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Endometrial receptivity markers, the journey to successful embryo implantation

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Human embryo implantation is a three-stage process (apposition, adhesion and invasion) involving synchronized crosstalk between a receptive endometrium and a functional blastocyst. This ovarian steroid-dependant phenomenon can only take place during the window of implantation, a self-limited period of endometrial receptivity spanning between days 20 and 24 of the menstrual cycle. Implantation involves a complex sequence of signalling events, consisting in the acquisition of adhesion ligands together with the loss of inhibitory components, which are crucial to the establishment of pregnancy. Histological evaluation, now considered to add little clinically significant information, should be replaced by functional assessment of endometrial receptivity. A large number of molecular mediators have been identified to date, including adhesion molecules, cytokines, growth factors, lipids and others. Thus, endometrial biopsy samples can be used to identify molecules associated with uterine receptivity to obtain a better insight into human implantation. In addition, development of functional *in vitro* systems to study embryo–uterine interactions will lead to better definition of the interactions existing between the molecules involved in this process. The purpose of this review was not only to describe the different players of the implantation process but also to try to portray the relationship between these factors and their timing in the process of uterine receptivity.

Key words: cell adhesion molecules/cytokines/embryo/endometrial receptivity/implantation

Introduction

Embryo implantation represents the most critical step of the reproductive process in many species. It consists of a unique biological phenomenon, by which the blastocyst becomes intimately connected to the maternal endometrial surface to form the placenta that will provide an interface between the growing fetus and the maternal circulation (Denker, 1993; Aplin, 2000). Successful implantation requires a receptive endometrium, a normal and functional embryo at the blastocyst developmental stage and a synchronized dialogue between maternal and embryonic tissues (Simon *et al*., 2000).

The process of implantation may be classified into three stages: apposition, adhesion and invasion (Enders, 1967). During blastocyst apposition, trophoblast cells adhere to the receptive endometrial epithelium. The blastocyst will subsequently anchor to the endometrial basal lamina and stromal extracellular matrix (ECM). At this point, the achieved embryo–endometrial linkage can no longer be dislocated by uterine flushing. This is followed by the invasive blastocyst penetration through the luminal epithelium (Enders, 1967).

Even though the blastocyst can implant in different human tissues, surprisingly in the endometrium, this phenomenon can only occur during a self-limited period spanning between days 20 and 24 of a regular menstrual cycle (day LH+7 to LH+11). Throughout this period, namely the window of implantation (Psychoyos, 1973), the human endometrium is primed for blastocyst attachment, given that it has acquired an accurate morphological and functional state initiated by ovarian steroid hormones (Finn and Martin, 1974; Yoshinaga, 1988; Paria *et al*., 2002).

Implantation involves a complex sequence of signalling events that are crucial to the establishment of pregnancy. A large number of identified molecular mediators, under the influence of ovarian hormones, have been postulated to be involved in this early feto–maternal interaction. These mediators embrace a large variety of inter-related molecules including adhesion molecules, cytokines, growth factors, lipids and others (Lessey *et al*., 1992; Simon *et al*., 2000). Endometrial receptivity consists in the acquisition of adhesion ligands together with the loss of inhibitory components that may act as a barrier to an attaching embryo (Aplin, 2000).

The relative inefficiency of the implantation process is paradoxical in view of the fact that reproduction is critical to species survival. Implantation failure remains an unsolved problem in reproductive medicine and is considered as a major cause of infertility in otherwise healthy women. Indeed, the average implantation rate in IVF is around 25% (de los Santos *et al*., 2003). Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures, whereas the embryo itself is

responsible for only one-third of these failures (Simon *et al*., 1998; Ledee-Bataille *et al*., 2002).

The recent discovery of molecules crucial for successful embryo implantation has offered researchers precious insight into this field. Nevertheless, important questions regarding the molecular mechanisms governing this process remain to be deciphered. A better understanding of the mechanisms regulating embryo implantation may improve the ability of clinicians to treat infertility, to prevent early pregnancy loss and to develop new contraceptive approaches. This knowledge might enable investigators to improve this critical step in modern reproductive therapies.

The purpose of this review was to describe the most important players of the feto–maternal crosstalk in the apposition and adhesion phases and to summarize the current knowledge as to their regulation, relationships and their involvement in physiological and pathological conditions.

Endometrial morphological features

Histology

The classical work describing the dating of the endometrium, by Noyes *et al*. (1950), dates from more than 50 years ago. Interestingly, this article was the most cited one in infertility literature for a long time (Key and Kempers, 1987). Current textbook recommendations on the evaluation of the infertile couple include routine luteal phase assessment of the endometrial histology. The rationale for this routine evaluation serves two purposes. The first is to ascertain that ovulation has occurred resulting in the development of an active corpus luteum releasing progesterone with its observed effects on the endometrial glands. The second is to ensure that the endometrial dating is in proper association with the embryonic age. In recent years, however, new and updated methods to evaluate the endometrium have been proposed making the classical criteria of Noyes somewhat outdated (Acosta *et al*., 2000; Lessey *et al*., 2000).

In some cases, the menstrual cycle date, which is based by the pathologist on Noyes' criteria, lags behind the actual cycle date. When this lag is of more than 2 days, the endometrium is considered to be 'out of phase'. Patients diagnosed with an 'out of phase' endometrium were counselled to treat this condition by hormonal means. The original Noyes' criteria compared endometrial dating with the estimated day of ovulation based on an increase in basal body temperature. This estimate was later shown to be accurate only in 77% of patients. In comparison, a better accuracy can be obtained by LH surge detection or by ultrasound demonstration of ovulation (85 and 96%, respectively; Shoupe *et al*., 1989). More recently, it was shown that the prevalence of an 'out of phase' endometrium in the fertile population is extremely high (49%). In fact, these investigators found that fertile women were more likely to have an 'out of phase' endometrium than infertile women (43%; Coutifaris *et al*., 2004). Moreover, the Noyes' criteria, even when examined in normal fertile women, lack the precision to be used to accurately date the endometrium (Murray *et al*., 2004). It can thus be concluded that histologic evaluation adds little significant information pertaining to the treatment of the infertile couple. More significant markers, discussed in this article, will surely replace histologic criteria in the near future.

Pinopods

Pinopods are bleb-like protrusions found on the apical surface of the endometrial epithelium (Usadi *et al*., 2003). These structures are several micrometers wide and project into the uterine lumen above the microvilli level. They were first described in mice (Nilsson, 1958) and later in human endometrium (Johannisson and Nilsson, 1972; Martel *et al*., 1987; Murphy *et al*., 1987). The term 'pinopod', from the Greek 'drinking foot', signifies their pinocytotic function in the mouse (Enders and Nelson, 1973). Nevertheless, this pinocytosis capacity was not detected in human (Adams *et al*., 2002). Electron microscopy is the major tool used to observe these structures (Johannisson and Nilsson, 1972; Martel *et al*., 1987). However, use of light microscopy has been proposed so as to facilitate their detection (Develioglu *et al*., 2000).

Pinopod expression is limited to a brief period of maximum 2 days in the menstrual cycle corresponding to the putative window of implantation (Nikas, 1999; Stavreus-Evers *et al*., 2001; Aghajanova *et al*., 2003). Others have detected that pinopods are present throughout the mid- to late-secretory phase, however, displaying cycle-dependent morphological changes. This suggests that morphology, rather than pinopod presence or absence, is of great significance (Usadi *et al*., 2003). The pinopod-regulated expression pattern throughout the menstrual cycle advocates their use as markers of implantation.

Pinopods appear progesterone dependant. Association between mid-luteal increase of progesterone level and the first appearance of pinopods throughout the menstrual cycle was noted (Stavreus-Evers *et al*., 2001; Usadi *et al*., 2003). Moreover, *HOXA-10*, a homeobox gene whose expression is necessary for endometrial receptivity to blastocyst implantation, has an essential role in pinopod development. Indeed, blocking *HOXA-10* expression dramatically decreases the number of pinopods. *HOXA-10* illustrates a dual role in the endometrium by regulating both endometrial stromal cell (ESC) proliferation and epithelial cell morphogenesis (Bagot *et al*., 2001).

