Potential biochemical markers of uterine receptivity

Linda C. Giudice

Division of Reproductive Endocrinology & Infertility, Department of Gynecology and Obstetrics, Stanford University Medical Center, Stanford, CA 94305–5317, USA

Implantation in humans is a complex process that involves embryo apposition and attachment to the maternal endometrial epithelium, traversing adjacent cells of the epithelial lining, and invasion into the endometrial stroma. These processes involve a variety of molecules which are not unique in themselves, but play unique roles in the process of implantation. The molecular dialogue that occurs between the implanting conceptus and the endometrium involves cell–cell and cell–extracellular matrix interactions, mediated by lectins, integrins, matrix degrading enzymes and their inhibitors, prostaglandins, and a variety of growth factors, cytokines, and angiogenic peptides, their receptors and modulatory proteins. It is likely that each of these, when appropriately expressed or inhibited, contributes to endometrial receptivity or non-receptivity to an implanting conceptus. Currently, a working definition of a receptive versus a non-receptive endometrium is incomplete. While histological normality of the endometrium does not necessarily imply functional normality, temporal and spatial expression of particular biochemical principles in the endometrium are highly suggestive of functional roles of these principles in implantation and endometrial receptivity. These potential markers of endometrial receptivity are discussed herein. It is envisioned that as regulation of these markers is elucidated, their expression may be manipulated to improve implantation rates and fertility or to limit implantation for successful contraception.

Key words: uterine receptivity/implantation/endometrium/decidua

Introduction

Histological examination of early human gestations reveals distinct patterns of blastocyst attachment to the endometrial surface and underlying stroma (Hertig and Rock, 1956; Lindenberg, 1991), which support a model of implantation (Figure 1). Attachment of the trophoblast to the endometrial epithelium is unique in that it occurs via respective apical cell membranes (Denker, 1994). It has been postulated that these apical plasma membranes express unique cell adhesion molecules that mediate this initial attachment that begins the process of implantation (Kliman et al., 1989). The invasive phase of implantation involves attachment of the invading trophoblast to the extracellular matrix, degradation of the matrix, migration, and eventually replacement of the decidual artery endothelium with a new trophoblast phenotype, the vascular trophoblast (Cross et al., 1994). Temporally, the window of implantation in humans is between cycle days 20 and 24 (Anderson, 1990). Endometrial receptivity is the temporally and spatially unique set of circumstances within the endometrium that facilitates successful embryonic implantation. It is generally discussed in terms of epithelial receptivity (Anderson, 1990), although it is likely that the stroma likewise displays receptivity or non-receptivity during the invasive phase of implantation.

In determining endometrial receptivity, several questions arise, including: (i) what should be measured and how? (ii) when in a woman’s cycle is the best time to perform the test? (iii) is receptivity an all-or-none event leading to implantation or no implantation, or are there gra-
Figure 1. Schematic representation of the phases of implantation in humans and the biochemical principles believed to be important in these processes. HB-EGF = heparin-binding epidermal growth factor-like growth factor; MUC-1 = mucin 1; LIF = leukaemia inhibitory factor; CSF-1 = colony stimulating factor-1; IL-1β = interleukin-1β; TGF-β = transforming growth factor-β; IGFBP-1 = insulin-like growth factor binding protein-1; MMP = matrix metalloproteinases; TIMP = tissue inhibitors of matrix metalloproteinases. (Reproduced from Giudice, 1998, with permission.)

