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Blastocyst–endometrial interaction: an appraisal of some old and new ideas

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The nature of the early interaction between blastocyst and endometrial epithelium is known to be highly specific within individual species. Despite this unique initial interaction, the trophoblast promptly establishes a common theme as invasive cells penetrate the endometrium and colonize its local blood vessels. In all animals with this type of implantation the blastocyst plays a more active role than the endometrial epithelium. The aggressive behaviour of the blastocyst may be induced by signals from the endometrium which has been primed with preimplantation ovarian steroids. Activation of the blastocyst may reflect triggering of synchronized paracrine loops, activated during the implantation window. Endometrial cytokines and eicosanoids are probably the primary signals that drive the interlinking paracrine loops and are essential for decidualization, trophoblast growth and invasion. Since the dominant feature of early implantation is rapid trophoblast migration, particularly in primates, the degree to which the blastocyst attaches to the apical surface of the luminal endometrial epithelium during implantation is uncertain. The thickness of the glycocalyx on the uterine luminal epithelium during the peri-implantation period varies considerably between species. Its role in blastocyst attachment, if any, and in trophoblast cell locomotion, requires further study. The molecular properties and functions of the uterine epithelial plasma membrane, and those of the interacting trophoblast at the site of implantation, have been largely neglected and require further extensive study.
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The endometrial receptivity concept

What is meant by the phrase 'endometrial receptivity'? Is it a concept describing a transient stage of endometrial function that can only be identified after implantation is initiated, or is it a biologically autonomous condition of the endometrium that can be recognized in the absence of an implanting blastocyst? Historically, 'endometrial receptivity' was developed as a concept which implied that the uterine luminal epithelium was favourable for blastocyst implantation during a limited period of time described as the implantation window (Psychoyos, 1976, 1986; Finn, 1977). From the outset to the present time it has been believed that the receptive phase can be explained in terms of specific biological changes which occur in the endometrium following priming with oestrogen and progesterone. It is disappointing, therefore, that despite a large amount of work on the cellular and molecular biology of the endometrium, a definitive marker of 'receptivity' has not been found (Rogers *et al.*, 1989; Sengupta *et al.*, 1995). A number of morphological (Nikas *et al.*, 1995) and molecular changes in the uterine epithelium have been found to be associated with the 'receptive' phase (reviewed in Sharkey, 1995; Tabibzadeh and Babaknia, 1995). However, none of these descriptive parameters when used alone, or in combination, can unequivocally identify a 'receptive' epithelium. 'Endometrial receptivity' has remained a concept linked to implantation, and in the absence of such blastocyst–endometrial interaction a receptive state cannot be identified with certainty. An alternative approach, therefore, is to consider that a suitably primed endometrium may be poised to become receptive, and that the changes associated with surface receptivity are only induced by a suitable embryonic stimulus. If such interaction with embryonic trophoblast is a prerequisite for inducing implantation it would, in turn, suggest that the induced receptivity is localized to the vicinity of the blastocyst. Elsewhere, the epithelium remains unaltered as it does not undergo the surface changes required for initiating implantation. The notion that the embryo induces functional receptivity also implies that the primed endometrium has a predominantly passive role while the blastocyst plays an active part in transforming the epithelial barrier into a localized gateway to the stroma.

Has 'endometrial receptivity' been a useful concept? The concept implies that following priming with ovarian steroids the endometrial epithelium becomes adhesive and hence capable of binding blastocysts, both those that have emerged from their zona and those that are still enclosed by this coat (e.g. guinea pig and rabbit; Enders and Schlafke, 1969; Enders, 1976). As already indicated, this has prompted a vigorous search for biological factors, presumed to be located on the surface of the apical epithelial plasma membrane, that may account for the transient receptive state. However, in the absence of a blastocyst capable of inducing a functionally receptive state at the implantation site, a search for interactive endometrial molecules may continue to be unrewarding.