Although the role of pinopods remains unknown, it seems that they are the preferred sites of embryo–endometrial interactions. Blastocyst attachment was shown to occur onto the top of endometrial pinopods (Bentin-Ley *et al*., 1994; Bentin-Ley *et al*., 1999). Hypothetically, the receptors required for blastocyst adhesion are located on the pinopod surface. Endometrial pinopods' development is associated with the mid-luteal phase increased expression of leukaemia inhibitory factor (LIF) and its receptor (Aghajanova *et al*., 2003), progesterone (Stavreus-Evers *et al*., 2001) and integrin αVβ3 (Lessey *et al*., 1992). The detection of pinopods during the mid-secretory phase may be extremely useful for the assessment of endometrial receptivity to optimize implantation rates.

Cellular adhesion molecules family

The cell adhesion molecule (CAM) family is composed of four members known as integrins, cadherins, selectins and immunoglobulins. These surface ligands, usually glycoproteins, mediate cell-to-cell adhesion. Their classical functions include maintenance of tissue integration, wound healing, morphogenic movements, cellular migrations and tumour metastasis.

Integrins

Integrins are a family of transmembrane glycoproteins, formed by the association of two different, non-covalently linked, α and β subunits. To date, 18 α and eight β chains have been identified in mammals. When paired, they form 24 distinct integrin heterodimers that differ in their function (Hynes, 2002). These subunits contain extracellular, transmembranal and intracellular domains. The extracellular domain enables integrins to act as a receptor to ECM components [fibronectin (FN), laminin and collagen type IV], complement and other cells. The intracellular domain, however, is able to interact with the cytoskeleton. Integrins participate in cell–matrix and cell–cell adhesion in many physiologically important processes including embryological development, haemostasis, thrombosis, wound healing, immune and non-immune defense mechanisms and oncogenic transformation. In response to ligand binding, integrins aggregate in discrete assemblies known as 'focal adhesion sites' (Gilmore and Burridge, 1996). This aggregation leads to the recruitment of a network of cytoskeletal proteins (e.g. α-actinin, talin and vinclulin that may act as an anchor for F-actin) and intracellular signalling complexes, mainly kinases [e.g. focal adhesion kinase (FAK), integrin-linked kinase (ILK), molecules of the mitogen-activated protein (MAP) kinase pathway and lipid kinases; Bowen and Hunt, 2000]. This network of cytoskeletal and signalling complexes within the focal adhesion site allows for a double modulation of the integrins action. Indeed, binding of the ligand to integrins activates classical intracellular signal transduction pathways and triggers cellular events (outside-in signalling). Moreover, the number and the affinity of integrins present on the cell surface are modulated in response to the ligand binding (inside-out signalling; Longhurst and Jennings, 1998).

A large variety of integrins have been described within the luminal and glandular endometrial epithelium (Lessey *et al*., 1992, 1994a; Klentzeris *et al*., 1993). Whereas the majority of the integrins are constitutively expressed throughout the entire menstrual cycle, others exhibit an interesting regulated pattern within the cycle (Lessey *et al*., 1992). Integrins whose expression is increased in the mid-luteal phase were proposed as markers for the frame of the window of implantation (Lessey *et al*., 2000). Three cycle-specific integrins are co-expressed by the human endometrium defined histologically on days 20–24 of the human menstrual cycle: α1β1, α4β1 and αVβ3, but only the β3 mRNA subunit expression was shown to increase after day 19 and is not detected beforehand. Moreover, αVβ3 integrin as well as its ligand osteopontin was positively detected by immunohistochemistry on the endometrial luminal epithelial surface, which first interacts with the trophoblast (Apparao *et al*., 2001). In regard to its expression pattern along with its epithelial localization, αVβ3 has been proposed as a potential receptor for embryonic attachment (Lessey, 2003).

Integrins are also expressed by the human trophoblast at the time of implantation (Wang and Armant, 2002). Trophoblastic receptors for ECM (essentially, integrins α1β1 and α5β1) increase in concert with the differentiation of human cytotrophoblast cells to invasive extravillous phenotype (Damsky *et al*., 1994). It is hypothesized that integrins, which are present on both the uterine epithelium surface and the trophoblast, bind to specific ECM components. These ligands typically include oncofetal FN that is secreted by the trophoblast and osteopontin secreted by the uterine epithelium. This provides the possibility of a sandwich model of embryonic adhesion.

The cycle-specific pattern of endometrial integrin expression is suggestive of hormonal regulation. Indeed, αVβ3 integrin expression is orchestrated in the human endometrium both by positive [e.g. epidermal growth factor (EGF), heparin-binding EGF (HB-EGF)] and negative [e.g. 17β-estradiol (E_2)] factors (Somkuti *et al*., 1997). During the proliferative phase, high estrogen levels act via the estrogen receptor-α (ERα) to inhibit integrin expression. The luteal progesterone rise subsequently down-regulates the number of those receptors, thus indirectly suppressing the inhibitory effects of E_2 on integrins. This results in a net integrin increase. Progesterone, probably, also acts positively by increasing paracrine stromal factors (e.g. EGF and HB-EGF) to induce epithelial β3 integrin expression that serves as the rate-limiting step in αVβ3 formation (Lessey, 2003). Progesterone also has a direct effect on osteopontin synthesis by stimulating its gene expression (Lessey, 2003). In addition to these factors, the homeobox gene HOXA 10 is implicated in the regulation of β3 subunit expression. Indeed, when treated with HOXA 10, cultured endometrial cell β3 expression was greatly augmented (Daftary *et al*., 2002). The embryo itself was shown to participate in this regulation. Human blastocysts were shown to up-regulate β3 integrins in cultured human endometrial epithelial cells (EECs). This effect seems to be partially mediated by the embryonic interleukin-1 (IL-1) system (Grosskinsky *et al*., 1996; Simon *et al*., 1997). This observation strongly suggests an active role for the blastocyst in the establishment of a receptive endometrium.

Aberrant αVβ3 integrin expression pattern has been associated with unexplained infertility (Klentzeris *et al*., 1993; Lessey *et al*., 1995; Tei *et al*., 2003), endometriosis (Lessey *et al*., 1994b), hydrosalpinx (Meyer *et al*., 1997), luteal phase deficiency (LPD; Lessey *et al*., 1992) and, more recently, polycystic ovarian syndrome (PCOS; Apparao *et al*., 2002). Other investigators could not, however, demonstrate different integrin pattern in endometriosis (Creus *et al*., 1998).

We found that the integrin mRNA level on day 21 could predict the IVF success rate. Patients with normal integrin levels had a double pregnancy rate as compared with patients with low levels. Implementation of integrin β3 expression may thus be a useful tool to predict success in an IVF program (Thomas *et al*., 2003; Revel, 2005). Considering the literature on integrin αVβ3 expression and regulation, this protein represents a promising clinical and research marker of the human implantation process.

Selectins

Selectins are glycoproteins which also belong to the CAM family. They include P-selectin, L-selectin and E-selectin. The human L-selectin, which is of importance in the implantation process, consists of a large, highly glycosylated extracellular domain, a single spanning transmembrane domain and a small cytoplasmic tail (Smalley and Ley, 2005). Selectins are known to play an important role in leukocyte transendothelial trafficking (Alon and Feigelson, 2002). Indeed, L-selectins are expressed on leukocytes and interact with their carbohydrate-based ligands on the endothelium. This interaction, termed tethering, allows the rolling of leukocytes on inflamed vascular endothelium before their firm adhesion and transmigration. The shear pressure exerted by blood flow is known

to be necessary for optimal L-selectin-mediated adhesion of leukocytes to the vasculature. A parallel can be made between the leukocytes' 'rolling' phenomenon and the blastocyst apposition to the endometrial epithelium (Genbacev *et al*., 2003; Dominguez *et al*., 2005).

The selectin adhesion system is well established at the maternal–fetal interface. On the blastocyst side, strong L-selectin staining has been observed over the entire embryo surface (Genbacev *et al*., 2003). On the maternal side, the expression of selectin oligosaccharide-based ligands, such as MECA-79 or HECA-452, is up-regulated during the window of implantation (Genbacev *et al*., 2003). Indeed, L-selectin ligand MECA-79 is immunolocalized in the luminal and glandular endometrial epithelium throughout the menstrual cycle, although the staining considerably intensifies during the mid-secretory phase. Additionally, the immunoreactivity appears to be stronger in the luminal epithelium as compared with the glandular epithelium (Lai *et al*., 2005). The physiological importance of the interaction between L-selectin and its oligosaccharide ligands was investigated in the human endometrium (Genbacev *et al*., 2003). It was shown that beads coated with specific selectin ligands bound avidly to trophoblast cells in the placental villous tissues under conditions of shear stress that mimic those of the uterus. In a reverse experiment, isolated trophoblasts adhere preferentially to epithelial cells from a receptive endometrium. The binding of L-selectin ligands is regulated by a sulphation mechanism among others. Sulphatases are capable of removing a sulphate moiety from natural sulphated oligosaccharides, which prevents selectin binding to its receptors (Rosen, 2004). These findings suggest that the interaction between L-selectin, expressed by trophoblast cells, and its oligosaccharide ligands, expressed by the endometrium, may constitute the initial step in the implantation process (Fazleabas and Kim, 2003).

