The Role of Integrins in the Human Reproductive Process

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Abstract. Integrins are heterodimeric transmembrane glycoproteins involved in cell-cell and cell-extracellular matrix adhesion. They also participate in cytoskeletal rearrangement, co-regulation of growth factor activities and activation of signal transduction. This review describes the available information regarding the role of integrins in human reproductive physiology and discusses their clinical implications. Integrins play important roles in several reproductive processes including fertilization, embryogenesis, and implantation. Disturbance of integrin expression in reproductive organs can be the cause, or the result of such reproductive disorders as endometriosis, unexplained infertility, hydrosalpinx, ectopic pregnancy, and preeclampsia. Further knowledge of the integrin-mediated regulation of cell growth and cell survival may facilitate our understanding of many key aspects of development and physiology in reproduction.

Key words: Integrin, Fertilization, Embryogenesis, Implantation, Infertility

The interactions of cells with the components of extracellular matrix (ECM) proteins participate in the control of cellular differentiation, morphogenesis, proliferation, and migration, thereby impacting on such processes as implantation, embryogenesis, wound healing, inflammation, and cancer [1, 2]. Many of the interactions with the ECM proteins are mediated by the integrin family of cell adhesion molecules. In addition to their role as adhesion receptors, integrins also function as signaling receptors and have been shown to regulate reorganization of the cytoskeleton, intracellular ion transport, lipid metabolism, kinase activation, and gene expression [3]. Integrins are now recognized as signaling molecules capable of transducing messages via classical signaling pathways, and integrin receptor function can be regarded as a key modulator of cellular behavior [2, 4]. A characteristic feature of certain integrins is their ability to modulate affinity for extracellular ligands in response to intracellular signals, a process termed activation or inside-out signaling [2, 5]. Integrins have been recently shown to play particularly important roles in the reproductive processes, including fertilization, embryogenesis, and implantation [6, 7]. In this review, we summarize what is currently known about the expression of cell adhesion molecules in the reproductive organs and discuss the critical roles of these molecules in reproductive physiology and medicine.

Integrins and Receptor Signaling

Integrins can mediate signaling by two mechanisms, so called “outside-in” signaling and “inside-out” signaling [2–4]. Outside-in signaling mediates signals from ECM proteins after integrin ligation and involves the regulation of many fundamental cellular processes [8]. It involves integrin-ligand binding and receptor clustering,
with subsequent assembly of the focal adhesion plaque—a complex of cytoskeletal proteins and signaling molecules including paxillin, talin, vinculin, α-actinin, tensin, and focal adhesion kinase (FAK) [9]. This process is dependent on GTPase Rho A [10]. Phosphorylated FAK can also lead to activation of the mitogen activated protein (MAP) kinase pathway, probably via Ras activation, which can then influence gene expression. Inside-out signaling is the mechanism by which a cell regulates the affinity state of its integrin receptors. It is thought to involve the propagation of conformational changes from the cytoplasmic domains of integrins to the extracellular binding site in response to intracellular signaling events.

FAK represents a new family of nonmyristylated tyrosine kinases (molecular radius of 125 kDa). FAK was first identified as a phosphotyrosine protein in chicken embryo fibroblasts transformed with v-src [12]. FAK-deficient mice, generated by targeted gene disruption, are embryonic lethal and display a general defect in mesoderm development [13]. FAK phosphorylation is induced by attachment of various cell lines to fibronectin and to other ECM proteins and clustering of β1 and β3 integrins also induces FAK phosphorylation. Integrin-stimulated FAK tyrosine phosphorylation correlates with an increase in the intrinsic kinase activity of FAK. Recent data indicate that FAK plays a role in cell motility. Embryonic mesodermal cells isolated from mouse embryos in which FAK was deleted by homologous recombination were less well spread than FAK-positive control cells [13]. While deletion of FAK inhibits motility, overexpression of FAK appears to be correlated with increased motility. Increased levels of FAK expression have also been correlated with the invasive and metastatic phenotype in solid tumors [14]. These data indicate that overexpression or perhaps activation of FAK plays a role in cell locomotion and invasiveness. Such an effect is consistent with the ability of FAK to modulate assembly/disassembly of focal contacts and actin filaments.

Less is known about regulation of downstream signaling components, but integrins have been shown to activate mitogen-activated protein (MAP) kinases [11, 15]. Activation of MAP kinase was observed when cells adhered to either fibronectin, laminin, collagen, or Arg-Gly-Asp(RGD)-containing peptides but not when cells adhered to poly-L-lysine. These results suggest that multiple integrins can activate kinases. Clustering of β1-integrins is sufficient to induce MAP kinase activation and has also been shown to activate another MAP kinase family member, jun kinase [15]. The activation of MAP kinases by growth factors and integrins appears to be quantitatively different. Integrin-mediated activation is slower yet persists longer than growth factor receptor-mediated activation [11]. This dual mode of activation may be required for stimulation of cell growth. Like FAK, MAP kinases may also act as a point of convergence between integrin-mediated signaling and growth factor receptor signaling.

**Integrins and Endometrium**

The recent discovery that certain endometrial integrins exhibit cycle- and cell-specific patterns of expression has generated intense interest in this family of cell adhesion molecules and has led to speculation that integrins may participate in the cascade of molecular events involved in the implantation process. Several studies have confirmed the presence of β1 integrins on human endometrium and demonstrate that integrin expression is a dynamic process related to the menstrual cycle [16–18]. The distribution of different α and β integrin subunits in human endometrial tissues at different stages of the menstrual cycle has been determined by using immunohistochemistry. These studies suggest that some integrins normally undergo spatial and temporal changes with expression in the cycling endometrium, and that disruption of this expression pattern may be associated with certain types of infertility in women [16–18]. The pattern of integrin expression in the endometrium during the secretory phase and implantation is most interesting [16–20]. Initial studies demonstrated that three integrins were coordinately expressed around the window of implantation [16–18]. The α1β1 and α4β1 integrins appear at the time of ovulation. The αvβ3 integrin appears on cycle day 20, at the opening of the putative window of implantation. Cycle days 20 to 24, corresponding to the time of endometrial receptivity, are marked by the co-expression of all three of these cycle-specific integrins. The loss of
the α4β1 integrin heralds the closure of this “window”. The expression of the endometrial αvβ3 integrin is aberrant in some women, and may be associated with certain infertility states including endometriosis, luteal phase defect, hydrosalpinges, and unexplained infertility [21]. Others have also noted that a decrease in α4β1 expression might serve as a marker for unexplained infertility [18].

