The Cytokine Network during Embryo Implantation

Hong-Yuan Huang[1,2], MD

Embryo implantation is an essential step in mammalian pregnancy. The molecular events of embryonic attachment to the endometrial epithelium and the subsequent invasion of the stroma have long been of interest. The process involves both the scientific interest of reproductive biologists and clinical interest of physicians that care for infertility couples. Understanding the molecular factors involved in each phase of the implantation process is critical to comprehending the mechanisms that control reproduction, especially as part of a human in vitro fertilization program. This review will focus on the role during early embryonic development and implantation of two distinct cytokines, the interleukin-1 (IL-1) system and interleukin-18 (IL-18) system; it will also examine the role of the matrix metalloproteinases (MMP) and their inhibitors as well as vascular endothelial growth factor (VEGF) system. The possible influence of the cytokines on other systems involved in embryonic implantation, including the embryonic-endometrial dialogue, the subsequent invasion and neoangiogenesis will be discussed. These suggest a role for the two cytokine families in early embryonic development. *(Chang Gung Med J 2006;29:25-36)*

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Successful embryo implantation requires an appropriate interaction between the blastocyst and a well-prepared uterine endometrium that has had adequate steroid hormonal stimulation during the luteal phase of the cycle. This involves the sequential action of estradiol and progesterone on the endometrium to induce secretory glandular epithelium and the subsequent decidual transformation of the stromal cells.[1] Although the factors that are involved in the regulation of blastocyst implantation are incompletely understood, increasing evidence suggests that growth factors and cytokines play a crucial role in the maternal-fetal interaction during embryonic implantation.[2] In addition, the human endometrium is known to be an active site for cytokine production and action.[3-5]

Human endometrium undergoes tissue-specific cyclic changes during proliferation and secretion that eventually result in the endometrium being shed and menstrual bleeding occurring when no embryonic implantation occurs. Uterine endometrium therefore is the anatomic prerequisite for the continuation of our species, and its main purpose during female reproductive age is to communicate with the embryo during the implantation process.[6]

The pre-implantation embryo produces several factors during its development to communicate and signal its presence to the maternal endometrium. Cytokines and growth factors and their corresponding receptors are the major candidates for these molecular events. The appropriate interaction between the preimplantation embryo and maternal endometrium is at least partly controlled by paracrine cytokines and this subject has been extensively covered by several reviews.[7-10] It is known that both the endometrium and the preimplantation embryo

From the [1]Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Taipei, [2]Department of Obstetrics and Gynecology, College of Medicine, Chang Gung University, Taoyuan.
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Correspondence to: Dr. Hong-Yuan Huang, Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital. 5, Fushing Street, Gueishan Shiang, Taoyuan, Taiwan 333, R.O.C. Tel.: 886-3-3281200; Fax: 886-3-3288252; Email: hongyuan@cgmh.org.tw

express several of these cytokines and growth factors and their corresponding receptors at the time of embryonic implantation. Recently, an increasing amount of knowledge about the actual role of these factors has become available. A better understanding of these factors during early embryonic development and implantation could lead to improved in vitro culture conditions and enhance the outcome of human in vitro fertilization (IVF) program.

The interleukin-1 (IL-1) system

Recently, the interleukin-1 system has also been implicated as a major factor in the events associated with embryonic implantation. IL-1 is a family of polypeptides that comprises two agonists; interleukin-1α and β (IL-1α and IL-1β) and an inhibitor, interleukin-1 receptor antagonist (IL-1ra).[11] Two receptors (IL-1 R) have been identified and characterized as type I (IL-1R tI) and type II (IL-1 R tII).[12,13] Both IL-1 agonist and antagonist are recognized by IL-1R tI, and they trigger signal responses in target cells.[14,15] IL-1 R type II is the major form found in many cells but primarily neutrophils, monocytes, and B-lymphocytes. Recent knowledge of IL-1 expression in both the human endometrium and the embryo has led to a better understanding of implantation events. The presence of the IL-1 system has been documented in human endometrium[16-21] and IL-1 mediated inhibition of endometrial stromal cell differentiation has also been described.[22,23]