Like most adhesion molecules, L-selectin function is regulated by a variety of mechanisms including gene transcription, post-translational modifications and association with the actin cytoskeleton. Another regulatory mechanism consists of modifications of the L-selectin topographic distribution by increasing or decreasing its availability at the cell surface. One of the down-regulation processes involves proteolytic cleavage, also termed ectodomain shedding. Sheddases such as TACE [tumour necrosis factor (TNFα)-converting enzyme]/ADAM 17 are able to proteolytically cleave the L-selectin ectodomain at the endothelial surface. This process of 'ectodomain shedding' results in the release of most of the extracellular portion of L-selectin from the cell surface while preserving the cytoplasmic, transmembrane and a small part of the extracellular domain on the cell. Shedding of L-selectin from the leukocytes surface seems to be required for their efficient migration through the endothelium (Smalley and Ley, 2005). Indeed, blocking L-selectin cleavage on antigen-stimulated lymphocytes, by gene targeting, allowed their continued migration to peripheral lymph nodes and inhibited their short-term redirection to inflammatory sites (Venturi *et al*., 2003). The question of whether selectin shedding is of relevance for embryonic implantation remains to be clarified.

In conclusion, very little is known about the involvement of selectins in embryo implantation. It appears, however, that selectins take part in the very early stages of blastocyst interactions with the uterine wall. Similar to a leukocyte stopping at a particular site on the endothelium, the blastocyst is expected to find the best location in the uterine cavity to ensure successful implantation.

Cadherins

Cadherins constitute a group of glycoproteins responsible for the calcium-dependent cell-to-cell adhesion mechanism. They are divided into subclasses E-, P-, and N-cadherins that are distinct in immunological specificity and tissue distribution. They promote cell adhesion via a homophilic mechanism. In regard to implantation, E-cadherin represents the most studied subclass.

E-cadherin is a cell surface transmembrane glycoprotein, which belongs to the family of calcium-dependant CAMs, that mediates cell–cell adhesion through homeotypic binding. E-cadherin is located in the adherens junctions that are specialized regions on the lateral side of the epithelial plasma membrane and is believed to be critical for the establishment and maintenance of these junctions in epithelial cells (Gumbiner, 1996; Huber *et al*., 1996). E-cadherin is expressed by a variety of tissues and plays an important role in embryogenesis formation during gastrulation, neurulation and organogenesis (Barth *et al*., 1997). Suppression of E-cadherin expression is regarded as one of the main molecular events responsible for dysfunction of cell–cell adhesion. In this regard, E-cadherin may contribute to malignant cell transformation and tumour development and progression.

Studies on mouse embryo implantation have shown that targeted mutations in the E-cadherin gene result in defective pre-implantation development (Riethmacher *et al*., 1995). The role of E-cadherin in human embryo implantation is not known, but based on its expression pattern, we suspect that it is of importance for this process. E-cadherin mRNA levels were shown to be significantly higher during the luteal phase (Fujimoto *et al*., 1996). Nevertheless, these menstrual cycle variations were not detected at the protein level by immunohistochemical studies (van der Linden *et al*., 1995; Beliard *et al*., 1997; Dawood *et al*., 1998; Poncelet *et al*., 2002).

The regulation of E-cadherin availability at the epithelial cell surface enables cellular adhesion control. Down-regulation of E-cadherin expression correlates with the acquisition of metastatic potential by carcinomatous cells. Subsequently, the tissue architecture is lost resulting in cell dissociation and dispersion (Batlle *et al*., 2000; Cano *et al*., 2000; Comijn *et al*., 2001). Intracellular calcium is essential in the E-cadherin regulation. Indeed, a rise in its concentration activates key signalling pathways that mediate cytoskeletal reorganization and disassembly of E-cadherin at the adherens junctions. Alterations in intracellular calcium concentrations affect epithelial cell adhesiveness and polarity by triggering CAMs redistribution (Gumbiner *et al*., 1988). This phenomenon could be of importance in EECs expressing E-cadherin. *In vitro* experiments on cultured Ishikawa cells demonstrated that a transient rise in intracellular calcium, triggered by calcitonin, suppresses E-cadherin expression at cellular contact sites (Li *et al*., 2002). Interestingly, calcitonin expression is induced by progesterone in the human endometrial epithelium specifically during the mid-secretory phase of the menstrual cycle (Kumar *et al*., 1998). Indeed, calcitonin is known to be a potential regulator of implantation (Ding *et al*., 1994; Zhu *et al*., 1998). Progesterone, probably via endometrial calcitonin induction leading to increased intracellular calcium, could regulate E-cadherin expression (Figure 1).

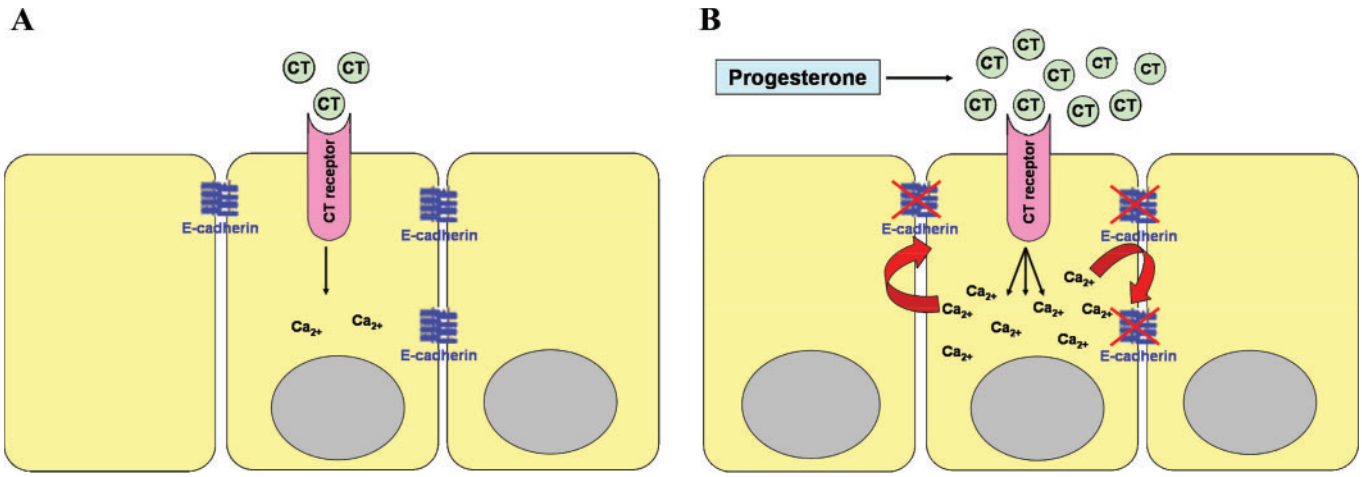

Figure 1. (**A**) Epithelial cell adhesiveness by E-cadherin is controlled by intracellular calcium. (**B**) Rising progesterone levels induce calcitonin expression and thus increase the concentration of intracellular calcium, which then suppresses E-cadherin expression at cellular contact sites.

Thus, it is possible that E-cadherin possesses a dual function. In the preliminary phases, its expression at the cell surface is required to ensure adhesiveness. In contrast, E-cadherin may be subsequently down-regulated to enable epithelial cells dissociation and blastocyst invasion.

Immunoglobulins

Among the CAMs family, the immunoglobulins superfamily is the most extensive. Intercellular adhesion molecule-1 (ICAM-1 or CD54) is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily and is constitutively expressed on the cell surface of a variety of cell types, such as fibroblasts, leukocytes, endothelial and epithelial cells. This molecule is up-regulated at the transcriptional level by both inflammatory and non-inflammatory cytokines. ICAM-1 mediates cell–cell adhesion since it constitutes a ligand for β2 integrin molecules expressed on many cell types. ICAM-1 adhesive interactions are essential for the transendothelial migration of leukocytes and for various immunological functions (van de Stolpe and van der Saag, 1996).

