner, thereby playing a crucial role in embryonic signaling (Simon & Valbuena 1999, Simon *et al.* 2000).

Role of hormones

The ovarian steroids, progesterone and estrogen, have a major regulatory role by mobilizing several molecular modulators in a spatiotemporal manner, which supports embryo

implantation (Carson *et al.* 2000, Lim *et al.* 2002). During the implantation period, the endometrium undergoes a transition and acquires an appropriate morphological and functional state under the influence of ovarian steroids, which facilitates the attachment of blastocyst. In addition, progesterone and estrogen are the dominant hormonal modulators of endometrial development. Progesterone is essential for implantation and pregnancy maintenance in all mammals, whereas the requirement for estrogen is species specific (Dey *et al.* 2004). The preimplantation estrogen surge is essential in mice, whereas ovarian estrogen does not play an obligatory role in the implantation of primates (Ghosh & Sengupta 1995). Various evidences suggest the presence of estrogen and progesterone receptors in stromal and epithelial regions. Thus, the levels of these receptors and concentrations of hormones are equally important for successful implantation (Lessey 2003, Ma *et al.* 2003). Integrin molecules play a pivotal role in the attachment of blastocyst to the uterine epithelium and their expression is shown to be regulated by an increased ratio of estrogen over progesterone (Basak *et al.* 2002). Therefore, further understanding of the molecules involved in the steroid hormone signaling pathways might be useful for improving the endometrial receptivity or embryo quality to increase implantation rates.

The intricate process of implantation also requires other key molecules in addition to hormones like progesterone and estrogen (Kodaman & Taylor 2004). It is well documented that prostaglandins (PGs) play an important role in various reproductive processes, including ovulation, implantation, and menstruation (Jabbour & Sales 2004, Kang *et al.* 2005). Cyclooxygenases (COX-1 and COX-2) are the crucial enzymes responsible for the synthesis of various PGs. The unique expression pattern of *Cox-1* and *Cox-2* genes in preimplantation mouse uterus suggests an important role for PGs in embryo implantation. This expression pattern suggests that COX-2 expression during the adhesion phase is critical for implantation (Chakraborty *et al.* 1996). Recently, we have reported that COX-2 expression is regulated by steroid hormones that play a crucial role in embryo implantation and decidualization through PG synthesis (St-Louis *et al.* 2010). Extensive research in past years provides crucial evidence confirming the role of PGs in implantation process. Achache *et al.* (2010) have reported that patients with recurrent pregnancy failure were shown to have very low levels of cPLA_{2α} and COX-2, possibly reducing further PG synthesis, which could be responsible for implantation failure. In rodents, PGs are known to facilitate increased vascular permeability during implantation (Kennedy 1979). Previously, we have shown that prostaglandin-D synthase and prostacyclin synthase are present in the rat uterus during pregnancy and high levels could be seen at the early stage of pregnancy. The steroids controlled the expression of various PG synthases, which further produce PGD₂ and PGI₂ at specific times during pregnancy in endometrium (Kengni *et al.* 2007). Blockade of PG synthesis before or during the time of implantation causes complete inhibition, a delay in

implantation or a reduction in the number of implantation sites with diminished decidual tissue. However, PG supplementation can partially restore a normal phenotype. Use of nonsteroidal anti-inflammatory drugs, also known as the inhibitor of COX-2 and PG biosynthesis, could lead to abnormal implantation that predisposes an embryo to peri-implantation loss due to reduced uterine decidualization (Li *et al.* 2003). Gene knockout studies suggest that COX-1-deficient female mice are fertile with specific parturition defects, whereas COX-2-deficient females are infertile with abnormalities in ovulation, fertilization, implantation, and decidualization (Dinchuk *et al.* 1995, Lim *et al.* 1997). Thus, targeted gene therapy with COX-2 may be necessary for better outcome of pregnancy.

