Factors controlling blastocyst implantation

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Abstract

Establishment of early pregnancy is promoted by a complex network of signalling molecules that mediate cell-to-cell and cell-to-extracellular matrix communications, in order to manifest controlled invasion of the trophectoderm and successful implantation. During the period known as the ‘window of implantation’, the endometrium expresses specialized proteins, many of which exhibit potential use as markers of endometrial receptivity. Trophoblast differentiation to the invasive phenotype also depends on the up-regulation of certain peptides and the down-regulation of others. Disruption of each pathway is theoretically possible, and studies in animal models suggest that implantation defects result when the function of these proteins is blocked. Indeed, the implantation process is currently considered the most important limiting factor for the establishment of viable pregnancy. The present overview of the literature reports critical families of molecules located at the embryo–maternal interface and describes the mechanisms of interplay and control. Since these factors are crucial to the process of implantation, targeting them might be a valuable contraceptive tool. Conversely, induction of specific peptides may prove to be beneficial in certain infertility cases.

Keywords: blastocyst implantation, CRH, cytokines, ECM-degrading enzymes, integrins

Introduction

Implantation is a dynamic process in which the blastocyst apposes, attaches and progressively invades into the endometrium to establish the placenta. It requires precisely coordinated development of a hormonally primed adhesive endometrium and a blastocyst competent to implant. Under the influence of the ovarian steroids, the endometrium undergoes a series of structural changes that render it receptive to the conceptus. The uterus is ready to accept the implanting embryo only during a limited period of time known as the ‘window of implantation’, outside of which the endometrium may be indifferent or even hostile to the embryo (Psychoyos et al., 1995; Klintzeris, 1997). Uterus and blastocyst must differentiate in synchrony so that they provide the genetically defined molecular repertoire required for adhesive interaction between the endometrium and the trophectoderm. This delicate process involves a complex sequence of signalling events, mediated by a large number of endocrine, paracrine and autocrine molecular regulators (Tabibzadeh, 1991; Guilbert et al., 1993; Stewart and Cullinan, 1997; Senturk and Arici, 1998).

In humans, the efficiency of the process is remarkably low. Once implantation begins, it has been estimated that it is successful in no more than 30% of cases (Miller et al., 1980). Disruption of events very early in pregnancy may be responsible for non-chromosomal pregnancy loss, which continues to be a major limiting factor in assisted reproductive therapies. Despite significant progress in reproductive research, many fundamental questions about implantation remain unanswered. Clearly, the driving force that initiates the
events is represented by the ovarian hormones. Thereafter, the exact mechanisms that promote implantation remain elusive. Several researchers have focused on multiple molecular pathways and postulated how these may facilitate the establishment of early pregnancy.

The present review will highlight the expression, regulation and possible roles of a number of molecules that have been localized at the maternal–fetal interface, namely the cytokines, the corticotrophin releasing hormone (CRH), and the families of integrins and matrix metalloproteinases (MMP).

Cytokines

During blastocyst implantation and early pregnancy, the immunological processes that take place within the uterus are to a great extent modulated by pro-and anti-inflammatory cytokines. Several cytokines have been localized in the human endometrium as well as in pre-implantation embryo, and are thought to act as mediators of cellular interactions between the maternal and fetal tissues.

The interleukin-1 (IL-1) family, which could perhaps be singled out as having the widest impact, consists of four related peptides: the agonists IL-1α and IL-1β, the inhibitor IL-1 receptor antagonist (IL-1Ra) and the signal transduction receptor (IL-1 t). All components of the IL-1 system have been localized in both the endometrium and the embryo, and represent modulators of differentiation and proliferation of several cell types (Simón et al., 1994a; De los Santos et al., 1996). A considerable number of functions have been attributed to IL-1 regarding the paracrine regulation of implantation. IL-1 was shown to stimulate the expression of several cytokines in the endometrium, including IL-6, IL-8, leukaemia inhibitory factor (LIF), tumour necrosis factor-α (TNFα), as well as the expression of prostaglandins PGE2, PGFαα, and their receptor EP1 (Jacobs et al., 1994; Arici et al., 1996; Laird et al., 1996; Tseng et al., 1996; Kniss et al., 1997; Spaziani et al., 1997; Knight et al., 1999). Remarkably, in-vitro experiments reveal that IL-1β can induce the expression of the cyclo-oxygenase 2 (COX-2) gene in cultured endometrial stromal cells, hence supporting the idea of an IL-1 mediated paracrine effect in the control of prostaglandin synthesis during the peri-implantation period (for review, see Pellicer et al., 2002). As mentioned above, IL-1 affects the expression of MMP and integrins. More specifically, epithelium derived IL-1α induces the production of MMP-1 in stromal fibroblasts and raises the activity of MMP-9 in trophoblasts (Meisser et al., 1999a; Singer et al., 1999). The second agonist, IL-1β, raises MMP-9 and reduces TIMP-1 and TIMP-3 mRNA concentrations in the endometrium (Huang et al., 1998). Moreover, IL-1 modulates the synthesis of steroids, human chorionic gonadotrophin (HCG) and CRH in trophoblasts and endometrial cells (Yanushpolsky et al., 1993; Feinberg et al., 1994; Makrigiannakis et al., 1999a). IL-1 has been shown to attenuate progesterone production by LH-stimulated as well as unstimulated granulosa cells (Fukuoka et al., 1988). Animal models have shown that blocking the IL-1 system in mice, either by deleting the relevant genes or by treating them with IL-1Ra during the pre-implantation phase, resulted in a significant cost in terms of the number of implanted blastocysts (Simón et al., 1994b; Zheng et al., 1995; Abbondanze et al., 1996). However, the animals were not totally infertile, an observation which suggested that essential functions may be preserved due to the utilization of multiple overlapping mechanisms.

