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ENDOCRINE AND PARACRINE CORRELATES OF ENDOMETRIAL RECEPTIVITY TO BLASTOCYST IMPLANTATION IN THE HUMAN

D. GHOSH* AND JAYASREE SENGUPTA

Department of Physiology,
All India Institute of Medical Sciences,
New Delhi – 110 029

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Abstract : Synchronous attainment of maternal endometrial receptivity allows implantation-stage adhesive blastocyst to undertake apposition, attachment and invasion. In the present essay, we propose a model according to which luteal phase progesterone induces a basic drive in endometrium toward receptivity and as a result, adequately primed endometrium differentiates through certain steps in a fixed action pattern. The implantation-stage embryo senses such endometrial responsiveness circumstantially by the factors secreted by maternal endometrium and undertakes differentiation to implant by secreting factors which act on maternal endometrial cells to further potentiate them to implantation stage-specific changes. Such a dynamic temporo-spatial manner of interaction involving a set of specific factors acting synchronously leads to the activation of innate releasing process in both compartments towards embryo attachment followed by successful intrusion and controlled invasion of trophoblast cells into maternal endometrium. In the present review we discuss the potential role of various endocrine and paracrine factors in the process of blastocyst implantation in the human.

Key words : cytokines endocrinology endomterium implantation paracrinology preimplantation embryo receptivity

INTRODUCTION

The term uterine 'receptivity' refers to a state when endometrium allows blastocyst to attach, penetrate and induce localized changes in the stroma resulting in decidualization (Fig. 1). These changes are initiated in the human by around day 7 after fertilization, and by day 16 secondary villi begin to form. Although the physiological and biochemical determinants which allow endometrium to enter into the state of receptivity for human embryo attachment and implantation remain poorly understood, various endocrine and paracrine correlates of endometrial receptivity and implantation

*Corresponding Author : Fax : 91-11-26588663; Tel. : 26594625; Email : dghosh_57@hotmail.com

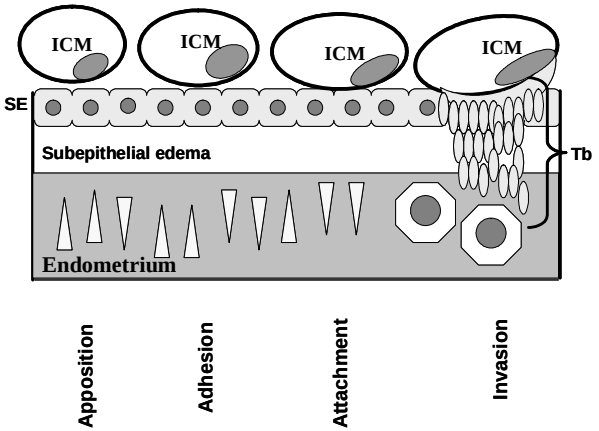

Fig. 1 : Schematic representation of the phases of implantation in the human. It is generally believed that zona-free blastocyst undertakes the process of implantation via a sequel of critical events like apposition, adhesion, attachment and invasion towards an increasing closeness to maternal endometrium which remains receptive to the blastocyst in synchronous manner. Subepithelial edema arising from increase in vascular permeability is initiated in receptive stage endometrium, and is further accentuated at implantation with venular dilatation. In primates, blastocyst implantation occurs with trophoblast cells (Tb) adjacent to embryonic pole of inner cell mass (ICM) orientating and apposing/adhering to luminal surface epithelium (SE). Following invasion of SE, endometrial stromal fibroblasts (*triangles*) transform into epitheloid decidual cells (*hexagons*) as decidualization ensues.

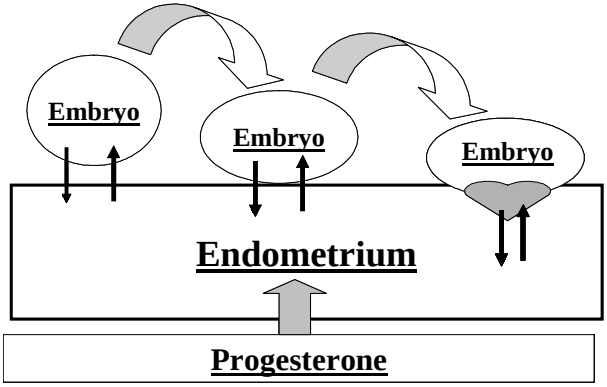

Fig. 2 : Schematic representation of a proposed model of blastocyst implantation in the human. According to this model, progesterone creates a basic drive in the endometrium resulting in its differentiation along with luminal secretion. The biomolecules in luminal secretion promote synchronous embryonic growth and differentiation, which in turn liberates specific molecules for releasing innate endometrial response for implantation. Such dynamic interaction between two heterogeneous compartments proceeds via synchronous exchange of stage-specific factors.

are well documented from studies in areas directly related to human reproduction, as well as, research in allied areas, and from studies using small animals and non-human primates. The present essay is an exercise to the effect of developing a tentative model of endocrine and paracrine correlates of endometrial receptivity toward blastocyst implantation in the primate (Fig. 2). In the present discussion, we shall attempt to examine this model in terms of the physiological nature of the functional modules, namely endometrial factors under endocrine influence, embryonic factors potentially involved and the dynamics of the chemical interaction along the process of increasing closeness between the two compartments - embryo and endometrium.