Cytokines

Cytokines are small multifunctional glycoproteins, whose biological actions are mediated by specific cell surface receptors and act as potent intercellular signals regulating functions of endometrial cells and embryo–maternal interactions. Entry of blastocyst into the receptive uterine is very important for the production of cytokines by trophoblastic cells and uterine epithelium that can modulate the endometrial receptivity by regulating the expression of various adhesion molecules (Simon *et al.* 2000). In mammals, deregulated expression of cytokines and their signaling leads to an absolute or partial failure of implantation and abnormal placental formation (Guzeloglu-Kayisli *et al.* 2009). Redundancy exists within the cytokine families, and several different cytokines often exert similar and overlapping functions on certain cells. In this review, we focus on cytokines known to be crucial and indispensable for the embryo implantation.

Leukemia inhibitory factor

Leukemia inhibitory factor (LIF), a polyfunctional glycoprotein, is a member of interleukin 6 (IL6)-type cytokine family (Hilton 1992). It is secreted from the uterus and is regarded as an important factor in embryo implantation. The pleiotropic effects of LIF are accomplished by binding to heterodimeric LIF receptor (LIFR), which consists of two transmembrane proteins, LIFR and gp130. The LIFR activates several signaling pathways in diverse cells types, including the Jak/STAT, MAP kinase (MAPK), and PI3-kinase (PIPK) pathways (Duval *et al.* 2000). The first evidence of the role of LIF in implantation came from the report that embryos failed to implant in LIF-deficient female mice. However, after LIF supplementation in the same mouse model, normal implantation was restored (Stewart 1994). Further studies have shown that in the LIF- and/or mutant gp130-deficient mice, the endometrial epithelial cells failed to respond to the embryo due to deregulation in STAT3 signaling even though these embryos are viable and can implant when transferred to wild-type recipients (Stewart

et al. 1992, Wang *et al.* 1998, Ernst *et al.* 2001). However, embryos lacking LIFR and/or gp130 show normal implantation but die during the perinatal period (Ware *et al.* 1995). Thus, LIF may signal to both embryonic and uterine cells during implantation (Laird *et al.* 1997). The potential role of LIF in decidualization was studied in mice, where authors have found that LIF receptors were expressed in decidualized stromal cells adjacent to the implanting blastocyst (Ni *et al.* 2002). LIF is the best example of how hormones act through cytokines in a manner that high levels of estradiol are coincident with upregulation of the uterine LIF during peri-implantation period. Most recently, it has been shown that LIF plays a role in both adhesive and invasive phases of implantation due to its anchoring effect on the trophoblast (Dimitriadis *et al.* 2010). These findings suggest that LIF plays a major role in both rodents and primate implantation. In the case of humans, endometrial biopsies obtained from women of proven fertility have shown LIF mRNA and protein expression throughout the menstrual cycle with pronounced levels in the middle- and late-secretory phase, where implantation occurs (Charnock-Jones *et al.* 1994, Arici *et al.* 1995). The role of LIF in human implantation is further proven by the fact that mutations in the *LIF* gene occur in women with unexplained infertility and repeated implantation failures (RIFs; Steck *et al.* 2004). It has been demonstrated that women with stronger LIF immunoreactivity during the window of implantation have greater probability of getting pregnant than those with weaker expression, thereby suggesting importance of LIF in IVF (Serafini *et al.* 2009). Treatment with a recombinant human LIF (r-hLIF) has been investigated in preclinical and clinical trials to improve endometrial receptivity in RIF patients. However, based on the above-discussed study, the authors suggested that compensating low levels of endometrial LIF expression may positively affect IVF outcome in women with recurrent implantation failure. Unfortunately, r-hLIF administration during luteal phase after embryo transfer failed to improve implantation rates in women with recurrent implantation failure (Brinsden *et al.* 2009). In view of the important role of LIF in implantation, administration of such r-hLIF could be valuable in future studies.