tions of epithelial and stromal receptivity which may lead to transient implantations and repetitive miscarriage? To date, the ‘gold standard’ of evaluating endometrial normality (and presumably therefore, receptivity) has been the luteal phase endometrial biopsy (Noyes et al., 1951). During recent years, it has become evident that normal endometrial histology on a given post-ovulatory day is not necessarily reflective of normal function of the endometrium or receptivity (Swiersz and Giudice, 1997). Traditionally biopsies have been performed late in the luteal phase, which is informative for the beginnings of the invasive phase of implantation, but gives no direct indication of events during the window of implantation. Blood tests of some endometrial proteins have been postulated to be of benefit (McRae et al., 1991), although this has not been realized. Uterine fluid lavage is another potential method to evaluate endometrial adequacy, although it is dependent on secreted markers, whose biochemical forms and concentrations may be altered by other components in the fluid and by fluid shifts within the endometrium and transudation from serum respectively. Also, some markers may not be secreted, and they would not be detected, or some may be secreted, but at concentrations so low that this method would be ineffective as a clinical screening tool. So far, the endometrial biopsy offers the most promise for evaluating endometrial receptivity. If there were a consensus on the ideal timing for this procedure, however, several problems still remain. For example, the biopsy specimen may not reflect the endometrium as a whole, especially in view of known microenvironments that exist within this tissue. Also, the biopsy is representative of the cycle in which it is taken, but how representative is it of past and future cycles? In evaluating therapies for endometrial inadequacy, one of the biggest questions is how long post therapy does the effect last? Does the endometrial functionalis have a memory?

The biochemical principles that will be evaluated by histological, immunohistochemical, or in-situ hybridization analyses of endometrial biopsy specimens from women with implantation failures (absolute or relative), are the subjects of scrutiny in the quest of evaluating endometrial receptivity, and are the subject of this review. Most of what we believe occurs is derived from animal models, many of which have different mechanisms of implantation, compared to primates. Nevertheless,
Transgenic and gene ‘knock-out’ models have been useful in elucidating the potential importance of some effector molecules in the process of implantation, which may have relevance to humans. Additional information has been derived from in-vitro studies with human endometrial and placental cells and explant cultures. As a consequence, our understanding of the mechanisms underlying coordinated development of the embryo and the endometrium and the establishment of successful implantation and pregnancy in humans is limited. Several reviews have been published recently, addressing the role of the endometrium in implantation and uterine receptivity (Bulletti et al, 1994; Bischof and Campana, 1996; Petraglia et al, 1996; Swiersz and Giudice, 1997; Tazuke and Giudice, 1996; Fazleabas, et al., 1998; Ghosh and Sengupta, 1998; Giudice, 1998).

**Apposition and attachment**

**Pinopodes**

Pinopodes, structures involved in endocytosis and pinocytosis, are present on the apical surface of endometrial luminal epithelium during the period of receptivity in rats (Psychoyos, 1973) and humans (Ferenczy et al., 1972; Martel et al., 1987a,b; Psychoyos and Martel, 1990). Their appearance is progesterone-dependent. Their function(s) is unclear, but they may be involved in implantation by mechanisms that involve uptake of macromolecules, withdrawal of uterine fluid, and/or by facilitating adhesion of the blastocyst to the luminal epithelium (Psychoyos and Nikas, 1994). Because of their temporal and spatial expression, it has been speculated that pinopode formation may define the development of uterine receptivity for blastocyst implantation (Martel et al., 1991). Scanning electron microscopy shows that 78% of endometrial biopsies obtained from normally cycling women on post-ovulatory day (POD) 6 have pinopodes, compared with rare pinopodes seen on POD 2 or 9. Also, only 15% of endometrial biopsies from women undergoing clomiphene citrate or menotrophin-stimulation for infertility have pinopodes on POD 6 (Martel et al., 1987a). These observations support the idea of a distinct period of endometrial development that coincides with the window of implantation. Furthermore, hormonal treatment used to induce ovulation can modify normal development of the pre nidatory endometrium and may have a negative effect on embryonic implantation.

**Cell adhesion molecules**

Common changes in luminal epithelial cells at the opening of the window of implantation include decreased surface negativity, increased thickness of PAS-positive material, and a more fibrillar glyocalyx structure seen under electron microscopy (Aplin, 1991). Removing carbohydrate moieties or decreasing the negativity of the surface charge is compatible with the acquisition of uterine receptivity to implantation. In addition, numerous cell adhesion molecules are expressed by the endometrial epithelium (and the embryo) during the window of implantation (Turpeenniemi-Hujanen et al., 1995; Yelian et al., 1995). Some of the glyocalices are receptors for extracellular matrix proteins, e.g. fibronectin, vitronectin, laminin, and collagen.