Human endometrium has also been shown to express more IL-1ra in the follicular phase compared to luteal phase.[24] In addition, repeated injections of the IL-1ra into pregnant mice, beginning two days before the onset of implantation, has been shown to result in an apparent failure of blastocyst to implant; this suggests an inhibitory effect of IL-1ra on embryo implantation.[20] The mechanism by which this occurs remains unclear, although it has been suggested that IL-1ra prevents embryo implantation by a direct effect through the microvilli of the mouse endometrium.[25] Interestingly, repeated injections of recombinant IL-1ra in pregnant wild type or IL-1R tI null female mice do not inhibit embryo implantation.[26] It has been previously demonstrated that the ratio of IL-1β to IL-1ra in human endometrial stromal cells remained constant even in the presence of increasing concentrations of IL-1β, suggests that an appropriate ratio of both agonist to antagonist may be relevant to embryo implantation.[27] On the other hand, it has been demonstrated that the prevention of embryonic implantation by IL-1ra in the mouse model may be mediated by a direct effect on the morphology of endometrial epithelium and not an effect on the pre-implantation embryo. This is because there is an inhibition of the transformation of the epithelial plasma membrane at the time of implantation, presumably related to the alteration in the α4, αv, and β 3 adhesion molecules.[9]

The results obtained from immunohistochemical studies have also localized the complete IL-1 system in human oocytes and embryos at all developmental stages.[28,29] Preimplantation human and mouse embryos are known to express IL-1 agonist, IL-1 receptor antagonist and their common receptor, the IL-1 receptor type I.[30-32] These results suggest that the entire IL-1 system may play an important role in embryo implantation and decidualization of stromal cells.

The human fallopian tube is the site of oocyte capture and migration, sperm migration, fertilization, and early embryonic development. The fallopian tube is also the most common implantation site for extra-uterine gestation. The fallopian tubes are regarded as being biologically active, providing an environment that sustains and enhances fertilization during early embryonic development as the embryo traverses the tubes and moves toward the uterine cavity.[7,33] The anatomy, histology and physiology of the human fallopian tube all contribute important information to our understanding of the role of cytokines in the human fallopian tube and their impact on embryonic development. Moreover, it has been shown that improved embryo morphology, development, and hatching as well as better implantation rates are obtained after embryos are cocultured on feeder layers of human oviduct cells.[34] Cytokines have recently been identified within human fallopian tubes, including EGF, TGF-α, IGF, TGF-β, GM-CSF, GnRH and LIF.[35,36]

It has been demonstrated that complete IL-1 system mRNA and protein expression occurs in fallopian tubes during ectopic pregnancies. When fallopian tubes with ectopic pregnancies are compared to normal control tubes, IL-1β expression is decreased and IL-1ra and IL-1R tI expression is increased. There is a lower ratio of IL-1β to IL-1ra at the mRNA level in fallopian tubes with ectopic preg-

nancies suggesting that an inappropriate ratio of IL-1agonist to antagonist and a higher expression of the receptor in fallopian tubes may be implicated in ectopic pregnancy implantation in the oviduct.[37]

Matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMPs)

The mechanism underlying cellular invasion includes tissue remodeling of the extracellular matrix, which is regulated in part by matrix metalloproteinases. This multigene family of endopeptidases is capable of degrading components of the extracellular matrix and is important to many physiological and pathological processes, including embryo implantation and cyclic endometrial breakdown. So far, at least twenty-five members of the MMP family have been identified in human beings.[38] Earlier studies showed that MMPs and their inhibitors, the tissue inhibitors of metalloproteinase, are crucial during implantation and mediate in vitro trophoblast penetration. They are regulated by several cytokines, including IL-1 and TGF-β, which are expressed by decidual stromal cells and trophoblast cells.[39]