Endocrinology of endometrial receptivity

Estrogen

Generally, synchronous development of embryo and endometrium, which is a prerequisite for blastocyst implantation, is dependent upon the actions of ovarian estrogen and progesterone. Normal implantation, gestation and delivery can be experimentally obtained from surrogate embryo transfer combined with estrogen plus progesterone in ovariectomized rhesus monkeys (1), and in women having

primary ovarian failure (2, 3). It is often pragmatically assumed that mid-luteal phase rise of estradiol is required for blastocyst implantation in the human. However, it has been reported that luteal support with progesterone alone to women with inadequate or absent ovaries led to normal secretory maturation of endometrium (4). In a study aimed to investigate whether luteal phase ovarian estrogen is essential for endometrial preparation for blastocyst implantation, rhesus monkey embryos were transferred to either acutely ovariectomized, or long-term ovariectomized, hormone-primed surrogate recipients; implantation and live births were obtained in both groups following supplementation with progesterone alone (5). Examination of endometrial histology also failed to indicate any insufficiency in glandular and stromal characteristics of mid-luteal phase monkey endometrium in the absence of mid-luteal phase ovarian estrogen (5). It appears that luteal phase ovarian estrogen is not essential for progesterone-dependent endometrial receptivity and blastocyst implantation and pregnancy maintenance in the rhesus monkey (5, 6). Luteal phase estrogen support is also not required for the establishment of pregnancy in women (7). High serum estradiol concentrations on the day of hCG administration in patients undergoing ovulation induction demonstrated that endometrial receptivity and not oocyte quality was affected (8). Furthermore, high estradiol may inhibit embryo adhesion for its direct toxic effect on cleavage stage embryo (9). Nevertheless, the possibility that the level of estradiol typically present in the mid-luteal phase endometrium may be permissive for endometrial factors required for blastocyst implantation cannot be ruled out based on available indirect evidence (10–12). Besides a potential permissive action, mid-luteal phase estrogen may balance the effect of relatively high level of mid-luteal phase progesterone on other tissue systems (13). Such a multi-event, multi-system based complex involvement may explain why the mid-luteal phase rise in ovarian production of estrogen had been selected in the human in the course of the natural selection. There are also reports that luteal phase treatment with anti-estrogen like tamoxifen or with anti-estradiol antibody may inhibit implantation (14, 15). Furthermore, a putative role of locally available estrogen, either from endometrial stromal cells (16, 17), or from blastocyst (18) around the time of implantation remains to be explored in primates.

Progesterone

Progesterone however is essential for endometrial preparation for blastocyst implantation in most mammals including primates. Inadequate endometrial maturation and progesterone insufficiency are well known causes of infertility. It is substantiated by the reports that application of a high affinity antiprogestin like mifepristone (RU486) and onapristone (ZK98 299) during early luteal phase can delay or inhibit endometrial maturation for implantation resulting in contragestion (19–22). Progesterone also maintains embryo viability, presumably indirectly through its action on the uterus (23). Pre-implantation stage embryo growth and viability is adversely affected by inadequate endometrial maturation following blockade

of progesterone action at the receptor level during the luteal phase by an antiprogestin like mifepristone (24, 25). Despite the fact that serum concentration of progesterone is very high during the mid-luteal phase of the ovulatory cycle, it appears possible that endometrial maturation towards receptivity does not require a very high concentration of progesterone in peripheral circulation, and implantation stage embryo can withstand a partial lack of progesterone for a limited period of time (26–28).

An apparent enigma in this process is that endometrial epithelial cells in the functionalis zone are generally progesterone receptor negative, however, stromal cells are progesterone receptor positive (29). It appears plausible that progesterone regulates endometrial epithelial differentiation during blastocyst implantation via a stromal cell mediated paracrine action of progesterone (23). Furthermore, it has been speculated that loss of progesterone receptor in surface and glandular epithelium during receptivity is an essential pre-requisite for the production of specific secretory proteins which may play critical role in embryo viability and implantation (23). Thus, a complex network of cytokines and other cellular factors involving endometrial cells and implantation stage embryo under progesterone dominance plays a cardinal role for endometrial receptivity. The nature of potential involvement of various endometrial bio-molecules in the process of endometrial receptivity toward ovo-implantation under progesterone dominance will be discussed in the section of *Functional correlates of endometrial receptivity for blastocyst implantation.*

Chorionic gonadotropin

Substantial evidence now suggests that chorionic gonadotropin (CG) from embryo can be regulated by paracrine factors secreted from mid-luteal phase endometrium under progesterone dominance, and embryonic CG potentially modifies the endometrial differentiation for blastocyst implantation and also up-regulates ovarian progesterone synthesis and secretion giving rise to a self-sustaining progressive flow-loop of synchronous development of endometrium and embryo (30, 31).

Embryonic CG thus exhibits two types of biological action: juxtacrine action on implantation stage endometrium, and endocrine action on corpus luteum. While the endocrine action of CG is well documented, its juxtacrine action on endometrium has only recently been receiving due importance. In the first type of action, CG acts on midluteal phase endometrium and promotes endometrial differentiation toward implantation (30, 31). For example, *in vitro* studies substantiate that CG can influence decidual transformation of endometrial stromal cells (32, 33). Uterine infusion of CG on post-ovulation day 10 of non-mated cycle in the baboon can trigger endometrial response (epithelial plaque reaction) typically seen in maternal endometrium around the time of blastocyst attachment and implantation (34). Uterine CG infusion during the midluteal phase of normal cycles in baboons and women typically regulate the secretion of endometrial factors related to its differentiation for receptivity and implantation (30, 35). It appears that CG

may have paracrine influence on endometrial matrix metalloproteineases (MMPs) synthesis and secretion under progesterone dominance and thereby play an important role in the tissue response during trophoblast invasion (30).

Relaxin

Relaxin is a luteal peptide hormone. It has been shown to increase at the time of implantation, and it is suggestively regulated at least partially by chorionic gonadotrophin, CG (36). In embryo transfer experiment using normally cycling rhesus monkeys, it was observed that the delay in the appearance of CG in circulation correlated well with a delay of appearance of relaxin (28).