During the last 10 years it has become clear that a major role of the uterine epithelium in implantation is linked to its capacity to produce an impressive range of bioactive signals

(Sharkey, 1995). When primed sequentially with oestrogen and progesterone, the uterine epithelium becomes primarily a powerful paracrine tissue, transmitting bidirectional signals between the embryo and stroma and transducing embryo–uterine dialogue, rather than just an adhesive surface. It is possible that the ability of the uterine epithelium to fasten the blastocyst is a secondary function, induced through feedback from the embryo. A primary function of the endometrial epithelium may be to activate the preimplantation blastocyst through the local action of one or more potent cytokines (growth and differentiation factors), or eicosanoids (Cross *et al.*, 1994; Sharkey, 1995; Tabibzadeh and Babaknia, 1995). Oestrogen may induce this secretory state while progesterone, or some other endocrine message, may down-regulate it to produce a paracrine window during which implantation may occur if a responsive blastocyst is present in the uterus.

The paracrine window of implantation

A good example of a paracrine window is the preimplantation window of leukaemia inhibitory factor (LIF) secreted by the uterine epithelium which is essential for inducing implantation in the mouse (Bhatt *et al.*, 1991; Stewart *et al.*, 1992). Although the mechanism of action of LIF is not known at present, it is likely that the cytokine acts on the blastocyst via LIF receptors that may be present on the trophectoderm and/or the inner cell mass (Fry, 1992). A similar paracrine window may exist during human implantation since the uterine epithelium produces LIF during the peri-implantation period and LIF receptors have been identified on the human blastocyst (Charnock-Jones *et al.*, 1994).

An even more precise paracrine window has been recently discovered in mouse implantation involving the epidermal growth factor (EGF) family of molecules (Das *et al.* 1994). These investigations have shown that several hours before implantation the mouse blastocyst induces the expression of heparin-binding EGF-like growth factor in the luminal epithelium in the vicinity of the preimplantation embryo. A further example is colony stimulating factor (CSF) which is maximally expressed in the uterine epithelium during the peri-implantation period and presumably acts on the embryo via CSF receptors shown to be present during cleavage and blastocyst stages of development (Sharkey, 1995). Before such paracrine windows open, and after they close, implantation may fail to occur even in the presence of healthy blastocysts. When compared to the multitude of studies dealing with surface changes on the uterine epithelium, investigations on the role of the activated embryo in inducing implantation are disappointingly scarce. Perhaps endometrial receptivity has been such an intellectually attractive premise that the specious concept of an adhesive surface has largely displaced alternative important ideas.

Blastocyst–endometrial interaction

It has been claimed almost universally that the blastocyst 'attaches', 'adheres' or 'binds' to the uterine luminal epithelial surface as a prerequisite for starting implantation. There is probably no need to attempt to differentiate between the words that have been used to describe the firm association between blastocyst and uterine epithelium for they all imply that the trophectoderm is materially fastened or stuck onto the luminal surface. What is the evidence on which these claims are based? The evidence is primarily based on the close apposition of embryonic and uterine apical plasma membranes, observed in electron micrographs of early stages of implantation in some species. Interpretations of this close association between apical surfaces are discussed in subsequent sections. Can the blastocyst begin to implant without attaching or adhering to the apical epithelial surface? My view is that epithelial penetration without attachment of the blastocyst to the luminal surface is a feasible process. This idea is also considered in later sections. It is known that the blastocyst becomes lodged at the site of implantation. If adhesion to the uterine apical surface occurs, it would be one of several transitory mechanisms that immobilize the blastocyst during early implantation.

It is generally accepted that implantation begins when the blastocyst becomes stationary as a result of the interaction of the trophectoderm with the endometrial epithelium. In some species the blastocyst also becomes restricted and enclosed within a tightly fitting pocket of endometrium. The idea of a stationary blastocyst may give a static impression of the beginning of implantation. It is possible to imagine the implanting blastocyst to be something like a small pulsating aquatic creature, which contracts and swells in its endeavour to explore the surface of the endometrial barrier that it encounters. Its long microvilli continuously sweep over uterine luminal microvilli, allowing the apposing micro-fronds to interlock and unwind as the blastocyst contracts and expands. At the same time waves of protruding trophoblastic fingers advance and retract, as they randomly probe the uterine surface in search of a potential passage through the endometrium.

