Integrins and human reproduction

Richard A. Bronson1,3 and Francesco M. Fusi2

1Departments of Obstetrics and Gynecology, Health Sciences Center, State University of New York, Stony Brook NY 11794-8091, USA and 2Hospital San Raffaele, University of Milan, Milano, Italy

To whom correspondence should be addressed

Introduction

Integrins are a family of cell surface glycoprotein receptors by which cells attach to extracellular matrices. The integrins also act as co-receptors in many cell-cell interactions. Cells vary their adhesive properties by changing the integrins expressed on their surface. Apart from functioning in adhesion, a second major property of integrins is their role in signal transduction. There is increasing evidence that several integrin receptors play major roles in the area of reproduction. These include events that result in the establishment of pregnancy, such as gamete interactions leading to fertilization, interactions between the preimplantation embryo and endometrium that lead to successful nidation, as well as later roles in trophoblast outgrowth during placentation. Evidence has also accumulated that alterations in the expression of certain integrins or their ligands are associated with impairment in the ability of spermatozoa to undergo an acrosome reaction, abnormalities in the receptivity of the luteal phase endometrium to embryo implantation, the development of endometriosis, and abnormalities of placentation associated with pregnancy-induced hypertension, and placenta accreta. Although integrins have yet to enter the lexicon of the clinician, they will most certainly do so in the near future. Our goal is to review how integrins function, as well as the relevant physiology of those reproductive events in which integrins appear to play an important role.

Several excellent reviews have recently appeared describing integrin biochemistry and function (Hynes, 1992; Diamond and Springer, 1994; Yamada et al., 1995). All integrins are glycoprotein heterodimers (Figure 1). The α subunits vary in size between 120 and 180 kDa and are each non-covalently associated with a β subunit. While initially three large families, defined by their β chains (β1, β2, β3) were recognized, there are now more than eight known β subunits and 14 α subunits. Many α subunits can associate with only a single β subunit, so that while these could, in theory, associate to give >100 integrin heterodimers, their actual number is much more limited. Both subunits of integrins are transmembrane proteins, each containing a single hydrophobic transmembrane segment. In most integrins, the cytoplasmic domains are short (<50 amino acids). β4 is an exception, in that its cytoplasmic domain comprises >1000 amino acids. The subunits of integrin receptors are glycosylated by asparagine-linked oligosaccharides which appear crucial for integrin function.

All α subunits contain a region that contributes to the divalent cation-binding properties of integrins, which are essential for receptor function. The N-terminal domains of α and β subunits combine to form the ligand-binding region of each integrin, while their two cytoplasmic domains interact with cytoskeletal proteins. Different α subunits have very different cytoplasmic sequences, which mediate differing cellular responses to common extracellular ligands.

Individual integrins can often bind to more than one ligand. The first binding site to be defined was the Arg-Gly-Asp (RGD) sequence present in fibronectin, vitronectin and osteopontin (Ruoslahti and Pierschbacher, 1986). While ~50% of the integrin receptors recognize RGD within their ligands, other integrins recognize different amino acid sequences.

Individual cells can vary their adhesive properties by selective expression of integrins and by modulating the binding properties of integrins through conformational changes in the receptor. For example, the platelet integrin αIIbβ3 increases its

Figure 1. Illustrates the overall integrin shape, deduced from electron microscopy. The shaded area represents the ligand binding region, formed by the heads of the α and β chains. M++ represents the divalent cation-binding region of the α chain. The α and β chains each contain a short cytoplasmic domain (adapted from Hynes, 1992).
affinity for soluble fibrinogen only after platelet activation by thrombin or collagen (Smyth et al., 1993). These results are associated with a conformational switch between states of the extracellular domain of the receptor. The β3 integrins expressed on leukocytes exhibit activation phenomena similar to those of αvβ3 (Diamond and Springer, 1994).