The questions whether relaxin is essential for blastocyst implantation, and what are the functional correlates of relaxin in this process in the primate by and large remain to be investigated. There is now evidence that relaxin can promote uterine growth and affect uterine contractility (37–39), and it presumably exerts permissive action to progesterone towards endometrial differentiation around the time of implantation (40, 41). Relaxin causes vasodilation (42), inhibits mast cell degranulation (43), depresses platelet activation (44) and counteracts immune reaction induced by antigen exposure (45) in various tissue systems. These functional properties of relaxin may be involved in endometrial preparation for embryo implantation.

There is now evidence that relaxin may also regulate endometrial paracrinology around the time of blastocyst implantation. Bryant-Greenwood et al. (41) have shown that implantation stage human endometrium sequentially express relaxin, prolactin and insulin like growth factor binding protein 1 (IGF-BP1) in a temporo-spatial manner. It is interesting to note that relaxin is a homologue member of insulin like growth factor (IGF) family and it stimulates IGF-BP production by human endometrial cells (40). Recently, relaxin has also been associated with neovascularization of the endometrial lining of the uterus, via specific induction of angiogenic growth factors (46). Whether such functions of relaxin are essential for blastocyst implantation remains only speculative at this time, because it has been observed that normal pregnancy in women can be achieved following ovum transfer with no detectable relaxin in peripheral circulation (47).

Functional correlates of endometrial receptivity for blastocyst implantation

Luminal epithelial pinopodes

Several endometrial functions are influenced by luteal phase progesterone and appear to be robust correlates of endometrial receptivity. The luminal surface is considered to play a critical role in embryo-uterine interaction. Pinopodes differentiate on apical surface of luminal epithelium in human receptive stage uterus under the influence of progesterone, and these may allow absorption of luminal fluid and thereby facilitate embryo adhesion (48). Although pinopode expression on surface epithelium is considered a reliable biological marker of endometrial receptivity (49, 50), its functional significance is open to

question (51). A high level expression of glutaredoxin in pinopodes may play an important role in endometrial epithelial cells during blastocyst implantation (52). A 24 kDa heat shock protein (HSP) that has been shown to exhibit discrete expression in luminal epithelial cells of human endometrium around the time of implantation (53) may be involved in pinopode differentiation through influencing the organization of microfilament resulting in at least two broad functions: facilitating embryo apposition and influencing signal transduction (26, 54). Features like glandular hyperplasia, and changes in vascular bed along with stromal edema in endometrium during receptivity and implantation in conception cycle under progesterone dominance are also evident (55–57).

Endometrial protein factors

The endometrial samples classified as normal based on histology may however be found abnormal on the basis of endometrial protein expression (58, 59). Progesterone during the luteal phase is known to modulate the synthesis and secretion of a number of endometrial proteins (60). Pregnancy associated endometrial alpha-2 globulin (α-2 PEG, also known as placental protein 14, PP14, or glycodelin) appears in mid-luteal phase and is a glandular marker of endometrial function (61). Estimates of uterine luminal concentrations of PP14 collected on LH+6 day have revealed significantly low levels of PP14 in washings collected from a group of women with recurrent miscarriage having both histologically normal and retarded endometrial development as compared with normal fertile women (62). The expression of endometrial PP14 during different phases of the menstrual cycle in the rhesus monkey is similar to those of human endometrium during the menstrual cycle (63). Furthermore, endometrial expression of PP14 decreased following a single dose early luteal phase administration of antiprogestin, a treatment that resulted in inhibition of blastocyst implantation in the rhesus monkey (63). Also, relaxin potentiates the expression of PP14 in receptive endometrium in the human (31). The functional relevance of PP14 in peri-implantation endometrium (64) is not known, however, there is evidence to suggest that this polypeptide may play a role in immunomodulation (65).

Furthermore, progesterone-induced uterine protein-1 (PUP-1) and prolactin secreted by endometrial cells during the luteal phase may be involved in embryo-endometrium interaction, implantation and decidualization (66, 67). Even in the absence of precise knowledge about the physiological significance, the expression of such proteins in endometrial cells may potentially be useful for identifying a receptive stage endometrium (48).

Progesterone inhibits superoxide radical formation (68,69) and tumour necrosis factor (TNF)-α in endometrium (70, 71), and thereby inhibits the occurrence of degenerative changes in the tissue. Superoxide dismutase (SOD) is a scavenging system for superoxide radicals and is highest in endometrium at mid-secretory stage of the cycle and SOD has been recovered in human uterine fluid in pre- and peri-implantation stage (72). SOD could

play a prominent role in protecting blastocysts from superoxide radical damage. Thus, luteal phase anti-progestin (mifepristone) treatment may result in increased superoxide radical formation and increase in TNF-α production compromising endometrial maturation, embryo viability and blastocyst implantation (73, 74).

In recent times, several other endometrial factors have been implicated in the process of blastocyst implantation. Endometrial epithelial cells synthesize a large amount of calcitonin (75, 76) and HOXA-10 (77, 78). While calcitonin may regulate calcium homeostasis in these cells and thereby may influence cell-cell adhesion at the time of blastocyst adhesion (76), HOXA-10 which belongs to the homeobox gene family may regulate the specific genomic expression around the time of implantation (79). Although calcitonin and HOXA-10 appear to be important for blastocyst implantation in small mammals, their significance in blastocyst implantation in the human is only suggestive at this point of time. The suggestion that leptin is involved in the process of blastocyst implantation and placentation in the human (80, 81) also needs to be examined closely.

Additionally, endometrial plasminogen activator inhibitor and other protease inhibitors are also increased around the time of blastocyst implantation (82–84). Furthermore, progesterone inhibits the expression of matrix metalloproteinases (MMPs) and stimulates the release of tissue inhibitors of metalloproteinases (TIMPs) in endometrium, and thereby influences ECM organization (85, 86). Administration of antiprogestin during the luteal phase results in high tissue plasminogen activity and high levels of MMPs causing dysregulation of endometrial maturation (87). With the event of trophoblast invasion into the maternal endometrium, manifestation of an ordered sequence of specific combination of MMPs and integrins, and TIMPs presumably under the influence of CG and local cytokines results in a controlled intrusion of luminal epithelium by trophoblast cells (30, 79, 88).