The idea that during early implantation the blastocyst adheres to the apical plasma membrane of the uterine epithelium may be valid in the rabbit but not necessarily in other species. In the rabbit, ultrastructural studies of early implantation have shown that uterine epithelial and trophoblast plasma membranes fuse (Larsen, 1970; Enders and Schlafke, 1971). The exact region on the apical epithelial surface where fusion, and hence adhesion, begins has not been clearly established. Membrane fusion may begin along the apico-lateral region, close to the junctions of epithelial cells, or on the apical surface *per se*. Regardless of the exact site of this true intermembrane adhesion the fused membranes break down to establish cytoplasmic continuity between trophoblastic and luminal cells, thus forming an invasive embryo–maternal hybrid tissue (Schlafke and Enders, 1975). However, blastocyst adhesion to the apical epithelial membrane may not occur in species such as the mouse and rat in which the endometrial epithelium degenerates before the blastocyst invades. Embryo adhesion to the apical surface may also not occur in the guinea pig, in carnivores (e.g. ferret), or in primates, the species in which implantation requires the invasive trophoblast cells to have aggressive and unhindered mobility.

The rapid penetration of the endometrium by trophoblast cells during blastocyst implantation has been considered to be

similar to the invasion of basement membranes and stroma by tumour cells (Yagel *et al.*, 1988; Graham and Lala, 1992). The migration of cancer cells into tissues and vessels is believed to involve a series of cell–matrix interactions. Attachment, primarily to laminin or fibronectin, is considered to be required for traction and stabilization of the migrating cell (Liotta *et al.*, 1983), as well as for inducing matrix metalloproteinase expression (Stetler-Stevenson *et al.*, 1993). Locomotion of the cell associated with detachment from and degradation of the extracellular matrix are subsequent stages in the invasive process (Liotta *et al.*, 1983). The exact sequence of events and the temporal activation of genes required for such cell migration has not been fully elucidated. It should be noted, however, that invasion by a malignant tumour usually also involves detachment of cancer cells from the primary neoplasm followed by their spread. This process does not occur during the early stages of blastocyst implantation. Whereas tumours tend to invade as individual migrating cells, the implanting trophoblast invades *en masse*. At the site of implantation, proliferation and differentiation of cytotrophoblasts, with formation of syncytiotrophoblast, and protrusion of cytoplasmic processes from the enlarging trophoblast, appears to be the main mechanism for initiating endometrial invasion. The cohesive trophoblast cells, unlike detached cancer cells, probably possess sufficient stability and traction from their intrinsic intercellular contacts, to initiate invasion without the need for adhesion to apical epithelial plasma membranes. The implanting trophoblast mainly requires a direction for movement, which it probably acquires by establishing junctional complexes with the lateral borders of uterine epithelial cells (Enders, 1976).

Differences in implantation between species

The nature of embryo–maternal interaction varies considerably between species. In animals in which the trophoblast gains access to the endometrial stroma three main mechanisms for breaching the epithelial barrier have been described (Schlafke and Enders, 1975).