It is well established that the endometrium, under normal conditions, contains a wide population of leukocytes, including macrophages, T lymphocytes and granulocytes (Kamat and Isaacson, 1987; Marshall and Jones, 1988), which are significant in many physiological mechanisms such as decidualization (King, 2000), menstruation (Salamonsen and Lathbury, 2000) and parturition (Yellon *et al.*, 2003). This population of leukocytes expresses ICAM-1 within the endometrium. Nevertheless, it has been demonstrated that this adhesion molecule is also expressed by other endometrial cell types. Indeed, ICAM-1 was immunolocalized, throughout the menstrual cycle, to the apical surface of the glandular and luminal EECs as well as in the stroma. Stromal cell expression of ICAM-1 is up-regulated at the time of menstruation (Thomson *et al.*, 1999). A soluble circulating form of ICAM-1 (sICAM-1) was also detected in human serum (Rothlein *et al.*, 1991) and in peritoneal fluid (PF; Somigliana *et al.*, 1996). This soluble form is proteolytically released from the cell surface by shedding of the transmembrane-bound ICAM-1. It was recently shown that endometrial cells in culture are able to constitutively express ICAM-1 mRNA and protein without hormonal supplement. Purified EECs are, however, able to produce more ICAM-1 than ESCs. In view of the fact that ICAM-1 is strongly expressed in both stromal and epithelial endometrial cells, it was suggested that ICAM-1 may play a role in the pathophysiology of the endometrium (Defrere *et al.*, 2005).

In cultured ESCs, ICAM-1 expression is up-regulated by interferon-γ (IFN-γ; Thomson *et al.*, 1999). This result was confirmed by the finding that expression of sICAM-1 is up-regulated after IFN-γ stimulation in eutopic ESCs in women with endometriosis. IFN-γ allows the accumulation of the soluble form of ICAM-1 by acting on its shedding at the ESC surface (Wu *et al.*, 2004).

ICAM-1 seems to play a role in the pathogenesis of endometriosis by acting at two different levels. On the one hand, it was suggested that an aberrantly high expression of ICAM-1, found in peritoneal cells of patients with endometriosis, could provide an adhesion potential to endometriotic cells and augments their interaction with the surrounding peritoneum. This phenomenon could explain the high recurrence rate of this disease (Wu *et al.*, 2004). On the other hand, it has been demonstrated that sICAM-1 interferes with immunological functions and its shedding may be one of the mechanisms of endometriosis pathogenesis, by which refluxed endometrial cells escape immunosurveillance (Defrere *et al.*, 2005). Moreover, it has been demonstrated that a genetic polymorphism in the ICAM-1 gene domain may be correlated with the susceptibility to endometriosis (Vigano *et al.*, 2003). Even before these results, sICAM-1 was proposed as a potential marker in the detection of endometriosis (Wu *et al.*, 1998).

The relationship between ICAM-1 expression and recurrent pregnancy loss (RPL) has been investigated. It was found that membrane-bound ICAM-1 was identically expressed on luteal phase endometrial cells of patients with and without unexplained RPL. However, the endometrial release of sICAM-1 was lower in RPL patients as compared with the control group. Because sICAM-1 is able to interfere with several immunological responses, the reduced protein levels observed in these patients may point towards an overactive immunological environment during the early phases of pregnancy (Gaffuri *et al.*, 2000).

Although ICAM-1 was not shown to be indispensable for the early steps of blastocyst interactions with the endometrium, it

could participate indirectly in this process by interacting with the immune system. A clearer picture of human endometrial pathophysiologies may be acquired by further studies of ICAM-1 expression and function.

Mucins

Mucins are high molecular weight (MW) glycoproteins, which contain at least 50% of carbohydrate O-linked to a threonine/serine rich peptide core (Gendler *et al*., 1990). Among the 14 cloned human mucins, only Mucin-1 (MUC1) and to a lesser extent MUC6 have been found in the human endometrium (Hey *et al*., 1994; Gipson *et al*., 1997). MUC1 is a large glycoprotein (MW>250 kDa), which is encoded by a gene comprising seven exons that span approximately 4–7 kb. The variable length of this gene depends on the number of 60 bp tandem repeats located in exon 2, and this leads to a polymorphism in the expressed gene product (Swallow *et al*., 1987). The MUC1 glycoprotein contains an intracellular cytoplasmic tail and a long extracellular part (ectodomain) consisting of a parallel variable number of identical tandem repeat (VNTR) domains of 20 amino acids. Each of these domains also contains five potential O-glycosyl sites (Gendler *et al*., 1990). When highly expressed on the cell surface, MUC1 interferes with cellular adhesion by a steric hindrance phenomenon. Cell–cell and cell–matrix adhesion are inhibited in direct correlation to the length of the MUC-1 ectodomain (Hilkens *et al*., 1992; Wesseling *et al*., 1996).

The apical surface of most epithelial cells is protected by a thick glycocalyx composed mostly of mucins that are believed to protect the cell surface from pathological processes (Strous and Dekker, 1992). In the endometrium, MUC1 extends beyond the glycocalyx and is probably the first molecule that the embryo encounters on its route to attachment. One could contemplate the possibility that endometrial MUC1 repels the blastocyst until it finds the correct time and place for implantation. The distribution and regulation of MUC1 vary through the menstrual cycle and among species. MUC1 is down-regulated before implantation in the receptive endometrium of mice (Braga and Gendler, 1993; Surveyor *et al*., 1995), rats (DeSouza *et al*., 1998) and pigs (Bowen *et al*., 1996). High-progesterone levels presumably reduce MUC1 expression, therefore, facilitating embryo–epithelial interactions by unmasking CAMs on the endometrial surface (Surveyor *et al*., 1995). Hence, MUC1 inhibits implantation and its down-regulation could contribute to the achievement of endometrial receptivity (Surveyor *et al*., 1995; Bowen *et al*., 1996; Hild-Petito *et al*., 1996; DeSouza *et al*., 1998). Surprisingly, human endometrial MUC1 was found to be up-regulated during the peri-implantation period (Hey *et al*., 1995; Aplin *et al*., 1998). Indeed, both MUC1 mRNA and protein show a several fold increase from the proliferative to the mid-secretory phase (Hey *et al*., 1994). This finding presents a paradox; one would expect inhibitory factors to decrease during implantation, as was described in other species. It was suggested that humans require a locally acting mechanism for the removal of the MUC1 barrier to the implanting embryo (Thathiah and Carson, 2004).

Immunohistochemistry on human endometrium, using monoclonal antibodies against the MUC1 ectodomain, could not detect noticeable variations in its localization on the apical surface of epithelial cells (Hey *et al*., 1994; DeLoia *et al*., 1998). Nevertheless, scanning electron microscopy combined with immunohistochemistry has succeeded in precisely consigning the MUC1 epitope only to ciliated cells. In contrast, MUC1 was missing from the surface of non-ciliated cells and from uterine pinopods (Horne *et al*., 2002). We suggest that the importance of pinopods is to supply an area, free of the widespread MUC1 inhibition to embryo–endometrial interaction. Indeed, human *in vitro* implantation models indicate that MUC1 is lost at the site of embryo attachment (Meseguer *et al*., 2001). In this model, human embryos were co-cultured to the blastocyst stage on a monolayer of EECs and then transferred in a three-dimensional system containing EECs and ECM gel. These embryos were observed to attach to the underlying epithelium. MUC1 staining was absent from epithelial cells beneath and in the immediate vicinity of the attached embryo, whereas it was unaffected at a greater distance from the implantation site. These findings may suggest that factors expressed on the blastocyst cell surface or secreted by the blastocyst itself trigger the local loss of MUC1 (Thathiah and Carson, 2004). Ectodomain shedding has a significant impact on the biological activity of integral membrane proteins and, therefore, regulates intracellular signalling cascades. One way to trigger the loss of MUC1, and thereby its anti-adhesive function, is to remove its cell surface ectodomain. The family of proteolytic enzymes termed ‘sheddases’ was shown to be capable of MUC1 ectodomain proteolysis (Thathiah *et al*., 2003). TNFα, a proinflammatory cytokine, secreted both by the endometrium (Hunt *et al*., 1992; Tabibzadeh *et al*., 1995; Bischof *et al*., 2000) and by the human blastocyst (Witkin *et al*., 1991), could play a role in locally removing the repelling MUC1 (Thathiah *et al*., 2004). Interestingly, TNFα has a dual effect. On the one hand, it increases MUC-1 gene expression. This stimulation seems to be mediated by the binding of nuclear factor κB to its site in the MUC1 gene promoter (Thathiah *et al*., 2004). On the other hand, TNFα was shown to markedly stimulate MUC1 shedding in human uterine epithelium. Indeed, in a human uterine epithelial cell line (HES), TNFα enhances the expression of a member of the sheddase family, the TNFα-converting enzyme TACE/ADAM17 (Thathiah *et al*., 2004). Other sheddases, in particular , also mediate *in vitro* MUC1 ectodomain release (Thathiah and Carson, 2004). Interestingly, *in vivo* MT1-MMP expression was shown to increase during the receptive phase in human endometrial biopsies (Zhang *et al*., 2000). Moreover, immunohistochemical stainings have demonstrated co-localization of MUC1 and MT1-MMP in human uterine epithelium at the time of implantation. Since this enzyme is not affected by TNFα, it was hypothesized that other factors could impact cell MUC1 stability (Thathiah *et al*., 2004).