Surface glycoproteins and cell adhesion molecules

Surface glycoproteins may play an important role in regulating maternal-fetal interactions at implantation. The composition and profile of glycoproteins, degree of glycosylation, charge properties of the glycocalyx and the proteoglycans secreted into luminal fluid are known to change during the receptive phase of the uterus (89). Antigens involving α1-3-fucosylated type 2 chain (Le^x and Le^y) secreted by glandular epithelium may have a potential in mediating embryo adhesion; Le^y antigen has also been detected in embryonic cells in a stage specific manner and could play a role in trophectoderm recognition during implantation (90). In the rhesus monkey, the expression of Le^y is maximal on luminal and glandular epithelium of endometrium around the time of implantation in normal menstrual cycles, as well as, in fecund cycles (91); its expression is inhibited by a single dose of early luteal phase anti-progestin (mifepristone) treatment, which also inhibits blastocyst implantation in this species (91).

Polymorphic epithelial mucin (MUC1) is a secretory product of endometrium and its synthesis and secretion is high in midluteal

phase endometrium, presumably under progesterone dominance (92). Given the fact that MUC1 is highly polymorphic in nature and generally anti-adhesive in function, the functional significance of high levels mucin production by receptive endometrium in primates is only speculative at this point of time. It seems likely that histo-blood group related antigens carried by mucin molecule may help in initial apposition of implanting blastocyst, and in the next step adhered blastocyst via paracrine factors results in selective break down of mucin at the specific site of nidus and thereby a cascade of increasing adhesion and attachment is initiated (93). This model helps to explain how uterine luminal surface allows for implantation process in an embryo stage-specific and site-specific manner in receptive endometrium, but does not allow intrusion of other infective agents. In mice however the mucin level is decreased in implantation stage endometrium (94).

Cell adhesion molecules involved in cell-cell and cell-matrix interactions have been recognized to contribute to cell migration, matrix organization and transduction of differentiation signals (95). The co-expression of $\alpha_v\beta_3$ and $\alpha_4\beta_1$ in human endometrium during the 'implantation window' has been documented and that lack of $\alpha_v\beta_3$ in luteal phase deficiency, minimal or mild endometriosis and infertility are consistent with the suggestion that these integrins are involved in the implantation process (96–98). Furthermore, there is evidence to suggest that several types of cell adhesion molecules including integrins, cadherins, CAM families and other adhesion molecules are expressed by preimplantation embryos and trophoblast cells (99–101), which may be involved in the process of interaction between embryo and endometrium during implantation in primates.

Endometrial vascular response

Non-invasive studies indicate that uterine blood flow is highest around the time of implantation in normal cycling women and that down-stream impedance is reduced with increased blood flow on the side of corpus luteum (102–104). Clinical studies have now shown that embryos fail to implant in women with impaired uterine perfusion (105, 106). Improvement in pregnancy rates in women with impaired uterine perfusion have been reported after the addition of low-dose aspirin to hormone replacement therapy (107); this could result from improved uterine blood flow since aspirin is known to shift the balance towards prostacyclin production (108, 109).

Increased endometrial vascular permeability is one of the earliest distinguishable features of implantation in several mammalian species. In the rhesus monkey, increased endometrial permeability occurs even prior to commencement of attachment and implantation when most blastocysts remain zona-encased (55, 110). Cellular mechanism responsible for increases in vascular permeability has not been clearly defined but endometrial prostaglandins (PGs) and platelet activating factor (PAF) have been suggested as possible candidates. A network of prostaglandins and PAF operative in endometrial cells and modulated by endocrine and paracrine factors at the time of implantation has been proposed (Fig. 3). According to this model,

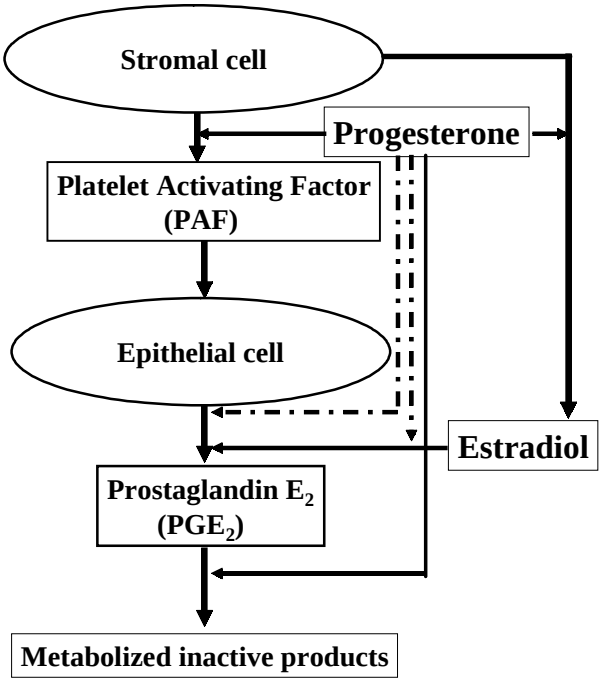

Fig. 3: Endocrine and paracrine regulation of prostaglandin (PG) E synthesis and metabolism in the receptive stage endometrium of the human. Major inactive metabolic end-product is 15-keto-13,14-PGE_2. *Solid line* indicates positive regulation and *broken line* indicates negative regulation. Various other factors like nitric oxide, interleukin-1, leukemia inhibitors factor, endothelin and epidermal growth factor may influence this network in the human endometrium during implantation. See the text for details.

progesterone stimulates PAF production by endometrial stromal cells; PAF along with oestradiol in turn acts on glandular epithelial cells and promotes PGE production; PGE in turn stimulates PAF production and aromatase activity in stromal cells. Thus, a positive feedback ensues. It now appears that various other factors like nitric oxide (NO), interleukin (IL-) 1, leukemia inhibitory factor (LIF), endothelin (ET)-1 and epidermal growth factor (EGF) may influence this network in the receptive and implantation stage human endometrium under progesterone dominance (31, 111–115). The net result is an increase in PGE to PGF ratio, which may mediate positive modulation of embryonic function, and vasodilatation, immunosuppression and decidualization in the endometrium at the time of implantation (31, 116, 117). When vascular impedance and blood flow are compromised around the time of implantation by the application of an anti-progestin, blastocyst fails to implant (21, 110, 117–119).