In rats and mice, the species in which implantation has been studied most extensively, migration of trophoblast into the endometrium does not involve movement of embryonic cells through the epithelium. In these rodents the uterine epithelium in the vicinity of the blastocyst undergoes apoptosis and sloughing. During this shedding of the uterine epithelium, which may be induced by decidual cells or their secretions specifically at the site of implantation (Finn, 1980), trophoblast cells have been observed to be present between the disrupting cells. Some of the disintegrating epithelium undergoes phagocytosis by neighbouring trophoblast cells (Enders and Schlafke, 1967). However, it is not until the denuded epithelial basal lamina is eroded by decidual cell processes that the blastocyst begins to venture into the endometrial stroma. The rodent blastocyst has been described as lacking aggressive invasive behaviour until its surrounding epithelium is stripped away (Finn, 1980). In essence this type of implantation does not involve trophoblast penetration through the epithelium since this layer breaks up and is removed before the blastocyst commences stromal invasion. The process has been described as 'displacement implantation' (Schlafke and Enders, 1975), presumably because the uterine epithelium is removed before invasion is initiated, but it could also be regarded as 'juxta-epithelial' or even 'sine-epithelial implantation' for the same reason. A number of early features of this type of implantation, prior to epithelial breakdown, are considered in a subsequent section.

A completely different process of trophoblast migration into the endometrial stroma has been described in primates, guinea pig and some carnivores. In these species cytoplasmic protrusions of trophoblast cells penetrate between epithelial cells to reach the basal lamina; they then degrade it and invade the stroma. This more aggressive intrusion by trophoblast cells displaces but does not damage the uterine luminal epithelium. This form of migration into the endometrium has been referred to as 'intrusive implantation', but it could also be regarded as being 'inter-epithelial implantation'.

An intricate and intriguing process of implantation has been described in the rabbit (Enders and Schlafke, 1971). In most regions of contact between blastocyst and uterine luminal cells the apical plasma membranes of the apposing epithelia fuse, the membranes subsequently break down to establish cytoplasmic continuity between embryonic and maternal cells. This hybrid tissue acquires a trophoblastic phenotype and becomes invasive. While fusion appears to be the predominant method of penetration, in some regions of blastocyst contact trophoblastic protrusions push between epithelial cells to reach their basal lamina (Enders and Schlafke, 1969). This odd bimodal method of trophoblast migration in the rabbit has been described as 'fusion implantation', but it could also be regarded as 'trans-epithelial implantation'.

Enders (1976) has proposed that during the early stages of implantation, adhesion of the rat blastocyst involves the apices of the free surfaces of trophectoderm and uterine epithelium. The progressive nature of this adhesion process was deduced from observations of implantation sites which had been carefully opened to expose the blastocyst and its enclosing epithelial chamber on days 5–7 of pregnancy. On day 5 some blastocysts came loose while others adhered to the uterine surface, by the middle of day 6 the blastocysts invariably adhered, while by the end of day 6 many became damaged after the implantation site was split, and by day 7 nearly all became damaged when the chamber was opened. In view of the rapid and drastic changes in the integrity of the uterine epithelium, known to occur at sites of implantation from early day 6 of pregnancy (Enders and Schlafke, 1967), and the interaction of the trophectoderm with the sloughing epithelium (Enders and Schlafke, 1967; Tachi *et al.*, 1970), it would be inaccurate to deduce that a single mechanism was responsible for the increasing fastening of the blastocyst to the uterus. For example, during the period under consideration, extensions of trophectoderm pass between the lateral borders of the epithelial cells and such protrusions become insinuated between the epithelium and its basal lamina. An opportunity therefore exists for the spreading trophectoderm to transiently adhere to lateral and basolateral epithelial surfaces, possibly through formation of junctional complexes, and to the underlying basal lamina,

through interactions with laminin, collagen type IV and other proteoglycans.

Clear-cut evidence is also lacking on whether blastocysts adhere to the apical membrane of uterine epithelial cells in species that have intrusive (inter-epithelial) implantation. Close and intimate apposition of trophoblast and apical epithelial surfaces, with no detectable specialization of their membranes at the ultrastructural level, is not necessarily proof of adhesion. Yet in descriptions of the early stages of implantation in the guinea pig, ferret and rhesus monkey (as well as rat, mouse and hamster, which have epithelial sloughing at implantation sites), the close association of embryonic and apical epithelial surfaces has been the major argument for early adhesion. It has also been proposed that interdigitating microvilli of apposing surfaces of trophoblast and uterine epithelium may also promote early attachment of the blastocyst (Schlafke and Enders, 1975). While such interactions are believed to be transitory, the membranes of the interlocking microvilli have been observed to be separated by <15 nm, and yet the curvature of their surfaces is considered to be unfavourable for adhesion (Enders, 1976).