Women with RPL were shown to express reduced endometrial MUC1, as compared with a normal group of patients. Indeed, using semi-quantitative immunohistochemistry, it has been demonstrated that mid-secretory phase levels of MUC1 core protein and mucin-associated glycans are reduced in RPL women (Serle *et al*., 1994; Aplin *et al*., 1996). Similar results were found in the uterine flushings of RPL patients after day LH+7, as compared with a fertile group (Hey *et al*., 1995; Aplin *et al*., 1996). The repellent effect of MUC-1 could be of importance in guiding the blastocyst to the precise area fittest for implantation. As previously described, the human gene sequence of MUC1 contains a variable number of tandem repeat regions. This polymorphism, characteristic of the human MUC1 gene, is relevant to normal implantation. A study, comparing the MUC1 polymorphism in

fertile versus recurrent implantation failure (RIF) patients, has shown a higher frequency of small MUC1 alleles in the infertile group. Hence, primary infertility due to implantation failure might be associated with a polymorphism in the MUC1 VNTR, resulting in a protein with a substantial reduction in the number of O-glycosylation sites (Horne *et al*., 2001).

In conclusion, MUC1 appears to be a negative factor for embryo implantation. Indeed, in the area where implantation takes place, MUC1 disappears. This effect was shown to be controlled *in vitro* mainly by the sheddase family enzymes that are modulated by blastocyst and endometrial derived factors. Because endometrial MUC1 increases at the time of implantation, we suspect this factor has a crucial role to direct the embryo temporally and spatially to effective implantation. This theory is consistent with the finding that MUC1 extends beyond the glycocalyx covering the endometrium and is the first molecule that meets the blastocyst entering the uterine cavity. Further research will better define its precise role in human embryo implantation.

Cytokines

Cytokines comprise a group of proteins that separately or in concert modulate a variety of cellular functions, such as cellular proliferation and differentiation. They play a major role in the reparative and inflammatory-like processes occurring every menstrual cycle in the human endometrium, but they are also implicated in critical reproductive events such as ovulation and implantation.

LIF

LIF is an IL-6 family pleiotropic cytokine which also includes oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin 1 (CT-1; Gearing, 1993). Differential glycosylation of 20 kDa peptide results in the secretion of the LIF glycoprotein with molecular weight ranging from 38 to 67 kDa. LIF has the classical four α-helix cytokine structure, characteristic of many haematopoietic factors (Tomida *et al*., 1984; Hilton *et al*., 1988a,b). LIF was first identified as a haematopoietic factor by its ability to induce macrophage differentiation of the mouse myeloid leukaemia cell line M1 (Hilton, 1992). The autocrine and paracrine effects of LIF, such as proliferation, differentiation and cell survival, have led researchers to investigate its role in blastocyst development and implantation. Evidences for LIF contribution to the implantation process derive from the finding that wild-type embryos failed to implant in the endometrium of female mice homozygous for LIF gene deficiency. Moreover, in this model, mouse embryo implantation was rescued after LIF supplementation to the gestational carrier (Stewart, 1994).

LIF acts through a cell surface receptor complex that comprises the LIF receptor (LIFR) and the gp130-receptor chain. Binding of LIF to LIFR induces the heterodimerization with gp130. A high-affinity receptor complex is thus formed, which allows for signal transduction to occur. LIFR activates several signalling pathways in diverse cell types (including JaK/Stat, MAP Kinase and PI3-kinase), whereas gp130 participates in the activation of STAT-1, STAT-3 and STAT-5b. The molecular characterization of the receptor complex has allowed a partial explanation of the functional pleiotropic and redundant effect of LIF. Indeed, other members of the LIF family such as OSM, CNTF, IL-6 and CT-1 possess structural similarities and, hence, are able to signal through the gp130 chain. In this way, those factors mediate similar physiological effects in a variety of biological systems including the human endometrium.

LIF expression has been demonstrated in the uterus of a variety of mammals. Although LIF mRNA expression in the proliferative to early-secretory phase is controversial (Charnock-Jones *et al*., 1994; Vogiagis *et al*., 1996), its expression at high levels is well established in the mid- to late-secretory phase. In endometrial biopsies obtained from women of proven fertility, LIF mRNA expression was observed from day 18 to 28 with a peak at day 20 of the menstrual cycle (Charnock-Jones *et al*., 1994; Vogiagis *et al*., 1996). LIF protein can be observed by immunohistochemistry in the luminal and glandular epithelium as well as in the stroma. Stromal staining was detected without noticeable cyclic variation. In contrast, epithelial staining is present throughout the cycle with an increase from the mid- to late-secretory phase. LIF secretion by cultured human endometrial explants was assessed by enzyme-linked immunosorbent assay (ELISA). Fertile patients demonstrate a 2.2-fold increase in LIF secretion between the proliferative and secretory phase (Hambartsoumian, 1998). A similar observation was reported *in vivo* in the human uterus. LIF production was shown to gradually increase from day LH+7 to LH+12 in uterine flushings from fertile patients (Laird *et al*., 1997).

IL-1α, TNF, platelet-derived growth factor (PDGF), transforming growth factor (TGF) and EGF are potent inducers of LIF expression in cultured ESCs in a concentration- and time-dependant manner. In contrast, IFN-α is a potent inhibitor of LIF production induced by this cytokine (Arici *et al*., 1995). Although LIF expression reaches maximal levels during the secretory phase of the cycle, when the endometrium is under the progesterone influence, its regulation by steroidal hormones is not yet established (Arici *et al*., 1995; Hambartsoumian *et al*., 1998; Hombach-Klonisch *et al*., 2005). On the one hand, the majority of studies did not reveal, *in vitro*, any direct stimulatory evidence of progesterone on LIF mRNA expression or protein production by cultured endometrial cells (Arici *et al*., 1995; Hombach-Klonisch *et al*., 2005). On the other hand, *in vivo* treatment with a progesterone antagonist, mifepristone, reduces endometrial glandular LIF expression at the expected time of implantation (Danielsson *et al*., 1997).

It has been recently hypothesized that the embryo, through hCG secretion, may actively participate in the control of endometrial LIF expression. Indeed, in cultured EECs, early embryonic signals such as hCG, insulin-like growth factor (IGF)-1 and IGF-2 stimulate LIF secretion in a dose-dependant manner (Perrier d'Hauterive *et al*., 2004). Different members of the TGF such as TGFβ and activin A also contribute to this control through increasing LIF secretion by cultured EECs (Perrier d'Hauterive *et al*., 2005).

The pivotal role of LIF in human embryonic implantation has been established based on abnormal LIF levels in infertile patients and especially in those with RIFs. Furthermore, a presumed role of LIF gene mutations in RIF patients has been investigated (Steck *et al*., 2004). In patients with unexplained infertility, LIF secretion by human endometrial explants only weakly increases from the proliferative to secretory phase. This was even worse in patients diagnosed with RIF (Hambartsoumian, 1998). Similarly, LIF concentration on day LH+10 in uterine flushings from unexplained infertility patients was significantly lower than those from fertile women (Laird *et al*., 1997). Uterine flushing, as compared with

endometrial biopsy, is advantageous because of its simplicity, speed and minimal invasiveness. Hence, this method has recently been proposed as a diagnostic tool in impaired implantation (Mikolajczyk *et al*., 2003). A recombinant human LIF (r-hLIF) has been investigated in preclinical and clinical trials to improve endometrial receptivity in RIF patients (Brinsden *et al*., 2003). In view of the important role of LIF in implantation, administration of such r-hLIF could be valuable in future studies.