**Mucins**

Mucins are highly glycosylated, large molecules found on many cell surfaces and on the apical side of endometrial epithelial cells. They are believed to play a role in embryo attachment (Carson et al., 1994). MUC-1 is a highly glycosylated mucin whose expression in endometrium is cycle-dependent. In mice, MUC-1 disappears on day 4.5, corresponding to the initial attachment phase of implantation (Surveyor et al., 1993). MUC-1 may be a barrier to implantation that must be removed in order to allow embryonic interaction with the apical surface of the luminal epithelium of the endometrium during the window of implantation. In contrast to the mouse, MUC-1 expression is up-regulated in human endometrium during the peri-implantation period (Hey et al., 1994), and alterations in carbohydrate structure may permit embryo attachment. Alternatively, up-regulation of MUC-1 in human endometrium may have functional significance per se for embryo attachment and nidation. Some electronegative mucins may act as specific ligands for the embryo, marking the initial point of attachment, leading to later interactions mediated by integrins (see below). Another mucin, MAG (mouse ascites Golgi), is expressed on the
luminal epithelial surface in human endometrium on cycle days 18–19 (Kliman et al., 1995; Feinberg and Kliman, 1997)). Of patients with unexplained infertility, 60% have abnormal MAG expression, suggesting that it may be a clinical marker for uterine receptivity (Kliman et al., 1996) in select patients.

Integrins

Integrins are ubiquitous transmembrane glycoproteins that belong to a family of cell adhesion molecules of the immunoglobulin superfamily, cadherins, and cell adhesion molecules with lectin-like domains (Albelda and Buck, 1990). In addition to a transmembrane domain, there is an extracellular domain which serves as a receptor for extracellular matrix (ECM) ligands, including fibronectin, collagen, and laminin (Albelda and Buck, 1990). Specific recognition and binding of ECM components transmit information to the cytoskeleton which may have major roles in promoting hormone responsiveness and genomic activation (Clark and Brugge, 1995). Endometrium is unique in that it expresses both constitutive and cycle-dependent integrins (Tabibzadeh and Sun, 1992; Lessey, 1992, 1994a, 1996a). Three integrins are constitutively expressed on endometrial epithelial cells: α2β1, α3β1, and α6β4 (Lessey et al., 1996a), similar to other tissues. These collagen/laminin receptors probably play a role in cell-substratum attachment. The fibronectin receptor, αvβ3, is the only integrin constitutively expressed by endometrial stromal cells. Other stromal integrins, α5β1, α6β1, α6β3, αvβ5, are cycle-dependent (Lessey, 1992). The presence or absence of individual integrins may serve to evaluate endometrial function during the window of implantation and act as a basis for a clinical test for uterine receptivity. A lack of endometrial expression of αvβ3 may be associated with histological delay, i.e. classical luteal phase (type I) defect, or with histologically normal endometrium, i.e. without histological delay or (type II defect) (Lessey et al., 1992). The latter suggests an intrinsic defect in endometrial function and is found in women with minimal or mild endometriosis (Lessey et al., 1994) or with hydrosalpinges (Meyer et al., 1997). The lack of αvβ3 and the loss of αvβ1 have both been associated with unexplained infertility (Lessey et al., 1995). Since regulation of αvβ3 is not precisely defined, pharmacological correction of this defect is empirical. However, preliminary studies suggest that ovarian suppression or laparoscopic ablation of pelvic endometriosis may restore αvβ3 expression in the eutopic endometrium (Lessey et al., 1996b). During the window of implantation, endometrial epithelial cells lack functional oestrogen receptors (ER) and progesterone receptors (PR) (Lessey et al., 1988). The decline in PR is specifically associated with initiation of αvβ3 expression. In infertile women with type I defect, the loss of PR is delayed, as is the expression of αvβ3. Medical therapy restores both the timely loss of PR and the appearance of αvβ3 (Lessey et al., 1992).