Other interleukins that are thought to be members of the paracrine and autocrine network of factors controlling implantation are IL-6, IL-10, IL-11 and IL-15. IL-6, which is constitutively expressed by trophoblasts, may modulate their invasive capacity by increasing the activity of MMP-9 and MMP-2 (Meisser et al., 1999b). IL-10, an anti-inflammatory and immunomodulatory cytokine, is produced in-vitro by first trimester cytotrophoblasts and these cells also express the IL-10 receptor mRNA. IL-10 down-regulates MMP-9 and cytotrophoblasts invasion in vitro and in vivo. Therefore, it is considered that this cytokine is an autocrine regulator of cytotrophoblast activity and invasiveness (Roth and Fisher, 1999; Szony et al., 1999). IL-11 may be important for the differentiation of endometrial stroma to decidua, since IL-11 receptor-null mice were infertile as a result of lack of optimum decidualization (Robb et al., 1998). IL-15, a cytokine produced by the trophoblast, has been reported to increase trophoblast invasion and migration, and modulate MMP-1 production by JEG-3 choriocarcinoma cells, but it has no effects on MMP-2 and MMP-9 (Zygmun et al., 1998). Latest reports propose that IL-15 could act as a mediator of cell-to-cell interaction between decidual cells and uterine natural killer cells (uNK), which represent the largest population of bone marrow derived cells in the decidua during the first trimester of pregnancy. It has been suggested that decidual cell-derived IL-15 might maintain the uNK cell population through stimulating proliferation (for review, see Dunn et al., 2003).

At this point, it should be stressed that increased expression of potent pro-inflammatory cytokines, such as IL-18, may be deleterious for the onset of pregnancy. Recently, it has been proposed that the detection of IL-18 in the uterine lumen at the time of oocyte retrieval, i.e. 48 h prior to embryo transfer, is associated with a poor implantation rate. Researchers have also found strongstromal anti-IL-18 staining with patients with implantation failure, thus suggesting that IL-18 might serve as a marker of endometrial receptivity (Lede-Bataille et al., 2004a,b). Notably, in order to facilitate the analysis of peptide patterns serving as receptivity markers during assisted reproduction treatment, van der Gaast et al. (2003) showed that endometrial secretion aspiration prior to embryo transfer provides sufficient material for protein pattern analysis and does not adversely affect the treatment outcome, i.e. the implantation rates.

Leukaemia inhibitory factor (LIF), a polyfunctional glycoprotein whose inducible production can occur perhaps in all tissues, appears to be irreplaceable for blastocyst implantation, in contrast with what happens with most of the other molecules examined. Female mice with no functional LIF gene (LIF-/-), when mated with wild-type or LIF-/- males were found to have normal blastocysts which failed to implant (Stewart et al., 1992; Escary et al., 1993). A possible explanation for this deficiency is the crucial lack of oestrogen-stimulated LIF secretion in the uterine wall at the time of blastocyst implantation, since LIF mRNA is shown to be induced by oestrogen in the uterus of ovariecetomized delayed implantation mouse model (Takabatake et al., 1997).
Investigators were able to observe viable pregnancies after injecting LIF−/− mice with LIF (Croy et al., 1991; Chen et al., 2000; Song et al., 2000). In humans, LIF acts on cytotrophoblasts, causing them to differentiate into the anchoring phenotype. This is achieved by increased synthesis of fibronectin and decreased production of HCG protein, whilst inducing the secretion of onfFN (oncofetal fibronectin) protein and expression of onfFN mRNA (Nachtigall et al., 1996). More recently, it has been reported that infertile patients with multiple implantation failure, compared with fertile women, have a severe dysfunction of LIF production in the endometrium during the menstrual cycle (Hambartsoumian, 1998). Hence, LIF may also play a role, as yet unclear, in the invasive phase of implantation.