Definitive attachment of the implanting rabbit blastocyst appears to occur only after projections of syncytiotrophoblast penetrate between healthy uterine epithelial cells and establish junctional complexes along their lateral borders (Enders and Schlafke, 1969). Clear evidence for blastocyst adhesion prior to this stage has not been presented. In fact it is likely that remnants of the blastocyst's extracellular coat (mostly zona pellucida), intervene between the trophoblast and epithelial surfaces except at sites where penetration has commenced. Less frequently, it was observed that the cell membranes of syncytiotrophoblast and apical epithelial surface appeared to have become fused, with establishment of cytoplasmic continuity between embryonic and maternal tissues. This type of blastocyst 'binding', involving the apical border of uterine epithelium, is now known to be unique to the rabbit. However, in terms of the time sequence of early events, it is likely that it occurs subsequent to inter-epithelial migration of sprouts of syncytiotrophoblast (Enders and Schlafke, 1969).

The interaction at apical surfaces

In the rat, mouse and hamster it is unlikely that early implantation involves direct interaction between the plasma membranes of outer trophectoderm and the uterine apical epithelial surface. In these species, the blastocyst becomes enclosed by uterine epithelium which forms an oval implantation chamber that closely embraces almost the entire mural trophectoderm, with only the embryonic and abembryonic poles remaining free. As implantation commences, the outer surface of mural trophectoderm is believed to become adherent over patches where intimate contacts are made. At such sites of apparent adhesion the interlocking microvilli of the apposing surfaces are flattened, or absorbed, producing plasma membranes that become closely approximated and run parallel to one another. Nevertheless, a narrow intercellular space, ~15 nm wide, remains evident between the membranes, even where 'tight adhesion' is considered to be present between the blastocyst and luminal epithelium (Enders *et al.*, 1980). Particles of cationized ferritin can diffuse into this intercellular gap and bind to the apposing membranes of trophectoderm and uterus, suggesting that both are negatively charged. Moreover, both surfaces appear to bind cationized ferritin to the same extent as non-adherent adjacent surfaces. At these sites of close association between trophectoderm and uterine epithelium there is also an absence of membrane specializations indicating that definitive junctional complexes are not formed between the apical plasma membranes (Enders *et al.*, 1980; Nilsson, 1975). It is of interest that similar regions of close apposition between blastocyst and uterine surface have been described during delayed implantation in the mouse (Nilsson, 1975) and even between zona-free unfertilized ova clasped by the epithelium during pseudopregnancy in hamsters (McLennan, 1974).

In studies on early implantation in the rat, close apposition between the cell membranes of trophoblast and uterine luminal epithelial cells have been described (Enders and Schlafke, 1967). The cytoplasm adjacent to such regions of close membrane apposition had no apparent specialization that could imply junctional complex formation. Enders and Schlafke (1967) nevertheless considered that these intercellular regions could represent primitive junctional complexes which possibly account for blastocyst adhesion during the early stages of implantation. Alternatively, it is possible that marked thinning of the uterine epithelial glycocalyx (Enders and Schlafke, 1967) and a mutual negative surface charge on apposing cells (Enders *et al.*, 1980), allow intimate apposition without adhesion of uterine and embryonic plasma membranes. In areas near such intermembrane apposition, some stability with minimal hindrance to trophoblast migration may be provided by progressive interdigitation of microvilli between the laterally mobile surface of trophoblast and the stationary underlying uterine epithelial apices. Such close intermembrane association could provide sufficient blastocyst stability to facilitate unrestricted migration of trophoblast cells across the luminal epithelium and for trophoblastic processes to intrude between the lateral borders of epithelial cells. In some species, such as guinea pig, ferret and primates, it is only when the invasive trophoblast cells begin to abut on the lateral epithelial membranes that junctional complexes become established between the epithelial cells and the intruding embryo. This process is likely to anchor the blastocyst to the endometrial surface and ensure further penetration rather than withdrawal of the probing trophoblast processes. The overall outcome is to firmly secure the blastocyst on the endometrium and to provide a direction that leads the trophoblast into the stroma.