Cytokines as paracrine regulators

In recent times, several cytokines have been implicated to be involved in the process of endometrial maturation, embryonic development and most importantly in the functional interaction between receptive endometrium and synchronous stage, blastocyst in primates. Of these, the ones appear to be important in the human are discussed below. Table I gives a synopsis of

TABLE I: Blastocyst implantation and pregnancy outcome in knockout mice.

Gene	*Phenotype*	*Reference*
CSF-1	Ovulation affected. Implantation normal.	208
GM-CSF	Implantation normal. Placental deficiency.	209
TGF-β1	Intrauterine and peri-natal lethality.	210, 211
LIF	Implantation failure	212
LIFR	Intrauterine lethality	213
IL-1Rt1	Implantation normal	214
IL-6	Fertility reduced. Implantation rate low.	215, 216
IL-11Ra	Implantation failure. Defective decidualization.	164
Gp130	Intrauterine lethality	217
STAT3	Intrauterine lethality	218
TNF-α	Normal implantation. Abnormal development.	219
VEGF	Intrauterine lethality	220
Flt-1	Intrauterine lethality	221
Flk-1	Intrauterine lethality	222

results obtained from different studies using knockout mice for different cytokines, growth factors and their receptors.

Leukemia inhibitory factor (LIF)

Leukemia inhibitory factor (LIF) is a pleotropic cytokine. Its secretion *in vitro* by human endometrium during different phases of cycle has been studied from tissue biopsies collected from normal fertile women. This cytokine is secreted throughout the menstrual cycle, and its expression is high in progesterone dominated implantation stage endometrium (See 120 for details). Endometrial LIF concentration is less in infertile women with recurrent embryo transfer failure after IVF and in women with unexplained infertility (121, 122). Blockade of progesterone receptor inhibits endometrial maturation along with repressed expression of mid-luteal phase endometrial LIF (123). It has been suggested that endometrial LIF influences blastocyst implantation through autocrine-paracrine interaction at the luminal epithelium level and blastocyst stage (124). For example, recent evidence suggests that LIF can functionally mediate progesterone dependent endometrial differentiation like pinopode formation (125). Pre-implantation stage embryo bears the machinery for both LIF and LIF receptor (126). It has also been demonstrated that LIF can influence endometrial angiogenesis which is, at least partially, dependent on progesterone (127, 128) and may influence the regulation of CG secretion from implantation stage embryo (Fig. 4). However, the level of LIF secretion by implantation stage endometrium may have questionable diagnostic value for predicting its pregnancy potential (129, 130). Nonetheless, a significant functional role of LIF in blastocyst implantation has been proposed based on evidence that: (i) failure of implantation takes place after the administration of polyclonal antibody against LIF in the uterus during the peri-implantation stage (131, 132); (ii) administration of LIF promotes blastocyst implantation *in vivo* (132) and *in vitro* (133); and (iii) defective production of LIF at the feto-maternal interface has been observed to be associated with pregnancy loss (134).

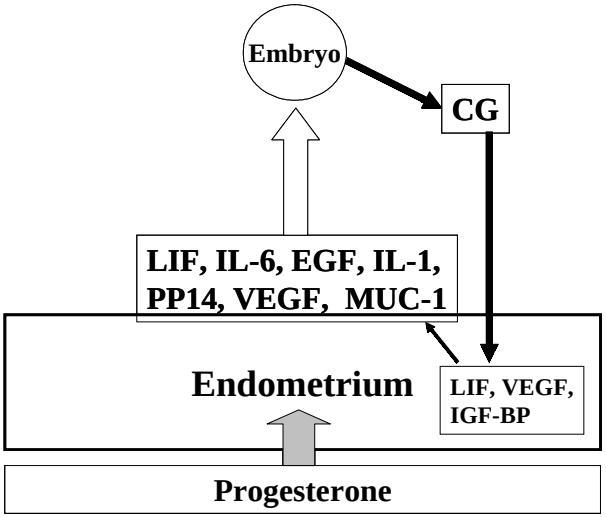

Fig. 4 : Biomolecules involved in embryo-endometrium dialogue during blastocyst implantation with potential embryotrophic action being elaborated by receptive stage endometrium. Indirect evidence suggests that endometrial interleukin-1 (IL-1), leukemia inhibitory factor (LIF), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), placental protein 14 (PP14) and mucin-1 (MUC-1) influence the synchronous growth, differentiation and attachment of implantation stage embryo. On the other hand, embryonic chorionic gonadotrophin (CG) can act on receptive stage endometrium in up-regulating LIF and VEGF and down-regulating IGF-BP.

Interleukins (ILs)

IL-1 family is comprised of IL-1α, IL-1β, IL-1 receptor antagonist and one class of signal transducing receptor, and they are expressed in the human endometrium (135) and human embryo (136). Although estradiol and progesterone only minimally modulates LIF gene expression and protein biosynthesis in human endometrium, LIF is stimulated by IL-1, TNF-α, PDGF, EGF and TGF-β in a time and concentration dependent manner (137). In fact, close synergism between IL-1α and TNF-α produced by decidual cells results in cooperative action towards an autocrine production of IL-6, IL-8 and LIF (138, 139). Since there is a minimal involvement of receptors for estrogen and progesterone in maternal endometrial cells of implantation zone in the Rhesus monkey (140), IL-1α from decidual-stromal cells may play a significant role in modulating LIF expression in maternal endometrial cells during early gestation (141).