The principal feature of β1 integrin expression is an apparent shift from epithelial to predominantly stromal expression. We demonstrated that expression of β1 integrins in the endometrium coincided with ovarian changes, allowing a distinction between the early proliferative and midsecretory phase [19, 20]. The expression of β1 integrins varied throughout the cycle, with predominant expression occurring during the secretory phase. The localization of β1 integrins in the early proliferative phase was restricted to the glandular epithelium, whereas stromal cells in the midsecretory phase also expressed β1 integrins. Several studies have demonstrated that the expression of β1 integrins in human endometrium increases at the time of implantation [16–20]. Thus, although certain β1 integrin moieties appear to be regulated throughout the endometrial cycle, the mechanisms responsible for β1 integrin regulation have yet to be established. Our previous study demonstrated that treatment of stromal cells in the proliferative phase with E2 and P increased the expression of β1 integrins in vitro [19], suggesting that β1 integrin expression in human endometrium may be progesterin-dependent. The differential expression of β1 integrins within different compartments of the endometrium reflects the distinct nature and function of these compartments.

The most commonly used method of assessing luteal function in infertility has been the direct evaluation of endometrial function by endometrial biopsy and histological dating [22], but such assessment represents a crude index of uterine receptivity. Integrin cell adhesion molecules have a housekeeping role in anchoring endometrial epithelium and do not appear to be useful markers of uterine receptivity [27]. Large studies of adequate statistical power on infertile patients and fertile controls, ideally investigating more than one menstrual cycle in the same women, should be undertaken in order to establish the usefulness of these molecular markers as clinical tools. In addition, basic research to establish a functional role for these integrins, in normal endometrial regeneration and maturation and in the adhesive events surrounding implantation of the trophoblast, needs to be performed.

The decidualized stroma also exhibit marked changes in the expression of several integrins (α2β1, α3β1, α6β1, αvβ3, and α5β1) during the late secretory phase and in early pregnancy. Our previous studies using flow cytometric analysis reveal that decidual cells express high levels of the α1 and α2 subunits and moderate levels of the α5 and α6 subunits [19]. In addition, the biosynthesis of β1 integrins has been demonstrated by metabolic labeling of cultured decidual cells and immunoprecipitation of the cell extracts with a specific antibody followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [19]. Grosskinsky et al. [28] have found that some of
these changes can be induced in vitro using human stromal cells and that growth factors appear to play a greater role than steroid hormones in eliciting these shifts in integrin expression. The expression of β1 integrins in human endometrium increases at the time of implantation and remains high in decidua during early pregnancy. This indicates the presence of a certain degree of specificity, potentially mediated by β1 integrins, which are the major family of ECM receptors present on the surface of decidual cells. Such changes suggest a potential role for the integrins in the later stages of implantation.

Human decidual cells synthesize and secrete prolactin (PRL) in vitro. Isolation of PRL-producing cells from human decidua has permitted identification of the specific cell found in the final preparation that is responsible for PRL secretion [19]. The enriched fraction of PRL-producing decidual cells isolated by Percoll gradients consists of a nearly homogenous population of large, round mononuclear cells (>25 mm diameter) which exhibit increased expression of β1 integrins on their cell surfaces [19]. These findings imply that the PRL-producing cells seen in human decidua during early pregnancy express cell surface β1 integrins in specific decidual subpopulations. Mature decidual cells may be responsible for this expression of β1 integrins leading to the deposition of ECM proteins at the implantation site. Alternatively, increased expression of receptors in PRL-producing cells may lead to the morphologic differentiation of decidual cells.

**Integrin Involvement in Fertilization**

Gamete interactions leading to fertilization can be divided into a series of discrete steps, starting from sperm-zona binding and ending with pronuclear formation. Evidence is accumulating that sperm-oolemmal adhesion and fusion may involve several combined receptor-ligand interactions including members of the family of integrins [29]. Many integrin-ligand interactions are mediated through the recognition of an RGD tripeptide sequence present in fibronectin, vitronectin, and osteopontin [30]. Oligopeptides containing the RGD sequence have been shown to competitively inhibit sperm-oolemmal adhesion and oocyte penetration in both heterologous (human-hamster) and homologous (hamster-hamster) gamete interactions, suggesting the involvement of integrins in fertilization [29].

Fertilization includes sperm-oocyte recognition, adhesion, binding, fusion and oocyte activation. Integrins, which are adhesion molecules, are expressed on sea urchin, mouse, hamster, and human unfertilized oocytes [31]. Several integrins are present on hamster unfertilized oocytes (α2, α4, α5, αv, and β1) and on human oocytes (α2, α4, α5, α6, αv, and β1). Expression of β1 class integrins (α3, α5, and α6) have been identified both at the protein and mRNA levels in unfertilized mouse oocytes with the α6 antigen mainly restricted to the micr villos area of the oocyte surface [31]. The mRNA transcripts of β1 integrins have also been found in ejaculated human spermatozoa [32], and their expression was enhanced during capacitation and modified in abnormal spermatozoa [33]. The αvβ3 integrin has been recognized on the inner acrosomal membrane and is exposed for recognition by the oocyte after the acrosome reaction. A relationship has also been established between sperm integrin expression and the penetration rate in the zona-free hamster oocyte sperm penetration assay [34]. Sperm integrins which are already present on spermatogenic cells [35] play a role in intratesticular cell-cell communication as well as in fertilization.

Ligands for integrin receptors have been detected on human spermatozoa. Fertilin is a sperm surface protein which also plays an important role in sperm-oocyte interaction in mammals through its interaction with oolemmal integrins. It is a heterodimer, initially identified in guinea pigs, that is formed by two distinct subunits, α and β, both glycosylated and structured with an extracellular domain and a cytoplasmic one. There is increasing evidence that fertilin is one of a conserved family of related cysteine-rich proteins, now designated as ADAM (a disintegrin and metalloproteinase domain), that contain metalloproteinase-like and disintegrin-like domains that play important roles in mammalian fertilization [36]. The fertilin-α chain contains a short sequence similar to the fusion peptides carried by most viral fusion proteins [37]. The N-terminal region of the fertilin-β chain possesses a 93 amino acid disintegrin-like domain [38]. Disintegrins are a class of soluble snake venom proteins that act as ligands for certain integrins, which induce defects in integrin-
mediated platelet function when they occupy integrin receptors. The disintegrin-like domain of fertilin-β is proposed as interacting with an oolemmal integrin [39], enabling the spermatozoa to adhere to the oocyte plasma membrane, leading to a conformational change in the fertilin-α subunit that reveals its hydrophobic fusion peptide. Evidence has been presented that the integrin α6β1 is the receptor for murine sperm-associated fertilin [40].