**Outside-to-inside signalling**

Integrins are not simply adhesion sites on cell surfaces. The integrins can be viewed as two-way signalling molecules (Figure 2). Although it was initially thought that the interactions mediated by the integrins were solely mechanical, there is increasing evidence that integrins transduce signals from the outside of the cell to the cytoplasm. Integrin-mediated signalling pathways identified include the regulation of a Na+/H+ antipporter (Schwartz et al., 1991), Ca2+ influx (Pardi et al., 1989; Miyauchi et al., 1991; Schwartz et al., 1991), stimulation of inositol lipid synthesis (McNamee et al., 1993) and protein tyrosine phosphorylation of a group of cytoplasmic proteins of 100–130 kDa (Kornberg et al., 1991, 1992; Guan et al., 1991; Burridge et al., 1992). The latter has been shown, in human umbilical endothelial cells, to be mediated via the β1 and β3 integrin families in response to various matrix proteins (De Filippi et al., 1994).

Integrins appear to transduce signals after receptor clustering. Integrin-mediated adhesion of cells to fibronectin and cross-linking of integrins trigger a rise in intracellular calcium in endothelial cells, due to influx of extracellular calcium. This process appears to be mediated by the αs subunit, in association with a putative calcium transporter (Lindberg et al., 1993; Schwartz and Denninghoff, 1994). Recent evidence points to a direct link between extracellular ligands, clustering of integrins, and organization of cytoskeletal proteins into submembranous aggregates. Binding of beads coated with ligands such as fibronectin leads to accumulation of cytoskeletal proteins on the inner surface of the plasma membrane (Plopper and Ingber, 1993), which appears to be mediated by a direct link between the β1 integrin tail and the cytoskeleton. Antibody-mediated clustering with certain anti-β3 and anti-β1 monoclonal antibodies will trigger the phosphorylation of the cytoskeletal protein focal adhesion kinase (FAK). A synergistic role of receptor occupancy and aggregation in transmembrane signalling by integrins has recently been demonstrated (Miyamoto et al., 1995).

Adhesion of integrins to different extracellular matrix components results in selective patterns of expression of specific genes in monocytes (Juliano and Haskill, 1993). Besides their effects on specific gene expression, integrins can also trigger cell proliferation (Han et al., 1993). For example, occupancy of the αβ5 receptor of an erythroleukemia cell line by the peptide Gly-Arg-Gly-Asp-Ser (GRGDS) stimulates kinase activities associated with cyclin A and cdc2 (Symington, 1992).

**Inside-to-outside signalling**

Evidence has accumulated that integrins can potentially exist in inactive, partially activated and highly activated states. This process involves 'inside-out' signalling resulting in a conformational change in the external domain of the receptor. This altered conformation leads to the presence of neoantigens which can be detected by monoclonal antibodies that bind only to activated receptor. Mutational analysis suggests that the cytoplasmic regions of both the α and β subunits may play a role in the modulation of integrin-ligand binding affinity. Recognition sequences within the cytoplasmic domain of the α subunit of the platelet integrin αIIbβ3, and perhaps of all integrins, regulate the integrin's activity. Although the detailed mechanisms of these activation events are not known, they appear to involve binding of one or more cytoplasmic effectors to the β tail (Du et al., 1991). This process then appears to be modulated by the α tail, resulting in a conformational change in the extracellular domain that permits the binding of ligands such as fibronectin.

![Figure 2](https://academic.oup.com/molehr/article-abstract/2/3/153/1056280)
Adhesion by integrins is dynamically regulated by cells on which they are expressed. This cycling between adhesive and non-adhesive states allows a cell the ability to rapidly regulate its binding to ligands on opposing cell surfaces or on matrices. Cross-linking of the cell-surface molecules CD2 or CD3 on lymphocytes, or treatment with phorbol esters, increases the adhesiveness of \( \alpha_b \beta_1 \), \( \alpha_c \beta_1 \), and \( \alpha_e \beta_1 \) integrins for laminin and fibronectin (Shimizu et al., 1990). Phorbol esters also induce the \( \alpha_b \beta_1 \) integrin on peripheral B cells to bind ligand and \( \alpha_e \beta_1 \) on macrophages to bind laminin.