IL-1β was localized predominantly in the maternal endometrium during blastocyst implantation (141). It has been reported by several groups that endometrial cells are involved in prostaglandin production under the influence of IL-1β (142–144) resulting in vascular and immuno-physiological responses to invading trophoblast cells. IL-1β may influence endometrial decidualization through the induction of insulin-like growth factor binding protein-1 (IGF-BP1), as shown in the baboon (145). Furthermore, IL-1 may induce vascular endothelial growth factor (VEGF), and thereby promote endometrial angiogenesis at the time of trophoblast invasion (146–149). Embryonic IL-1 has been shown to up-regulate integrin expression by endometrial epithelial cells, thereby facilitating adhesion of blastocyst to maternal endometrial mucosa at a time when the IL-1 receptor is high in human endometrium (150). Moreover, it has been shown to increase the expression of laminin and collagen receptors by trophoblast cells (151). It is also possible that IL-1β influences local concentration of hepatocyte growth factor activator inhibitor type I (HAI-1) which has been localized in proliferating type of trophoblastic stem cells, Langhan's cells of first trimester human placenta, but not in syncytiotrophoblast cells, and thereby regulate proliferation and invasion of trophoblast cells as shown in other cell types (152). It therefore appears feasible that IL-1 influences a multi-factorial process involving adhesion molecules, IGFs, VEGF, PGs and HAI (Figs. 4 and 5). It is of interest that IL-1 receptor antagonist can block embryo implantation in mice (153). Although similar evidence is not available for any primate species, human embryos secreting relatively higher amount of IL-1β show higher probability of successful evolutive pregnancy upon embryo transfer (see 154 for details).

It also appears possible that production of IL-6 by endometrial epithelial cells and decidual-stromal cells during implantation may be regulated by IL-1α and IL-1β (141). IL-6 is produced by epithelial and stromal cells of human endometrium, and epithelial cell production of IL-6 is increased during the secretory phase by IL-1 (155–157). Furthermore, estrogen has been shown to be a powerful suppressant of IL-6 production (158), and this inhibition occurs at the transcriptional level (159). Thus, a relative

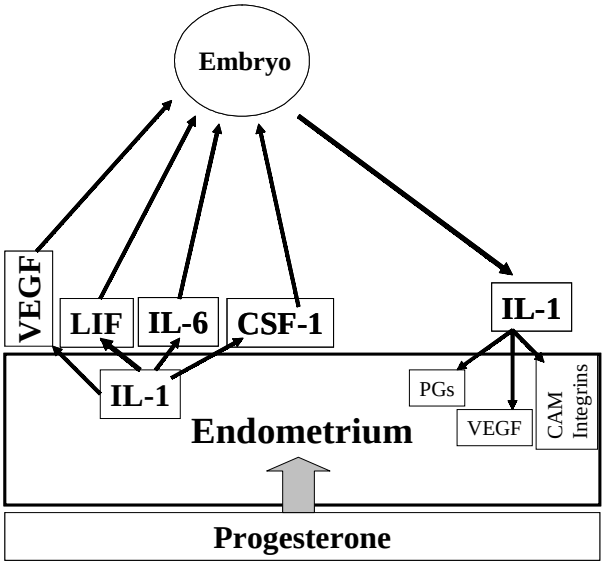

Fig. 5 : Biomolecules with potential embryotrophic effect elaborated by implantation stage endometrium. Indirect evidence suggests that interleukin-1 (IL-1) under progesterone dominance up-regulates several endometrial cytokines like leukemia inhibitory factor (LIF), interleukin-6 (IL-6), colony stimulating factor-1 (CSF-1) and vascular endothelial growth factor (VEGF), which influence the synchronous growth and differentiation of preimplantation embryo. On the other hand, embryonic IL-1 acts on endometrial factors like prostaglandins, cell adhesion molecules (CAM), integrins and VEGF.

absence of estrogen receptor at implantation site (140) may facilitate an IL-1 mediated positive action on IL-6. Based on available reports based on experiments using several species, it appears that IL-6 is important in the process of blastocyst implantation (141, 160–162), however the nature of the functional role of IL-6 in implantation remains conjectural. It is possible that IL-6 along with LIF, CSF-1, and other cytokines and growth factor regulates trophoblast differentiation and CG synthesis (Figs. 4 and 5). Based on data from mouse knockout model, it appears that IL-6 is not essential for normal fertility, while LIF is essential for the establishment of successful pregnancy (Table I). Although it has been reported that the level of endometrial IL-11 is high during mid- to late-secretory phase in the human (163), and mice lacking receptors for IL-11 show defective endometrial response to implanting blastocyst (164), whether IL-11 indeed plays any critical role in the process of attachment and invasion of blastocyst in primates is not known. Interestingly, mice with defective gp-130 mediated signal transducer and activator of transcription (STAT) signaling which is the down-stream mediator of activities of cytokines of IL-6 family (IL-6, IL-11, LIF, oncostatin M, ciliary neurotrophic factor, and cardiotrophin-1) show failure in blastocyst implantation (165; Fig. 6). Although there is no comparable evidence for any primate species, it has been demonstrated that luminal secretion of gp130 is significantly higher during receptive stage in fertile women compared with patients with primary unexplained infertility, while secretion of IL-6, soluble-IL-6R, and LIF did not differ between the two groups (130).