The MMPs share several important characteristics, such as common mode of activation, a conserved amino acid sequence at the putative metal binding-active site region, and inhibition by specific proteinase inhibitors known as tissue inhibitors of metalloproteinases.[40] MMP-9, an important enzyme involved in the degradation of the basement membrane (primarily collagen type IV), is crucial to the invasive ability of trophoblast cells.[41-43] MMP-2 (72 kd) and MMP-9 (92 kd) have been shown to cleave native collagen into two fragments at a single site[44,45] and are expressed by migrating trophoblasts from outgrowth mouse blastocysts.[46] The regulation of MMP-2 and MMP-9 expression may play a central role in embryo implantation and placentation.[47] The naturally occurring specific inhibitors, tissue inhibitors of metalloproteinases, of decidual or trophoblast cell origin also have an important physiological role in regulating trophoblast invasion.[48] TIMP-1 is a glycoprotein with a molecular mass of 28.5-kDa that binds in a stoichiometric manner to form a complex with activated interstitial collagenase, stromelysin and MMP-9.[49] TIMP-2 is closely related in activity to TIMP-1.[50] TIMP-3, a novel member of the TIMP family, has been shown to have inhibitory activity against stromelysin-1, collagenase-1 and MMP-9.[51] Studies have demonstrated that the expression of TIMP in mouse uterine wall[52] and hatching blastocysts[46] results in an attenuation of trophoblast penetration in vitro. Significant expression of TIMP-3 is seen in maternal cells in the area surrounding invading mouse embryonic tissue.[53]

Simultaneous expression of MMP-9 and its inhibitors in early human decidua suggests that the activity of MMP-9 is also regulated by TIMP and this may play an important role in the myometrial invasion of trophoblast cells.[54] Human endometrium is an active site for cytokine production and action.[55] Both IL-1 and tumor necrosis factor (TNF-β) stimulate the secretion of MMPs by the cultured stromal cells in a concentration dependent manner.[56] Furthermore, TGF-β, a putative regulator of trophoblast function, and IL-1 may also play an intermediary role in trophoblast invasion by regulating trophoblast expression of MMP-9.[57] MMP-9 and its inhibitors mRNA expression has been demonstrated in cultured human endometrial stromal cells.[39] It has further been hypothesized that both IL-1 and TGF-β may play a crucial role in embryo implantation at the embryo-maternal interface by regulating stromal cell expression of MMP-9 activity as well as TIMP-1 and TIMP-3 protein expression.

The vascular endothelial growth factor (VEGF) system

Mammalian embryonic development and growth depends on the trophoblast cell and the embryo must be capable of adhering to the maternal uterine surface to allow the rapid invasion that ensures successful implantation.[58] After invading the maternal endometrium, embryonic development is characterized by a dramatic growth of vascular membranes and the formation of the placenta; this is regulated mainly by VEGF system.

The VEGF system is composed of one agonist, two transmembrane receptors, the kinase insert domain containing receptor (KDR) and the fms-like tyrosine kinase (Flt-1), as well as one soluble receptor (sflt) that acts as antagonist. The agonist, VEGF, is a dimeric heparin-binding glycoprotein that has been purified as a vascular permeability factor from various tumor cell lines and that has been shown to increase the proliferative ability of vascular endothelial cells in vitro by acting as a highly specific mito-

gen in these cell types.[59-62] The VEGF ligand system is composed of five isoforms created by alternative splicing of the VEGF mRNA. The human VEGF proteins have been characterized to consist of 121, 145, 165, 189 and 206 amino acids.[63-65] All isoforms contain exon 1 to 5 and 8. They differ only in having, in addition, various combinations of the other exons. Biologically, $VEGF_{121}$, $VEGF_{145}$ and $VEGF_{165}$ are secreted forms, while $VEGF_{189}$ and $VEGF_{206}$ appear to be bound to the cell surface.[66,67]

There are two major transmembraneous tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR) that bind to all the secreted VEGF-isoforms. Binding of VEGF to either of the receptors induces autophosphorylation and signal transduction but the initiation of a biological response is facilitated through the binding of VEGF to KDR rather than to Flt-1.[68-70] There is also a soluble form of the Flt-1 receptor called sflt, which is generated by alternative splicing of the Flt-1 mRNA and this mRNA encodes a protein similar to the Flt-1 protein but without the transmembraneous region and the intracellular kinase-insert domain. This soluble receptor acts as a specific high-affinity antagonist of VEGF function by competitively binding with VEGF; this prevents the agonist-receptor interaction and thus the induction of a biological response.[71-73]