Trophinin/tastin

Trophinin is an intrinsic membrane protein that is important in trophoblast cell adhesion, and tastin (trophinin assisting protein) is a cytoplasmic protein necessary for trophinin to function as an adhesion molecule (Fukuda et al., 1995). The cytoplasmic domain of trophinin associates with tastin, which in turn, associates with the cytoskeleton. The net result is a restricted distribution of trophinin in the plasma membrane, creating highly concentrated areas of trophinin ‘patches’ that may function as efficient embryonic adhesion sites (Fukuda et al., 1995). In human endometrium, trophinin and tastin are uniquely expressed in the epithelium during the window of implantation. In addition, trophinin has been detected in trophoblasts and endometrial epithelium in the monkey blastocyst implantation site (Fukuda et al., 1995). Spatially- and temporally-restricted expression of this novel cell adhesion complex suggests that it is important in the process of blastocyst attachment in primate implantation, although its precise role remains to be elucidated.

Growth factors and cytokines

Growth factors and cytokines comprise families of peptides and proteins that bind to specific cell surface receptors, resulting in cellular mitosis or differentiation by autocrine, paracrine, juxtacrine, or endocrine mechanisms. While these factors are
expressed in most tissues, in endometrium many growth factors, cytokines, their receptors, and their binding proteins exhibit cycle-dependence (Tabibzadeh and Sun, 1992; Hamovici and Anderson, 1993; Giudice, 1994; Tazuke and Giudice, 1996). Many of these effector molecules are differentially expressed in endometrium during the secretory phase of the cycle and, for many, their expression continues during early pregnancy, suggesting a role for them in the molecular dialogue between the decidua and conceptus. Some of these effector molecules participate in the apposition/attachment phase, some in the invasive phase, and some in both phases of implantation (Tazuke and Giudice, 1996).

Epidermal growth factor (EGF)
EGF, transforming growth factor-α (TGF-α), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, and betacellulin comprise the ‘EGF family’ (Carpenter, 1987; Massague and Pandiella, 1993; Johnson et al., 1993; Watanabe et al., 1994) which interact with the EGF receptor (Carpenter, 1987). EGF mRNA is not detectable in mouse uterus on the day prior to implantation and, although TGF-α stimulates mouse blastocyst development in vitro (Paria and Dey, 1990), implantation occurs normally in TGF-α deficient mice (Leutteke et al., 1993; Mann et al., 1993). The weight of evidence favours HB-EGF as the endogenous ligand stimulating the embryonic EGF receptor during the process of implantation. The HB-EGF gene is expressed in mouse uterine luminal epithelium surrounding the blastocyst just prior to implantation and, although TGF-α stimulates mouse blastocyst development in vitro (Paria and Dey, 1990), implantation occurs normally in TGF-α deficient mice (Leutteke et al., 1993; Mann et al., 1993). The weight of evidence favours HB-EGF as the endogenous ligand stimulating the embryonic EGF receptor during the process of implantation. The HB-EGF gene is expressed in mouse uterine luminal epithelium surrounding the blastocyst just prior to implantation and, although TGF-α stimulates mouse blastocyst development in vitro (Paria and Dey, 1990), implantation occurs normally in TGF-α deficient mice (Leutteke et al., 1993; Mann et al., 1993). The weight of evidence favours HB-EGF as the endogenous ligand stimulating the embryonic EGF receptor during the process of implantation.

Colony stimulating factor-1 (CSF-1)
CSF-1 concentrations in mouse uterus increase ~1000-fold during pregnancy (Pollard, 1990). In human endometrium, CSF-1 is preferentially expressed in endometrial glands during the mid-proliferative and mid-secretory phases (Bartocci et al., 1986). With the onset of pregnancy, CSF-1 concentrations increase several-fold in endometrial decidua and are expressed in glandular epithelium and endothelium (Daiter et al., 1992; Kauma et al., 1991). The CSF receptor (the gene product of the proto-oncogene c-fms) is not cycle-dependent in endometrium (Kauma et al., 1991). However, during pregnancy, expression in decidual glandular epithelium is highest during the first trimester (Pollard et al., 1987). Placenta also expresses CSF-1 and its receptor, which increase during gestation (Kauma et al., 1991; Daiter et al., 1992). While it is likely that CSF-1 may play a role in endometrial cellular proliferation and differentiation, the role of this cytokine in implantation has been underscored by the osteopetrotic (op/op) mouse model (Pollard et al., 1991). Animals that are homozygous for a naturally occurring null mutation in the CSF gene are toothless, have multiple skeletal defects, decreased macrophages, and decreased implantation rates and fetal viability. When homozygotes are treated with exogenous CSF-1, their fertility is restored (Wiktor-Jedrzejczak et al., 1991). Thus CSF-1, produced by uterine epithelium, may interact with the CSF-1 receptor on the trophectoderm and promote blastocyst attachment. Abnormal CSF-1 expression in humans as a cause for implantation failure has yet to be established.