Interleukin 6

Fewer reports are available regarding the role of IL6 in pregnancy. IL6 can exert both pro-inflammatory and anti-inflammatory response through gp130. Expression of IL6 was found to be present during mid-secretory phase and is mostly localized in the epithelial glandular cells (Tabibzadeh & Babaknia 1995). The crucial role of IL6 during implantation was defined using IL6-deficient mice, which showed reduced implantation sites and reduced fertility. Furthermore, the presence of receptors both on endometrium and on blastocyst is suggestive of paracrine/autocrine role for IL6 during implantation in murine species (Robertson *et al.* 2000). In humans, IL6 receptor (IL6-R) is found to be expressed

during the menstrual cycle, thus providing evidence that the role of IL6 in controlling endometrial receptivity and implantation is not species specific (Tabibzadeh *et al.* 1995, Vandermolen & Gu 1996). In spite of that, there are some exceptions, with studies using targeted disruption of the IL6 gene in mice showing normal blastocyst implantation; however, the blastocyst was found to be underdeveloped (Kopf *et al.* 1994, Salamonsen *et al.* 2000). Thus, this suggests that IL6 might be useful as a predictor of blastocyst quality. However, abnormal expression of IL6 was reported during mid-secretory phase in patients with recurrent abortions (Lim *et al.* 2000, von Wolff *et al.* 2000). IL6 is predominantly expressed in the endometrium in mid- to late-secretory phase, the time when the endometrium is exposed to the highest progesterone and estradiol concentrations suggesting that steroid hormones might regulate IL6 expression.

Interleukin 11

Together with LIF and IL6, IL11 belongs to the gp130 cytokines (i.e. cytokines that share the gp130 accessory signal-transducing subunit). IL11 and its receptor (IL11R α) have recently been observed in the human endometrium. All the major cell types in endometrium express IL11 with cyclical variation. The most prominent immunoreactivity and mRNA expression is in the decidualized stromal cells late in the menstrual cycle (Dimitriadis *et al.* 2000, Cork *et al.* 2001, von Rango *et al.* 2004). Despite this, there is still uncertainty regarding the time of maximal production of IL11 in the epithelial cells, possibly because of differing protocols for immunohistochemistry in these studies. The presence of mRNA *IL11* and *gp130* during decidualization in stromal cells and glandular epithelial cells suggests the importance of IL11 in decidualization of stromal cells (Dimitriadis *et al.* 2000). Mice lacking the IL11R α have a fertility defect because of defective decidualization, and in normal uterus, maximal level of IL11 was observed at the time of decidualization (Robb *et al.* 1998). Interestingly, recent evidence in mice shows that IL11 signaling is required for decidual-specific maturation of NK cells. It has also been suggested that defective decidual vasculature is present in the IL11R α mutant uterus compared with wild-type mice (Ain *et al.* 2004). As for human, increasing evidence indicates that IL11 has an important function in implantation. Linjawi *et al.* (2004) studied the expression of IL11 and IL11R α in biopsies from normal fertile women and recurrent miscarriage women. In the latter study, the authors have shown that the IL11 expression was low in epithelial cells in endometrium from recurrent miscarriage women compared with normal fertile women. This suggests that administration of IL11 can possibly reduce miscarriage rates. Taken together, all these evidences indicate that IL11 may be important in the establishment of viable pregnancies. As regards for the regulation of IL11 expression by steroid hormones in primary cultures of human endometrial and decidual epithelial cells, estrogen induced, whereas progesterone reduced, IL11

secretion, and IL11 signaling was found to participate in the regulation of trophoblast invasion (von Rango *et al.* 2004). However, more comprehensive studies are needed to broaden our knowledge about the role of IL11 in human implantation.

Growth factors

The term growth factor stands for a family of secreted signaling molecules capable of inducing proliferation and differentiation in mammalian cells. These factors bind to specific cell surface receptors, which contain a tyrosine kinase domain in their C-terminus. Upon ligand binding, the receptors further initiate signaling cascade by phosphorylation of various MAPK and Smad proteins, as examples.