Transforming growth factor beta (TGF-β)

Despite the fact that expression of TGF-β is high in preimplantation stage endometrium of mouse (166), and its expression is enhanced by estradiol and progesterone in human endometrial stromal cells (167), the level of TGF-β1 in primate luteal phase endometrium is low at the time of implantation (128). Indeed, administration of luteal phase antiprogestin like mifepristone results in increased endometrial TGF-β associated with inhibition of epithelial cell maturation, and increased epithelial cell degeneration *in vitro*, and failure of implantation in monkeys (128, 168, 169). It

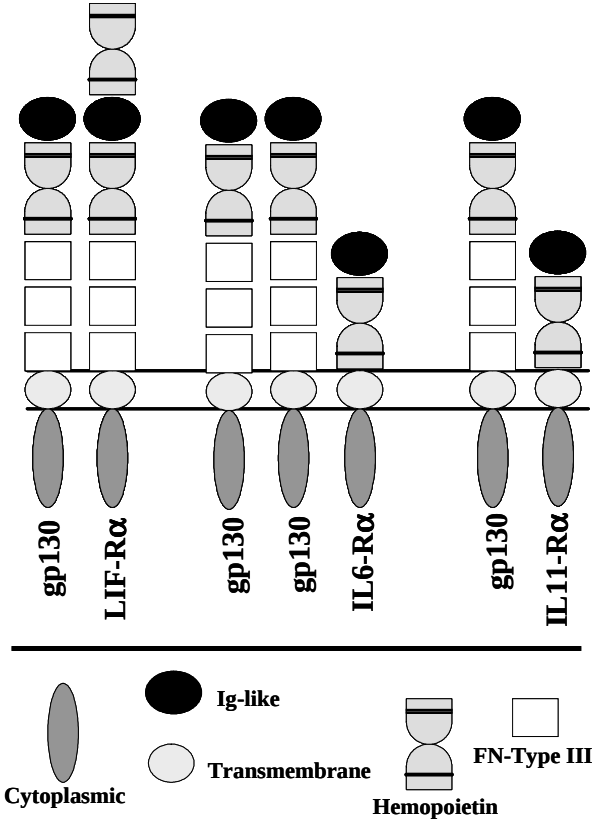

Fig. 6 : Receptors for gp130 cytokines. Leukemia inhibitory factor (LIF), interleukins-6 (Il-6) and -11 (IL-11) bind with their specific low affinity receptor alpha chains, which induce hetero-dimerization with gp130 resulting in activation of signal transduction pathways. Intrauterine lethality is high in gp130 knockout mice. Also, defective gp130-mediated signal transducer and activator of transcription (STAT) signaling results in failure of uterine implantation.

is evident that a '*time window*' of TGF-β synthesis and secretion is important. High levels of endometrial TGF-β during receptivity may adversely affect endometrial preparation, while decidual TGF-β at the time of invasion is important to restrict trophoblast invasion (Fig. 7).

Growth factors

Of several growth factors, peptides belonging to the family of insulin like growth factors (IGFs) and peptides belonging to the family of vascular endothelial growth factor (VEGF) appear important for endometrial receptivity and embryo growth and implantation.

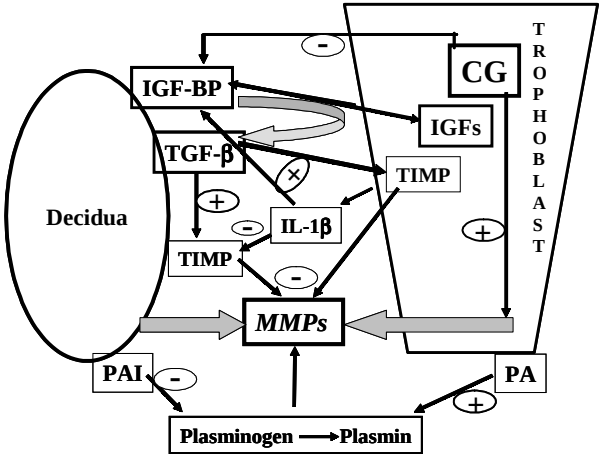

Fig. 7 : A model of paracrine interaction between embryonic trophoblast cell and endometrial decidual cell during trophoblast invasion. Decidual cells secrete insulin-like growth factor (IGF-BP) which regulates trophoblast invasion through its interaction with IGFs. Trophoblast cells also secrete chorionic gonadotrophin (CG) which down-regulates IGF-BP and up-regulates matrix metalloproteases (MMPs). Decidual cells secrete transforming growth factor β (TGF-β) which up-regulates tissue inhibitor of metalloproteases (TIMP), while trophoblast cells secrete interleukin-1β (IL-1β) to down-regulate TIMP expression in decidual cells. Laminin produced by trophoblast cells and fibonectin produced by decidual cells also play important role in regulating the process of trophoblast invasion (*not shown*). PA, Plasminogen activator. PAI, Plasminogen activator inhibitor.

Preimplantation embryos express mRNAs encoding insulin like growth factor-II (IGF-II) and receptors for insulin like growth factor-I (IGF-I) and IGF-II (170). It now appears that IGFs alone cannot effectively stimulate embryo growth, it requires insulin like growth factor binding proteins (IGF-BPs) which are primarily

