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The Role of Growth Factors and Cytokines during Implantation: Endocrine and Paracrine Interactions

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Abstract

Implantation, a critical step for establishing pregnancy, requires molecular and cellular events resulting in uterine growth and differentiation, blastocyst adhesion, invasion, and placental formation. Successful implantation requires a receptive endometrium, a normal and functional embryo at the blastocyst stage, and a synchronized dialogue between maternal and embryonic tissues. In addition to the well-characterized role of sex steroids, the complexity of embryo implantation and placentation is exemplified by the number of cytokines and growth factors with demonstrated roles in these processes. Disturbances in the normal expression and action of these cytokines result in an absolute or partial failure of implantation and abnormal placental formation in mice and human. Members of the gp130 cytokine family, interleukin-11 (IL-11) and leukemia inhibitory factor, the transforming growth factor beta superfamily, the colony-stimulating factors, and the IL-1 and IL-15 systems are crucial molecules for a successful implantation. Chemokines are also important, both in recruiting specific cohorts of leukocytes to the implantation site and in trophoblast trafficking and differentiation. This review provides discussion of the embryonic and uterine factors that are involved in the process of implantation in autocrine, paracrine, and/or juxtacrine manners at the hormonal, cellular, and molecular levels.

Keywords

Blastocyst; endometrium; implantation; pregnancy; uterine biology

The term *growth factor* corresponds to a family of secreted signaling proteins capable of inducing proliferation and differentiation in cells. Growth factors typically act as initiators of signaling cascades in cells by paracrine and endocrine interactions.¹ Examples are cytokines and hormones that bind to specific receptors on the surface of their target cells, typically initiating a variety of cellular processes.² They often promote cell differentiation and maturation, whose specificity varies among growth factors. For example, bone morphogenic proteins stimulate bone cell differentiation, and fibroblast growth factors and vascular endothelial growth factors stimulate blood vessel differentiation (vasculogenesis and angiogenesis).³

Cytokines, a family of soluble proteins, were thought to be produced only in the bone marrow, immune system, and circulating blood cells when first identified.^{3,4} Later, it was shown that these proteins are produced in many tissues including reproductive and embryonic tissues.^{5,6} The term *growth factor* is sometimes used interchangeably with the

term *cytokine*. Whereas growth factor implies a positive effect on cell division, cytokine is a neutral term with respect to whether a molecule affects proliferation. In this sense, some cytokines can be growth factors, such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor.⁷ However, some cytokines have an inhibitory effect on cell growth or proliferation. Yet others, such as the Fas ligand, are used as “death” signals; they cause target cells to undergo programmed cell death, or apoptosis.

Growth factors can be classified according to both structural and evolutionary associations, ordering them into larger families of proteins. Recent years have seen a great increase in the number of growth factors identified and growth factor families such as bone morphogenic protein (BMP), fibroblast growth factor (FGF), transforming growth factor beta (TGF- β), neurotrophins (nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], and neurotrophin [NT3]), colony stimulating factors (CSF), platelet-derived growth factor (PDGF), erythropoietin (EPO), thrombopoietin (TPO), myostatin (GDF-8), growth differentiation factor 9 (GDF-9), epidermal growth factors (EGFs), hepatocyte growth factor (HGF), and more.⁸ Their role in implantation is becoming increasingly well understood.

Implantation necessitates complex interactions among the developing embryo, decidualizing endometrium, and developing maternal immune tolerance and/ or alterations in cellular and humoral immune responses. All of these cellular and molecular events are controlled by endocrine hormones, including sex steroids and human chorionic gonadotropin (hCG), and by local paracrine factors. Such factors are produced by the endometrial epithelium, decidualized stromal cells, and immune cells; they are secreted locally into the uterine lumen and the area of the decidual reaction, where they affect not only blastocyst development, attachment, and invasion, but also each others’ cellular functions and the transformation of the decidualizing extracellular matrix. These locally produced signaling molecules consist of growth factors, cytokines, and chemokines.^{5,9}

Development of the embryo to the blastocyst stage, its implantation into the endometrium, and the formation of a functional placenta are essential steps in the establishment of pregnancy. Like many developmental processes, pregnancy involves a complicated series of genetic, molecular, and cellular interactions, all of which must be executed within an optimal time frame. In mammals, the fertilized egg undergoes many cell divisions to form a blastocyst, which is able to attach to the uterine epithelium. The implantation process starts when a free-floating blastocyst communicates with the endometrium. The adhesive interactions between the trophoblasts and the endometrial surface epithelium are followed by local invasion.^{5,10,11} Therefore, implantation requires the synchronous development of a blastocyst competent to implant and an endometrium able to respond to signals from the blastocyst. Countless endocrine, paracrine, and autocrine interactions during implantation occur among maternal-maternal, embryo-embryonic, and maternal-embryonic cells that mediate a complex dialogue between endometrium and the conceptus. These developmental events are orchestrated by sex steroids, hCG, growth factors, cytokines, adhesion molecules, the extracellular matrix (ECM) proteins, and prostaglandins.⁵

Many crucial growth factors and cytokines affect blastocyst implantation and/or endometrial receptivity; however, the molecular mechanisms regulating this process in human is still poorly understood and needs further study to have clinical treatment options for infertility patients related to implantation failure or embryo rejection. The incidence of early pregnancy loss during or immediately after implantation is high, estimated at 25 to 40%.¹² Of the pregnancies that are lost, most are lost very early and represent a failure of implantation. Failed implantation is also a major limiting factor in assisted reproduction.¹³ Implantation failure has been estimated to be related to inadequate endometrial receptivity in up to two thirds of cases.¹⁴ Endometriosis, endocrine abnormalities, thrombophilias,

immunological factors, and congenital and acquired anatomical factors may contribute to implantation failure.¹⁵ In this review, we focus on recent developments in implantation biology and discuss the significance of growth factors and cytokines involved in the implantation processes.

EBRYONIC DEVELOPMENT IN PREIMPLANTATION PERIOD

Successful embryonic development in the preimplantation period in many species depends on timely activations and spatial control of maternal and embryonic genes. Maternally derived mRNAs in the oocyte are translationally activated while transcription is silent during oocyte maturation. Zygotic gene activation (ZGA) refers to the first activation of gene expression after fertilization. Following fertilization, ZGA occurs within a short time interval depending on the species; for example, it occurs at the two-cell or four- to eight-cell stage in mouse and human, respectively.^{16,17} Until ZGA, maternal proteins and transcripts stored in oocytes control embryonic development, indicating the importance of maternal factors in early reproductive events.¹⁸ Transcription from the newly formed zygotic genome establishes the gene expression patterns that are required for continued development. At the morula stage the embryo undergoes a reorganization processes at the cellular level, called compaction. This is the first stage during which obvious morphological differentiation can be observed. The appearance of an inner cavity within the mass of cells corresponds to the initiation of the blastocyst formation;¹⁹ this results in the appearance of two distinct types of cells: the outer cell mass, also called trophectoderm (TE), and the inner cell mass (ICM). TE gives rise to the trophoblasts and extraembryonic structures such as placenta; the ICM gives rise to the embryo.^{20,21} Several genes have been identified in the mouse that are crucial for this cell lineage segregation, including *Oct4*, *Sox2*, *Nanog*, *Cdx2*, and *Eomesodermin* (*Eomes*).²² Fig. 1 and Table 1 summarize their cell-specific expression and functions.