The question that needs to be answered, therefore, is whether it is likely that cell membrane receptors, or other macromolecules incorporated within the facing membranes, could interact directly across the intercellular gap that exists between uterine and embryonic surfaces. Are these intercellular gaps involved in adhesion between the membranes at the embryo–uterine interface? It would also be interesting to know whether the thin residual glycocalyx between the membranes has the capacity to bind together the apposing genetically different epithelia. Or alternatively, whether this kind of interface would be more suited for locomotion of one epithelium over the other, since components of the glycocalyx may

promote trophoblast cell migration via a mechanism similar to the stimulated cell mobility induced by hyaluronan (McCarthy and Turley, 1993; Hall *et al.*, 1994). It may also be pertinent to note that the glycocalyx becomes more abundant on the surface of the implantation stage endometrium of the cynomolgus monkey (Anderson *et al.*, 1990), a species in which polar trophectoderm would be expected to spread aggressively and migrate through the epithelium. In contrast, thinning of the epithelial mucoprotein coat occurs at implantation in rats and mice, the species in which mural trophoblast remains relatively passive until the epithelium degenerates.

As implantation begins, cytoplasmic flow of trophoblast cells may follow paths of least resistance and sites of greatest attraction. Recent studies have suggested that such routes could correspond to intercellular borders of uterine epithelial cells. One of the earliest interactions could involve laminin and fibronectin, expressed at the outer surface of trophoblastic extensions, and integrins located at the lateral borders of epithelial cells, that bind these glycoprotein ligands. This reversed association of extracellular matrix proteins with the apico-lateral rather than baso-lateral region is believed to contribute to the loss of epithelial polarity and a loosening of lateral borders (Denker, 1990). The passage of trophoblast cell processes through such mutually interacting sites would be expected to be further stabilized by the establishment of junctional complexes with adjacent maternal epithelial cells (Enders and Schlafke, 1972; Enders, 1976; Enders *et al.*, 1983).

The apical surfaces of columnar epithelia lining the genital tract and the gastro-intestinal system are specialized for secretion, absorption and non-adhesion to neighbouring epithelial folds. Whereas the apical epithelial surface faces the luminal cavity of these organs the apical plasma membrane of trophectoderm cells faces away from the blastocyst cavity. Unlike the luminal epithelium, specific regions of trophectoderm are specialized for adhesion, locomotion and invasion, in addition to secretion and absorption which appear to be a more general property of the blastocyst epithelium. The interaction of the apposing apical surfaces of the 'receptive' uterine epithelium with trophectoderm at the commencement of implantation, and the apparent adhesion of the two surfaces, has been considered to be a biological paradox (Denker, 1990). If adhesion between these genetically disparate apical plasma membranes does occur, it would only be a partial paradox because binding would have taken place between an epithelium with a tendency for non-adhesion, and an epithelium with a propensity for physiological adhesion (Carson *et al.*, 1990). It is clear that such interaction between uterine and embryonic epithelia is strictly limited to the site of blastocyst implantation. Elsewhere, the 'receptive' uterine epithelium is non-adherent since folds of epithelium that are in close contact do not bind to one another, for example following absorption of uterine fluid by pinopods and closing down of the uterine lumen at implantation (Enders, 1976). Nevertheless, in such areas of intimate contact between endometrial epithelial surfaces (Finn, 1982), the apposing apical membranes are interlocked to a remarkable degree, as if they were zipped together by some mechanism.