The α6β1 integrin on mouse oocytes is thought to function as the receptor for sperm because of the following evidence: 1) the major integrins found to date on the mouse oolemma are α6β1 and αvβ3; 2) a function blocking antibody against integrin α6 inhibits sperm-oocyte binding; and 3) a peptide analog of the fertilin β disintegrin loop as well as a function blocking anti-α6 antibody inhibits binding of sperm to α6 transfected cells [40]. Evans et al. [41] have demonstrated that the integrin β1 is responsible for sperm (fertilin β) binding to egg, based on the observation that an antibody that is reactive with several β1 integrins inhibited binding of sperm or recombinant fertilin protein produced by E. coli to the surface of oocytes. Furthermore, recombinant fertilin β with the disintegrin domain deleted does not inhibit sperm-oocyte binding [42]. Binding of recombinant fertilin β is reduced by the anti-β1 polyclonal antibody, whereas binding of recombinant fertilin β to mouse eggs is not reduced by the anti-α6 monoclonal antibody. There is agreement therefore that sperm fertilin β binds to a β1 integrin on the egg surface. The identity of the partner α subunit has not been clearly established. However, these studies do not eliminate the possibility that other extracellular proteins in oocytes are required for binding of fertilin β and sperm to the oocyte surface integrin.

Of thirteen testis-specific ADAMs, five (ADAMs 1, 2, 3, 5, and 18) have been shown to be proteolytically processed during spermatogenesis and epididymal transport such that their forms on mature fusion-competent sperm lack pro and metalloprotease domains and therefore begin with a disintegrin domain [43]. Recombinant forms of the disintegrin domain from the testis-specific ADAM2 (fertilin β) have been shown to bind to the oocyte and to inhibit sperm-oocyte binding and fusion. An aspartic acid at position 9 of the ADAM2 disintegrin loop has been found to be important for these activities [44, 45]. Similar to ADAM2, ADAM3, a sperm surface protein that is also known as cyritestin, has been found in the equatorial region of mature fusion competent sperm [46]. The equatorial region is the microdomain of the sperm plasma membrane that makes initial contact with the oocyte during sperm-oocyte binding and fusion [47]. Peptide analogs of the disintegrin loop of ADAM3 inhibit sperm-oocyte binding and fusion [46]. Antibodies against the disintegrin loop of ADAM3 inhibit in vitro fertilization [46]. Male mice lacking ADAM3 are infertile [48], as are male mice lacking ADAM2 [49]. Whereas sperm from ADAM2 null mice are significantly compromised (80% reduced) in their ability to bind to the oocyte plasma membrane, sperm from ADAM3 null mice are competent to bind to the oocyte plasma membrane [48].

Recent studies raise the question of the role of the α6β1 integrin in sperm-oocyte binding and fusion. All the binding and fusion assays of Almeida et al. [40] were done with oocytes from which the zona pellucida has been removed by protease digestion. Evidence suggests that the inhibitory effects of the anti-α6 monoclonal antibody on sperm-egg binding and fusion depend on zona removal techniques [41]. In addition, the monoclonal antibody did not inhibit sperm-oocyte fusion in in vitro fertilization assays using zona intact oocytes [50]. The protease treatment may modify oocyte plasma membrane proteins, resulting in a loss of protein functions and/or a modification of critical protein interactions. In oocytes from cultured ovaries of mice lacking the α6 integrin subunit, the fertilization rate and sperm binding were not impaired compared with wild-type or heterozygous controls [51]. Normal sperm fusion with an oocyte lacking the α6 integrin subunit implies that another integrin or receptor can substitute for the α6β1 integrin in knockout oocytes, suggesting that the α6β1 integrin is not essential for sperm-oocyte binding and fusion.

SP-10 is a sperm intra-acrosomal protein, specific to the testis, that is believed to play an important role in egg-sperm binding. While the molecular characterization of the SP-10 protein has been clarified [52], little is yet known of its functional role in fertilization. We therefore established a monoclonal antibody (mAb pep-SP10) against a peptide (pep-SP10) that included the most hydrophilic portion of human SP-10 between the 135th and 149th amino acids [53]. Human SP-10...
was found to be localized in the equatorial region of acrosome-reacted sperm by immunofluorescent staining using our mAb pep-SP10. Monoclonal Ab pep-SP10 inhibited sperm-oolemma binding in the zona-free hamster egg penetration test, but it did not inhibit sperm-zona binding in the hemizona assay. Furthermore, we demonstrated that the oolemmal ligands of human SP-10 did not include β1 integrins, the most promising candidates for oocyte ligands involved in sperm-oolemma binding, based on the findings of a human sperm-cultured cell binding assay using F9 mouse embryonal carcinoma cells and F9-transformed cells lacking β1 integrins. These findings suggest that human SP-10, expressed on the equatorial region of acrosome-reacted sperm, indeed mediates sperm-oolemma binding in a β1 integrin-independent manner, but not sperm-zona binding.

Recently, another oocyte surface protein, CD9, has been discovered to play an essential role in sperm-oocyte fusion [54–57]. CD9 is a member of the transmembrane 4 superfamily, also called the tetraspanin family. Transmembrane 4 superfamily proteins contain four transmembrane domains, two extracellular loops, one cytoplasmic loop, and cytoplasmic amino and carboxyl termini. Chen et al. [54] found that the anti-CD9 monoclonal antibody inhibited sperm-oocyte binding and fusion. The involvement of CD9 in sperm-oocyte fusion has been confirmed using CD9 knockout mice [55–57]. The phenotype of the CD9 mutant mouse is a redistribution of CD9. Oocytes from the CD9 knockout mice bind sperm normally, but are severely inhibited in their ability to fuse with sperm [55–57]. In addition, the anti-CD9 monoclonal antibody completely blocks with either wild-type oocytes or oocytes lacking α6β1 [51]. These findings suggest that CD9 may act by itself, or interact with oocyte protein(s) other than the α6β1 integrin, to function in sperm-oocyte fusion.