Transforming growth factor- β

Transforming growth factor- β (TGF- β) exists in three different isoforms (TGF- β 1, TGF- β 2, and TGF- β 3), which have profound effects on ECM production and degradative enzymes. In addition, TGF- β isoforms are found to be present at the maternal fetal interface, thus implying their critical role in the implantation process. The members of TGF superfamily are importantly expressed in the endometrium and have active roles in modulating cellular events involved in the regulation of proliferation, decidualization, and implantation process (Jones *et al.* 2006b). We have previously shown that individual TGF- β isoforms are differently expressed in the uterus during rat pregnancy (Shooner *et al.* 2005); higher expression of TGF- β 1 and - β 2 are present during implantation and decidua basalis regression, whereas TGF- β 3 isoform is only present during DB regression and parturition. Recently, it has been demonstrated that TGF- β signaling pathway plays an important role in remodeling rat endometrium by modulating the activity of PI3-K/AKT survival pathways, along with the inhibition of anti-apoptotic protein XIAP levels, thus inducing apoptosis in decidual cells (Caron *et al.* 2009). In addition, TGF- β isoforms inhibit trophoblast proliferation and increase invasive property of HRP-1 and RCHO-1 rat trophoblast cells by activating Smad and p38 MAPK pathways (Lafontaine *et al.* 2011).

In human endometrium, TGF- β protein and mRNA are localized in endometrial stromal, epithelial, and decidual cells (Bischof & Campana 2000). Previously, it has been demonstrated that TGF- β 2 expression is more intense in stroma cells, whereas TGF- β 1 and TGF- β 3 are equal in intensity in stromal and epithelial cells (Godkin & Dore 1998). During the menstrual cycle, only TGF- β 3 expression varies, being more intense in glandular epithelium during the late-secretory phase. Thus, endometrium is prepared for implantation in the secretory phase through production and secretion of TGF- β isoforms by epithelial cells. Moreover, TGF- β s may play a role in human implantation via their stimulation of fibronectin or vascular endothelial growth

factor production (Feinberg *et al.* 1994, Chung *et al.* 2000) and by promotion of adhesion of trophoblast cells to the ECM (Irving & Lala 1995). Specifically, TGF- β 1 increases ECM oncofetal fibronectin and stimulates trophoblast adhesion to the ECM; therefore, TGF- β 1 is implicated in trophoblast attachment to the endometrium during implantation (Tamada *et al.* 1990, Feinberg *et al.* 1994). Other TGF- β superfamily member activins are expressed by decidualized stromal cells and are involved in decidualization (Jones *et al.* 2000, 2002). The known action of maternally derived activin is tissue remodeling by upregulating the expression of matrix metalloproteinases that promotes trophoblast invasion (Jones *et al.* 2006a). Dysregulation of activin A affects the adhesive property of trophoblast leading to implantation failure (Stoikos *et al.* 2006). Although NODAL protein was shown to be localized throughout the endometrium during the peri-implantation period, the precise role of NODAL in implantation is yet to be established (Park & Dufort 2011). The above reviewed literature indicates that the members of TGF- β superfamily play a major role in implantation in mice and humans. Its deregulated expression and action could lead to absolute or partial failure of embryo implantation.