IL-6

IL-6 is a pleiotropic cytokine, originally identified as a factor inducing immunoglobulin production in activated B cells and initially designated as IFN-β2 and B-cell differentiation factor or B-cell stimulatory factor-2. This factor was found to exhibit a wide range of biological functions in cells beyond the B-lymphocyte system (Revel, 1989; Akira *et al*., 1993). The complex of IL-6 and IL-6 receptor (IL-6R) associates with the signal-transducing membrane protein gp130, thereby inducing its dimerization and initiation of signalling (Taga, 1997; Rose-John, 2001). A soluble form of the IL-6R (sIL-6R) has been found in various body fluids (Novick *et al*., 1990; Lust *et al*., 1992) and acts as an agonist of IL-6 (Novick *et al*., 1992).

Within the human endometrium, IL-6 expression follows a regulated temporal pattern with highest detected levels during the luteal phase (Tabibzadeh *et al*., 1995; Vandermolen and Gu, 1996; von Wolff *et al*., 2002a). Endometrial IL-6 mRNA expression increases progressively during the mid- to late-secretory phase and decreases in the late-secretory phase (Vandermolen and Gu, 1996; von Wolff *et al*., 2002a). Strong immunoreactivity for IL-6 becomes detectable during the putative window of implantation. The protein quantity gradually increases during the secretory menstrual phase and is most pronounced in the epithelial and glandular cells, as compared with the stroma (Tabibzadeh *et al*., 1995).

The IL-6 receptor was found to be expressed by the blastocyst, the trophoblast and the endometrium (Sharkey *et al*., 1995). In the endometrium, mRNA expression of IL-6 receptor and gp130 remained constant throughout the menstrual cycle (von Wolff *et al*., 2002b). The IL-6 receptor and gp130 were immunolocalized mostly in the luminal and glandular epithelium and to a lesser extent in the stroma (Sherwin *et al*., 2002). The fact that IL-6 is maximally expressed during the window of implantation and that its receptor is found both in the blastocyst and in the endometrium suggests a paracrine/autocrine role for IL-6 in the peri-implantation period. Experiments using mice with a targeted disruption in the *IL-6* gene have shown that blastocyst implantation is not impaired. Nevertheless, the development of the blastocyst is compromised (Kopf *et al*., 1994; Salamonsen *et al*., 2000). IL-6 thus seems to be important but not essential in the mouse implantation process.

The regulation of IL-6 by steroid hormones is controversial. Stimulation and suppression of endometrial IL-6 secretion by E_2 and progesterone have indeed both been described (Tabibzadeh *et al*., 1989; Laird *et al*., 1993; Tseng *et al*., 1996). No direct effect of E_2 and/or progesterone treatment on endometrial IL-6 secretion by cultured EECs could be established. In another study, however, it was recently shown that E_2 mediates up-regulation of IL-6 in immortalized EECs, whereas E_2 and progesterone mediate up-regulation of its receptor. Nonetheless, IL-6 is undeniably expressed at maximum levels in EECs in the mid- to late-secretory phase, at the time when the endometrium is exposed to the highest progesterone and E_2 concentrations. It can therefore be speculated that even if IL-6 is not directly regulated by E_2 and progesterone, the action of these hormones could be indirect via other mediators that are expressed at maximum concentrations in the late-secretory phase (von Wolff *et al*., 2002a). IL-1β stimulates endometrial IL-6 protein production in a time- and dose-dependent manner. Human endometrial IL-6 may therefore mediate some actions of IL-1β involving the endometrium and trophoblast (Vandermolen and Gu, 1996).

Recent findings support a role for IL-6 in the early pregnancy stages because endometrial mRNA is suppressed in the mid-secretory phase of patients with recurrent abortions (Lim *et al*., 2000; von Wolff *et al*., 2000).

IL-1

The family members of IL-1, key mediators of the inflammatory and immunological response, include three polypeptides: IL-1α, IL-1β and a natural inhibitor, IL-1 receptor antagonist (IL-1ra; Dinarello, 1988). Two IL-1 receptors, IL-1R type I (IL-1RtI; Sims *et al*., 1988) and IL-1R type II (IL-1RtII; Horuk and McCubrey, 1989), were identified and characterized. IL-1RtI is expressed by nearly all cells and is crucial for the IL-1 signal transduction. IL-1ra can bind to IL-1RtI so as to prevent signalization by IL-1 (Hannum *et al*., 1990).

Relevance of IL-1 in the implantation process was established by mouse experiments. Surprisingly, although IL-1 knockout mice were shown to be fertile, an intraperitoneal injection of IL-1ra at the appropriate time is able to prevent blastocyst implantation. This was attributed to the down-regulation of critical integrins at the luminal epithelial surface (Simon *et al*., 1994). Such a phenomenon appears to also occur in human. Indeed, supplementation of IL-1 in the culture media of EECs leads to the increase of integrin β3 expression and thereby to enhanced blastocyst implantation (Simon *et al*., 1997). Furthermore, IL-1β stimulates the secretion of leptin and up-regulates its receptor Ob-R in EECs. Interestingly, leptin is able to trigger increase of β3 integrin expression as well as the components of the IL-1 family (Gonzalez and Leavis, 2001).

IL-1 was detected in the human endometrium throughout the menstrual cycle, both in stromal and glandular cells, although macrophages of the mononuclear phagocytic system (MPS) have been suggested to be an important reservoir of this cytokine. IL-1RtI mRNA and protein are localized in the human endometrial epithelium and reach maximal levels during the luteal phase of the menstrual cycle. IL-1 system may be an important paracrine/autocrine mediator of local intercellular interactions in the endometrial tissue (Simon *et al*., 1993).

Interestingly, it was found that expression of IL-1 antagonist is reduced for the duration of the implantation window. This suggests the existence of specific mechanisms of regulation that, by down-regulating the IL-1 antagonist expression, alleviates IL-1 inhibition and facilitates IL-1 preimplantation actions (Boucher *et al*., 2001).

IL-1α and IL-1ra levels in the PF and serum of women with endometriosis were found to be higher than in the control group. Impairment of regulation IL-1 activity in the PF and serum of

women with endometriosis may play an important role in the pathogenesis and development of the disease (Kondera-Anasz *et al*., 2005).

Prostaglandins

The process of implantation can be thought of as a proinflammatory reaction (McMaster *et al*., 1993), given that embryo attachment and invasion into the endometrium require connection to the maternal vascular system. In many species, this process involves increased vascular permeability at the site of blastocyst implantation (Chakraborty *et al*., 1996). It has long been speculated that prostaglandins (PGs), as vasoactive factors, play an important role in ovulation, fertilization and in late-pregnancy processes leading to the onset of labour (Espey, 1994). Moreover, PGs were recently demonstrated to be crucial for successful embryo implantation (Song *et al*., 2002; Ye *et al*., 2005).

PGs are members of the 'eicosanoids' family, which also comprises leukotrienes (LTs) and thromboxanes (TXa). They consist of four members, named PGD_2, PGE_2, $PGF_{2\alpha}$ and prostacyclin (PGI_2), which are generated from the membrane phospholipids by the consecutive action of two enzymes, cytosolic phospholipase A_2 ($cPLA_2$) and cyclooxygenase (COX). To date, three isoforms of COX have been reported, COX-1, COX-2 and COX-3 (Smith and Dewitt, 1996; Vane *et al*., 1998; Chandrasekharan *et al*., 2002). Although the expression of COX-1 is constitutive and mediates normal physiological functions, that of COX-2 is inducible by growth factors, cytokine, oncogenes and inflammatory stimuli (Smith and Dewitt, 1996; Vane *et al*., 1998). $cPLA_2$ acts on membranal phospholipids to release arachidonic acid (AA), which will then be oxygenated and reduced by COX enzymes to the intermediary prostaglandin H_2 (PGH_2). This intermediate subsequently serves as a substrate for PG synthase (PGS) in the generation of the four PGs, PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2. PGS enzymes are termed according to the PGs they produce; prostaglandin D synthase (PGDS) generates PGD_2 (Kanaoka *et al*., 1997), prostaglandin E synthase (PGES) generates PGE_2 (Forsberg *et al*., 2000), prostaglandin F synthase (PGFS) generates $PGF_{2\alpha}$ (Suzuki-Yamamoto *et al*., 1999) and prostaglandin I synthase (PGIS) generates PGI_2 (Miyata *et al*., 1994).