### Integrin as Regulators of Embryogenesis

Integrins play an important role in cell adhesion, migration, and differentiation during embryonic development by mediating cell-cell and cell-matrix interactions [58, 59]. In the mouse, a basic repertoire of integrin heterodimers, α5β1, α6β1, and αvβ3, is expressed by the embryo throughout early development, whereas five other β1-associated α subunits, α1, α2, α3, α6A and α7, show developmentally regulated expression [58]. Thus the embryo always has the potential to interact with fibronectin via α5β1 and αvβ3 integrins, laminin via α6β1 and αvβ3 integrins, and vitronectin via αvβ3 integrin as well as with other αvβ3 receptor ligands, notably thrombospondin. The major point of regulation of integrin expression is the late blastocyst stage [58]. There is little change in integrin expression during preimplantation stages, and none that correlates with any of the major developmental events of this period such as compaction and blastocoeI formation. α3 mRNA is detected during early preimplantation stages but the protein is detectable only in embryo outgrowth or postimplantation embryos. Expression of α2, α6A and α7 mRNA begins at the late blastocyst stage, before implantation, whereas α1 expression begins with embryo outgrowth or implantation [58]. At least three subunits, α1, α6A, and α7, are specific to extraembryonic tissues, reflecting the fact that trophoblast and endoderm cells have the most extensive interactions with ECM proteins at this time, and thus may require the greatest diversity of receptors.

The variety of integrins and ligands expressed in the preimplantation periods are likely to be important for basement membrane formation on the inner surface of the trophectoderm and between the primitive ectoderm and primitive endoderm of the late blastocyst. The anti-integrin antibody has no effect on preimplantation morphogenesis, whereas antibodies against the cell-cell adhesion molecule, E-cadherin, interfere with both compaction and primitive endoderm segregation. The anti-integrin antibody blocks trophoblast outgrowth and parietal endoderm migration in vitro, suggesting that integrin-mediated cell-matrix interactions are critical to the function of extraembryonic cell types. Thus, integrin-mediated interactions may not be involved in other aspects of preimplantation development.

Using explant cultures of epiblast or mesoderm, as well as inhibition experiments with antibodies, Burdsal et al. [60] have shown that α6β1 integrin and both β1 and β3 subunits mediate primitive strike mesoderm adhesion to fibronectin, laminin, vitronectin and type IV collagen. Null mutations of integrin α5 and β1 subunits have been generated by gene targeting in mouse embryonic stem cells [61]. Homozygous β1 subunit null embryos develop
normally until the blastocyst stage, and initiate implantation, but die thereafter [61]. Analysis of the \( \beta 1 \)-null blastocyst shows a delayed growth of the inner cell mass, and the absence of primitive endoderm migration [62]. Although the reason for the deterioration of the inner cell mass is not yet understood, it has been proposed that the defective position of endoderm layers may in turn affect the inner cell growth. The absence of endodermal cell migration can be attributed to a defective extracellular matrix provided by trophectodermal cells. However, experimental approaches using \( \beta 1 \) integrin deficient ES cells to generate chimeric embryos have demonstrated that \( \beta 1 \) null cells differentiate and migrate in normal tissues except in the liver and spleen [61]. Furthermore, in null mutations of \( \alpha 5 \) subunit, Yang et al. [63] observed that early development and gastrulation proceed at least in the anterior part of the embryo. Thus, most knock-outs of \( \alpha \) subunits of the \( \beta 1 \) family result in perinatal lethality, suggesting that migration of the cells can at least partially occur. These knockout experiments suggest the presence of redundancy and compensatory mechanisms among integrins. Alternatively, it is possible that other adhesion molecules and growth factors which are present in vivo but not in vitro, may compensate for the deficient integrin.

Less information is currently available concerning cell adhesion molecules during preimplantation embryogenesis in humans. Campbell et al. demonstrated that six integrin subunits, \( \alpha 3, \alpha v, \beta 1, \beta 3, \beta 4, \) and \( \beta 5 \), are consistently expressed throughout the preimplantation period [64]. Evidence has also been obtained for the presence of integrin subunits, \( \alpha 2, \alpha 4, \alpha 6, \beta 2, \) and \( \beta 7 \), on a small number of oocytes. Based on these subunit localization data, it is possible that at least four integrin complexes, \( \alpha 3 \beta 1, \alpha v \beta 1, \alpha v \beta 3, \) and \( \alpha v \beta 5 \), may be expressed on the embryos during preimplantation development. Recently, the \( \alpha v \) integrin subunit has been shown to be expressed in human embryos throughout early development, from the two cell-stage up to the blastocyst [65]. The expression of the \( \alpha v \) integrin subunit gradually increased throughout embryo development as measured quantitatively by image analysis [65]. The increased expression may have been due to increases in the number of cells in the developing human embryo. The exact roles and functions of integrins are still unknown in humans, but further study using in vitro models to evaluate the functions of these receptors may help advance our understanding of the role of integrins in embryonic development.

**Integrin-Mediated Regulation in Implantation**

Implantation of the embryo is mediated by trophoblasts, a specialized population of cells, which arise from the trophectoderm, the outer layer of epithelial cells that encloses the blastocoele cavity and the inner cell mass of the preimplantation blastocyst. Conversion of the epithelial trophectoderm to invasive trophoblast begins at the late blastocyst stage and comprises both a change in adhesive behavior and the onset of motility. The apical surface of the mural trophectoderm indeed possesses functional integrins and provides a novel experimental model for exploring the initial interactions between an implanting blastocyst and adhesive components of the ECM. At least three integrin \( \alpha \) subunits, \( \alpha 2, \alpha 6A, \) and \( \alpha 7 \) are newly expressed when the blastocyst becomes attachment-competent [58]. The \( \alpha 1 \) integrin subunit begins to be detectable only after trophoblast outgrowth, suggesting that its expression is a response to contact with the ECM. The \( \alpha 1, \alpha 6A \) (an alternatively spliced form of the \( \alpha 6 \) subunit), and \( \alpha 7 \) subunits are detected in the extra-embryonic ectoplacental cone and differentiating secondary trophoblast [58]. Thus, differentiation of trophoblasts is accompanied by the onset of expression of three additional integrins in these cells, providing expanded ability of trophoblasts to interact with ECM proteins.