Several lines of data propose an elegant communication between maternal and fetal tissues, which may begin even before initial attachment of blastocyst to the endometrium. Cytokines and LIF along with other molecules such as growth and stimulating factors, appear to serve as signals directed from the embryo to the mother and vice versa, facilitating cellular interactions which are essential to accomplish implantation. Interestingly, before the embryo hatches, that is before day 6, these signals have to pass through the extra-embryonic matrix (EEM, zona pellucida) to reach their destination. Therefore, studying the proteinaceous contents of EEM of preimplantation primate embryos may prove an invaluable tool in deciphering the embryo–maternal dialogue (for review, see Herrler et al., 2003).

**Corticotrophin releasing hormone**

The hypothalamic neuropeptide CRH is produced by several tissues outside the central nervous system, including the reproductive tract. The ‘reproductive’ CRH has been localized in endometrial epithelium, decidualized stroma, trophoblast (Figure 1), syncytiotrophoblast and placental decidua (Suda et al., 1984; Usui et al., 1988; Makrigiannakis et al., 1995b). The type 1 CRH receptor (CRH-R1) is the principal mediator of CRH action in human endometrium. It has been identified in endometrial epithelial and stromal cells, as well as in human placental trophoblast, amnion/chorion and decidua (Florio et al., 2000; Karteris et al., 2004). Therefore, CRH produced by both maternal and fetal sides exerts its local effects in a paracrine and autocrine manner.

The regulation of the CRH gene promoter in the endometrium appears to be similar to that in the hypothalamus. Known inducers of the hypothalamic CRH, such as forskolin and EGF, stimulate the activity of the CRH promoter in transfected human endometrial cells (Makrigiannakis et al., 1996). Oestrogens suppress the activity of the CRH promoter in endometrium in the same way that they affect the transcription of hypothalamic CRH. Furthermore, oestradiol is not capable of inhibiting forskolin- or EGF-induced CRH promoter, acting directly on pairs of functional half-palindromic oestrogen responsive elements (ERE), present in the promoter region of the CRH gene. Although glucocorticoids decrease the activity of the CRH promoter, the absence of the consensus glucocorticoid response element in CRH promoter and the inhibition of forskolin- or EGF-induced CRH promoter point to an effect via activation of cAMP- and EGF-dependent pathways. Indeed, the inhibitory effect of glucocorticoids in endometrium is in agreement with that described for hypothalamus, and exactly opposite to what has been found to take place in human placenta, suggesting that regulation of the transcription of the CRH gene is cell-specific, depending on the presence or absence of certain specific transcription factors (Makrigiannakis et al., 1996). Finally, PGE2 and the cytokines IL-1 and IL-6 have been shown to stimulate the activity of the CRH promoter, introduced into human endometrial cells (Makrigiannakis et al., 1999a). This effect appears to be mediated via prostaglandins, in accordance with what has been described in the hypothalamus and placenta.

It is well established that CRH possesses potent proinflammatory/procytokine properties, since the peptide is secreted at the sites of inflammation and its

![Figure 1](image_url)
immunoneutralization appears to drastically attenuate the inflammatory response (Karalis et al., 1991). One of the early effects of ‘immune’ CRH is the degranulation of mast cells and the release of histamine and several inflammatory cytokines, including TNFα and IL-6 (Theoharides et al., 1998). The differentiation of endometrial stroma to decidua shares a lot of similarities with an aseptic inflammatory reaction. More specifically, during decidualization the endometrial stroma is subjected to numerous functional changes, including an increase in its vascular permeability, remarkable cell growth, and remodelling of its extracellular matrix. A number of observations suggest that CRH participates as a local modulator in an endometrial network of neuropeptides, prostanoids and cytokines, which appears to be responsible for the fine tuning of the decidualization process. It has been shown that CRH induces the decidualization of endometrial stroma and that it potentiates the decidualizing effect of progesterone. Furthermore, progesterins stimulate the expression of endometrial CRH in a cAMP-dependent manner (Ferrari et al., 1995; Makrigiannakis et al., 1999b). In addition to progesterone, several proinflammatory immune factors, which are locally produced and secreted by stromal cells, also exert a decidualizing effect. In humans, PGE2 enhances, while IL-1 inhibits, the decidualizing effect of progesterone (Kariya et al., 1991; Frank et al., 1994, 1995; Psychoyos et al., 1995). It is postulated that during the decidualizing process, CRH interacts with these local factors. For instance, it has been shown that CRH inhibits the production of PGE2 and stimulates the production of both IL-1 and IL-6 in human endometrial stromal cells (Zoumakis et al., 2000). Additionally, progesterone per se induces the expression of the CRH gene in the stromal cells of human endometrium (Makrigiannakis et al., 1999b). Thus, CRH may exert its decidualizing effect as a principal regulator of locally produced factors and/or as a modulator of progesterone, the classic decidualizing effector.