During the early stage of blastocyst formation, an ICM-specific gene, named *Lefty-1*, was described in mouse embryos. Surprisingly, *Lefty* expression is restricted to a small subset of cells in the ICM of the early blastocyst, and the expression is localized to a region of the primitive endoderm (PE) in late-stage blastocyst.²³ Although the ICM is initially a mosaic of epiblast (EPI) and PE cells, these observations support the notion that patterning of the embryonic axis may exist as early as the blastocyst stage.²⁴ The asymmetries of the blastocyst and the later axes of the embryo suggest that early asymmetries in the relationships between the different lineages of the blastocyst will play roles in establishing later embryonic patterning. During the blastocyst stage, the TE starts to hatch from its glycoprotein coat, the zona pellucida, and acquires the ability to attach to the endometrium. At the same time, the uterine environment is able to support blastocyst growth, attachment, and the subsequent events of implantation.²⁵ Therefore, a successful implantation depends on two important factors: blastocyst competency and endometrial receptivity. Together these are responsible for the embryo–maternal interaction necessary for the attachment and invasion of the blastocyst into the endometrium.

Recent studies on mice have identified several genes involved in the differentiation of trophoblast cell subtypes. *Hand1*, *Stra13*, and *Imfa* are transacting factors that are expressed specifically in trophoblastic giant cells, the main invasive cell type mediating implantation in the mouse, as well as the main endocrine cells of the placenta. These cells produce several hormones that regulate the maternal endocrine and immune systems and promote maternal blood flow to the implantation site. Decidual prolactin like A (*Prlpa*) gene expression decreases in the uterus adjacent to *Hand1*- and *Ets2*-deficient embryos, suggesting that normal trophoblast giant cells in the placenta are required for the conceptus-dependent effects on *Prlpa* expression in the mesometrial decida. Moreover, these cells also express placental lactogen 1 and 2, a type 1 interferon-like molecule that has the ability to induce

Glip2 expression in endometrial stromal cells.²⁶ These results suggest that molecular signals from the mouse embryo have local effects on uterine gene expression during decidualization. However, these genes remain to be investigated in human embryos and decidual tissues to determine if their human homologs are expressed and function as in mouse embryos and decidua.

IMPLANTATION PROCESS

The human endometrium undergoes a complex series of organized proliferative and secretory changes in each menstrual cycle.²⁷ When implantation does not occur, there is a deliberate and timely destruction of the fully developed endometrium, leading to menstruation, only for the same cycle of events to be repeated once again to prepare for the next blastocyst. However, if implantation does occur, the endometrium continues to grow and undergo further morphological and molecular changes to provide sufficient support for the growing embryo.^{27,28}

Blastocyst implantation in the uterine endometrium is a highly coordinated event that depends on an intrinsic embryonic program operating in conjunction with extrinsic signals from the receptive uterus. In the past decade, many factors influencing the implantation process have been identified, and many researchers have focused on embryonic and maternal factors such as calcitonin, growth factors, integrins, cytokines, and ovarian hormones. For mammalian development to proceed beyond the first week, the embryo must establish intimate contact with uterine tissues and form a placenta that will provide an interface between the growing fetus and the maternal circulation.²⁹ Uterine-derived signals, for example calcitonin, heparin-binding epidermal growth factor-like growth factor (HB-EGF), insulin-like growth factor binding protein-1 (IGFBP-1), and lysophosphatidic acid (LPA) maintain the pace of blastocyst development and mobilize embryonic receptors used for subsequent signaling. The blastocyst is primed to advance in development only after it receives the necessary external signals from the decidua.³⁰

The process of implantation is classified into three phases: apposition, attachment (adhesion), and penetration (invasion). Apposition is identified as an unstable adhesion of the blastocyst to the endometrial surface. During this stage, the trophoblast becomes closely apposed to the luminal epithelium.³¹ This is followed by a stable adhesion or attachment phase. Local paracrine signaling between the embryo and endometrium is believed to occur to trigger a stronger attachment. The first sign of the attachment reaction occurs on the evening of day 4 in mice, or day 20 to 21 in humans, and it coincides with a localized increase in the stromal vascular permeability at the site of blastocyst attachment.³² Penetration involves invasion of the embryo through the luminal epithelium and its basal lamina into the stroma, to establish a vascular relationship with the mother, and this activity is mainly controlled by trophoblasts; however the decidua also limits the extent of invasion.³³ In response to this invasion and the presence of constant progesterone stimulation, the endometrial stromal cells and endometrial extracellular matrix undergo decidualization. The timely completion of attachment and decidualization are essential for the viability of the pregnancy. Human chorionic gonadotropin is one of the molecules that stimulate decidualization.^{34,35} In most species, hCG secreted by the embryo is detectable in maternal serum within 8 to 10 days of ovulation.^{32,36}

HORMONAL AND MOLECULAR DETERMINANTS OF IMPLANTATION

Endometrial receptivity can be defined as the capacity of the uterine mucosa to facilitate successful embryonic implantation. The timing of implantation has now been firmly established, occurring ~7 to 10 days after ovulation, corresponding to days 21 to 24 of an idealized 28-day menstrual cycle.³⁷ Endometrial receptivity is manifested by molecular and

genetic markers including cytokines (interleukin-6 [IL-6], IL-11), growth factors (EGF, TGF- β , FGF), transcription factors (*Hox*), and ovarian hormones (Table 2).³⁸

Hormones

SEX STEROIDS—Progesterone and estrogen have important interrelated roles in many systems and processes required for mammalian reproduction. The female reproductive tract, including the uterus, ovaries, as well as mammary glands, are all targets of both estrogen and progesterone action. Progesterone is essential for implantation and pregnancy maintenance in all mammals, whereas the requirement for estrogen is species specific.⁵ Estrogen is important for the proliferation of the uterine epithelium and enhances progesterone action via induction of the progesterone receptor (PR) gene. Progesterone plays a role in proliferation, differentiation, and maintenance of endometrial stromal and glandular cells and myometrial cells.³⁹