Mouse blastocyst outgrowth on ECM proteins is mediated by trophoblast expression of several integrin receptors, possibly in concert with one another. Fibronectin-mediated trophoblast cell adhesion and migration appear primarily to involve the interaction of cellular receptors with the RGD recognition site in the central cell-binding domain [66]. Comparable outgrowth is observed on substrates consisting of intact fibronectin, a fragment of fibronectin containing the central cell-binding domain, or a recombinant protein constructed with multiple copies of the decapetide encompassing the RGD cell recognition site. The mechanism of this interaction with fibronectin clearly involves the RGD
recognition site, and may not require other regions of the fibronectin polypeptide. By using trophoblast cells adherent to fibronectin, Yelian et al. [66] identified subunits of several RGD-dependent integrins that recognize fibronectin, including the \( \alpha_3, \alpha_5, \alpha_v, \alpha_{IIb}, \beta_1 \) and \( \beta_3 \) subunits. Blastoct outgrowth on fibronectin is inhibited partially by antibodies against the \( \beta_1, \beta_3 \) and \( \alpha_5 \) subunits [66], suggesting that integrins of the \( \beta_1 \) and \( \beta_3 \) classes play a functional role in trophoblast adhesion to fibronectin. In a recent study, function-blocking antibodies specific for the \( \alpha_5, \alpha_v, \beta_1 \) or \( \beta_3 \) integrin subunits were shown to inhibit the fibronectin-binding activity of intact blastocysts [67], indicating that initial recognition of fibronectin by primary trophoblast cells requires apically located integrins composed of the \( \alpha_5 \beta_1 \) and \( \alpha_v \beta_3 \) heterodimers. Because mouse primary trophoblast cells are capable of adhering to substrates through interactions with other adhesion-promoting peptide sequences [68], the array of ECM receptors expressed by invasive trophoblast cells may be dependent on the molecules that are present in the substrates. Thus, trophoblast cells may express constitutively a variety of receptors that permit recognition of many ECM components. During implantation, the ability of trophoblast cells to adhere to many ECM components may be advantageous because the composition of the ECM that the trophoblast cells engage changes as they penetrate the basement membrane, invade stroma, and infiltrate blood vessels.

We have developed assays for the attachment of mouse embryos and trophoblastic spreading on cultured human decidual cells [69]. Attachment of blastocysts to the cultured decidual cells appears to be a prerequisite for further outgrowth of trophoblasts. Outgrowth, but not attachment, of embryos on decidual cells is inhibited in a dose-dependent manner by adding an antibody that recognizes the \( \beta_1 \) subunit, suggesting that \( \beta_1 \) integrins are important in blastocyst development and differentiation following attachment [69]. Amino acid residues 140–164 of integrin \( \beta_1 \) comprise a RGD cross-linking region. A peptide corresponding to integrin \( \beta_1 \) (DDL) on decidual cells has been shown to be critical in embryonic development and differentiation following attachment [70]. In addition, monoclonal antibodies directed against the \( \beta_1, \alpha_1, \alpha_2, \alpha_5, \) and \( \alpha_6 \) subunits affect embryo outgrowth, but not attachment, suggesting that blastocyst attachment and outgrowth may be mediated by different mechanisms [69]. Subsequent spreading of trophoblasts involves a number of cellular events that are necessary to produce morphological changes and cell migration.

The integrins themselves have no enzymatic activity and therefore must rely upon interactions with accessory proteins for generation of cytoplasmic signals. Two pathways have been proposed for integrin activation. One is outside-in signaling, in which binding of integrin with ECM components activates integrin and triggers signaling events based on the formation of focal adhesion structures. In the other pathway, inside-out signaling, stimuli such as growth factor signals are transmitted within cells. Recent evidence suggests that the integrins are transducers of cytoplasmic signals, and activation of this pathway is linked to one or more protein tyrosine kinase (PTK) [2, 3]. A candidate PTK for a mediator of integrin signaling is FAK which colocalizes with integrins at sites of cell-ECM contact [3], and we demonstrated the co-localization of FAK and \( \beta_1 \) integrin in human decidual cells to the regions known as focal adhesions. \( \beta_1 \) integrin-cytoskeletal linkage in focal contacts on human decidual cells may be important in mediating implantation [70]. Integrin-mediated activation of FAK is an early step in a signal transduction cascade that permits flow of information from the ECM proteins to the cell interior. Thus, an outside-in signaling cascade is important in mediating implantation. Rho A plays a prominent role in regulating organization of the cytoskeleton by promoting the assembly of focal adhesion and actin stress fibers and by activating FAK [71]. Aggregation of integrins induces the recruitment of Rho A to sites of integrin clustering, and activation of Rho A, in turn, regulates signaling downstream from integrins [72, 73]. Recent evidence indicates that Rho A modulates initial steps in integrin signaling by regulating integrin clustering [71], most likely through changes in cell contractility [74]. We have demonstrated that expression of Rho A coincides with integrin expression in human endometrium and decidua, and that Rho A in decidual cells is important for embryonic development and differentiation after attachment. In this interaction Rho A acts as the key protein in inside-out
signaling connected with integrin activation. Activated integrins then bind to the ECM, and this process implicates cell motility. Thus, the inside-out signaling cascade is also important in mediating implantation. These findings indicate that both pathways of integrin activation are important in mediating implantation.

Mouse embryos carrying homozygous null mutation for the β1 subunit die shortly after implantation probably because the inner cell mass degenerates [61, 62]. However, the β1-null trophoblast cells invade the uterine stroma and survive longer than the inner cell mass, suggesting that integrins of the β1 family are not absolutely required for initial implantation. These results suggest that the inner cell mass requires β1 integrins for survival while the trophoblast cells do not. In a strain of β3-null mice, implantation appears to be unaffected, suggesting that αvβ3 integrin is not required for early implantation [75]. Thus, several integrins are involved in implantation, or β1 and β3 integrins may functionally replace each other.

At placentation, major transitions in the expression of integrins have been described. In vitro, the adhesion of blastocysts to fibronectin-coated microspheres induces a translocation event that up-regulates β1 and β3 integrins on the apical surface of trophoblast cells [67]. Trafficking of α5β1 integrin and possibly other integrins to the apical surface of trophoblast cells has shown to be a critical step in placentation. The αv subunit expressed during placentation binds, at least, five different β subunits, β1, β3, β5, β6, and β8, forming five receptors which recognize the RGD sequence. The αv-null embryos, which do not express these five integrins, show defects in the development of the placenta leading to embryonic lethality on day 12, a stage at which placental functions are essential for development [76]. The placental defects affect the formation of interdigitations between fetal and maternal vessels and could arise from defective invasion, suggesting a critical role for αv integrins in placentation [76]. In a strain of β3-null mice, similar placental defects occur and lead to fetal mortality [75]. Taken together, the αvβ3 integrin may regulate adhesive and invasive events required for placentation.