Interestingly, the implanting blastocyst secretes several inflammatory mediators, including IL-1 and PGE2, which are inducers of CRH expression in human endometrial cells, as mentioned above. Therefore, the blastocyst may modulate the expression of maternally and fetally derived CRH through IL-1 and/or PGE2 produced by both sides at the very site of nidation. It has been proposed that CRH participates in local inflammatory phenomena, which take place at the implantation site, rendering the endometrial surface ‘adhesive’ for the attachment of the blastocyst. This suggestion is supported by several lines of data. The content of CRH mRNA and its peptide product was found higher in the implantation sites of early pregnant rat uterus, compared with the inter-implantation regions (Makrigiannakis et al., 1995a). Implantation in mice can be impeded by administration of polyclonal anti-CRH rabbit serum. Intraperitoneal injections of anti-CRH antibodies on day 2 of pregnancy resulted to a 60% reduction of the number of murine fetuses (Athanassakis et al., 1999). Furthermore, rats that received antalarmin, a specific CRH-R1 blocker, on the day of conception, showed a 70% reduction in the number of implantation sites (Makrigiannakis et al., 2001).

Recent experimental findings show that CRH produced by decidual and EVT cells attenuates the maternal immunological attack to the invading embryo. The latter represents a semi-allograft, to which the endometrium responds with an acute, aseptic, inflammatory-like reaction. The data suggest that CRH potentiates the ability of decidual and EVT cells to induce apoptosis of the surrounding maternal T lymphocytes, activated by the presence of the embryo. CRH may inhibit local maternal immune response through stimulation of the expression of the pro-apoptotic Fas ligand (FasL) peptide in decidual and trophoblastic cells (Makrigiannakis et al., 2001). Fas ligand binds to its receptor Fas, which belongs to the death receptors family (DR), and induces the apoptosis of activated cells bearing the Fas receptor on their surface (Runic et al., 1996) (Figure 2). Maternal and fetal FasL may also participate in the regulation of cytotrophoblast migration into maternal tissue, and vice versa.

The intrauterine presence of CRH, both in the maternal (decidual) and fetal (trophoblast) sites, suggests that locally produced CRH regulates FasL production, thus affecting the invasion process through a local auto/paracrine regulatory loop of cytotrophoblast cells, regulating their own apoptosis. Increased CRH concentrations have been reported in products of conception from women with habitual spontaneous abortions (Madhappan et al., 2003). Therefore, aberrant CRH-mediated self-induction of FasL in EVT might be involved in the pathophysiology of infertility and recurrent fetal resorption or miscarriage (Makrigiannakis et al., 2001). Abrogation of immune privilege at the placental-uterine interface or unbridled invasion of the trophoblast may have deleterious consequences for the developing fetus, as evidenced by the high rates of fetal and maternal morbidity and mortality, observed in pregnancies complicated by inflammation at the maternal–fetal interfaces and pre-eclampsia/eclampsia (Lockwood, 1994; Makrigiannakis et al., 2001).

Integrins

Integrins represent a large family of heterodimeric (α-chain and β-chain) transmembrane adhesion receptors that mediate cell attachment to a variety of ligands including extracellular matrix (ECM) proteins and cell surface molecules (De Arcangelis and Georges-Labouesse, 2000). To date, 18 α and eight β subunits have been identified. These subunits form 24 known heterodimers and many of them are ubiquitously expressed among cellular populations. Usually, a single integrin recognizes a number of distinct ECM proteins, even though a particular integrin may not exhibit all its specificities in a given cell (Kirchhofer et al., 1990; Ruoslahti, 1991; Hynes, 1992). Recently, research interest has been focusing on the participation of integrins in the implantation process.

Integrins are considered to provide an intersection where mechanical forces, cytoskeletal organization, biochemical signals and adhesions meet during blastocyst implantation (Schwartz and Ginsberg, 2002). The latter is a dynamic process, occurring by a number of steps, in which trophoblast undergoes a series of distinct interactions with the underlying endometrial surface. It is initiated by apposition, which involves close proximity between trophoblast and endometrial epithelium, followed by attachment and concluded by invasion of trophoblast into the decidualized stroma (Schlafke and Enders, 1975; Blankenship and Given, 1992). It has been proposed that integrins are utilized by both endometrium and blastocyst and play a major role during the attachment,
Figure 2. Model suggesting CRH action during early phases of human implantation. CRH secreted locally by EVT and decidual cells acts in an autocrine–paracrine manner, through CRH-R1, to stimulate FasL expression and to potentiate the ability of these cells to induce apoptosis of surrounding T lymphocytes bearing the Fas receptor on their surface. CRH = corticotropin-releasing hormone; CRH-R1 = Corticotropin releasing hormone receptor type 1; DC = decidual cell; EVT = extravillous trophoblast; Fas = Fas receptor; FasL = Fas ligand; T = activated T lymphocyte.
enabling the blastocyst to firmly adhere to the uterine lining (Aplin, 1997; Yoshimura, 1997). This may be accomplished by synchronous and bilateral integrin–ECM recognition, where integrins expressed by both sides (endometrium and trophoblast) bind to proteins found in the intercellular space (Bischof et al., 1993; Tabibzadeh and Babaknia, 1995).