Leukaemia inhibitory factor (LIF)
LIF is a pleiotropic cytokine that has both proliferative and differentiative effects on a variety of cells (Hilton and Cough, 1991). In mouse endometrium, LIF is expressed prior to ovulation and also on day 4 of pregnancy or pseudopregnancy (Stewart et al., 1992; Stewart, 1994). The role of LIF in implantation has been shown conclusively in a mouse model lacking a functional LIF gene, achieved by gene targeting and homologous
recombination. Homozygous females produced normal blastocysts that were recoverable on day 4 from uteri, and i.p. administration of LIF partially restored implantation. In addition, transfer of recovered embryos from homozygotes to wild-type pseudopregnant females resulted in implantation and pregnancy (Stewart, 1994). Although the mechanisms underlying maternal endometrial LIF expression and its role in embryonic implantation await further investigation, it is likely that LIF plays an important role in embryonic attachment to the epithelium and perhaps intrusion through the epithelium. Recent evidence suggests that LIF is also important in the process of decidualization in the mouse (Stewart and Cullinan, 1997). In humans, LIF is expressed in endometrium and decidua (Charnock-Jones et al., 1994; Kojima et al., 1994; Cullinan et al., 1996). LIF mRNA is preferentially expressed in the secretory endometrium and is three-fold higher in glandular epithelium, compared with stroma. Regulation of LIF in endometrium by cytokines and steroid hormones (Arici et al., 1995) supports the importance of this cytokine in the process of implantation. Effects of LIF on cytotrophoblasts have been reported, including diverting them to differentiate to the anchoring phenotype (Nachtigall et al., 1996) or along the invasive pathway (Bischof et al., 1995). Thus LIF may also play a role, as yet undefined, in the invasive phase of implantation. A recent study reported a decrease in LIF in medium conditioned by explants from endometrium of women with unexplained infertility, compared with fertile women (Harmbartsoumian, 1998). These findings suggest an important role for LIF in human implantation, although mechanisms underlying LIF action in the implantation process await further investigation.

**Interleukin-1 (IL-1)**

The IL-1 ‘family’ is comprised of IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra), and one signal transducing receptor (IL-1R tl) (Dinarello, 1988). The entire IL system is expressed in human endometrium (Simón et al., 1995) and in human embryos (De los Santos et al., 1996). In the mouse, i.p. injection of high concentrations of IL-1ra on day 3 of pregnancy results in a significant decrease in the number of implantation sites (6.7%), compared with non-injected and buffer-injected animals (59 and 74%, respectively) (Simón et al., 1994). However, fertility is normal in similar studies using different strains of mice, IL-1R tl-deficient mice, and IL-1 knock-out mice (Abbondanza et al., 1996), as well as in the absence of IL-1 converting enzyme (ICE) that converts the IL-1β precursor into its active form (Zheng et al., 1995; Kuida et al., 1995; Li et al., 1995). These observations suggest that IL-1 may not play a key role in implantation in the rodent or that there is redundancy in the regulation of embryonic attachment. Recently, it has been reported that IL-1ra prevents embryonic implantation by a direct effect on endometrial integrins, α3, α6, and β3 (Simón et al., 1998), which are believed to be important in the process of attachment. While this is an exciting finding, the ratio of IL-1ra to IL-1 agonists in the endometrial environment of normal versus abnormal implantation remains to be determined.