Clinical Implications of Integrins in Reproduction

Endometriosis

Retrograde menstruation and peritoneal adhesion of shedded endometrial tissue are essential elements in the pathogenesis of endometriosis according to Sampson’s classical theory of implantation. Glandular epithelium and stroma of the human endometrium undergo a monthly cycle of first proliferative, and then secretory activity. Breakdown and tissue shedding ensues in the absence of embryo implantation. These changes are driven primarily by two ovarian steroid hormones, estrogen and progesterone, mediated by their respective receptors, which are expressed in epithelial and stromal cells. The presence of viable endometrial tissue fragments in peritoneal fluid has been demonstrated during the early follicular phase of the cycle in women with patent tubes. Cell adhesion molecules, such as cadherins and integrins, have been shown to be involved functionally in the shedding of endometrium during menstruation and in the adhesion of endometrial cells to the peritoneum [6, 24, 77, 78].

Of the constitutive epithelial and stromal integrin subunits, α2, α3, α5, α6, and β4, are found to be normally expressed in endometriotic cells [6]. With the exception of α2, which is weakly expressed in endometriosis, these cells exhibit a spatial distribution of constitutive integrins similar to that of normal endometrium [6]. Interestingly, α3 expression, normally exhibited in decidualized eutopic endometrium, is also observed in endometriotic implants decidualized by progestin therapy [6]. Differences in the expression of E-cadherin in normal and ectopic endometrium have been noted [77]. Thus, these integrins expressed in endometriotic lesions are involved in the development of endometriosis [6, 77, 78]. In peritoneal fluid, α4β1 is present frequently in women with endometriosis, but is absent in patients without endometriosis [77], suggesting a potential role for integrin α4β1 in endometriosis. These integrins may be involved in the shedding of endometrial tissue during menstruation and the attachment of endometrial tissue fragments to the peritoneum.

In contrast to the similar expression of
constitutive integrins in eutopic endometrium and endometriosis, cycle-dependent integrins are often absent in endometriotic implants. Specifically the \( \alpha_1, \alpha_4, \) and \( \beta_3 \) integrins are frequently absent in endometriosis implants from the secretory phase [77]. Rai et al. [79] have documented an increase in stromal \( \alpha_3 \beta_1 \) and a decrease in \( \alpha_6 \beta_1 \) in ectopic endometrium compared with eutopic endometrial expression, as well as a loss of cyclicity in the expression of epithelial \( \alpha \beta_3 \). The expression of \( \alpha \beta_3 \) integrin is significantly less in patients with endometriosis than in either fertile controls or infertile controls without endometriosis [78]. The severity of endometriosis appears to correlate with the presence or absence of the \( \beta_3 \) integrin subunit after cycle day 19. The expression of \( \alpha \beta_3 \) in the groups with stage I and stage II (minimal and mild) is significantly less than in patients with either stage III (moderate) or stage IV (severe), suggesting that aberrant \( \beta_3 \) expression is most commonly associated with milder forms of endometriosis [78]. Although the relationship between minimal endometriosis and infertility remains controversial, these findings suggest that there is a subgroup of patients with endometriosis who exhibit abnormal patterns of \( \beta_3 \) expression and whose infertility thus may relate to defective uterine receptivity. Alternatively, loss of this specific integrin that is normally expressed in the native endometrium, as the putative window of implantation opens, is highly associated with minimal and mild endometriosis. In melanoma cells, an association has been reported between the expression of \( \alpha \beta_3 \) and metastatic behavior [80], adding support to the speculation that expression of this integrin in eutopic endometrium and metastatic behavior is significantly elevated in the endometrium of women with endometriosis compared with normal controls. The increased expression of vascular \( \alpha \beta_3 \) integrin is shown to be associated with angiogenesis in other tissues [83]. These findings provide evidence for increased endometrial angiogenesis in patients with endometriosis, suggesting that angiogenic factors may play a significant role in the etiology of endometriosis. This concept of endometriosis provides novel approaches to medical treatment [84]. These potential medicines include various fragments of known proteins. Angiostatin, a 38 kD fragment of plasminogen, selectively inhibits endothelial proliferation. Endostatin, a related 20 kD fragment of collagen 18, also appears to be a potent antiangiogenic substance. The 16 kD and terminal PRL inhibits endothelial cell migration and increases plasminogen activator inhibitor-1 expression in endothelial cells. If any of the above potential medicines for endometriosis are worthy of further study, stained release via micro delivery systems under laparoscopy to areas of endometriosis would be possible.

Shed menstrual endometrium is viable, and the endometrial cells are found in the peritoneal fluid. In order to implant and grow, the endometrial cells need to establish cell-to-cell or cell-to-ECM interactions with the peritoneal lining. Endometrial stromal cells are involved in stimulating and inhibiting the growth of glandular epithelium [85]. The stromal cells are actively involved in the adhesion of endometrial implants to the peritoneum. Early endometriosis lesions have been shown to invade the ECM of the peritoneum after the initial attachment [86]. Witz et al. [87, 88] have recently demonstrated that the mesothelial cells express the \( \alpha_2 \beta_1 \) and \( \alpha_3 \beta_1 \) integrins \textit{in vivo} and \textit{in vitro}. Furthermore, intraperitoneal administration of neutralizing antibody to \( \alpha \beta_3 \) integrin reduces the incidence of adhesion formation [89]. The levels of \( \alpha \) and \( \beta_3 \) mRNA expression vary among the serosal tissues of intraperitoneal organs and adhesions; expression of \( \alpha \) mRNA is highest in the uterine serosa and lowest in the small bowel, and expression of \( \beta_3 \) mRNA is highest in the fallopian tubes and lowest in the uterine serosa [90]. On the basis of such variation and the knowledge that tissue injury alters local integrin expression, integrins may play
a key role in adhesion development in patients with endometriosis particularly in tissues with higher integrin expression.

Activated macrophages are a dominant feature of the inflammatory reaction and frequently contribute to the pathogenesis of the underlying disease. Leukocyte chemotaxis in the inflammatory lesion is mediated by several factors. Monocyte chemotactic protein (MCP)-1 is one potent chemotactic factor for monocytes/macrophages. MCP-1 is secreted by a number of cell types including endometrial stromal cells [91], and its levels in the peritoneal fluid are elevated both in women with endometriosis [92] and in women with abdomino-pelvic adhesions [93]. The endometrial stromal cells adhesion to various ECM proteins induces an up-regulation in MCP-1 gene expression and protein secretion [94]. The rise in MCP-1 expression is mediated by β1 integrin. These findings have important implications for integrins in the pathogenesis of endometriosis.