In humans, integrins $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_2$, $\alpha_6\beta_1$, $\alpha_4\beta_3$, $\alpha_6\beta_3$, and $\alpha_4\beta_3$ have been localized on the uterine luminal epithelium. The glandular epithelium expresses the same proteins with the exception of $\alpha_5\beta_2$ and $\alpha_6\beta_3$, and the addition of $\alpha_1\beta_1$ and $\alpha_6\beta_1$ heterodimers (Tabibzadeh, 1990; Aplin et al., 1994; Lessey et al., 1996; Reddy and Meherji, 1999). At the mRNA level, all integrin subunits examined ($\alpha_2$, $\alpha_3$, $\alpha_4$, $\alpha_5$, $\alpha_6$, $\alpha_7$, $\beta_1$, $\beta_2$, $\beta_3$, and $\beta_4$) were shown to increase in the secretory phase. Most of the peptides, however, show little menstrual cycle regulation (except for $\alpha_4\beta_1$, $\alpha_1\beta_1$ and $\alpha_1\beta_3$), which suggests differential control of transcription and translation (Dou et al., 1997a).

A well documented temporal and spatial expression of the three specific integrins mentioned above, which coincides with the ‘window of implantation’ (days 20–24 of an ideal cycle), is considered to be crucial to endometrial receptivity. The $\alpha_1\beta_1$ and $\alpha_1\beta_3$ integrins can be detected in the endometrium just after ovulation (day 14), and disappear on days 24 and 28 respectively. The $\alpha_3\beta_3$ molecule appears for the first time on luminal epithelial cells on day 20, thus signalling the beginning of the optimal uterine receptivity period (van der Linden et al., 1994; Sueoka et al., 1997; Bowen and Hunt, 2000). These observations led to the suggestion that a functional ‘window of implantation’ requires the concurrent expression of these three integrins. The window ‘opens’ with the appearance of $\alpha_1\beta_3$ and ‘closes’ with the loss of $\alpha_1\beta_1$. Another finding that adds to the importance of a functional ‘window of implantation’ during the peri-implantation period is the appearance of characteristic protrusions on the surface of endometrial epithelial cells during the luteal phase, known as pinopodes. Fully developed pinopodes appear for a restricted period in the mid-luteal phase that corresponds to the ‘window of implantation’ and they have been proposed as markers of endometrial receptivity (Nikas and Psychoyos, 1997; Nikas, 1999, Nikas et al., 1999). Recently, Pantos et al. (2004) investigated the clinical value of the detection of pinopodes in women with a history of multiple implantation failures after application of assisted reproduction treatment, in order to ideally plan their artificial cycle in donation programmes. The authors concluded that detection of pinopodes in mock cycles by examining endometrial samples under scanning electron microscopy allowed precise determination of the window of implantation and might improve the endometrial preparation regime, resulting in a favourable outcome (Pantos et al., 2004).

At the pre-implantation phase, the human blastocyst expresses a number of integrin subunits, including $\alpha_5$, $\alpha_3$, $\beta_1$, $\beta_3$, $\beta_4$ and $\beta_6$. During this stage, integrins may be important for blastocyst cleavage and proper development, as well as initial adhesion to the uterine surface (Campbell et al., 1995). In addition to what has been observed in the endometrium, cytotrophoblasts also exhibit temporal and spatial regulation of integrin expression. Interestingly, the differentiation of trophoblasts to an invasive phenotype seems to be accompanied by pre-programmed switching of their integrin repertoire. In the first trimester human placenta, the cytotrophoblast stem cell population, which is anchored to the trophoblast basement membrane, stains strongly for the $\alpha_5\beta_3$ integrin. The first step in differentiation, the formation of cell columns, is accompanied by up-regulation of $\alpha_5\beta_3$ integrin. When the extravillous trophoblast cells (EVT cells) leave the columns and invade the uterine wall, they up-regulate $\alpha_5\beta_3$, $\alpha_5\beta_1$ and $\alpha_6\beta_1$ integrins, whereas $\alpha_5\beta_3$ is no longer detected. Further observations using antibody perturbation of interactions between $\alpha_1\beta_1$, $\alpha_1\beta_3$ integrins and their ligands (laminin and fibronectin respectively), suggested an invasion-promoting role for $\alpha_1\beta_1$ and an invasion-restraining role for $\alpha_1\beta_3$. Therefore, down- or up-regulation of adhesion receptors, such as $\alpha_1\beta_1$ and $\alpha_1\beta_3$, may contribute to the regulation of cytotrophoblast invasion.