Evidence has recently been reviewed in support of the concept that the morphological and molecular changes occurring in the endometrial epithelium in preparation for implantation, are widespread and involve the entire surface of the uterus (Murphy and Shaw, 1994; Murphy, 1995). The transformation of the apical epithelial membranes, as defined by these authors, is impressive and involves protein and lipid changes in the plasmalemma and reorganization of the underlying microfilaments with associated absorption of microvilli and the appearance of pinopods, as well as changes in the carbohydrate composition of the glycocalyx. However, any additional modifications that occur in the surface membranes at sites of blastocyst contact are still largely unknown. Comparisons of differences between the surfaces at implantation sites with those of non-implantation regions of uterine epithelium are scattered in the literature. For example, prospective implantation sites in the rat have more flattened and irregular microvilli, and are covered with a thinner glycocalyx, than the surface of non-implantation epithelium (Salazar-Rubio *et al.*, 1980). Similarly, during early pregnancy in rabbits, a large increase in cholesterol content was detected in the apical plasma membrane of uterine epithelial cells at implantation sites compared with non-implantation regions or endometrial epithelia of pseudopregnant animals (Winterhager and Denker, 1990). In the mouse, microvilli are largely absent from regions of close apposition between blastocyst and uterine epithelium, and where apposed endometrial epithelial surfaces meet to obliterate the lumen (Pollard and Finn, 1972), the distance between all juxtaposed apical membranes being ~15 nm. Yet it is only where the blastocyst is present that an implantation reaction is induced (Finn, 1982), suggesting that a special relationship has been established here, and we can no longer assume that the luminal surface properties in the vicinity of the embryo are the same as elsewhere in the uterus.

Molecular basis for blastocyst adhesion

It is assumed almost universally that the blastocyst adheres to the apical surface of the uterine epithelium at the start of implantation in all species. Almost invariably such claims are based on previously reported assertions and no supporting proof is provided. In a growing number of studies the molecular basis of endometrial 'receptivity' and the initial stages of blastocyst attachment are being investigated. The major concept being pursued is that the interaction between the apical uterine epithelial membrane and the implanting trophectoderm surface involves complementary molecules, that bind the opposing membranes either directly, or via a bridging molecule (Coutifaris *et al.*, 1994; Yoshinaga, 1994). Although this idea involves the association of two membranes through a mechanism similar to ligand-receptor binding, in most studies either the endometrium alone, or isolated implantation stage embryos, are investigated for potential ligands or receptors. Very little information is currently available on the molecular changes at implantation sites, which are the specialized regions where the affinity of embryonic and uterine membranes becomes evident. Yoshinaga (1994) has raised a further crucial requirement before the ligand-receptor model of implantation can be accepted. The apposing embryonic and endometrial membranes must be close enough to permit the binding of the

ligand and its receptor. The closest distances observed between trophectoderm and apical uterine epithelial membranes, at the time of implantation, have ranged from 7.5–15 nm (Enders, 1976).

The glycocalyx covering the apical uterine epithelial membrane and the apical membrane of the trophectoderm may participate in the initiation of implantation. In many studies, where the general uterine surface was examined at the time of implantation, the glycocalyx coat was found to decrease in thickness, and its negative charge was reduced largely due to the loss of sialic acid residues. These changes are considered to promote membrane apposition prior to the postulated adhesion of the apical membranes at the onset of implantation. It should be noted, however, that the intermembrane glycocalyx probably does not disappear completely, except in the rabbit in regions of epithelial–trophectoderm fusion, and possibly at some foci during implantation of the ferret blastocyst. Moreover, in some species, such as the cynomolgus monkey, the glycocalyx coat becomes thicker over the surface of the implantation stage uterine surface, and this is associated with an increase in D-galactose revealed by lectin binding studies (Anderson *et al.*, 1990). But the nature of the glycocalyx at implantation sites in these monkeys was not examined.