Epidermal growth factors

The members of epidermal growth factor (EGF) family interact with an *ErbB* gene family of tyrosine kinase receptors: ErbB1 (EGF-R), ErbB2, ErbB3, and ErbB4. These receptors share common structural features but differ in their ligand specificity and kinase activity (Dey *et al.* 2004). The presence of EGF in proliferative phase of human endometrium and its localization to stromal cells in secretory endometrium and trophoblastic cells is suggestive of its involvement in embryo implantation (Hofmann *et al.* 1991). The effect of exogenous EGF on implantation rate of *in vitro* developing blastocysts in rats was studied previously (Aflalo *et al.* 2007). It was found that the implantation rate of developing blastocysts was significantly higher in the presence of EGF compared with the control blastocysts *in vitro* (Aflalo *et al.* 2007). The tyrosine receptors for EGF are found to be present at the early stages in the developing embryo and during peri-implantation period in the uterus (Wiley *et al.* 1992). Interestingly, knockout of the EGF receptor (*ErbB1*) gene in mice leads to preimplantation death of the embryos (Threadgill *et al.* 1995). In the case of rats, higher transcript levels of the EGF ligand and its receptor were present at the time of implantation after which their expression decreases gradually (Byun *et al.* 2008). The presence of EGF in human preimplantation embryo was suggested as unique to mammals and may be important for human preimplantation embryo development (Chia *et al.* 1995). The presence of EGF and EGF-R in human endometrium was also revealed by Haining *et al.* (1991). Using a different approach, Paria & Dey (1990) demonstrate that blastocyst development from eight-cell embryo cultured in the presence of EGF showed higher chances of zona hatching than when cultured in the absence of EGF. They

further confirmed the presence of EGF receptors on morula and blastocyst stage and showed a beneficial effect of EGF on preimplantation embryo development. In a recent study, RNA interference approach was used to confirm that down-regulation of embryonically expressed EGF and EGF-R impaired survival and delayed preimplantation of embryo development (Dadi *et al.* 2009). This study confirmed authors previous work (Dadi *et al.* 2007), in which *in vitro* supplementation of growth factors improved the maturation and survival of cloned embryos. This cumulative evidence, therefore, suggests that the expression of EGF and EGF receptor is important for implantation and embryo development.

Heparin binding-epidermal growth factor

Heparin binding-epidermal growth factor (HB-EGF) is a transmembrane protein that binds to its receptors in a juxtacrine manner and activates the signaling cascade (Riese & Stern 1998). HB-EGF shares a common receptor with EGF and TGF- α . HB-EGF requires heparin sulfate proteoglycan as a cofactor for binding to its receptors. The first evidence that HB-EGF plays a crucial role in implantation was given from the studies conducted in mice and rats (Das *et al.* 1994, Birdsall *et al.* 1996). Similar to other factors involved in the implantation process, HB-EGF is also regulated by steroid hormones estrogen and progesterone (Wang *et al.* 1994). HB-EGF is expressed in endometrial stromal and epithelial cells and has been demonstrated to regulate endometrial cell proliferation, glandular epithelial secretion, and decidual transformation (Simon *et al.* 2000). HB-EGF was found to be expressed in human endometrium at the time of implantation (Birdsall *et al.* 1996, Lessey *et al.* 2002). Growth factor-soaked beads of about the size of blastocysts were transferred to the uteri of pseudopregnant mice. Different factors including HB-EGF and insulin-like growth factor 1 (IGF1) efficiently elicited discrete local implantation-like response, such as increased vascular permeability, decidualization, and expression of implantation markers (Paria *et al.* 2001). In a separate study, authors showed that HB-EGF-soaked beads induced the expression of HB-EGF itself in the luminal epithelium, suggesting an auto-induction loop in regulation of HB-EGF during implantation (Hamatani *et al.* 2004). The presence of high-expression *HB-EGF* mRNA prior to the implantation window indicates that this growth factor could directly control blastocyst implantation (Yoo *et al.* 1997). Gene knockout studies revealed that *Hbegf*^{-/-} female mice were found to be sub-fertile with reduced litter size. In the same study, the authors have shown that HB-EGF could regulate ovarian and uterine functions (Xie *et al.* 2007).