**Calcitonin**

Calcitonin mRNA is specifically expressed in the endometrial glandular epithelium of the rat between days 3–5 of pregnancy (Zhu et al., 1998). In the endometrium it is regulated by progesterone, and its secretion into the uterine lumen on day 4 suggests that it may be a marker of uterine receptivity (Zhu et al., 1998). The temporal expression of calcitonin suggests it has an important function in the implantation process, and it has been suggested that it may alter calcium homeostasis in the implantation site. The change in calcium signalling by calcitonin may result in redistribution or expression of critical cell adhesion molecules or junctional complexes that control the polarized epithelial phenotype, preparing the apical cell pole for contact with the trophoblast (Zhu et al., 1998). Expression, regulation, and function of calcitonin in human endometrium is an exciting area of research and may be a marker of uterine receptivity in humans.

**HOXA-10**

Homeobox (Hox) genes encode a class of highly conserved transcription factors that control embry-
Endometrial receptivity to embryonic implantation

Prostaglandins are important mediators in the process of endometrial decidualization and in increased vascular permeability in the endometrium during implantation (Chakraborty et al., 1996). Cyclo-oxygenase is the rate-limiting enzyme in prostaglandin biosynthesis and exists in two isoforms, COX-1 and COX-2. Targeted disruption of the COX-2, but not the COX-1, gene in mice has resulted in multiple failures of reproductive processes, including ovulation, fertilization, implantation, and decidualization (Lim et al., 1997). Expression of LIF and expression of amphiregulin, both believed to be important in the implantation process (see above), were not compromised in the COX-2 deficient homozygous mouse, and still implantation did not occur (Lim et al., 1997), suggesting that expression and/or action(s) of COX-2 are downstream to the actions of LIF and amphiregulin. While implantation in the rodent and human have their own unique features, many similarities exist and many processes would be expected to be conserved from an evolutionary perspective. The challenge now is to extend the findings on COX-2 and other biochemical principles to uterine receptivity in the human.

Invasive phase of implantation

During the invasive phase of implantation, several events occur, including cytotrophoblast adhesion to extracellular matrix via cell adhesion molecules, local extracellular matrix proteolysis by matrix metalloproteinases (MMPs), cellular migration, and inhibition of these processes (Fisher et al., 1989; Bischof et al., 1991; Damsky et al., 1992; Stetler-Stevenson et al., 1993; Bischof and Campana, 1996). While the cytotrophoblast has been considered the kingpin of the invasive process of implantation, the maternal endometrium plays a major, active role in limiting and accommodating this invasion. Stromal ‘receptivity’ is rarely cited, but logically, this compartment of the endometrium must be prepared for, and modulate, the invading trophoblast in specific regions and at the appropriate time. It accomplishes this by several mechanisms, including producing inhibitors of trophoblast differentiation into the invasive phenotype, inhibition of autocrine or paracrine stimulators of invasion, direct inhibition of invasion, and inhibition of trophoblast-derived matrix degrading enzymes.

Enzyme inhibitors

Tissue inhibitors of metalloproteases (TIMP) and broad spectrum protease inhibitors (e.g., α2-macroglobulin) of primarily decidual (and cytotrophoblast) origin are important in limiting cytotrophoblast invasion and in endometrial stromal receptivity. Rodent decidua expresses TIMPs (Waterhouse et al., 1993), and TIMPs abolish trophoblast invasion in vitro (Librach et al., 1991; Behrendtsen et al., 1992). However, mice
with a null mutation in the TIMP-1 gene have normal fertility (Cross et al., 1994), suggesting TIMP-1 is not a critical inhibitor of trophoblast invasion. In humans, endometrial TIMP-1 and TIMP-2 mRNA expression is not cycle-dependent (Rodgers et al., 1994; Hampton and Salamonsen, 1994), and recent evidence supports a major role for TIMP-3 in limiting invasion. TIMP-3 mRNA in decidualized endometrial stromal cells is up-regulated by progesterone in vitro and in vivo (Higushi et al., 1995), and in mice, TIMP-3 mRNA is expressed in decidua immediately adjacent to the implanting embryo (Harvey et al., 1995). Human trophoblasts with the invasive phenotype up-regulate MMP-9 and TIMP-3 (Bass et al., 1994b), and TIMP-3 mRNA is expressed in decidua (as well as the invading trophoblast) (Higushi et al., 1995). IL-1β inhibits TIMP-3 expression in human decidualized endometrial stromal cells, suggesting that the trophoblast promotes its own invasiveness by inhibiting a maternal restraint on invasion (Huang et al., 1998).