In the human endometrium, VEGF has been detected at the mRNA and protein levels throughout the menstrual cycle with maximal expression during the secretory phase endometrium. The protein is predominantly localized in glandular epithelial cells, suggesting VEGF may play a central role during embryo implantation. The level of mRNA expression of the transmembraneous receptors Flt-1 and KDR as well as the soluble receptor (sflt) in human endometrium has been determined throughout the menstrual cycle. The results show that while mRNA for the transmembrane receptors Flt-1 and KDR was expressed at an almost constant level throughout the menstrual cycle, the soluble receptor shows a three-time higher level of expression during the proliferative phase compared to the secretory phase.[74,75] This down-regulation of sflt, which functions as a VEGF antagonist during the secretary phase of menstrual cycle may result in the initiation of the maternal endothelial receptors to angiogenetic stimuli secreted by the implanting embryo. In addition, VEGF mRNA expression has been demonstrated during the first cleavages of human preimplantation embryo development. Furthermore, it was demonstrated in another study that blastocysts express the mRNAs encoding for the free VEGF proteins, enabling the implanting embryo to immediately induce angiogenesis at the implantation site by VEGF proteins binding to the endometrial receptors.[76,77]

Gonadotropin releasing hormone (GnRH) system

GnRH regulates gonadotropin biosynthesis and release from the anterior pituitary via a specific receptor. Expression and action of GnRH has been demonstrated in several extrapituitary organs.[78,79] Recently, a possible role for GnRH in pre-implantation embryonic development, endometrial preparation and implantation has been suggested. It has been shown that both GnRH and its receptor are expressed by the human endometrium at the mRNA and protein level *in vivo* throughout the entire menstrual cycle.[80,81] Moreover, it has also demonstrated that preimplantation embryos (mouse and human) and the fallopian tube also express this hormone and its receptor, suggesting that GnRH may play a role in the embryonic/endometrial dialogue during early implantation.[36,82]

GnRH agonists are now used routinely in conjunction with exogenous gonadotropins in most ovulation induction protocols during human in vitro fertilization (IVF). The benefits of using these analogues are an increase in the overall pregnancy rate by preventing premature LH surge and an increase in the number of retrieved oocytes and embryos transferred.[83] In addition, inadvertent GnRH administration during early pregnancy has suggested that there may be a role for extrapituitary GnRH in human early embryonic implantation.[84,85]

GnRH is the hypothalamic hormone that controls secretion of both FSH and LH from the anterior pituitary.[86] It is also one of the paracrine/autocrine regulators of human trophoblast HCG secretion during early pregnancy.[87] Embryo implantation is the result of an embryonic-maternal dialogue, in which the embryo and the endometrium induce changes in each other to promote successful placentation and pregnancy. The role of GnRH in controlling placental HCG production and secretion has been fully demonstrated both in vitro[88] and in vivo,[89] especially in the first-trimester placenta. An increasing body

of evidence indicates that, in addition to this central action, a variety of human tissues express extra-hypothalamic GnRH that is immunological, biologically, and chemically identical to the hypothalamic hormone.[90]

Recent studies have demonstrated the expression of human GnRH-receptor mRNA in several extrapituitary organs such as the placenta, myometrium, breast, prostate, and ovary.[79,91-94] Studies have shown that the expression of extra-hypothalamic GnRH is regulated by IL-1β and this is assumed to be of embryonic origin in cultured human endometrial stromal cells; this demonstrates a possible role for this decapeptide in the embryonic-endometrial dialogue necessary for embryo implantation.[95] This is compatible with the physiological level of IL-1 during embryo peri-implantation interface.[96] These results are concordant with an earlier report that infertile women undergoing IVF have a significantly higher success rate and implantation rate if the administration of the GnRH agonist is maintained during the early stages of embryo development and implantation.[84,97]

Interleukin-18 (IL-18) system

The immune system's action on the endometrium could be modulated by various locally produced cytokines, such as TNF, IL-1, IL-16, IL-8 and IL-15.[27,98-101] Recently, the existence for the possible roles of the interleukin-18 (IL-18) system throughout the human reproductive system has been amply documented; however, IL-18 expression does not seem to show any characteristic variations during the menstrual cycle.[102] IL-18 is a new member of the interleukin family.[103-107] The IL-18 system is a family of polypeptides comprised of IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP) and IL-18 precursor (proIL-18). IL-18 was originally identified as a circulating molecule in endotoxin-challenged mice following bacterial priming, and was cloned from activated macrophages as interferon-γ inducing factor (INF-γ). IL-18 is produced as a biologically inactive precursor, and active IL-18 is secreted after cleavage by caspase-1 or other caspases.[108,109] IL-1 is also processed by the same pathways.[110,111] Structurally, IL-18 is similar to the IL-1 family of proteins. Functionally, IL-18 promotes a T-helper 1 (TH1) response through induction of INF-γ.[112,113] In addition, IL-18 stimulates the production of IL-1β, which exerts pro-inflammatory effects through the IL-18 receptor that are identical to IL-1 receptor binding protein.[114]