Hb-egf gene was found to be expressed at the site of attachment of blastocyst prior to its attachment in mouse uterine luminal epithelium, strongly indicating its involvement in embryo implantation (Das *et al.* 1994). The ability to inhibit apoptosis and induce invasiveness in human

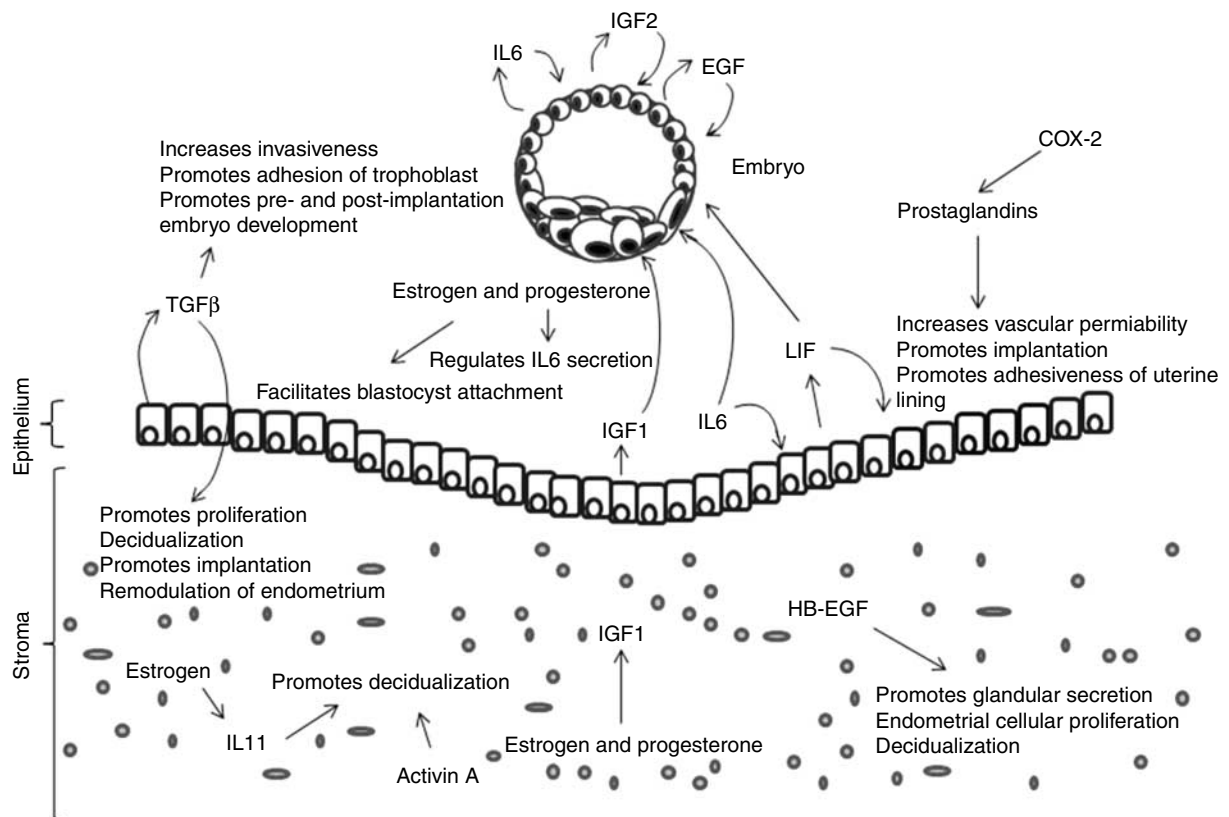


Figure 1 Summary of the various growth factors, cytokines, and hormones involved in implantation process.

endometrium qualifies HB-EGF as a vital candidate during embryonic implantation (Martin *et al.* 1998). Electron microscopy together with immunohistochemistry demonstrated that the expression of HB-EGF in luminal and glandular epithelium is highest when fully developed pinopodes are present, emphasizing the role of HB-EGF in the attachment and invasion processes of human implantation (Stavreus-Evers *et al.* 2002). Higher levels of HB-EGF are present in the apical surface of the luminal epithelium prior to implantation in humans (Yoo *et al.* 1997). Taken together, HB-EGF is a critical signaling molecule for successful pregnancy by regulating the endometrial receptivity and implantation.