**Unexplained infertility**

Although the reproductive failure of women with unexplained infertility remains enigmatic, increasing evidence indicates that the endometrium of women with unexplained infertility differs from that of normal fertile women with respect to both endometrial stromal leukocyte populations and glycoconjugate profiles of endometrial epithelial cells during the peri-implantation phase of the menstrual cycle. The expression of α4β1 integrin was detected in the endometrial epithelial cells of fertile women, but was absent in the endometrium of women with unexplained infertility [18]. The α4β1 integrin interacts with fibronectin and is a proposed cell-to-cell adhesion receptor. Feinberg et al. [95] demonstrated that oncofetal fibronectin characterizes a differentiated form of trophoblast capable of penetrating and anchoring to endometrial ECM proteins. Endometrial α4β1 integrin may participate in the recognition of trophoblastic tissue, acting as a receptor for oncofetal fibronectin. The absence of the α4β1 molecule in the endometrium of infertile women may result in incomplete embryo-maternal recognition, consequently resulting in implantation failure.

In the extensive evolution of integrins in human endometrium [6, 7, 16–21], α4 and β3 subunits are specifically coexpressed only during the time of maximum uterine receptivity. Especially, αvβ3 integrin is a much more reliable marker of the opening of the window of implantation (present after preovulatory day 5), and its expression extends into pregnancy [16, 21, 24]. Although all endometrial biopsies from parous controls contain positive immunostaining for the α1 and β3 integrin subunits in glandular epithelium, some samples from parous controls lack the α4 subunit [96]. In contrast, biopsy specimens from women with unexplained infertility demonstrate significantly reduced β3 expression with similar expression of α1 and α4 subunits. Thus, a majority of unexplained infertility patients may have alterations in uterine function that can be detected using the αvβ3 integrin as a marker protein. The temporal and spatial distribution of αvβ3 during the window of implantation makes this integrin uniquely important in the assessment of the endometrium [96]. The α1 integrin subunit, a component of the collagen/laminin receptor (α1β1), has been shown to exhibit the highest expression in both endometrial stromal and epithelial cells [97]. Fertile and infertile women show similar expressions of this integrin during the time of the implantation window. However, infertile women express lower concentrations of α1 in the epithelium and stroma during the late secretory phase. A lack of stromal α1 expression in infertile women may be related to the absence of laminin and collagen IV. The molecular significance of these changes in infertile patients is unknown. The low expression of both the receptor and ligands may influence trophoblast invasion. These findings suggest that the defective expression of the α1 integrin in endometrial stromal cells found in the late secretory phase may be associated with the poor fertility outcome of women with unexplained infertility. We determined the effect of danazol on in vitro fertilization-embryo transfer (IVF-ET) patients who failed to conceive in previous attempts despite having embryos with optimal morphology [98]. Danazol treatment (400 ng/day for 12 weeks) following unsuccessful IVF-ET cycles increased the pregnancy rate in the subsequent cycle. We recently found that the expression of epithelial αvβ3 integrin in fertile control women did not differ significantly from that found in the patients with explained infertility including tubal factors and male factors (unpublished data). However, the
expression of αvβ3 integrins was significantly lower in patients with unexplained infertility compared to that in fertile controls and explained infertility patients. An immunohistochemical study using the mid-secretory endometrium revealed that the intensity of glandular αvβ3 integrin staining was enhanced by danazol treatment. These studies demonstrate aberrant expression of αvβ3 integrin in the endometrium of repeatedly unsuccessful IVF-ET patients and a positive correlation in the expression levels with danazol administration. We postulate that danazol may have therapeutic potential for improving endometrial receptivity through possible up-regulation of αvβ3 integrin expression.

**Tubal diseases**

While IVF-ET was initially developed as a treatment for women with tubal factor infertility, recent clinical studies have suggested that the presence of hydrosalpinges lowers implantation and pregnancy rates [99–101]. These studies, complemented by reports demonstrating increased implantation rates and decreased miscarriage rates after surgical extirpation, drainage, or proximal ligation of hydrosalpinges [102, 103] suggest that retrograde spillage of hydrosalpingeal tubal fluid may affect either embryo growth and/or endometrial receptivity. Meyer et al. [104] evaluated endometrial histology and integrin expression during the mid-luteal phase in women with persistent hydrosalpingeal disease and compared them with those in fertile controls. Women with hydrosalpinges expressed significantly less αvβ3 integrin compared with controls. There was no difference in expression of α1β1 or α4β1 among the groups. A significantly greater number of cases had out-of-phase histology and missing αvβ3 and absent integrin expression despite normal histological maturation defects, compared with controls. In addition, hydrosalpinx surgery in patients with impaired endometrial receptivity increased the expression of αvβ3 integrin in the endometrium. These findings demonstrate that inflammatory hydrosalpinges have an adverse effect on endometrial receptivity, which in some cases may be overcome by surgical treatment of the hydrosalpinx.

The integrins expressed in the receptive endometrial epithelium are also present in the epithelial cells of the normal Fallopian tube [105]. The αv subunit is expressed in the Fallopian tube epithelium during both the non-receptive period (luteal phase days 2–4) and receptive period (luteal phase days 6–8) in a pericellular distribution. The β3 subunit is also expressed in the same location, but it is up-regulated during the period of endometrial receptivity. The other subunits are expressed in localizations which are not relevant to trophoblast adhesion and exhibit little or no difference in the level of expression between the non-receptive and receptive periods. These findings suggest that the expression of the β3 subunit in the human tubal epithelium is regulated by the same systemic control signals as in the endometrium and that the normal tubal epithelium may have an implantation window, at the same time as the endometrium, that affords an opportunity for trophoblast attachment. This implies that the adhesion of embryos to the endosalpinx in ectopic pregnancy shares the same molecular basis as adhesion to the endometrium.

**Contraception**

Contraceptive efficacy of oral contraceptives (OC) likely involves several complementary mechanisms. These include central inhibition of ovulation, altered cervical mucus characteristics, and interference with normal tubal motility. Clearly, pharmacologic levels of steroids in OC formulations also may affect endometrial development and function. Oral contraceptives induce characteristic morphological changes in the endometrium but the functional significance of these effects has not been investigated previously. Somkuti et al. [106] have demonstrated that constitutive integrin expression in the endometrium (α2β1, α3β1, α5β1, α6β1, and α6β4) is similar in OC users and normally cycling individuals, except for an increase in epithelial α3β1 in OC users. OC use is associated with significant alterations in cycle-dependent integrin expression (α1β1, α4β1, and αvβ3). Especially, increased stromal αv and β3 and decreased α3β1 and α6β1 staining are observed in OC users. These alterations in epithelial and stromal integrin expression suggest that impaired uterine receptivity is one mechanism whereby OCs exert their contraceptive actions. However, high doses of oral contraceptives in emergency contraceptives do not alter endometrial α1 and αvβ3 integrins in the late implantation window [107].
After oral contraceptives, the intrauterine device (IUD) probably is the most common contraceptive method used in the world. However, its mechanism of action is controversial and as yet not well understood. It has been suggested that IUDs act by interfering with sperm transport, fertilization, and implantation. It is also believed that IUDs stimulate an inflammatory response in the uterus. Savaris et al. [108] evaluated whether copper intrauterine devices induce abnormal integrin expression during the window of implantation. No difference in \( \alpha 4 \) integrin expression was found between IUD users and fertile controls in both the luminal and glandular epithelium, whereas significantly fewer women using copper IUD had positive \( \alpha v \beta 3 \) immunoreactivity in the glandular epithelium of mid-secretory endometrium.