The uterine effects of estrogen and progesterone are primarily executed by nuclear estrogen and progesterone (ER and PR) receptors.⁴⁰ The discovery of ER (ER α and ER β) and PR (PR-A and PR-B) isoforms and studies of the effects of their selective deletion provide evidence for their isoforms-specific functions in uterine biology and implantation. The uterus of ER α knockout (α ERKO) mice develops normally but remains immature, remaining hypoplastic, and is unable to support implantation (Table 3).⁴¹ The phenotype of the α ERKO uterus indicates that ER α does have an essential role in uterine physiology. Studies using tissue recombination have indicated that ER α need only be present in the stromal compartment for epithelial mitogenesis to occur because stromal cells from wild-type mice cultured together with epithelial cells from α ERKO uterus are capable of estrogen-dependent induction of DNA synthesis in the epithelial cells.⁴² In contrast, the ER β ^{-/-} mouse uterus retains biological functions that allow normal implantation.⁵

Interestingly, progesterone is sufficient for decidualization in ER α ^{-/-} mice in response to artificial stimuli, which indicates that ER α might be essential for blastocyst attachment but is dispensable for subsequent decidualization.^{43,44} Analysis of the reproductive phenotypes of PR knockout mice (PRAKO or PRBKO) has indicated that PR-A and PR-B mediate mostly distinct but partially overlapping reproductive responses. Selective ablation of the PR-A protein (PRAKO) shows a normal mammary gland response to progesterone but severe uterine hyperplasia and ovarian abnormalities leading to female infertility (Table 2). In contrast, ablation of PR-B protein (PRBKO mice) does not affect biological responses of the ovary or uterus to progesterone but results in reduced pregnancy-associated mammary gland morphogenesis. These tissue-selective activities of PR-B are due to this isoform's ability to regulate a subset of progesterone-responsive target genes in reproductive tissues rather than to differences in its spatiotemporal expression relative to the PR-A isoform.⁴⁵

Growth factors, particularly EGF and transforming growth factor alpha (TGF α), have gained attention as mediators of estrogen mitogenic action. In vivo experiments showed a mitogenic action of EGF on the reproductive tract of estrogen-deprived mice.⁴⁶ Subsequent studies found that an antiestrogen compound blocked the mitogenic effect of EGF on the mouse reproductive tract, suggesting that EGF was acting via the estrogen receptor.⁴⁷ Johnson and Chatterjee showed that EGF initiates implantation under some conditions. Although ineffective in delayed implanting ovariectomized or hypophysectomized rats treated with progesterone, a single intravenous dose of EGF initiated implantation of blastocysts that were transferred to progesterone-primed hypophysectomized rats.⁴⁸ Uterine EGF and its receptor are important factors in initiating implantation and increase in response to estrogen. Even though many of the physiological roles of estrogen and progesterone have been outlined, the molecular networks that mediate their actions other than their receptor-mediated genomic activation are largely unknown. Further studies are required to determine

the possible interaction among the growth factors and cytokines-mediated intracellular signaling cascades and estrogen and progesterone pathways leading to successful implantation.

HUMAN CHORIONIC GONADOTROPIN—Human chorionic gonadotropin belongs to the family of glycoprotein hormones that include follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH). It plays an important role in the implantation process and is principally produced by syncytiotrophoblasts and some tumors.⁴⁹ It affects several processes during pregnancy, besides the well-known maintenance of the corpus luteum; these include cell growth, differentiation, and immunosuppression. The trophoblasts themselves express a truncated and inactive hCG receptor until the 9th week of gestation, and then switch to expression of the full-length receptor, allowing hCG autocrine regulation of various functions including cell differentiation in the trophoblasts.⁵⁰ Functional hCG/LH receptors are also expressed on both cytotrophoblasts and syncytiotrophoblasts and stimulate the differentiation of cytotrophoblasts into syncytiotrophoblasts in response to hCG. In vitro hCG increases invasiveness of trophoblastic choriocarcinoma cell lines;⁵¹ however, in vitro studies of primary trophoblast showed the opposite effect.^{52,53} Therefore, the influence of hCG on trophoblast in vivo invasion remains unclear. In addition to its effect on decidualization,⁴⁹ hCG administration modulates the secretion of endometrial paracrine factors, leukemia inhibitory factor (LIF), macrophage colony stimulating factor (M-CSF), and IGFBP-1.⁵⁰ Expression of vascular endothelial growth factor (VEGF) was significantly stimulated by hCG, suggesting a role for hCG in the control of endometrial vascularization and placentation.⁵⁴ In summary, hCG seems to have a variety of local and systemic functions both in and outside the embryo-endometrial microenvironment.

Cytokines

LEUKEMIA INHIBITORY FACTOR—LIF is a secreted glycoprotein first described as a factor that induces macrophage differentiation of the myeloid leukemia cell line M1. LIF, a member of the IL-6 type cytokine family, has a variety of functions, including stimulation of cell proliferation, differentiation, and survival—all functions that are essential for blastocyst development and implantation.⁵⁵ The pleiotropic effects of LIF are accomplished by binding to heterodimeric LIF receptor (LIFR), which consists of two transmembrane proteins, LIFR and gp130. The LIF receptor activates several signaling pathways in diverse cells types, including the Jak/STAT, MAPK, and PI3-kinase (PIPK) pathways.⁵⁶ In the endometrium of a fertile woman, LIF protein and mRNA are expressed throughout the menstrual cycle with a striking increase in the mid- and late-secretory phase and in early pregnancy.^{57,58} LIF mRNA and protein are localized mostly in the glandular and luminal epithelium as well as in stroma.^{59–61} Strong expression of LIF mRNA has also been detected in human decidual leukocytes, which are abundant at the implantation site, suggesting that LIF may mediate interactions between maternal decidual leukocytes and invading cytotrophoblasts.⁶² In humans, progesterone is likely one of the regulators of LIF expression, although locally produced factors, including HB-EGF and TGF- β 1, regulate LIF secretion in cultured endometrial stromal cells in vitro in a concentration- and time-dependent manner^{57,63} and may also be relevant in vivo. Song et al showed that the expression of several members of the EGF family are downregulated in LIF-deficient uteri during implantation, although their receptors (erbBs) are sufficiently expressed.⁶⁴

LIF protein has been detected in human uterine flushings during the time of expected implantation in fertile women, with gradually increasing concentrations from days LH + 7 to LH + 12. Interestingly, the concentration of LIF in flushings from women with unexplained infertility was significantly lower than those obtained from fertile women at the same

time.^{65,66} Furthermore, it has been shown that mutations in the LIF gene may decrease the biological activity of LIF in the endometrium and cause an implantation failure.⁶⁷ However, screening of women with unexplained infertility and with recurrent failure of implantation after in vitro fertilization (IVF) treatment for LIF mutations did not reveal that LIF gene mutations play any crucial role in the etiology of unexplained infertility and IVF failure.⁶⁸