Insulin-like growth factors

IGFs have high homology with insulin. They exert their biological effects by binding to distinct transmembrane receptors (IGF-R) on the surface of target cells (Rechler & Nissley 1985). IGF1 and IGF2 are known mitogenic and differentiation-promoting growth factors. However, IGF1 is known to be even more potent than insulin to induce mitogenic effect and cellular proliferation (Lammers *et al.* 1989). During the peri-implantation period in mouse uterus, higher expression of *Igf1* mRNA was observed at days 4–6

(Kapur *et al.* 1992). The basal levels of IGF1 are low in ovariectomized rats and treatment with sex hormones estrogen or progesterone showed a sudden increase in the transcript level of IGF1, suggesting regulation of IGF1 by steroid hormones in the peri-implantation uterus (Kapur *et al.* 1992). IGF1 was found to be immunolocalized in stromal cells at day 5 of pregnancy in rat uterus and was suggested to be involved in decidualization of stromal cells (Oner & Oner 2007). In another study, when trophoblast from female gerbils were cultured *in vitro* in the presence of IGF1, it enhanced embryo development and improved embryo quality by increasing the cell numbers of trophectoderm and inner cellular mass (Yoshida *et al.* 2009). Accordingly, when extravillous trophoblast cells (EVT) were cultured in the presence of IGF1, it induced migration of EVT cells during the implantation process (Kabir-Salmani *et al.* 2004). Supplementation of the culture media with physiological concentration of IGF1 during IVF significantly increased blastocyst formation (Lighten *et al.* 1998). The presence of IGF1 in human reproductive tract fluid and maternally produced IGF1 has been shown to play a role in human preimplantation development (Lighten *et al.* 1998). IGF2 is also an important member of this family, and it has been demonstrated that addition of IGF2 to culture medium stimulated the formation of blastocyst from two-cell embryo

(Harvey & Kaye 1992). Preimplantation mouse embryos express IGF2, and production of IGF2 by embryos has been shown to enhance the formation of blastocyst in an autocrine manner. However, when expression of IGF2 was down-regulated using antisense IGF2 oligonucleotides, the rate of progression to the blastocyst stage and cell numbers in blastocysts were found to be decreased (Rappolee *et al.* 1992). In order to establish the *in vivo* role of both the IGF1 and the IGF2 in humans, more detailed studies are required.

Future directions

The current knowledge of preimplantation and implantation physiology is the result of observations gathered by many researchers and physicians through these years. Successful implantation is a result of complex autocrine, paracrine, and/or juxtacrine interactions of factors and their receptors at the site of implantation. Collectively, present literature suggests the role of a variety of molecules as potential mediators of embryo–uterine interactions during implantation (Fig. 1). It is impossible to locate the actual site of implantation in humans due to ethical and technical issues. Moreover, studies using human samples are generally confined to tissue biopsies and limited number of embryos that are discarded with the consent of patients who undergo IVF. Therefore, studies on embryo–uterine interactions usually start in mouse models and other species. The prerequisite in this direction is to describe an expression pattern of a molecule followed by more mechanistic approaches such as gene targeting to correct the abnormality. Previous studies have established the role of various molecules as implantation regulators but the list is still expanding each year. To fully understand this intricate process of implantation, investigations are required to decipher the signaling pathways and mechanism of action of these newly identified regulators. It is of paramount importance to define the precise hierarchical arrangements of the genes involved in implantation through gene deletion and DNA microarray approaches. Use of high-end tissue profiling technologies at genomic, transcriptomic, and proteomic levels will shed more light on the implantation process, which will bring new strategies in treating implantation failure and will usher a new era of successful pregnancies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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