Extracellular matrix proteins
The extracellular matrix (ECM) likely plays a major role in trophoblast invasiveness, because it is the substratum that supports cellular adhesion and cell–cell interactions and because interactions with the ECM result in changes in trophoblast invasiveness. Endometrial stromal cells, decidualized in vitro, secrete laminin and fibronectin (Irwin et al., 1991; Zhu et al., 1992), and progestins stimulate fibronectin expression in these cells (Zhu et al., 1992). Laminin production increases during decidualization of rat uterine stromal cells (Glasser et al., 1987). Laminin production by human endometrial stroma is more abundant in secretory, than in proliferative, endometrium and it increases with implantation and in early pregnancy (Zhu et al., 1992). Laminin decreases prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) which binds specific integrins, including α5β1 which is uniquely expressed by the invading trophoblast (Damsky et al., 1994). Adhesion receptors change during cytrophoblastic differentiation along the invasive pathway in vivo, from α4β1 to α5β1 and α1β1 fibronectin receptors. Fibronectin inhibits trophoblast invasion in vitro (Damsky et al., 1994), and RGD-containing peptides inhibit cytrophoblastic attachment to fibronectin-coated culture dishes (Kao et al., 1988; Irwin and Giudice, 1998). Thus, cytrophoblast interactions with fibronectin, through the α5β1 integrin, restrains rather than promotes, invasion. Mechanisms underlying this inhibition are not well understood, but fibronectin is likely to be one of several members of the ‘maternal restraint’ on trophoblast invasiveness at the maternal–fetal interface and of endometrial stromal receptivity (Giudice, 1998).

Insulin-like growth factor binding protein-1 (IGFBP-1)
IGF-II is abundantly expressed by the invading cytrophoblast (Han et al., 1996). In addition, IGF-II stimulates human trophoblast migration in vitro (Irving and Lala, 1995). IGFBP-1 has high affinity for the IGF peptides and primarily inhibits IGF actions at their target cells (Jones and Clemons, 1995). IGFBP-1 is a major product of secretory endometrium and decidua (Zhou and Bondy, 1992; Giudice et al., 1994; Han et al., 1996; Rutanen et al., 1985, 1986a,b), and maternal serum concentrations increase throughout gestation (Rutanen et al., 1984), probably derived from the decidua (Rutanen et al., 1982; Giudice et al., 1994, 1997). IGFBP-1 is well situated to interact with the IGF-II-expressing invading cytrophoblast (Zhou and Bondy, 1992; Han et al., 1996). IGFBP-1 also has IGF-independent actions, binding to cell membranes, altering cellular motility (Jones et al., 1993). Like fibronectin, it contains the Arg-Gly-Asp tripeptide motif which interacts with the α5β1 integrin which is uniquely expressed by the invading cytrophoblast at the maternal–fetal interface (see above). Thus, IGFBP-1 is also well situated to interact with the cytrophoblast to affect the implantation process by IGF-dependent and/or independent actions. For example, IGFBP-1 competes with endometrial membrane receptors for
binding IGF-I (Rutanen et al., 1988). It also inhibits the binding and biological activity of IGF-I on choriocarcinoma cells, suggesting a role in regulating trophoblast-derived IGF autocrine and paracrine actions (Ritvos et al., 1989). IGFBP-1 binds to human cytotrophoblasts and binds to the $\alpha_5\beta_1$ integrin in the cytotrophoblast membrane (Irwin and Giudice, 1998). In vitro, it inhibits human cytotrophoblast invasion into decidualized human endometrial stromal co-cultures (Irwin and Giudice, 1998), although another group has reported that IGFBP-1 stimulates trophoblast invasiveness in an in-vitro Matrigel assay (Irving and Lala, 1995; Hamilton et al., 1998). The former studies cumulatively suggest that this abundant maternal decidual product interacts with the invading trophoblast and may be one of several ‘maternal restraints’ to curb placental invasion into the maternal host. Whether an excess of IGFBP-1 at the maternal-fetal interface is detrimental to cytotrophoblast invasion, resulting in shallow implantation or early pregnancy loss, is not certain. However, IGFBP-1 concentrations are elevated at the maternal-fetal interface and in the circulation of women with severe pre-eclampsia, a disorder of shallow implantation (Than et al., 1984; Iino et al., 1986; Giudice et al., 1997). The roles of IGF peptides and IGFBP-1 in normal endometrial development and early human pregnancy, in occult endometrial defects, and in uterine receptivity and non-receptivity await further investigation.