The uterine milieu in LIF mutant mice fails to induce implantation irrespective of the blastocyst genotypes because LIF^(-/-) blastocysts can implant after transfer to wild-type pseudopregnant recipients (Table 2).^{69,70} These reciprocal embryo transfer experiments suggest that maternal LIF is essential for blastocyst implantation. However, a role for this cytokine in embryonic functions cannot be ignored because LIFR and gp130 are expressed at the blastocyst stage, and administration of exogenous LIF improves embryo viability and hatching in several species.^{71,72} Taken together, these data suggest that both the preimplantation embryo and the uterus are sites of LIF action. However, embryos lacking either LIFR or gp130 develop to the blastocyst stage and implant normally but die during the perinatal period.⁷³ Thus LIF may signal to both embryonic and uterine cells during implantation.⁶⁵ Gp130 deficiency in mice leads to embryonic lethality.⁷⁴ Gp130 mRNA localizes predominantly to glandular and luminal epithelium in human endometrium⁶⁰ and has also been demonstrated in human embryos from three-cell stage onward.⁷⁵ Soluble gp130 is secreted from endometrial tissues obtained from women between days 20 and 26 of the menstrual cycle at a 20-fold higher concentration to the proliferative phase. Importantly, secretion of soluble gp130 from endometrial tissues obtained between days LH + 6 and LH + 13 is reduced in infertile women compared with fertile women.⁷⁶ These data cumulatively show that the presence of such soluble receptors in the endometrium has important implications in cytokine action.

INTERLEUKIN-6—IL-6 is a multifunctional cytokine that plays important roles in host defense, immune response, acute phase reaction, and hematopoiesis. IL-6 has some functional redundancy with IL-11 and LIF. IL-6 exerts its activity through binding to a high-affinity receptor complex consisting of two membrane proteins (IL-6R and gp130).⁷⁷ IL-6-deficient mice have reduced fertility and a decrease in viable implantation sites⁷⁸ (Table 2).

In human endometrium, IL-6 is weakly expressed during the proliferative phase, but strong epithelial immunoreactivity is present during the midsecretory phase when the implantation window opens.³¹ Furthermore, IL-6 secretion by endometrial stromal cells is enhanced by interferon- γ .⁷⁹ In first-trimester trophoblasts, IL-6 protein and mRNA are present in both cytotrophoblastic and the syncytiotrophoblastic cells. Expression of this cytokine decreases significantly during differentiation of cytotrophoblastic cells into syncytiotrophoblast and that IL-6 concentration is found to be higher in decidual tissue than in placental tissue.^{80,81} Moreover, the IL-6-R is predominantly localized in glandular epithelium and to lesser extent in the stroma throughout the menstrual cycle.³¹ Therefore, a role in human implantation could also be postulated for this cytokine as for LIF. When levels of IL-6 secretion were measured from endometrial biopsies obtained between days LH + 6 and LH + 13 from infertile women compared with fertile women, no difference was found.⁷⁶ However, a recent study showed that endometrial expression of IL-6 and IL-1 is reduced in a group of women experiencing unexplained recurrent miscarriage compared with fertile women.⁸²

INTERLEUKIN-11—IL-11 was initially described as growth factor acting at multiple stages during hematopoiesis, synergizing with other factors.⁸³ IL-11 has important anti-inflammatory activities⁸⁴ as well as pleiotropic actions in several cell types.⁸³ IL-11 knockout mice have a fertility defect because of the postimplantation response to the implanting blastocysts⁵⁵ (Table 2). Increasing evidence indicates that IL-11 has an important function in human implantation. IL-11 and its receptor (IL-11R α) have recently been

demonstrated in human endometrium. Although all the major cell types in endometrium expressed IL-11 with cyclical variation, the most prominent immunoreactivity and mRNA expression is in the decidualized stromal cells late in the menstrual cycle.^{85–90} There is no cyclical variation in IL-11R α expression, and thus the expression pattern of ligand may be critical for IL-11 function in the endometrium. Furthermore, invasive trophoblast cells are a source of IL-11 and IL-11R α during early pregnancy in primates, suggesting involvement in placentation.⁸⁸ IL-11 is involved in both in vivo and in vitro decidualization.⁹⁰ Upregulation of IL-11 mRNA was detected by gene array during progesterone or cyclic adenosine monophosphate (cAMP)-induced in vitro decidualization of endometrial stromal fibroblasts.^{91,92} Interestingly, recent evidence in mice shows that IL-11 signaling is required for decidual-specific maturation of natural killer (NK) cells.⁹³ This evidence indicates that IL-11 may be important in the establishment of viable pregnancies. To date, knowledge on IL-11 function in implantation is only at an early stage. Future studies should validate and further investigate the importance of this cytokine in human implantation.

INTERLEUKIN 15—IL-15 is a 14- to 15 kDa member of the four α -helix bundle cytokine family that includes IL-2 and promotes activation of neutrophils, macrophages, and T cells, but importantly is a core chemokine that controls lymphocyte function and maintenance.⁹⁴ IL-15 is essential for NK cell development in bone marrow and stimulates the proliferation, cytokine production, and cytotoxicity of activated blood NK cells. IL-15 is reported to be essential for type 2 cytokine production by the uNK cells.⁹⁵ Unlike its effects on blood NK cells, it does not transform the uNK cells into potent cytolytic cells.⁹⁶ This is critically important for a cell that is present at the maternal–fetal interface where cytolytic activity would destroy trophoblast. It is therefore reasonable to assume that in the human uterus, IL-15 may play a role in promoting the uNK cell survival and expansion. IL-15 mRNA and protein have been demonstrated in non-pregnant human endometrium, decidua, and placenta⁹⁶ with the protein localized perivascularly in secretory phase stromal fibroblasts, in glandular epithelial cells during the proliferative phase, and in decidua in the first trimester of pregnancy.⁹⁷ IL-15 knockout mice are fertile, but they display impaired decidual integrity, unmodified spiral arteries, and lack of uNK at the implantation sites⁹⁸ (Table 2).

Human endometrial stromal fibroblasts decidualized using either cAMP or progesterone show enhanced IL-15 mRNA expression and protein secretion⁹⁷ that is further enhanced in the presence of interferon- γ , although the latter cytokine alone cannot stimulate IL-15 production. The probable source of interferon- γ in the endometrium is the uNK cells,⁹⁹ and thus the likelihood exists of enhancement of IL-15 production from decidualizing cells by adjacent uterine NK cells. IL-1 β appears to play an opposing role because it acts as a negative regulator of IL-15 mRNA and protein during in vitro decidualization.