The numbers of available integrins on the cell surface can also be increased as well. For example, activation of neutrophils with chemotactic peptides or with phorbol esters rapidly induces their binding of particles coated with inactivated complement component C3 (iC3b), fibrinogen, or intercellular adhesion molecule (ICAM)-1 to the leukocyte integrin \( \alpha_m \beta_2 \). A greater than 10-fold quantitative increase in levels of \( \alpha_m \beta_2 \) present on the leukocyte surface occurs rapidly after stimulation and is the result of the translocation of secretory granules that contain \( \alpha_m \beta_2 \) integrin to the cell surface. This increase is transient, and within minutes, there is a reduction in binding to these ligands (Miller et al., 1987; Diamond and Springer, 1994). In addition to an increase in the population of \( \alpha_m \beta_2 \) receptors that occurs after leukocyte activation, the increase in binding strength of this integrin may be due to a conformational change in the ligand-binding region on a sub-population of \( \alpha_m \beta_2 \) molecules.

The recognition of neoepitopes by monoclonal antibodies and changes in the apparent affinity between specific integrins and their ligands after cell activation can also be explained by a receptor multimerization model. As suggested by Diamond and Springer (1994), redistribution of integrins so that their local concentration increases the area of ligand apposition could strengthen attachment of the integrin to any ligand, without requiring a change in affinity of an individual integrin. It has been shown that treatment of myeloid cells with phorbol esters induces a clustering of \( \alpha_m \beta_2 \) integrins that correlates with their increased adhesiveness for erythrocytes coated with the complement component iC3b.

Divalent cations also play a role in the adhesiveness of integrins. Integrin \( \alpha \) subunits contain repeated motifs that have homology to the divalent cation binding sites of Ca\(^{2+} \) binding regulatory proteins. Occupancy of these binding sites alters the adhesiveness of integrins in vitro, and detection of several neo-epitopes present on active integrins requires the presence of Ca\(^{2+} \).

The role of integrins in reproduction

Integrin-mediated adhesion plays roles in a wide variety of physiological and pathological functions in human reproduction, at the level of fertilization, embryo implantation and placentation. Based on progress in our laboratory as well as others, the mammalian egg plasma membrane has been shown to contain integrin receptors, and spermatozoa display on their surface fibronectin and vitronectin following capacitation, adhesion molecules that bind to these receptors. Integrins have also been detected on human spermatozoa, and their expression varies with the functional state of spermatozoa. Integrins are expressed on human endometrial epithelium, and the expression of specific integrins varies throughout the menstrual cycle. Evidence has accumulated suggesting they play a role in establishing the implantation window of uterine receptivity. Abnormalities of integrin expression by eutopic endometrium have been documented in association with endometriosis that could play a role in both nidatory failure leading to infertility as well as in the mechanisms that lead to implantation of ectopic endometrium within the peritoneal cavity. Integrins are also expressed by trophoblast. They are modulated during trophoblast outgrowth, and a complex relationship exists between the endometrial extracellular matrix, decidualized stromal cells, and integrin expression by trophoblasts, that is required for normal placentation. It has been suggested that abnormalities in this interaction could lead to placenta accreta, pregnancy-induced hypertension and intrauterine growth retardation.

Sperm-egg interactions leading to fertilization

It has been >40 years since the observation that freshly ejaculated spermatozoa in mammals cannot penetrate eggs, but acquire the ability to do so during their residence within the female reproductive tract (Chang, 1951; Austin, 1952). The molecular basis of this process, termed capacitation, remains largely unknown, but appears to involve loss of cholesterol from the sperm plasma membrane and the expression of mannoside-binding lectins (Benoff et al., 1993), membrane associated progesterone receptors (Meizel and Turner, 1991; Blackmore et al., 1991; Baldi et al., 1991), and glyco-protein adhesion molecules (Fusi et al., 1992a, b) on the sperm surface.