In support of this notion, later gestation cytotrophoblasts, whose invasive capacity is greatly reduced, fail to up-regulate $\alpha_1\beta_1$ complexes (Damsky et al., 1992, 1993, 1994; Aplin, 1993). Moreover, integrin $\alpha_4\beta_3$ is considered to act as a mediator of IGF-I induced migration in EVT cells (Kabir-Salmani et al., 2003).

Little is known about the detailed mechanisms that regulate the expression of integrins at the endometrium and trophoblast. It is recognized, however, that they are subject to the endocrine and paracrine effect of the ovarian steroid hormones and a network of cytokines and growth factors. Progesterone stimulates the synthesis of $\alpha_4\beta_1$ and $\alpha_1\beta_1$, thus accounting for the up-regulation of the two integrins in the endometrium just after ovulation (Shiokawa et al., 1996). Conversely, progesterone and oestrogens inhibit the synthesis of $\alpha_1\beta_3$. As a result, the $\alpha_3\beta_3$ peptide appears on day 20, after the down-regulation of progesterone receptors, which is induced by high progesterone concentrations. The growth factors EGF, TGF-α and b-FGF strongly up-regulate the expression of $\alpha_3\beta_3$ in Ishikawa cells (Tabibzadeh, 1991; Castelbaum et al., 1997; Somkuti et al., 1997). In human endometrial adenocarcinoma cells, TGF-α increased the expression of $\alpha_1\beta_1$ and $\alpha_1\beta_3$ integrins. Treatment with interleukin-1α (IL-1α) or IL-2β induced the expression of $\alpha_3$, $\alpha_4$ and $\beta_3$ integrins in endometrial epithelial cells in culture (Somkuti et al., 1997; Simón et al., 1999). Cytokines IL-1 and IL-6 stimulated $\alpha_1$ and $\alpha_3$ integrins in human trophoblast cells (Das et al., 2002).

The regulation of integrin expression has also been studied in several other cell lines. In human osteosarcoma cells, TGFβ1 was shown to up-regulate the cell surface expression of a variety of integrins, including subunits $\alpha_1$–$\alpha_6$, $\beta_1$, $\beta_3$, $\beta_4$ and $\beta_6$ (Heino and Massague, 1989). Inhibition of TGFβ1 in human promonocytes decreased the mRNA concentrations of $\alpha_2$, $\alpha_3$, $\alpha_4$, $\alpha_2\beta_1$ and $\beta_3$ and increased the relative amount of $\alpha_4$ and $\beta_3$ mRNA (Dou et al., 1997b). Inflammatory mediators IL-1β and IFN-α increase the expression of $\alpha_5\beta_1$ integrin in fibroblasts and the cytokine GM-CSF regulates the expression of $\alpha_5$ in peripheral blood monocytes and T lymphocytes (Santala and Heino, 1991; Novella et al., 1997). The growth factor TGFβ1 was also found to decrease $\alpha_3$ mRNA concentrations and increase the $\alpha_6$ and $\beta_1$ expression at mRNA and protein concentrations in alveolar epithelial cells (Kumar et al., 1995).

A number of reports have connected aberrant expression of integrins with fertility defects. Subunits $\beta_3$, $\alpha_4$ and $\alpha_6$ are absent in menopausal endometrium. Subunit $\beta_3$ is also lacking
in endometriosis and it is expressed late in delayed endometrial differentiation in cases of unexplained infertility. The expression of \( \alpha_v \beta_3 \) was found to be lower in the mid-luteal phase endometrium of women with hydrosalpinges, a condition adversely affecting endometrial receptivity (Lessey \emph{et al.}, 1994, 1995a,b; Meyer \emph{et al.}, 1997). Integrin concentrations have been reported to be associated with subsequent treatment success in women undergoing IVF–intracytoplasmic sperm injection (ICSI). Specifically, patients who finally became pregnant showed a statistically significant increase in the luminal expression of the \( \alpha_v \beta_3 \) integrin, in support of the protein’s usefulness as a marker of endometrial receptivity. However, it should be noted that the same study reported successful IVF outcome in a few patients with negative expression of the \( \alpha_v \beta_3 \) peptide (Thomas \emph{et al.}, 2003). In pregnancies with pre-eclampsia, which is a condition adversely affecting endometrial receptivity (Lessey \emph{et al.}, 1995; Blobel, 1997; Hurskainen \emph{et al.}, 1999). ADAM-TS5 and ADAM-TS6 are expressed at low concentrations, primarily in the placenta. Using northern blot analysis, it has been demonstrated that ADAM-TS5 has prominent expression on day-7 embryos, but lower expression thereafter (Hurskainen \emph{et al.}, 1999). This suggests that this enzyme may play a role in proteolytic processing mostly during the peri-implantation phase.