A soluble binding protein for IL-18 (designated IL-18BP) identified in human urine, acts as a natural inhibitor of IL-18-induced IFN-γ, and suppresses the TH1 response.[115] IL-18BP has been shown to neutralize IL-18 function, suggesting that the IL-18 system operates via collaboration between its receptor and binding protein. IL-1β and IL-18 are structurally homologous proteins, as are their receptors, and together they are members of the IL-1R/toll-like receptor superfamily. This superfamily exhibits similar signal and signal transduction mechanisms.

Similarities between IL-1β and IL-18 exist at several levels. Unlike the IL-1 system, the IL-18 system does not have an antagonist. A secreted soluble binding protein for IL-18 has been recently characterized in human urine as IL-18 binding protein and acts as a natural inhibitor of IL-18-induced IFN-γ, and suppresses the Th1 response. Similar to the effect of neutralizing antibodies of IL-18, the IL-18BP prevents LPS-induced IFN-γ production. In addition, similar to anti-IL-18 antibodies, IL-18BP does not affect IFN-γ production after stimulation with mitogens such as concanavalin A. It has become clear that IL-18 is a proinflammatory cytokine and that its mechanism of action may be independent of its ability to induce IFN-γ. IL-18BP circulates in healthy humans at a concentration of 2.15 ± 0.15 ng/ml (range 0.5-7).[116] In general, the molar excess of IL-18BP to IL-18 is on the order of 20-30-fold. It seems that IL-18BP is an effective inhibitor of immune and inflammatory disease that may be associated with increased production of IL-18.

The presence of the complete IL-18 system, including mRNA expression and protein production has been demonstrated in both the proliferative and secretory phases of the endometrium. Moreover, the steady state amount of human endometrial IL-18 system mRNA expression and ratio of IL-18 to its inhibitor, IL-18BP, in the human endometrium at different times during the menstrual cycle has been measured. Both IL-18 and IL-18R mRNA expression decreased, and, IL-18BP expression increased, in human endometrium during the secretory phase compared to the proliferative phase. In addition, there is a significantly lower ratio of IL-18 to IL-18BP pro-

teins in secretory endometrium compared to proliferative phase endometrium. The presence of a complete IL-18 system in human endometrium may play a crucial role in embryo-maternal interactions during the process of early embryonic implantation. Expression and the appropriate ratio of IL-18 agonist to IL-18 BP during the implantation period of secretory endometrium may modulate the TH1/TH2 cytokine networks during embryo implantation.[117]

In summary, embryo implantation is a progressive process that requires communication between two different organisms, the embryo and endometrium, and consists of three consecutive phases; apposition, adhesion and invasion. It must be realized that the period called the "window of implantation" is characterized by morphological and biochemical changes to the endometrium including plasma membrane transformation as well as the presence of various specific adhesion molecules, cytokines, growth factors, proteinases and neoangiogenesis factors, all of which can have a wide range of paracrine, autocrine and endocrine activities (Fig. 1). Further studies on cytokine and growth factor expression in the endometrium and in preimplantation embryos will provide a better understanding of their roles in human infertility and pregnancy loss; this should open the way to the identification of new IVF treatment options.