During the normal processes of fertilization in situ, the spermatozoon penetrate through specific receptors to ligands on the zona pellucida (Wasserman, 1990; Yanagimachi, 1994). This event triggers, through the mediation of G proteins, an acrosome reaction (Leyton and Saling, 1989; Kopf and Wilde, 1990). The spermatozoon penetrates the zona pellucida, entering the perivitelline space. Hence, under normal conditions, only acrosome-reacted spermatozoa encounter the oolemma. While the spermatozoon is the active partner in its penetration of the egg vestments, once having entered the oolemma, the egg plays an active role in the subsequent processes leading to sperm entrance of the ooplasm.

We have recently studied the kinetics of binding of fresh versus capacitated human spermatozoa to the oolemma of zona-free hamster eggs, utilizing matched ejaculates of known fertile men. This heterologous interaction was chosen given evidence that human spermatozoa must undergo those same processes (capacitation and the acrosome reaction) required for their penetration of human eggs (Yanagimachi et al., 1976; Yanagimachi, 1988), but avoids the ethical considerations of fertilization associated with performing such experiments in a homologous human system. These experiments indicate that uncapacitated, acrosome-intact human spermatozoa recovered fresh from ejaculates exhibit a markedly diminished ability to bind to and fuse with the oolemma, compared with capacitated...
spermatozoa. The rate of sperm binding to the oolemma was seven-fold greater for capacitated versus fresh spermatozoa (Table I) reflecting their increased adhesiveness. Very few fresh, un capacitated spermatozoa that bound to the egg surface fused with the oolemma. While rapid binding of capacitated spermatozoa to the egg surface was noted within seconds of gamete mixing, gamete fusion was delayed, with a latent period varying 20–90 min, depending upon the semen donor.

Previous ultrastructural studies have shown that the vast majority of human spermatozoa adhering to zona-free hamster eggs are acrosome-reacted (Talbot and Chacon, 1982). The molecular processes whereby capacitated, acrosome-reacted spermatozoa increase their adhesiveness to eggs, undergo gamete membrane fusion, and enter the cortical ooplasm remain unknown. Ultrastructural analysis indicates that these events involve different regions of the acrosome-reacted spermatozoa, and that the process of sperm incorporation by the oocyte is quasi-phagocytic in appearance (Thomson et al., 1974; Huang and Yanagimachi, 1985; Sathananthan and Chen, 1986). Gamete membrane fusion appears to take place at the equatorial segment of the acrosome, while the rostral portion of the sperm head (limited by the inner acrosomal membrane) is incorporated in an oolemmal-derived vesicle. Scanning electron micrographs, at this time, revealed an apparent elongation of oolemmal microvilli over the rostral portion of the acrosome-reacted sperm head, in contact with the inner acrosomal membrane (Bronson et al., 1990a). Subsequently, microvilli elongated over the equatorial segment of the acrosome, as these spermatozoa appeared to drop into the cortical ooplasm (Figure 3).

In rodents, sperm tail beating ceases at the time of gamete membrane fusion. Hence, sperm motion is not required for incorporation of the sperm head into the egg. That this appears to be the case in humans has been suggested by our own unpublished experiments, in which spermatozoa were immobilized with sperm tail-directed antisperm antibodies and complement. Such immotile spermatozoa were capable of penetrating zona-free hamster eggs. Further supporting evidence that sperm motility is not required for sperm entry to the human egg has been obtained through observations at the time of laboratory assisted reproduction. When immotile spermatozoa from men with dynein arm abnormalities are injected into the perivitelline space, fertilization and pregnancy have been documented (Wolf et al., 1993).