MMP, secreted by cytotrophoblast, can be classified into four different sub-families according to their substrate specificity and structure: gelatinases (A or MMP-2 and B or MMP-9), collagenases (MMP-1, MMP-8 and MMP-13), stromelysins (MMP-3, MMP-7, MMP-10, MMP-11 and MMP-12), and a sub-family containing membrane metalloproteinase types (MMP-14, MMP-15, MMP-16 and MMP-17). Studies in animals have concluded that MMP-2 and MMP-9 are the most important MMP expressed by invasive trophoblasts (Salamonsen \emph{et al.}, 1995; Alexander \emph{et al.}, 1996; Das \emph{et al.}, 1997; Rechtman \emph{et al.}, 1999; Riley \emph{et al.}, 2000). Similarly, human trophoblasts isolated from first-trimester placenta, were shown to constitutively express both MMP-2 and MMP-9 in vitro. Importantly, the levels of expression and activity of MMP-9 were higher compared with third-trimester trophoblasts. Furthermore, blocking of MMP-9 prevented invasion of first-trimester trophoblasts into the artificial matrix Matrigel (Librach \emph{et al.}, 1991; Shimonovitz \emph{et al.}, 1994). Recently, investigators studied the mRNA levels of MMP-1 and MMP-2 in eutopic and ectopic endometrium, suggesting a possible contribution of the two molecules to the angiogenic process. Endometrium was cultivated in chorioallantoic membrane of chick embryos, thus establishing a model of endometriosis. The eutopic proliferative endometrium strongly expressed MMP-2 mRNA, whereas MMP-1 mRNA was marginally expressed. The ectopically cultivated endometrium expressed high concentrations of MMP-1 mRNA, which persisted at least 72 h after implantation on chorioallantoic membrane, whereas MMP-2 mRNA concentrations were unaltered (Wolber \emph{et al.}, 2003).

The regulation of MMP expression involves multiple pathways of autocrine and paracrine effectors. The proinflammatory cytokines IL-1\(_\alpha\) and TNF\(_\alpha\) increased MMP-9 expression in first-trimester cytotrophoblasts (Meisser \emph{et al.}, 1999a). Treatment of human trophoblasts with progesterone, down-regulated steady state concentrations of MMP-9 mRNA and peptide, an effect that was reversed by the addition of the progesterone receptor antagonist onapristone (Shimonovitz \emph{et al.}, 1998). Leptin, which is secreted by cytotrophoblasts, increased the activity of MMP-9 and MMP-2, whereas LIF decreased metalloproteinase secretion (Bischof \emph{et al.}, 1995; Nachtigall \emph{et al.}, 1996; Castellucci \emph{et al.}, 2000). Interestingly, it has been shown that MMP-2 binds and becomes activated by \( \alpha_v \beta_3 \) integrin on the surface of cultured melanoma cells. Further evidence supporting a correlation between integrins and MMP is presented by a recent report which demonstrated that human cervical tumour cell surface \( \alpha_v \beta_3 \) integrin was able to modulate MMP-2 activity (Brooks \emph{et al.}, 1996).

In addition to the regulation of MMP synthesis and activation, MMP-induced ECM degradation is modulated by locally

**Matrix metalloproteinases**

The matrix metalloproteinases (MMP), a family of extracellular matrix (ECM)-degrading, zinc-dependent endopeptidases, are considered to be crucial to the invasive capability of trophoblasts. MMP can degrade a variety of ECM substrates, including collagens and non-collagenous molecules, suggesting that they function as ECM-clearing enzymes during cell migration. Recently, it has been proposed that MMP not only remodel the ECM, but also influence cellular behaviour by altering the ECM microenvironment and modulating, through cleavage, the activity of biologically active molecules (Vu and Werb, 2000). Two groups of zinc metalloproteases, in particular ADAM and MMP, seem broadly relevant to extracellular proteolysis as well as invasive properties of blastocyst.