produced by endometrial cells and other cells (171, 172). IGF-BPs 1, 2 and 3, as well as, and IGF-I and IGF-II are differentially expressed in secretory endometrium under progesterone dominance (173), while embryos can secrete IGF-II and accumulate IGF-BPs (172, 174). Furthermore, the production of IGF-BP by human oviductal cells, endometrial cells and *Vero* cells was stimulated by embryo co-culture (171, 172). However, the nature of interaction between IGFs and IGF-BPs during blastocyst implantation remains still controversial. It appears that a relatively low level of IGF-BPs from mid-secretory phase endometrium allows unhindered action of IGFs on preimplantation stage embryo, while viable growing embryo after morula to blastocyst transition secretes CG which in turn actively down-regulates IGF-BP secretion (Fig. 5); IGF-BP secretion on the other hand increases progressively with decidualization (175). Increasing production of IGF-BPs along with the progression of implantation process results in a shift in the balance of interaction between IGF-BP and IGFs. Giudice (173) has proposed that IGF-BP1 at this stage may serve as one of several "*maternal restraints*" to curb placental invasion into maternal host uterus. IGF-BP1 has been shown to bind to $\alpha_5\beta_1$ integrin on the cytotrophoblast cell membrane to inhibit cell invasion into decidualized human endometrial stromal co-cultures (176). IGF-II has been suggested to serve as a modulator of maternal restraint of trophoblast invasion through dose-dependent inhibition of tissue inhibitor of metalloproteinease-3 and IGFBP-1 in decidualized stromal cells *in vitro* (177). On the other hand, Gleeson et al. (178) reported that IGF-BP1 stimulates human extravillous trophoblast migration *in vitro* by the binding of its RGD domain to the $\alpha_5\beta_1$ integrin leading to activation of focal adhesion kinase and stimulation of the mitogen activated protein kinase pathway. Previously, Hamilton et al (179) demonstrated that IGF-II and IGF-BP1 individually and their combination enhanced trophoblast invasiveness in serum depleted culture. We have demonstrated a temporo-spatial, as well as, cell-type specific localization of IGF-BP1 in stromal-decidual cells surrounding invading fronts of trophoblast cells, around spiral arterioles and engorged blood vessels during the early stages of gestation in the rhesus monkey (180). Our studies support the hypothesis that a close paracrine type of interaction between IGF-II present in trophoblast cells with IGF-BP1 in stromal-decidual cells is important for modulating placental development in the primate (180, 181). The function of IGF-II:IGF-BP1 at the feto-maternal interface may involve a delicate balance between invasion and its suppression to achieve normal implantation and placental development (182–184). It is interesting to note that over-expression of human IGF-BP1 in transgenic mice led to placental insufficiency with altered trophoblast invasion and differentiation (185), and in preeclampsia, a disorder characterized by shallow uterine invasion and altered placental development, increased IGF-II mRNA has been reported in intermediate trophoblast surrounding placental infarcts along with decreased IGFBP-1 mRNA expression in basal plate decidua (186).

Vascular endothelial growth factor

(VEGF) promotes angiogenesis and vascular permeability. VEGF expression is high in secretory phase endometrium in women (187–190) and monkeys (191), and it is inhibited by an early luteal phase administration of antiprogestin in monkeys (128, 191). There is indirect evidence that endometrial VEGF may have embryotropic action (192), while embryonic CG (30) and IL-1 (193–197) acting locally may up regulate uterine VEGF secretion into luminal fluid (Figs. 4 and 5). A balance between VEGF, its receptors- both soluble and membrane bound may determine the endometrial and embryonic responses during receptivity and placentation (198–202). However, administration of monoclonal antibody against VEGF during days 0–10 of the luteal phase of mated, potentially fecund cycle in the female marmoset monkeys could not significantly inhibit blastocyst implantation and pregnancy establishment (203).

A high degree of association has been observed between endometrial maturation and the levels of several cytokines in endometrial cells under progesterone dominance. The hypothesis that IL-1:VEGF:LIF:TGFβ in a network involving IGFs and IGF-BPs can influence endometrial vascular function towards receptivity and blastocyst implantation is supported by indirect evidence (204–207) and needs to be further examined for attending issues related to endometrial failure of blastocyst implantation under conditions like progesterone insufficiency, diabetes, nutritional deficiencies, inadequate perfusion of uterus, dysregulated glandular maturation and vascular competence.

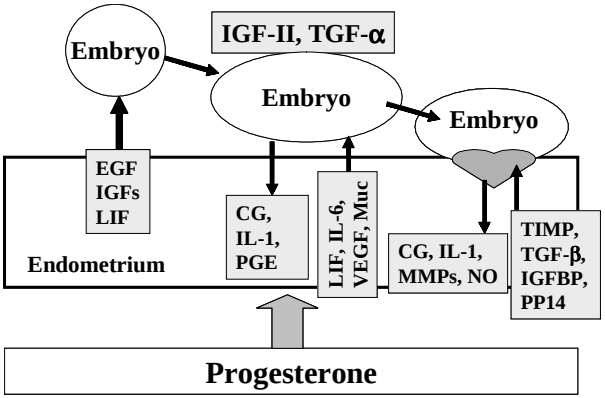

Fig. 8: A schematic model which describes the nature of progesterone-induced endometrial responsiveness to embryo derived signals during the pre- and the peri-implantation stages of gestation in the human.

A tentative integrated model

Fig. 2 proposes a model to explain the actions of luteal phase progesterone inducing a basic drive in endometrium for receptivity which proceeds through certain steps in a fixed action pattern so that even a vacuum stimulus such as an oil droplet can induce decidualization. Implantation stage embryo senses as a field signal endometrial responsiveness circumstantially via factors released by maternal cells, and in turn undertakes differentiation and secretes factors acting on endometrium. This dynamic interaction leads to activation of an innate release process in both compartments resulting in blastocyst attachment followed by trophoblast invasion and decidualization. In other words, the state of dynamic and interactive preparedness of maternal endometrium (*endometrial receptivity*) and implantation stage embryo (*embryonic adhesiveness*) is driven by progesterone and is sustained for a defined time period (*window of*

implantation) during the luteal phase of a potential conception cycle. During this period, even a vacuum stimulus can mimic endometrial and embryonic responses in a fixed action pattern. Initiation of embryo implantation is thereafter supported by successful interaction between embryo and endometrium involving a different set of specific factors interacting synchronously in autocrine, as well as, in paracrine modes (Fig. 8). It appears that this mode of factorial regulation allows a robust set of safety limits for blastocyst implantation, and that implantation fails only when the interaction is inadequate and/or non-synchronous beyond the sustainable safety limit.

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