### Gamete receptors and ligands play a role in sperm oolemmal adherence and fusion

Initial evidence that oolemmal receptors could play a role in sperm binding to the egg came from experiments performed by Boldt et al. (1988, 1989). They observed a time-dependent loss in the ability of trypsinized mouse eggs to bind capacitated mouse spermatozoa. Sperm–egg fusion was also inhibited. Four specific protein bands, identified following surface iodination of oolemmal glycoproteins and one-dimensional gel electrophoresis, were lost during egg exposure to proteases. Two of these bands exhibited molecular weights similar to those of the α and β chains of integrin receptors. Subsequent incubation of these trypsinized eggs resulted in reappearance of the specific oolemmal proteins in association with restored ability of these eggs to bind spermatozoa. Further evidence that oolemmal receptors and their ligands on spermatozoa play a role in fertilization comes from studies of Rochwerger et al. (1992). They showed that the epididymis, in the rat, secretes a glycoprotein acquired by spermatozoa during their epididymal passage which acts as an oolemmal receptor ligand. This protein has been purified and found to block subsequent sperm binding to eggs that have been pre-incubated in its presence.

Evidence has accumulated that oolemmal integrins and their ligands on spermatozoa play a role in sperm adherence to the oocyte. We have demonstrated that oligopeptides containing the RGD integrin-binding sequence inhibit the binding of human and hamster spermatozoa to zona-free hamster eggs (Bronson and Fusi, 1990). This inhibition was concentration dependent and occurred at a range (30–100 μM) which had been shown to inhibit the binding of fibroblasts to vitronectin and fibronectin-coated microwells (Figure 4). When several different RGD-containing oligopeptides were compared, they varied in their ability to inhibit sperm oolemmal binding, suggesting the stereospecificity of a receptor (Table II).

Although we were unable to perform similar experiments utilizing human eggs for ethical reasons, the presence of active
Integrins and human reproduction

Figure 3. Scanning electron micrograph of human spermatozoa on the surface of a zona-free hamster egg. Apparent adherence and elongation of oolemmal microvilli is seen over the equatorial segment of the sperm head (Bronson et al., 1990a).

Figure 4. Inhibition of the adherence of human spermatozoa to the oolemma of zona-free hamster eggs by Asp-Gly-Arg (RGD)-containing oligopeptides (adapted from Bronson and Fusi, 1990). *Control consisted of gamete incubation in the absence of peptides; bP < 0.01, Student’s t-test; dP < 0.005; cP < 0.001.

RGD recognition sites has been documented on these eggs by studying the specific oolemmal rosetting of immunobeads coated with PepTite 2000, a synthetic RGD-containing oligopeptide (Fusi et al., 1993). This rosetting could be inhibited by RGD-containing proteins but not by a peptide that did not contain RGD (Figure 5).

The presence of integrins on mammalian eggs has been documented, in several species. Using dot blot analysis, we have shown the presence of α5β1 and α5β3 integrins in lysates of hamster eggs (Fusi et al., 1992a). We have detected α2, α5, α6, α8, β1, and β3 integrin chains on the oolemma of human eggs through the use of a rosetting technique in which anti-integrin monoclonal antibodies were coupled to immunofluorescent covaspheres (Figure 5). These results indicate that integrin receptors that recognize fibronectin (α5β1) and vitronectin (α5β3) are present on the egg surface. Fibronectin and vitronectin have both been detected on human spermatozoa (see below). The integrins α5β1 and α5β3 have also been detected on unfertilized mouse eggs by indirect immunofluorescence while mRNA encoding the integrin subunits α5, α6, α8, β1 and β3 was detected by polymerase chain reaction (PCR) (Tarone et al., 1993; Almeida et al., 1995).

β1 integrin chains have been detected on ejaculated human spermatozoa, as well as spermatogenic cells within the testis, and exhibit alterations in their expression during an induced acrosome reaction in vitro (Schaller et al., 1993; Glander and Schaller, 1993). Klementzis et al. (1995) have compared the expression of β1 class integrins on human spermatozoa, determined by immunohistochemistry, with their in-vitro fertilizing ability. Expression of α5β1, α5β3, and α8β1 by spermatozoa from men with abnormal ejaculates was significantly lower than from men with normal semen specimens, and most spermatozoa with abnormal morphologies lacked expression of these integrins. Compared with the tubal infertility group, males with unexplained infertility had a significantly lower proportion of spermatozoa expressing α5β1. Expression of α4, α5, and α8 chains correlated significantly with the incidence of fertilization in vitro.