ADAM, also referred to as metalloprotease–disintegrins with cysteine-rich domains (MDC), have catalytic domains with zinc-binding signatures and disintegrin domains that are very similar to the MMP. This quite recent family of proteolytic enzymes includes seven members (ADAM-TS–ADAM-TS7) all exhibiting a similar domain organization (Wolfsberg \emph{et al.}, 1995; Blobel, 1997; Hurskainen \emph{et al.}, 1999). ADAM-TS5 and ADAM-TS6 are expressed at low concentrations, primarily in the placenta. Using northern blot analysis, it has been demonstrated that ADAM-TS5 has prominent expression on day-7 embryos, but lower expression thereafter (Hurskainen \emph{et al.}, 1999). This suggests that this enzyme may play a role in proteolytic processing mostly during the peri-implantation phase.
produced MMP inhibitors. Tissue inhibitors of metalloproteinases, or TIMP, bind to and specifically inhibit the active forms of MMP and ADAM in the extracellular space. This family consists of four members (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) (for review, see Baker et al., 2002). In humans, endometrial TIMP-1 expression is not cycle dependent, and TIMP-2 is also relatively constant throughout the menstrual cycle. TIMP-3 appears to particularly accumulate scientific interest, since it has been found to be highly expressed in maternal cells in the area surrounding the invading murine embryonic tissue (Reponen et al., 1995; Leco et al., 1996). Moreover, TIMP-3 mRNA is up-regulated by progesterone in vitro in the decidualized stroma (Higuchi et al., 1995).

Evidently, the colocalization of MMP and TIMP at the maternal–fetal interface serves to promote and at the same time limit the invasive behaviour of the trophoblast. In support of this suggestion, high concentrations of both protein and mRNA of MMP-9 and TIMP-3 were reported to be expressed after differentiation of cytotrophoblasts to their fully invasive phenotype (Bass et al., 1997). Briefly, trophoblast invasion into the decidualized stroma requires structural alterations in adjacent tissue architecture. It is plausible that MMP, ADAM and TIMP orchestrated by various hormones, growth factors and cytokines, facilitate the invasion and play their part in the implantation process.

Table 1 summarizes the expression of major integrins, MMP and TIMP at the human implantation site and presents the available data on the correlation between expression patterns of the molecules and IVF treatment outcome.

**Conclusions**

Implantation is a prerequisite for furtherance of the species and therefore it is not surprising that high levels of redundancy and compensatory gene expression have been developed to ensure its success. Nevertheless, the process is characterized by remarkably low efficiency, indicating how easily it may be disturbed. Both the endometrium and the blastocyst have to develop in synchrony, since temporal and spatial limits are placed on the endometrial receptivity. So far, studies propose that a multifactorial, complex and dynamic regulation, which involves hormones, cytokines, neuropeptides, adhesion molecules and ECM-degrading proteases, is operative between embryonic and endometrial compartments. Intriguing findings suggest multiple interrelationships between the implicated factors. Thus, it would be tempting to further investigate the possible connections between molecules such as integrins, MMP and locally produced modulators of the inflammatory phenomena. Additionally, newly applied approaches that utilize molecular biology technologies such as macroarray, microarray and differential display polymerase chain reaction analysis are most promising in elucidating the expression patterns of genes implicated in the process of endometrial receptivity (Domínguez et al., 2003).

Table 1. Summary of the expression of major integrins, MMP and TIMP at the human implantation site and available data on the correlation between expression patterns of the molecules and IVF treatment outcome. A statistically significant \( (P \leq 0.05) \) increase in \( \alpha_v \beta_3 \) integrin was found in the luminal epithelium of patients who became pregnant after IVF treatment. There was also a statistically greater expression in the glandular localization of the \( \alpha_4 \beta_1 \) integrin in patients whose treatment was unsuccessful \( (P \leq 0.05) \). Additionally, intrauterine MMP9 activity was increased in women with recurrent failed embryo transfer. Data accumulated from Curry and Osteen (2003); Hwang et al. (2002); Inagaki et al. (2003); Kimber and Spanswick (2000); Ruck et al. (1994); Thomas et al. (2003).

<table>
<thead>
<tr>
<th>Decidua</th>
<th>Trophoblast</th>
<th>Favourable prognosis</th>
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<tbody>
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<td>( \alpha_1 )</td>
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<td>( \alpha_4 )</td>
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<td>( \alpha_5 )</td>
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<td>MMP2</td>
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<td>MMP9</td>
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<td>TIMP1</td>
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<td>TIMP2</td>
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<td>TIMP3</td>
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</table>

Relative concentrations of MMP and TIMP protein or mRNA are expressed as + (focal) or + + (moderate). Relative levels of integrin subunit fluorescence intensity in decidual cells are expressed as + (low), + + (moderate) or + + + (intense). Arrows indicating up- or down-regulation of the respective integrin expression levels during trophoblast differentiation to the invasive phenotype.
In conclusion, the majority of pregnancy losses occur prior to or during implantation and progress towards improving early pregnancy success rates is one of the most enticing aims of reproductive medicine. In this direction, a better understanding of the mechanisms involved in endometrial function and blastocyst implantation is required. Presumably, when the mechanisms underlying normal embryonic and endometrial development are elucidated, fertility defects will be decoded and hence effectively treated.

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