**Preeclampsia**

In normal human pregnancy, invasion of the uterus and its arterial system by cytotothrophoblasts extends through the entire decidua and the adjacent third of the myometrium. During the first trimester of pregnancy, invasion is accompanied by a marked change in the expression of cell adhesion molecules by invasive cytotothrophoblasts. Placental cytotothrophoblasts that invade the uterus down-regulate the expression of adhesion receptors that are characteristic of their epithelial origin, and up-regulate the expression of adhesion receptors that are expressed by vascular cells [109]. Two patterns of cytotothrophoblast integrin switching occur in tissues from normal pregnancies [110]. One transition takes place in the cell column of the anchoring villus and is characterized by a down-regulation of \( \alpha 6 \beta 4 \) integrin and an up-regulation first of \( \alpha 5 \beta 1 \) integrin and then of \( \alpha 1 \beta 1 \) integrin. The second transition occurs as a function of gestational age. From the second trimester onward \( \alpha 3 \) integrin is expressed by cytotothrophoblasts in all locations. At term, placental bed cytotothrophoblasts express \( \alpha 6 \), but not \( \beta 4 \) integrin. This transformation is critical to endovascular invasion, the process whereby cytotothrophoblasts invade the uterine spiral arterioles and line their walls. Thus, during normal trophoblast invasion, the cytotothrophoblasts up-regulate the expression of \( \alpha 5 \) integrin, that inhibits invasion, and then \( \alpha 1 \) integrin, that promotes invasion. Correctly balancing these mechanisms is likely to be crucial to normal placental development.

Preeclampsia is a leading cause of maternal death and contributes significantly to premature deliveries and increased perinatal mortality. An abnormality of the placenta has been implicated as the underlying cause of preeclampsia [111]. Cytotrophoblast expression of \( \alpha 6 \beta 4 \) and \( \alpha 1 \beta 1 \) integrins is dramatically and consistently disregulated in preeclampsia [110]. Placental bed cytotrophoblasts from preeclamptic patients express the \( \alpha 6 \) integrin at a time during the second trimester when expression of this integrin subunit is normally not observed. Furthermore, cytotrophoblast staining for \( \alpha 4 \) integrin, which is never detected on cells within the uterine wall, is prominent on cytotrophoblasts of the preeclamptic placental bed. Cytotrophoblasts in the uterine wall in preeclampsia also fail to up-regulate the expression of \( \alpha 1 \beta 1 \) integrin.

Cytotrophoblasts in cell columns show reduced E-cadherin staining and express VE-(endothelial) cadherin, platelet-endothelial adhesion molecule-1, vascular endothelial adhesion molecule-1, and \( \alpha 4 \) integrins [112]. Cytotrophoblasts in the uterine interstitium and maternal vasculature continue to express these receptors, and like endothelial cells during angiogenesis, also stain for \( \alpha v \beta 3 \). In functional studies, \( \alpha v \beta 3 \) and VE-cadherin enhance cytotrophoblast invasiveness, while E-cadherin restraints. Cytotrophoblasts expressing \( \alpha 4 \) integrins bind immobilized VCAM-1 in vitro, suggesting that this receptor-pair could mediate cytotrophoblast-endothelium or cytotrophoblast-cytotrophoblast interactions in vivo, during endovascular invasion. In preeclampsia, invading cytotrophoblasts retain expression of \( \alpha v \beta 6 \), which is transiently expressed in remodeling epithelium, and fail to up-regulate \( \alpha v \beta 3 \), which is characteristic of angiogenic endothelium. Therefore, as is the case for integrin \( \alpha 1 \) [110], the analyses of the expression of \( \alpha v \)-family members suggest that in preeclampsia, cytotrophoblasts start to differentiate along the invasive pathway but cannot complete this process. Preeclampsia also has a striking effect on cytotrophoblast expression of E-cadherin and VE-cadherin [109]. Cadherin modulation by cytotrophoblasts in preeclampsia is defective, as shown by the persistence of strong E-cadherin staining and the absence of VE-cadherin staining of cytotrophoblasts in columns and in the superficial decidua.
Vascular endothelial cells express several members of the Ig superfamily of adhesion receptors, many of which interact with leukocytes [25]. In control pregnancy at 26 weeks of gestation, vascular adhesion molecule-1 (VCAM-1), which interacts with integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$, is not detected on villus cytotrophoblasts within the uterine wall. Staining is particularly strong on endovascular cytotrophoblasts. When the pregnancy is complicated by preeclampsia, staining for VCAM-1 is not detected on cytotrophoblasts in the decidua [109]. The few cytotrophoblasts that reach the termini of spiral arterioles show only weak antibody reactivity. Thus, in the pregnancy disorder preeclampsia, in which endovascular invasion remains superficial, cytotrophoblasts fail to express most endothelial markers, suggesting that an adhesion phenotype switch is required for successful endovascular invasion and normal placentation.

**Conclusions**

The ability of cells to successfully integrate signals arising from soluble factors, cell-matrix adhesion and cell-cell adhesion, is at the heart of multicellularity. Correct integration of these signals allows appropriate cellular growth, differentiation, and ultimately tissue morphogenesis. The integrins play significant roles in all processes important for human reproduction. The participation of integrins in fertilization, implantation and placentation raises the possibility of discovering naturally occurring defects in the receptor structure and function that may play a role in impaired reproductive performance. Knowledge of the integrin-mediated regulation of cell growth and cell survival may facilitate our understanding of many key aspects of development and physiology in reproduction. Although substantial progress has been made toward understanding the integrin function, many questions remain for the future. The elucidation of the integrin-mediated signal transduction pathway provides insights into the regulation of the reproductive processes.

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