We have demonstrated the presence of α5, α6, β1 and β3 integrin chains on ejaculated human spermatozoa, noting that their expression varied with the functional state of spermatozoa (Fusi et al., 1995a). α5β3 appeared following capacitation, while α5β1 was only observed following exposure of capacitated spermatozoa to a calcium ionophore. The proportion of living spermatozoa that expressed α5β3, as determined by flow
Table II. Effects of Arg-Gly-Asp (RGD)-containing peptides on hamster spermatozoa/hamster egg co-incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. eggs</th>
<th>Egg penetration (%)</th>
<th>Penetration index</th>
<th>Mean no. adherent spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>86</td>
<td>1.07</td>
<td>37.7 ± 6.2</td>
</tr>
<tr>
<td>GRGES (2 μM)</td>
<td>13</td>
<td>69</td>
<td>0.77</td>
<td>37.2 ± 5.1</td>
</tr>
<tr>
<td>(NS*)</td>
<td>13</td>
<td>54</td>
<td>0.61</td>
<td>31.6 ± 5.3</td>
</tr>
<tr>
<td>GRGES (75 μM)</td>
<td>13</td>
<td>46</td>
<td>(P &lt; 0.01)</td>
<td>26.4 ± 5.2</td>
</tr>
<tr>
<td>GRGES (200 μM)</td>
<td>13</td>
<td>38</td>
<td>(P &lt; 0.0005)</td>
<td>19.1 ± 7.3</td>
</tr>
<tr>
<td>GRGDTP (2 μM)</td>
<td>13</td>
<td>7</td>
<td>(P &lt; 0.0004)</td>
<td>4.6 ± 3.8</td>
</tr>
<tr>
<td>(P &lt; 0.001)</td>
<td>13</td>
<td>0</td>
<td>(P &lt; 0.00001)</td>
<td>1.8 ± 1.9</td>
</tr>
<tr>
<td>GRGDTP (75 μM)</td>
<td>13</td>
<td>0</td>
<td>(P &lt; 0.00000)</td>
<td>1.8 ± 1.9</td>
</tr>
<tr>
<td>(P &lt; 0.00001)</td>
<td>13</td>
<td>0</td>
<td>(P &lt; 0.00000)</td>
<td>1.8 ± 1.9</td>
</tr>
</tbody>
</table>

GRGES = Gly-Arg-Gly-Glu-Ser.
GRGDTP = Gly-Arg-Gly-Asp-Thr-Pro.
*Student’s t-test compared to control; NS = not significant.

These results suggest that α3β3, a vitronectin receptor, is expressed on the inner acrosomal membrane, while α5β1, a fibronectin receptor, is present on the plasma membrane.

Human spermatozoa contain the adhesion proteins fibronectin and vitronectin, which could act as ligands for oolemmal integrins. Miranda and Tezon (1992) have documented that the human epididymis secretes fibronectin, which is acquired by spermatozoa during their epididymal passage. Both fibronectin and vitronectin have been extracted from human ejaculated spermatozoa (Vuont et al., 1984; Fusi and Bronson, 1992). We have shown that fresh spermatozoa do not express fibronectin on their surface, but they do so during capacitation (Fusi et al., 1992b). Vitronectin is also expressed on living human spermatozoa during capacitation. In contrast to fibronectin, vitronectin appears to be an intrinsic protein of spermatozoa, synthesized during spermatogenesis. Vitronectin has been localized to the acrosomal cap of acetone fixed spermatozoa and is released during a calcium ionophore-induced acrosome reaction (Figure 6). Vitronectin message has been detected in whole testis poly(A) mRNA by Northern blot analysis (Fusi et al., 1994). The stage specific expression of vitronectin message within spermatocytes of the human testis has also recently been documented (Nuovo et al., 1995).