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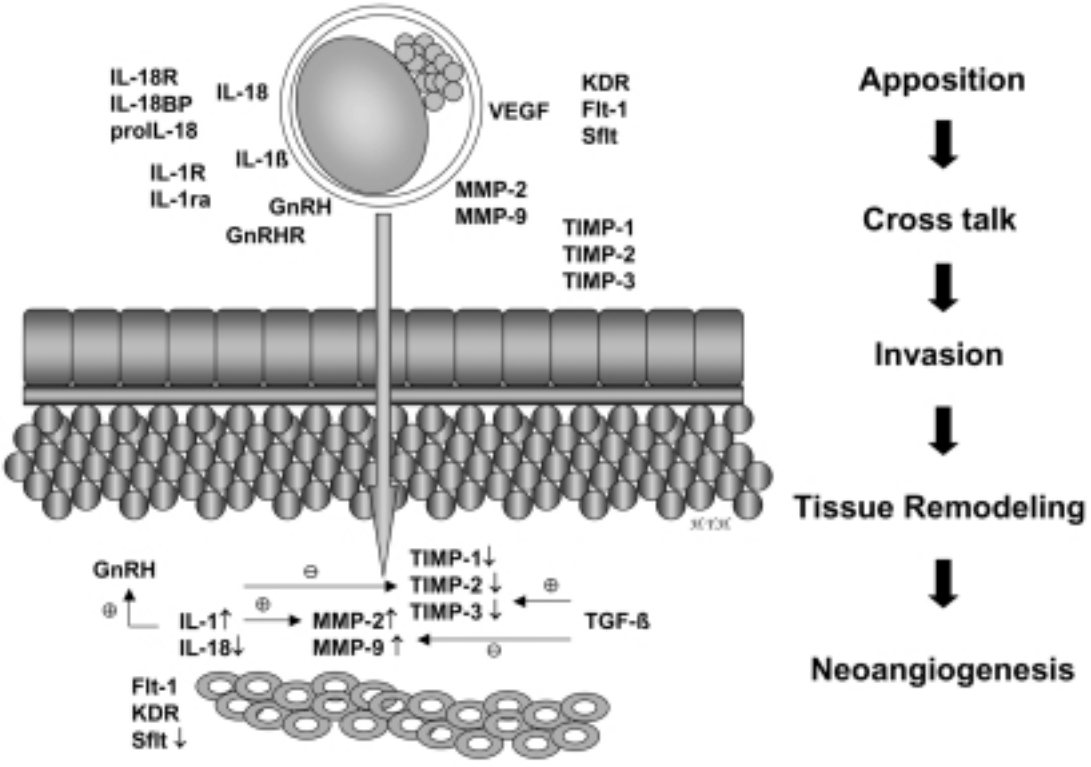

Fig 1. Schematic illustration of the cytokine network during embryo implantation.

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胚胎著床之分子生物機轉

黃泓淵[1,2]

胚胎著床在懷孕的過程中是一相當重要的環節，因此若能瞭解胚胎著床的機轉以及其所涵蓋之生子生物因子調節的作用，將有助於我們如何來改善對於不孕症患者接受人工生殖科技，胚胎植入後著床失敗的可能原因。成功的胚胎著床有賴於胚胎與其對應的母體子宮內膜間有一適當的分子生物調節者來扮演對話者的角色，而此一調節者，細胞動力素，特別是介白質系統則是在此一胚胎與母體子宮內膜的互動介面上，其中一相當重要的調和者，藉由細胞間或細胞內而達到生物訊息傳遞的目的。其次胚胎在孵化後，其滋養層細胞必須對子宮內膜上皮細胞進行附著並穿透基底膜進入子宮內膜基質細胞層，以達到建立與母體的血液循環之目的，而使胚胎能順利生長，繼續懷孕的過程。此一滋養層細胞穿透子宮內膜的過程與細胞外母質的組織重整有密切關聯，而此一組織重整的生理現象則是受到基母質金屬蛋白酶 (MMP) 與其抑制劑，金屬蛋白酶組織抑制劑 (TIMP) 的控制與調節。當滋養層細胞侵入母體子宮內膜伴隨著胚胎的發育，血管快速的增生形成血管床而完成胎盤之生成以利於懷孕之進行，這些血管生成則是由血管內皮生長因子系統調節與控制。本篇綜論著重於在胚胎著床機轉中母體子宮內膜與胚胎間藉由介白質系統、基母質金屬蛋白酶系統、促性腺激素釋放荷爾蒙系統與血管內皮生長因子系統調控之分子生物機轉。(長庚醫誌 2005;29:25-36)

關鍵字：介白質，胚胎著床，基母質金屬蛋白酶，血管生成，子宮內膜。

[1]長庚紀念醫院 台北院區 婦產部，[2]長庚大學 醫學院 婦產科
受文日期：民國94年11月6日；接受刊載：民國94年12月8日
通訊作者：黃泓淵醫師，長庚紀念醫院 婦產部。桃園縣333龜山鄉復興街5號。Tel.: (03)3281200; Fax: (03)3288252; E-mail: hongyuan@cgmh.org.tw