Studies in female mice lacking $cPLA_2$ or COX-2 enzymes have established the central role of PGs in implantation (Song *et al*., 2002). The lack of either of these enzymes leads to an absence of PG synthesis, which then results in several implantation defects. More precisely, $cPLA_2$ knockout mice exhibit pregnancy failure and smaller litter size, both secondary to delayed implantation (Song *et al*., 2002). Lysophosphatidic acid receptor-3 (LPA3)-deficient mice show similar problems to $cPLA_2$-deficient mice (Ye *et al*., 2005). Exogenous PG administration can however restore embryo implantation at the correct time (Song *et al*., 2002; Ye *et al*., 2005).

Expression of PGs, and the enzymes implicated in their synthesis, has been well demonstrated throughout the menstrual cycle in human endometrium. Indeed, PGES expression and PGE_2 synthesis were detected in human endometrium at all stages of the menstrual cycle with apparent reduced expression during the late-secretory phase. It was then proposed that PGE_2 might induce proliferation of glandular epithelial cells during the proliferative phase (Milne *et al*., 2001). Other PGs such as PGF2 and PGI_2, implicated in vascular function, were shown to play an important role in epithelial cell proliferation (Milne and Jabbour, 2003) and in the menstrual process (Battersby *et al*., 2004). COX expression is maximal during the menstrual and proliferative phases and is localized to epithelial and perivascular cells (Rees *et al*., 1982; Rees *et al*., 1984; Marions and Danielsson, 1999). There is no evidence that defective PG expression will prevent human fertility. Because mice lacking PGs are fertile but present fine-tuning defects, we suspect that a similar role pertaining to the window of implantation could be found in humans. Indeed, delayed human embryo implantation results in increased early pregnancy loss (Wilcox *et al*., 1999). Further research on the role of PGs at the time of implantation may shed light on a ripple effect leading to late-pregnancy abnormalities.

Following their synthesis, PGs are rapidly transported of the cells by the means of a specific PG transporter (PGT). This transporter belongs to the family of 12-transmembrane organic anion-transporting polypeptides (Schuster, 1998; Schuster, 2002). Expression of the PGT was assessed in the human endometrium across the menstrual cycle. Human PGT expression is elevated in the proliferative and early-secretory phase and low in the mid- to late-secretory phase, as shown by quantitative RT–PCR. Moreover, this transporter was immunolocalized to luminal, glandular epithelium and stromal cells. PGT modulation in epithelial cells during the menstrual cycle suggests an important role in the regulation of PG action in the human endometrium. This may concern regulation of local PG availability (Kang *et al*., 2005) although their specific role in human implantation is still not well defined.

Once released out of the cells, PGs exert their autocrine and paracrine effects by binding to cell surface G-protein-coupled receptors in the vicinity of their sites of production (Coleman *et al*., 1994). Seven different receptors, encoded by four genes, exist and are termed DP, EP1–4, FP and IP. EP2 and EP4 are the two receptors for PGE which have been studied in human endometrium. EP2 mRNA expression does not change across the menstrual cycle. However, EP4 mRNA expression is significantly higher in the late-proliferative phase. Moreover, EP2 and EP4 expressions were localized by *in situ* hybridization (ISH) in epithelial and vascular cells at all stages of the menstrual cycle. These receptors were demonstrated to be functional in human endometrium, because cyclic adenosine monophosphate (cAMP) increases *in vitro* in response to PGE_2 stimulation. This effect is more pronounced in proliferative phase endometrium (Milne *et al*., 2001). FP receptor is predominantly expressed in human EECs throughout the menstrual cycle and is up-regulated during the mid- to late-proliferative phase. This receptor was demonstrated to be functional in human endometrium *in vitro*, because treatment with exogenous $PGF_{2\alpha}$ activates the phospholipase C (PLC) pathway and the release of inositol phosphate (Milne and Jabbour, 2003). IP receptor mRNA expression was detected in human glandular epithelial and stromal endometrial cells throughout the menstrual cycle with a significant increase at menstruation. Functionality of the IP receptor was assessed by measuring cAMP generation following treatment with exogenous administration of an analogue of PGI_2, iloprost. cAMP generation was significantly higher in endometrial tissue collected during the proliferative phase compared with the secretory phase of the menstrual cycle. Increased expression and signalling of the IP receptor during the menstrual phase imply a role for PGI_2 in normal and dysfunctional

menstruations rather than in implantation (Battersby *et al*., 2004). Collectively, it appears that further research to better define the role of PGs in timing human implantation should focus on the receptors that show a regulated expression pattern and are preferentially expressed before the mid-secretory phase corresponding to the putative window of implantation.

PG production in human endometrium has been reported to be up-regulated by oxytocin. Moreover, progesterone affects oxytocin-induced PGE_2 production *in vitro*. Indeed, PGE_2 production in Ishikawa cells was weakly increased by oxytocin and significantly increased by progesterone (Kotani *et al*., 2005). Moreover, COX-2 expression is induced by IL-1β in human normal ESCs and in endometriosis (Wu *et al*., 2005). We have previously described in this review the major role of IL-1 in the establishment of endometrial receptivity. Apart from its action on αVβ3 integrin expression, IL-1 could also enhance PGs expression via increase of COX-2. Cytokines such as IL-1 would then play a role not only in setting adhesion molecules necessary for blastocyst adhesion but would also control the initiation of the window of implantation.

The pathology of endometriosis is associated with aberrant biosynthesis of COX and PG. Immunohistochemical studies have shown that COX-2 is up-regulated in endometriotic endometrium (Ota *et al*., 2001; Matsuzaki *et al*., 2004). The increase of COX-2 expression in endometriotic tissue may result from increased sensitivity of ectopic endometrium to proinflammatory cytokines such as IL-1β, which is consistently present in the PF of endometriosis patients. Regulation of *COX-2* gene by IL-1β may play a critical role in the pathophysiology of endometriosis (Wu *et al*., 2005). Moreover, increased PG concentration has been reported in the PF of infertile women with endometriosis, suggesting that ectopic endometrium directly synthesizes and releases prostanoids into the PF (Haney, 1993).

PGs were shown to be essential for embryo implantation. Their role consists in timing the window of implantation. Delayed timing of blastocyst implantation has a ripple effect that presents in mice as embryo crowding near the cervix, abnormal placentation and fetal resorption. PGs supplementation can partially restore a normal phenotype. Whether PGs have a similar role in human implantation should be further explored.

Discussion

Embryo implantation is the result of a well-orchestrated sequence of events including cellular adhesion, invasion and immune regulatory mechanisms, some of which are controlled through genetic processes by the ovarian hormones. It is proposed that embryo implantation is a well-defined and precise process, in which various factors come into play one after the other, yet remaining in close collaboration. It is rather surprising that during most days of the menstrual cycle, the endometrium is essentially hostile towards the embryo. A major physiological endeavour is thus needed by the endometrium so as to reverse this paradoxical condition.

The rising estrogen level during the first part of the menstrual cycle enhances endometrial cell proliferation. Following ovulation, progesterone levels secreted by the luteinized follicles lead to the differentiation of these cells. At this point, the endometrium is mature and primed for embryo implantation. This process is rigorously controlled both temporally and spatially. The fine-tuning of the window of implantation timing is crucial and seems to be partially under the influence of PGs. When the blastocyst enters through one of the Fallopian ostia, 4 days after ovulation, it appears to move freely in the uterine cavity. Selectins were proposed to have an important role in this phase to ensure suitable rolling of the blastocyst. Because the human embryo is required to attach to the endometrium in a polarized way and because the embryo is looking for the best area in the endometrium for implantation, this 'rolling' phenomenon is strictly regulated to ensure that the blastocyst will eventually settle in the proper spot and in the correct orientation. To prevent the blastocyst from adhering to an area with poor chances of implantation, an important role is played by the repellent activity of MUC-1. As detailed above, MUC-1 is widely expressed throughout the endometrium and, surprisingly, even increases before implantation. This phenomenon seems to be crucial in preventing the embryo from adhering to the wrong location.