INTERLEUKIN-1—IL-1 is a proinflammatory cytokine with multiple functions in a range of tissues.¹⁰⁰ The IL-1 system includes two ligands, IL-1 α and IL-1 β , the cell surface receptors, IL-1 receptor type 1 (IL-1R1) and IL-1R2, a non-binding receptor accessory protein (IL-1RAcP), and the naturally occurring receptor antagonist (IL-1ra), which competes with IL-1 for receptor binding.¹⁰¹ All components of the IL-1 system have been examined in the human endometrium and at the maternal–trophoblast interface during implantation. IL-1 is present at the feto-maternal interphase; trophoblastic cells and decidual cells produce IL-1, and the IL-1 receptor is present in endometrial epithelial cells as well as in trophoblasts.¹⁰² IL-1 may be one of the first signals of the blastocyst acting on the endometrium because in vitro IL-1 increases endometrial secretion of prostaglandin E₂, LIF, and integrin β 3 subunit expression.¹⁰³ Moreover, in mice, IL-1 receptor antagonist given before implantation significantly reduces the number of implanted embryos, indicating a role for IL-1 in embryo implantation.¹⁴

Growth Factors

The expression of various growth factors and their receptors in the uterus is cell specific and temporal during the preimplantation period, suggesting that some of these factors are important for the implantation process.¹ Several well-known growth factors are members of the EGF, TGF- β , and VEGF families. IGFBP-I, a member of the EGF family, is also important for implantation.

EPIDERMAL GROWTH FACTOR FAMILY OF GROWTH FACTORS—Members of the EGF family of growth factors are hypothesized to participate in multiple developmental, physiological, and pathological processes. The EGF family of growth factors includes EGF itself, TGF- α , HB-EGF, amphiregulin, β -cellulin, epiregulin, and neuregulins. The EGF-like growth factors interact with receptor subtypes that belong to an ErbB gene family of tyrosine kinase receptors: ErbB1 (EGF-R), ErbB2, ErbB3, and ErbB4. They share common structural features but differ in their ligand specificity and kinase activity.⁵

EGF has been localized in human endometrium throughout the menstrual cycle, in gestational decidua, and in first-, second-, and third-trimester placenta. In the proliferative phase of the human endometrium, moderate EGF immunostaining is observed in the cytoplasm of stromal cells, whereas in secretory endometrium, EGF immunostaining is intense and localizes mostly to stromal cells. EGF expression is also found in both decidual and trophoblastic cells.¹⁰⁴ Previous studies have shown that EGF plays a critical role in trophoblast invasion,¹⁰⁵ differentiation, and proliferation¹⁰⁶ suggesting that EGF may affect implantation in several different ways and that this growth factor is a significant regulator of the implantation process.

Studies in mice demonstrate that EGF deficiency during pregnancy causes intrauterine growth retardation or abortion.^{107,108} By replacing estrogen, EGF can initiate embryo implantation in the rat.^{48,109} Knockout of the EGF receptor (EGFR or ERBB1) gene in mice leads to preimplantation death of the embryos.¹¹⁰ Thereafter, several studies have demonstrated that EGF affects mouse embryo development in vitro^{111,112} and promotes trophoblast differentiation into the invasive type, a crucial cell type for implantation.^{113,114} EGF receptors are expressed in the mouse embryo at very early stages¹¹⁵ and also in the peri-implantation uterus.¹¹⁶ The spatiotemporal expression patterns of EGF gene family members and ErbBs in the uterus during the peri-implantation period suggest compartmentalized functions of EGF-like growth factors in implantation.

HEPARIN BINDING EGF-LIKE GROWTH FACTOR—HB-EGF shares a common receptor with EGF and TGF α . 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.⁵⁴ The expression of HB-EGF reaches a peak level in the midsecretory phase in uterine epithelial cells, signifying that this growth factor may also be involved in the regulation of blastocyst implantation. The two soluble and transmembrane forms of HB-EGF were found to be the earliest molecular marker of local endometrial receptivity in mouse uterus, expressed exclusively in the location of active blastocysts before the attachment reaction. These data suggest paracrine and/or juxtacrine interactions with embryonic ErbBs, as well as autocrine, paracrine, and/or juxtacrine interactions with uterine ErbBs that are expressed in a spatiotemporal manner during the peri-implantation period.¹¹⁷ This induction is followed by the expression of β -cellulin, epiregulin, neuregulin-1, and COX-2 around the time of the attachment reaction.¹¹⁸ Strong expression of amphiregulin in the luminal epithelium is found only around the implanting blastocysts, and this expression is absent by day 5 of pregnancy. Mice with targeted disruptions of EGF/TGF- α /amphiregulin do not exhibit implantation defects because HB-

EGF, betacellulin, epiregulin, neuregulin, and amphiregulin all show overlapping uterine expression patterns around the implanting blastocyst at the time of attachment.^{118,119}

In humans, HB-EGF appears to play a role in implantation and embryonic development. Its expression is maximal during the period of receptivity.^{120,121} Martin et al showed that the soluble form of HB-EGF increases the rate of blastocyst hatching,¹²² and Chobotova et al reported that human endometrial epithelial cells, expressing the transmembrane form of HB-EGF, adhere to trophectoderm of human blastocyst displaying cell surface ErbB4.¹²³ Thus HB-EGF is one of the most potent growth factors for the blastocyst and has a significant role in preimplantation embryo development and implantation in a paracrine fashion in various species.

TRANSFORMING GROWTH FACTOR BETA—The TGF- β family of growth factors contains three isoforms. TGF- β has pleiotropic effects, playing key roles in tissue morphogenesis and growth. Detection of TGF- β isoforms at the maternal–fetal interface, together with the known roles of TGF- β in the regulation of proliferation, differentiation, extracellular matrix production, and in migration, clearly implicate it in the cellular events underlying implantation.¹²⁴

In human endometrium, TGF- β protein and mRNA are localized in endometrial stromal, epithelial, and decidual cells.¹⁰² Previously it has been demonstrated that TGF- β 2 expression is more intense in stroma cells, whereas TGF- β 1 and - β 3 are equal in intensity. Only TGF- β 3 expression varies during the menstrual cycle, being more intense in glandular epithelium during the late secretory phase. 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 the implantation.^{125,126} TGF- β 1, - β 2, and - β 3 and their receptors have also been detected at the maternal–fetal interface during the first trimester of pregnancy. TGF- β s modulate maternal immunotolerance during implantation and regulate, in vitro, several implantation-related molecules, such as VEGF, MMP-9, IGFBP-1, and LIF.¹²⁷ TGF- β 1 knockout mouse embryos die either in the uterus or early after birth,¹²⁸ suggesting a potential role in implantation. A study of TGF- β receptor types I and II and their signal transducers, Smad2 and Smad4, in rat endometrium during the estrous cycle and early pregnancy suggest that functional TGF- β signaling is spatiotemporally associated with the onset of uterine receptivity and embryo attachment, whereas it diminishes when trophoblast invasion is initiated. Alternatively, TGF- β also inhibits trophoblast proliferation and invasion by stimulating tissue inhibitor of metalloproteinase (TIMP) secretion and decreasing matrix metalloproteinase (MMP) activation through downregulation of plasminogen activators.¹²⁹ Elevated TGF- β activity has been reported in the plasma of preeclamptic mothers and may be implicated in the impaired implantation associated with preeclampsia.^{130,131} Thus TGF- β may be considered as an endometrial signal, which is able to up- or downregulate trophoblast invasion during implantation and placentation.

INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 AND INSULIN-LIKE GROWTH FACTOR 1

IGFBP-1 is one of six homologous proteins that specially modulate the mitogenic and metabolic effects of insulin-like growth factors IGF1 and IGF2, which have an important role in growth, apoptosis, metabolism, and development. IGFBP-1 is expressed primarily in the liver, kidney, decidualized endometrium, and luteinizing granulosa cells.¹⁰² Both IGFBP-I protein and mRNA have been localized to the predecidual stromal cells in late secretory-phase endometrium and to decidual cells during pregnancy.^{132,133} IGFBP-I increases the gelatinolytic activity of t3. IGFBP-1 also inhibits trophoblast migration into decidualized stromal multilayers.¹³⁴ The patterns of IGFBP expression suggest that IGFBP-1 is the predominant IGFBP synthesized by decidual cells;

the IGFBP-1 interacts with the IGF2 synthesized by trophoblasts. It is proposed that IGF2 and IGFBPs are used for cell-to-cell communication between trophoblasts and decidua, regulating invasion.¹³⁵ IGF-II and IL-1 β are inhibitory to IGFBP-1, with IL-1 β inhibiting decidualization, whereas other growth factors such as TGF- β , stem cell factor (SCF), colony-stimulating factor-1 (CSF-1) and LIF have not been reported to affect IGFBP-1 expression.¹³⁶

In vitro mouse embryo studies demonstrated that the addition of IGF1 to the culture medium resulted in a significant decrease in the number of apoptotic cells per embryo, mostly in cells of the ICM lineage,¹³⁷ whereas supplementation with growth hormone exerted similar effects targeting the trophectodermal cells.¹³⁸ A similar effect was shown in the human cells as well. Spanos et al found that the percentage of apoptotic nuclei in human blastocysts was decreased by ~50% upon addition of IGF1 to culture.¹³⁹

VASCULAR ENDOTHELIAL GROWTH FACTORS—VEGF is an endothelial cell-specific mitogen in vitro and is the main factor responsible for de novo blood vessel formation (vasculogenesis) and angiogenesis in a variety of in vivo models.¹⁴⁰ VEGF (also referred to as VEGF-A) is a member of a growth factor family that includes placenta growth factor (PlGF),¹⁴¹ VEGF-B,¹⁴² VEGF-C,¹⁴³ and VEGF-D.¹⁴⁴ VEGF directly binds to its surface receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR) that are type 3 receptor tyrosine kinases.¹⁴⁵ VEGFR-1 also binds the PlGF, a protein closely related to VEGF and expressed only in a restricted set of tissues such as the placenta.^{146,147} This growth factor is reviewed extensively in other articles in this issue of *Seminars in Reproductive Medicine*.

It is well known that implantation occurs under hypoxic conditions. VEGF (VEGF-A) gene transcription is stimulated in hypoxic conditions. VEGF is involved in embryo–endometrium interactions by regulating increased endometrial vascular permeability and endothelial cell proliferation at implantation sites in rodents.^{148,149} Moreover, expression of VEGF-A shows menstrual cycle dependence in human and monkey endometrium.^{150,151} Expression of VEGF189, an extracellular matrix-bound isoform, was found to be progesterone dependent in cynomolgus monkey¹⁵² and in the human uterus.¹⁵³ The expression of VEGF mRNA and protein has been demonstrated in endometrial stromal, glandular, and endothelial cells during the mid-secretory stage in the human, although the highest level of expression is observed in the mid to late proliferative phase.¹⁵⁴ Both VEGFR1 and VEGFR2 are present in microvessels during the midsecretory period, emphasizing a correlation between the increased microvascular density and vascular permeability observed at this time.¹⁵⁵ Additionally, secretion of VEGF into the lumen of endometrial glands in polarized human endometrial cell cultures suggests that apically secreted VEGF may also function as an endometrial signal for blastocyst development, implantation, or both.¹⁵⁶ Trophoblast cells and endometrial epithelium in the rhesus monkey also express VEGF during early villous stages of implantation and placentation. Moreover, mifepristone treatment during early secretory phase causes pregnancy failure, endometrial dyssynchronization, and downregulation of VEGF expression and secretion in the rhesus monkey.^{157,158} A recent study has shown that administration of an immunoneutralizing monoclonal VEGF antibody at 10 mg on days 5 and 10 after ovulation inhibited pregnancy establishment significantly.¹⁵⁹ These results reveal two basic roles for VEGF in endometrial tissue: First, VEGF regulates endometrial vascularization and vascular permeability, and second, VEGF establishes a receptive endometrium to support blastocyst implantation and trophoblast invasion.

Interactions between Growth Factors and Molecular Regulator of Implantations

Integrins are a family of cell adhesion molecules intensively studied in endometrium. Heterodimers of α and β integrins serve as receptors for ECM ligands such as collagen, laminin, and fibronectin, as well as transducing signals from soluble ligands such as osteopontin.²⁹ Some integrins such as $\alpha 4$ and $\beta 3$ exhibit cycle-dependent changes in gene expression in both the stroma and epithelium throughout the cycle.¹⁶⁰ In addition to the endometrium, the cytotrophoblasts also exhibit temporal and spatial regulation of integrin expression. In normal fertile women the $\alpha 4$ subunit is upregulated in the glandular epithelium at the time of ovulation and disappears around cycle day 24. In contrast, $\beta 3$ expression in both glandular and luminal epithelium is upregulated on day 19 or 20.^{161,162} Because $\beta 3$ upregulation and $\alpha 4$ downregulation coincide with the window of receptivity, this has led to the conclusion that these integrins could serve as markers of functionally receptive endometrium. At the preimplantation phase, the human blastocyst expresses several integrin subunits, including αv , $\alpha 3$, $\beta 1$, $\beta 3$, $\beta 4$, and $\beta 5$.¹⁶³ Cyclic-specific expression of integrins appears to be regulated by ovarian hormones and growth factors. The $\alpha v \beta 3$ integrin expression in the human endometrium is regulated both positively by EGF and HB-EGF, and negatively by estrogen.¹⁶⁴ During the proliferative phase, estrogen inhibits integrin expression; however, in the luteal phase, progesterone reverses the effect of estrogen, thus indirectly increasing integrin expression. Progesterone also acts positively by increasing EGF, HB-EGF, and HOXA10 to induce epithelial $\beta 3$ integrin expression.¹⁶⁵ Similarly, blastocyst-derived IL-1 α and -1 β upregulate endometrial epithelial $\beta 3$ integrin subunit expression, suggesting a paracrine mechanism for the IL-1 system in implantation.¹⁴