For adhesion to occur between implanting trophectoderm and the apical plasma membrane of uterine epithelium, adhesion-promoting molecules must be embedded within the membranes, they must be capable of reaching and interacting with one another, and such adhesive determinants must be expressed or unmasked on each apposing surface (but limited to mural or polar trophectoderm depending on the species) specifically during the peri-implantation period. It has been proposed, for example, that an H-type I blood group antigen, which is detectable on the surface of mouse uterine epithelium during early pregnancy, may be involved in the initial stages of blastocyst attachment (Kimber *et al.*, 1994). These investigators obtained evidence that the complementary binding ligand (receptor) was a lectin-like molecule which was localized specifically to the mural trophectoderm of hatched blastocysts, and appeared on day 5 of pregnancy. Although the H-type I epitope appeared to be present on the apical surface of uterine epithelial cells using immunofluorescence, more precise examination of its distribution by electron microscopy revealed that the antigen was predominantly localized within the glycocalyx (Kimber *et al.*, 1993). The H-type I determinant was not expressed in the apical plasma membrane of uterine epithelium during the 'receptive' phase. If, therefore, the glycoprotein carrying the H-type I epitope were to be involved in the initial stages of murine implantation, its effects could be mediated via the glycocalyx rather than the apical epithelial membrane. Although the proposed adhesion system was detected at implantation sites, the influence of blastocysts could not be determined.

Another example of the involvement of adhesion-promoting molecules in the initiation of mouse blastocyst implantation is the proposed role of heparan sulphate (HS) binding proteins and heparan sulphate proteoglycans (HSPGs) in embryo–uterine attachment (Carson *et al.*, 1990). The postulated interactions are complex because the localization of HS binding proteins at the apical region of uterine epithelial cells has not been clearly defined, and HSPGs are expressed and secreted at both the uterine and trophectoderm cell surfaces. The embryonic and maternal proteoglycans may, therefore, compete for the same complementary adhesion molecules in the glycocalyx or the apical epithelial membrane. Furthermore, it is not clear whether in attachment-competent blastocysts HSPG is expressed throughout the trophectoderm, or only in the region of the apical surface that establishes contact with the uterine epithelium. There is also a lack of information on whether the proposed adhesion systems are expressed at implantation sites.

Both mouse and human uterine luminal and glandular epithelium produce a cell surface associated mucin, MUC-1, which is regulated by ovarian steroids (Aplin *et al.*, 1994; Surveyor *et al.*, 1995). It has been proposed that the MUC-1 epitope, embedded in the apical plasma membrane of the endometrial epithelium, has a highly extended conformation, and that its polypeptide core reaches further into the surface glycocalyx than other glycoproteins, such as the integrins and the CD44 family of molecules (Aplin *et al.*, 1994). The expression of MUC-1 at the cell surface has been reported to inhibit cell–cell and cell–matrix interactions. It is likely that if MUC-1 is expressed at the epithelial cell surface during the peri-implantation period, its steric properties could hinder the approach of cell receptors, postulated to be required for embryo–endometrial attachment. At the same time, the rheological properties of MUC-1, and its polyanionic character, may facilitate the mobility of the negatively charged trophoblast surface over the endometrial surface at the site of implantation. In contrast, the absence of MUC-1 along lateral epithelial cell borders could promote receptor mediated attachment and junctional complex formation.

In view of the potential inhibitory role of MUC-1 on blastocyst attachment to the luminal surface, it is of interest that MUC-1 transcript expression peaks in the implantation stage human uterine epithelium (Aplin *et al.*, 1994). Distinctly different findings have been reported for mouse uterine epithelium, in which MUC-1 mRNA falls to minimum levels during the implantation phase of pregnancy (Braga and Gendler, 1993). At this stage, further studies are required on MUC-1 expression at sites where blastocysts interact with the uterine epithelium during implantation. Nevertheless, it was of interest to note in a recent paper, reviewing the biochemistry and physical properties of endometrial MUC-1, the following comment: 'It is by no means certain that embryo attachment is mediated by direct binding of trophectoderm to maternal epithelial cells' (Aplin *et al.*, 1994). It is encouraging that the old dogmas related to blastocyst adhesion are beginning to be questioned from various directions.

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