Given the increased expression of these cell adhesion molecules on the sperm surface during capacitation, a reasonable hypothesis is that they play a role in the increased ability of capacitated, acrosome-reacted spermatozoa to adhere to the egg surface. The inhibition of sperm-oolemmal binding through the use of RGD containing oligopeptides supports this hypothesis. The effects of anti-integrin antibodies in murine in-vitro fertilization (IVF) provide further evidence that specific integrins are involved in gamete interactions leading to fertilization. A rabbit polyclonal antibody recognizing the integrins α5β1, α5β3, α6β1 and α6β3 has been shown to inhibit mouse sperm binding to zona-free mouse eggs by 80%, but had no effect on gamete fusion (Almeida et al., 1995). A function-blocking monoclonal antibody against the α6 integrin subunit abolished sperm binding to zona-free mouse eggs in a dose-dependent fashion. In contrast, a function-blocking antibody...
against α5β3 and a non-function-blocking anti-α6 antibody had no effect (Almeida et al., 1995).

Further support for the role of integrins in gamete interactions comes from the recent evidence that echistatin, a snake venom disintegrin, inhibits the adhesion of human spermatozoa to zona-free hamster eggs (Bronson et al., 1995). Disintegrins are a family of naturally occurring peptides isolated from the venoms of several pit vipers that bind with high affinity to the platelet integrin αIIbβ3, and inhibit the aggregation of platelets (Scarborough et al., 1991). The disintegrins are 500-1000 times more potent than linear RGD containing oligopeptides at inhibiting platelet aggregation. The disintegrin echistatin also blocks the adhesive function of RGD-dependent integrins, such as the vitronectin receptor αvβ3 and the fibronectin receptor α5β1. The number of spermatozoa adhering to the oolemma was significantly reduced at nanomolar concentrations of echistatin, in a dose-dependent manner. In the presence of echistatin 0.00, 0.5 and 10 μg/ml, the rates of increase of sperm–oolemmal binding were found to be significantly different from each other (P < 0.001, Table III). In contrast, echistatin did not inhibit the penetration of oocytes by spermatozoa that had become adherent to the oolemma. The rate of sperm penetration, while increasing with time, was independent of whether echistatin was either present or not. Using a cell matrix adhesion assay, we confirmed that echistatin strongly inhibited fibroblast adhesion to vitronectin and fibronectin coated microwells. A concentration of 1 μM virtually eliminated attachment to either substrate. Other disintegrins that blocked the platelet receptor αIIbβ3, but did not block the fibronectin receptor α5β1, as judged by fibroblast adhesion to fibronectin coated microwells, did not block sperm–oolemmal adhesion (Table IV).

These results support the hypothesis that integrins and their ligands play a role in sperm–oolemmal adhesion. They suggest that two processes occur in the binding of spermatozoa to the oolemma, one echistatin-sensitive and probably involving integrin receptors that recognize fibronectin and vitronectin. Another mechanism that is insensitive to echistatin appears to be independent of RGD and involves the adherence and subsequent penetration of only a minority of spermatozoa to the zona-free egg. These spermatozoa are capable of fusing with the oolemma.