Transforming growth factor-β (TGF-β)

In humans, TGF-β1 is equally distributed in endometrial glands and stroma, with highest concentrations in secretory endometrium and decidua (Chegini et al., 1994). The cycle-dependency of TGF-β1 and its abundance in decidua also suggests a possible role for it in implantation (Graham et al., 1992, 1993). TGF-β1 inhibits proliferation of cytotrophoblasts and promotes differentiation into the non-invasive phenotype. The invading trophoblast secretes a variety of proteases, including plasminogen activators and MMPs. TGF-β1 induces plasminogen activator inhibitor (PAI) mRNAs and TIMP-1 secretion and decreases MMP-2 in cytotrophoblasts (Graham et al., 1992). In addition, the latent form of TGF-β1 is activated by plasmin. Thus, it is possible that maternal decidua stores latent TGF-β in the extracellular matrix, waiting for the invading trophoblast and its production of plasmin. TGF-β could subsequently increase PAIs and inhibit trophoblast-derived MMPs (by increasing TIMP-1), limiting trophoblast invasion (Lala and Graham, 1990). Support for this hypothesis derives from the observation that TGF-β produced by first trimester decidual cells inhibits the in-vitro invasiveness of first trimester human trophoblasts, due to the induction of TIMP-1 (Graham et al., 1992, 1993).

Conclusions

Endometrial receptivity is an important concept in the biology of implantation, for it permits a framework in which temporal and spatial interactions occur between the endometrium and the conceptus. It is also important as a clinical tool to help explain why women with implantation failure and perhaps abnormal implantation, including shallow implantation and pre-eclampsia and repetitive miscarriage not due to genetic causes or phospholipid antibody syndrome, have not had successful pregnancies. However, the molecular players that comprise a receptive endometrium are just beginning to be understood, although the clinical translation is far from complete. Assuming that the endometrial biopsy is the ‘ideal test’ for endometrial receptivity (see above), two biopsies would need to be obtained: one during the window of implantation (for apposition and attachment) and one in the late secretory phase (for invasion). During the window of implantation, screening could be performed for some of the known ‘likely’ players, including pinopodes, mucins, $\alpha_3\beta_3$, trophinin/tastin, EGF, HB-EGF, amphiregulin, CSF-1, LIF, IL-1β, calcitonin, Hoxa-10, and COX-2. During the late luteal phase, screening would include TGF-β1, IGFBP-1, fibronectin, laminin, and TIMP-3. Appropriate cellular expression would be assessed, as would, ideally, amounts of mRNA and protein. Advances in molecular biology may permit less invasive screening than an endometrial biopsy and screening of not only the female partner, but also the male partner and the conceptus. Consider the new DNA arrays and chip technology, where hundreds of mRNAs can be screened in a given
tissue. This could prove to be very valuable in the near future for small amounts of tissues and large numbers of molecules to be screened. In addition, could reproductive biology ignore the human genome project and the wealth of information available from that valuable resource? Within the next 5 years it is anticipated that all human genes will be sequenced. Surely some of them must be relevant for endometrial receptivity, for information exchange between the decidua and the conceptus which carries genetic information from its father, and for screening for implantation failure and success. In addition to diagnosis, another major challenge for the millennium is to define successful therapies aimed at aberrantly expressed molecules in the endometrium or in response to an abnormal stimulus. It is within the realm of possibility that within the next decade, endometrial defects and abnormal maternal–conceptus interactions will be diagnosed and treated for successful implantation or for preventing implantation for contraceptive purposes.

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