MMPs are key modulators of many biological processes during angiogenesis, wound healing, and cellular migration. Twenty-four members of the MMP family have been identified in humans, degrading many components of the extracellular matrix, cellular receptors, and cytokines. Tissue remodeling and angiogenesis are hallmark events during implantation and decidualization. The changing endocrine state of the female during the reproductive cycle and pregnancy results in extensive remodeling of the uterine tissue.¹⁶⁶ MMPs and TIMPs are thought to be key mediators for matrix degradation during implantation and decidualization.¹¹⁹ Evidence for an *in vivo* role for MMP-9 in implantation comes from studies of the Ets-2- and MMP-9-null mice, as well as the TIMP-1 overexpressing mouse, and pharmacogenetic studies using chemical inhibitors. Ets-2 is a member of the Ets family of transcription factors that regulate the transcription of diverse genes, particularly MMPs. Interestingly, in trophoblasts, expression of MMP-9, which is a transcriptional target of Ets-2, is significantly decreased in the Ets-2 null mice. Thus the placental phenotype in Ets-2 deficiency may be due to insufficient MMP-9 activity in the trophoblast. In support of this model, TIMP-1 overexpressing metalloproteinase inhibitor-treated mice¹⁶⁷ and MMP-9-null mice on some genetic backgrounds¹⁶⁸ display a similar placental phenotype, even though these do not lead to lethality. Mechanisms regulating the MMP and TIMP genes during the peri-implantation period are not clear, although growth factors and cytokines including the EGF and TGF- β family and LIF modulate MMPs and TIMPs.¹⁶⁹ VEGF, FGF-4, and FGF-10 stimulate MMP-9 expression in trophoblast cells.¹⁷⁰

Transcription Factors

All genes are transcriptionally regulated. Thus all cytokines and growth factors are regulated by transcription factors. In addition to ER and PR, some of the well-characterized transcriptional regulators of implantation are described next.

HOMEBOX GENES—The homeobox (*Hox*) genes encode transcription factors that guide embryological development as well as regulate differential gene expression within the endometrium with each menstrual cycle.¹⁷¹ *Hox* genes are arranged in a cluster within

chromosomal loci. There are 39 Hox genes arranged in four parallel clusters (termed A, B, C, and D), which are located on four separate chromosomes. The transcription factors encoded by the *Hox* genes may activate or repress downstream targets, both of which define body segment identity. The DNA-binding domain of these transcription factors contains a 60 amino acid helix-turn-helix motif that is conserved evolutionarily, suggesting common ancestry as well as evolutionary conservation and genetic importance.¹⁷²

Although animal species differ widely in appearance, they all use *Hox* genes to establish their body plans.¹⁷³ There are two *Hox* genes (*Hoxa10* and *Hoxa11*) that are thought essential for implantation in mice because homozygous mutants of either of these genes are infertile due to loss of endometrial receptivity. The genes *Hoxa10* and *Hoxa11* are expressed in the endometrial glands and stroma of the uterus throughout the menstrual cycle, and their expression levels increase dramatically during the midsecretory phase at the time of implantation, and thereafter remain elevated throughout the remainder of the cycle as well as in the decidua of pregnancy in humans.¹⁷⁴ Taylor et al showed that ovarian hormones regulate the expression of both *HOXA10* and *HOXA11* in human endometrium.¹⁷⁵ Peak *HOXA10* and *HOXA11* expression is first obtained at the window of endometrial receptivity. Furthermore, there is a predictable concentration-dependent response of *HOX* gene expression to progesterone, which increases over the physiological range. These data demonstrate that progesterone modulates the peri-implantation increase in *HOX* gene expression.¹⁷⁵

This pattern of expression in the adult suggests that *HOX* genes play a role in human implantation, as seen in mice.^{174,176} The mechanisms by which the *HOX* genes interfere with implantation are not fully characterized, but mice homozygous for *Hoxa10* deficiency show oviductal transformation of the proximal third of the uterus, defective implantation, and embryonic reabsorption in the early postimplantation period, suggesting that *HOXA10* may be important during morphogenesis for proper patterning of the reproductive tract and in adult endometrium for adequate implantation events.¹⁷⁴ In addition to its effect during embryonic development, *HOXA10* is essential for normal endometrial development in each menstrual/estrus cycle. Decreased *HOXA10* expression obtained with the use of siRNA leads to decreased implantation in the mouse; humans with implantation defects, including those induced by endometriosis, fibroids, and polycystic ovary syndrome (PCOS) have diminished *HOXA10* expression. Uterine stromal cells in *HOXA10*-deficient female mice show reduced proliferation in response to progesterone, leading to decidualization defects.^{174,176} Furthermore, because several progesterone-responsive genes are dysregulated in the uterine stroma of *HOXA10* mutant mice, *HOXA10* may convey progesterone responsiveness in the uterine stroma by regulating gene expression. *HOXA10* has been demonstrated to regulate the expression of β 3 integrin as well as several other genes involved in endometrial receptivity. Similarly, *HOXA11*-deficient mice are infertile due to endometrial implantation defects. *HOXA11*^{-/-} mice have reduced expression of LIF.¹⁷⁷ A gene therapy approach that involves the manipulation of the expression of *Hox* genes may have a role in the enhancement of endometrial receptivity and implantation.

FORKHEAD BOX O TRANSCRIPTION FACTOR—FOXO transcription factors are critical mediators of cell fate decisions in response to growth factor, hormonal, and environmental cues.¹⁷⁸ FOXO is involved in the control of cell cycle arrest and the induction of apoptosis. FOXO1 expression is dramatically stimulated upon decidualization both in vivo and in vitro, and it is involved in regulating the expression of decidual marker genes, such as PRL and IGFBP-1. The promoters of both genes are activated by multimeric transcription factor complexes containing FOXO1, which assemble in response to the interplay of cAMP- and progesterone-dependent signals.¹⁷⁹ A known FOXO target gene is BIM (BCL2 L11), which encodes the proapoptotic Bcl-2 homology 3 domain-only protein

Bim.^{180,181} Bim expression increases in the endometrium before menstruation and in culture upon activation of the cAMP pathway in a FOXO1-dependent manner.¹⁸² Another FOXO target gene, Fas ligand (FASLG), has also been implicated in regulating endometrial stromal cell apoptosis.¹⁸³ These findings suggest a critical role for FOXO1 in mediating the proapoptotic pathway initiated by progesterone withdrawal at the end of the menstrual cycle.