In particular endometrial areas, secretion of chemokines and growth factors will attract the blastocyst to landing platforms known as pinopods. These pinopods are fully developed for only 1 or 2 days and extend over the tips of the microvilli expressing the repellent MUC-1. At this stage, adhesion molecules such as integrins and cadherins intervene to ensure adhesiveness between the embryo and the endometrium. Although this view of the described series of events (depicted in Figure 2) could appear somewhat simplistic, it nevertheless helps to realize that different markers of endometrial receptivity are crucial at different times.

Endometrial receptivity now appears to be the bottleneck of the reproductive process. Basic and clinical research will help to better understand the events of uterine preparation for embryo implantation. This knowledge could significantly improve the treatment of female infertility. Novel *in vivo* approaches, including additives to the embryo culture or intrauterine flushing with putative adhesion promoting factors, could potentially increase implantation rates especially in repeated implantation failure. As an example, we have shown that supplementation of recombinant heparanase to the embryo culture medium before transfer into mouse uteri significantly increases implantation rates (Revel *et al*., 2005). Research on embryo implantation depends heavily on animal experiments. Animal data, however, are not always transposable to the human model of implantation. Thus, endometrial biopsy samples can be used to identify molecules associated with uterine receptivity to obtain a better insight into human implantation. In addition, development of functional *in vitro* systems to study embryo–uterine interactions will lead to better define the interactions existing between the molecules involved in this process. Up to date, only a few modalities have been employed to treat failures of conception, despite the repeated transfer of apparently good-quality embryos. Table I summarizes the methods reported in the literature including medium supplementation by hyaluronic acid (Simon *et al*., 2003), systemic administration of LIF (Brinsden *et al*., 2003), progesterone (Nosarka *et al*., 2005), non-steroidal anti-inflammatory drugs (NSAIDs; Rubinstein *et al*., 1999; Pakkila *et al*., 2005) or heparin (Stern *et al*., 2003) and others.

With the exception of luteal phase support by progesterone administration, none of the treatments cited above was shown to be efficient in increasing implantation or pregnancy rates. Future research, therefore, must be directed towards deciphering the functional, rather than the morphological, characteristics of endometrial receptivity. The knowledge, acquired from this line of research, will surely assist investigators in the development of specific

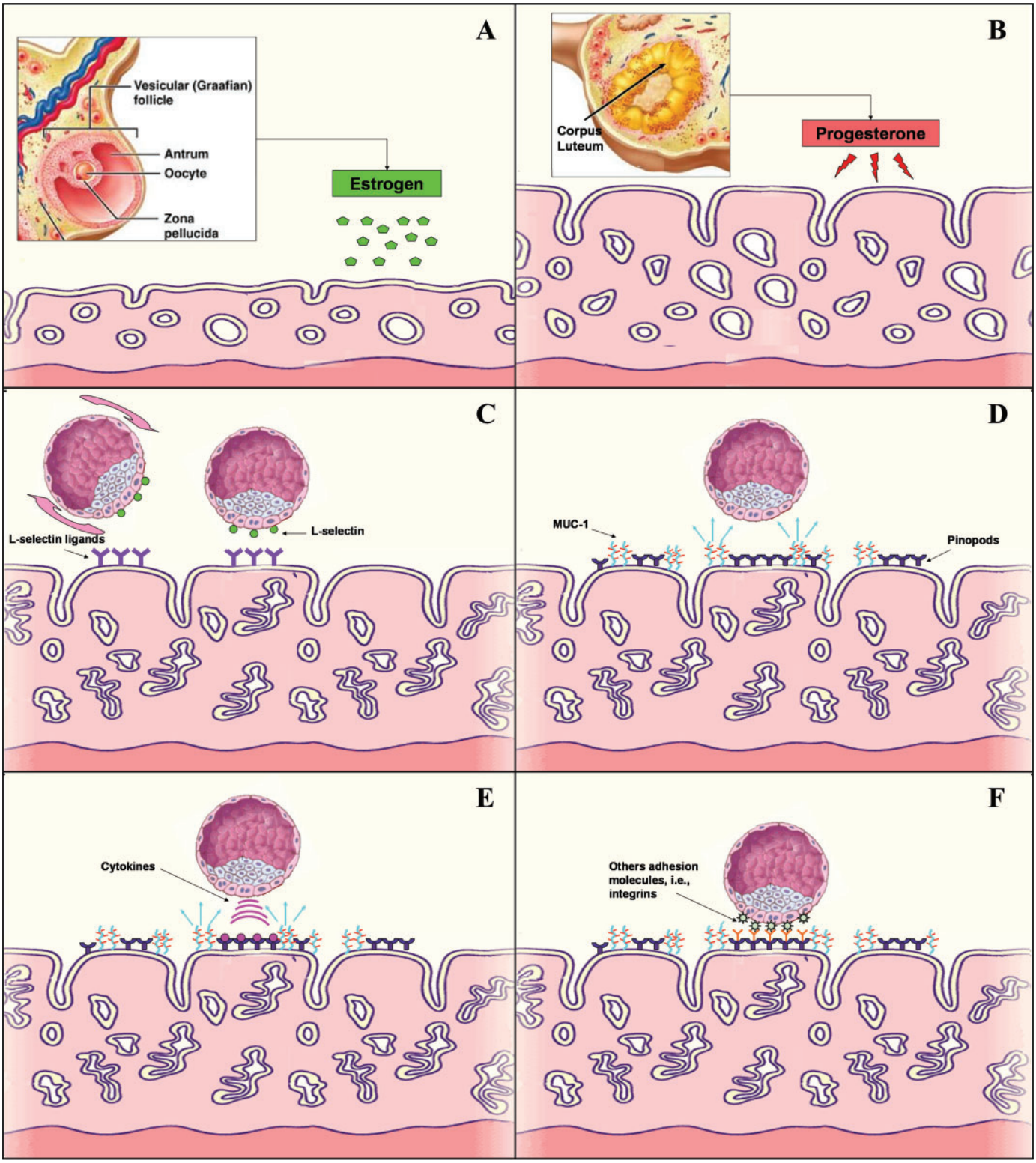

Figure 2. Human embryo implantation in the uterus. (**A**) Endometrium proliferates under estrogen enhancement. (**B**) Progesterone from luteinized follicles leads to endometrial differentiation. (**C**) The blastocyst enters the uterus through the ostia and rolls freely over the endometrium under signals by L-selectin. (**D**) Mucin-1 (MUC-1) repels the blastocyst and prevents its adhesion to endometrial areas with poor chances of implantation. (**E**) Chemokines and cytokines attract the blastocyst to the optimal implantation spot. (**F**) Adhesion molecules (e.g. integrins and cadherins) firmly attach the blastocyst to the endometrial pinopods to ensure further successful implantation.

Table I. Methods used to treat conception failures

Molecules	Intervention	Proposed mechanism	Result
Progesterone (Nosarka *et al*., 2005)	Vaginal/IM/oral	Decreases uterine contractions Improves endometrial receptivity	Two-fold increase in PR (meta-analysis)
Hyaluronic acid (Simon *et al*., 2003)	Embryos culture medium supplement	Promotes cell–cell and cell–matrix adhesion	No significant increase in PR or IR
Heparin (Stern *et al*., 2003	Subcutaneous	Inhibits binding of phospholipids with antibodies Protects the trophoblast from injury	No significant increase in PR or IR
Aspirin (Rubinstein *et al*., 1999; Pakkila *et al*., 2005)	Oral	Increases endometrial blood flow and tissue perfusion Decreases uterine contractions	Controversial results
LIF (Brinsden *et al*., 2003)	Subcutaneous	Improves endometrial receptivity	No significant increase in PR or IR
Prostaglandin E and TGF-β (Tremellen *et al*., 2000)	Intercourse around embryo transfer	Semen components may induce embryo cleavage and immunotolerance	No significant increase in PR Significant increase in IR
IVIG (Coulam and Goodman, 2000; Stephenson and Fluker, 2000)	Intravenous IgG administration	Reduces detrimental natural killer (NK) cells activation	Controversial results

PR, pregnancy rates; IR, implantation rates; LIF, leukaemia inhibitory factor; TGF-β, transforming growth factor-β; IM, intramuscular; IVIG, intravenous IgG.

therapeutics measures that will optimize embryo implantation but also lead to the development of new and improved contraceptive methods.

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