JAK/STAT INTRACELLULAR SIGNALING—The importance of the JAK/STAT signal-transduction pathway in embryo implantation has been demonstrated by the embryonic lethality of STAT3-deficient mice. Interestingly, STAT3-deficient embryos implant but die rapidly due to placental defects.¹⁸⁴

LIF and IL-11 induce activation of the Stat family of signal transducers via the Jak/Stat pathway. In the mouse uterus, LIF acts primarily through the activation of STAT3.¹⁸⁵ Similarly, in human endometrial stromal cells, IL-11 acts via activated STAT3.^{186,187} It remains to be determined when phosphorylated-STAT3 can be detected in human endometrium because this will indicate when the signal-transduction pathway is activated. Interestingly, STAT3 protein production is stimulated by progesterone¹⁸⁸ and is activated by IL-11 in human endometrial stromal cells in vitro.^{187,188} Furthermore, a role for STAT3 activity in trophoblast invasiveness has also been proposed.¹⁸⁹

CONCLUSION

Understanding coordinated interaction among growth factors, hormones, secondary signaling cascades, and transcription factors during endometrial receptivity and trophoblast adhesion and invasion will improve not only the pregnancy rate of assisted reproductive techniques but also will deepen our understanding of several other pathophysiological processes, such as tumor development, angiogenesis, and tumor metastasis. It is clear that both endometrial receptivity and blastocyst implantation are regulated by growth factors (e.g., HB-EGF, amphiregulin) and adhesion molecules (e.g., integrins, E-cadherin, laminin, fibronectin) that are necessary for embryo attachment. However, further studies are required to understand growth factor- and cytokine-mediated signaling cascades and their pathophysiological regulation during implantation. Many cytokines are expressed during the implantation window, but only a few are required for embryonic implantation, such as LIF, IL-6, and IL-11. A significant advance has been made in recent years toward understanding the molecular mechanisms governing trophoblast and ICM differentiation. Appearance of early blastocyst formation is the first morphological sign of proper differentiation. *Oct-4*, *Nanog*, *Sox2*, and *Gata6* are involved in ICM and its subsequent differentiation to epiblast and primitive endoderm. *Cdx-2*, *Eomes*, *Stra13*, *Hand1*, and *Tpbpa* are involved in trophoblast development and subsequent differentiation to trophoblastic giant cells, spongiotrophoblasts. These genes are detected through the generation of knockout mice, and further studies are required to confirm their relevance in human embryos. Studying regulation of these genes by implantation-related growth factors and cytokines and vice versa will increase our knowledge on implantation biology and may enable novel clinical approaches to implantation-related infertility.

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Figure 1.

Lineage differentiation during 8-cell to blastocyst transition and gene expression patterns during mouse blastocyst formation. *Oct4* is expressed throughout the embryo before the late morula and crucial for the formation of inner cell mass. The expression of *Nanog* is induced in the inside cells of the late morula. *Cdx2* is expressed in trophectoderm. *Gata6* is expressed in the primitive endoderm of the late blastocyst. 2PN, two pronuclei.

Table 1

Function of Transcription Factors Known to Be Important for the Fate of Trophectoderm and the Inner Cell Mass

Gene	Function	References
<i>Oct4</i>	Expressed in nuclei of all cells of the early embryo, but its expression becomes restricted to the ICM upon blastocyst formation, preventing TE formation	Kirchhof et al, 2000 ¹⁹⁰
<i>Sox2</i>	A similar expression profile to Oct4, preventing trophoblast specification	Avilion et al, 2003 ¹⁹¹
<i>Nanog</i>	Sustaining the self-renewal capacity of embryonal stem cells and inhibiting their differentiation, ICM formation, and visceral-parietal endoderm formation	Mitsui et al, 2003 ¹⁹²
<i>Cdx2</i>	Segregating ICM and TE lineages by ensuring the suppression of <i>Oct4</i> and <i>Nanog</i>	Niwa et al, 2005 ¹⁹³
<i>Eomes</i>	Required for TE proliferation and differentiation at the blastocyst stage	Simmons and Cross ²⁶
<i>Lefty-1</i>	Determining the left–right axis in mouse embryos	Takaoka et al ²³

ICM, inner cell mass; TE, trophectoderm.

Table 2**Reproductive Function in Animals with Targeted Disruption of Genes Important in Implantation**

Gene	Reproductive Function	References
<i>LIF</i>	Failure of implantation, unable to induce decidualization	Stewart et al ⁷⁰
<i>IL-6</i>	Reduced fertility; viable implantation sites decreased 48%	Robertson et al ⁷⁸
<i>IL-11</i>	Fertility defect, which, unlike that in the LIF-deficient mice, occurs in the postimplantation response to the implanting blastocyst	Laird et al ⁵⁵
<i>IL-15</i>	Fertile, but display impaired decidual integrity, unmodified spiral arteries, and lack of uNK at the implantation sites	Ashkar et al ⁹⁸
<i>LIFR</i>	Intrauterine lethality	Ware et al ⁷³
<i>TGF-β1</i>	Intrauterine and early postnatal lethality	Kulkarni and Karlsson ¹²⁸
<i>HOXA10</i>	Oviductal transformation of the proximal third of the uterus, defective implantation and embryonic reabsorption in the early postimplantation period	Benson et al ¹⁷⁴
<i>HOXA11</i>	Diminished uterine glands; partial homeotic transformation of uterus to oviduct, defective implantation	Hsieh et al ¹⁹⁴

Table 3Reproductive Function in Animals with Targeted Disruption of *ER* and *PR* Genes in Implantation

Gene	Reproductive (Uterine and Implantation) Phenotype	References
<i>ERa</i>	Infertile owing to loss of ovarian function, immature uteri, unresponsive to estrogen	Hewitt and Korach ⁴¹
<i>ERb</i>	Subfertile owing to reduced ovarian efficiency, enhanced response to estrogen	Harris, 2007 ¹⁹⁵
<i>PRA</i>	Infertile, subovulatory, unable to induce and support decidualization	Mulac-Jericevic, 2000 ¹⁹⁶
<i>PRB</i>	Fertile, normal ovarian and uterine response to progesterone	Mulac-Jericevic, 2003 ¹⁹⁷
<i>PR (PR A and PR B)</i>	Infertile, anovulatory	J.P. Lydon, 1995 ¹⁹⁸