This paradigm is consistent with the known ability of integrins to act as accessory receptors in a number of cell–cell binding events. The specificity of adhesion between T lymphocytes and antigen-presenting cells (APCs) comes from the T cell receptor, which recognizes antigenic peptides bound to major histocompatibility molecules. However, lymphocyte–APC adhesion also relies on the integrin αvβ3, and can be blocked by antibodies to this integrin (Dustin and Springer, 1991). The specificity of leukocyte adhesion to endothelium, at sites of inflammation, comes from the involvement of multiple receptors. During this process, however, an adhesive cascade leads to the function of the β2 integrins, which have been shown to provide strong adhesion (Butcher, 1991). Further supporting evidence that integrin–RGD ligand interactions could play a role in the entry of the spermatozoon into the oocyte comes from recent studies indicating that the entry of cells by viruses is mediated through an RGD signal sequence. Attachment and entry of Coxsackie virus A9 to GMK cells has also been shown to be dependent on an RGD sequence in the viral capsid protein, and antibodies specific for the αv and/or β3 integrin subunits protect GMK cells from A9 infection (Roivainen et al., 1994). Human adenovirus type 2 enters host cells by receptor-mediated endocytosis, an event mediated by the virus penton base binding to cell surface integrins αvβ3 and αvβ1 via an RGD amino acid sequence. Membrane permeabilization appears to be mediated through the αvβ3 integrin (Wickham et al., 1993, 1994; Nemerow et al., 1994). Signal transduction mediated via integrins appears to play a role as well in the attachment and entry of mammalian cells by certain pathogenic bacteria. Yersinia enterocolitica and Y. pseudotuberculosis are enteropathogens that infect Peyer’s patches and cause gastroenteritis and mesenteric lymphadenitis. Following their attachment to the cell surface, the entry of enteropathogenic Yersinia into cultured mammalian cells involves a process closely resembling phagocytosis, during which cytoskeletal elements respond to signals from transmembrane receptors that recognize the bacterial ligand invasin. The ability of invasin to trigger reorganization of the cytoskeletal elements actin and talin is mediated through the αvβ3 integrin. Blockers of actin polymerization, such as cytochalasin D, inhibit the entry of Yersinia into mammalian cells in vitro, suggesting the need for an intact cytoskeleton (Bliska et al., 1993). It should be noted, however, that while sea urchin eggs treated with cytochalasin D are unable to incorporate spermatozoa, implicating cytoskeletal elements in that process (Schatten and Schatten, 1981), this is not the case in mammals. Maro et al. (1984) have shown that cytochalasin D does not block gamete fusion or incorporation of the sperm nucleus by the egg in the mouse, although spindle rotation, polar body formation and migration of pronuclei are inhibited.
reverse transcriptase-PCR was identical to that of T and a class II-like major histocompatibility molecule (MHC) on the oolemma of mouse eggs by immunocytochemistry, lymphocytes.

1990). The nucleotide et al., located on the sperm head (Mori 1991, 1992) have detected the presence of a CD4-like molecule 46) and the oolemma (binding CR3), in this manner playing bridge between acrosome-reacted spermatozoa (binding CD 1993). Mori a role in sperm adhesion (Anderson et al., 1993; Taylor reaction (Cervoni et al., 1993). Several complement regulatory proteins have been detected on sperma- et al., et al., 1991) and CR3 (Anderson 1993). Several Fey receptors for immunoglobulin (Ig) G immunoglobulins (Bronson et al., 1995).

**Table III. Effect of echistatin, a disintegrin that blocks Arg-Gly-Asp (RGD)-mediated integrin adhesion on sperm–oolemmal binding and egg penetration**

<table>
<thead>
<tr>
<th>Time of gamete coculture (min)</th>
<th>Echistatin*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherent Mean ± SD</td>
<td>Penetrating Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Adherent Mean ± SD</td>
<td>Penetrating Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6.5 ± 3.4</td>
<td>0.00</td>
</tr>
<tr>
<td>40</td>
<td>11.5 ± 2.6</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>60</td>
<td>14.8 ± 5.7</td>
<td>2.5 ± 1.5</td>
</tr>
<tr>
<td>80</td>
<td>16.6 ± 4.4</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>10.8 ± 0.8</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>9.7 ± 4.1</td>
<td>1.8 ± 1.8</td>
</tr>
<tr>
<td>40</td>
<td>23.2 ± 5.7</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>60</td>
<td>47.7 ± 13.8</td>
<td>2.6 ± 0.8</td>
</tr>
</tbody>
</table>

*Zona-free hamster eggs were preincubated in Biggers–Whitten–Whittingham medium containing 30 mg/ml human serum albumin and 5 µg/ml echistatin for 10 min. Capacitated spermatozoa (250 000 motile spermatozoa/ml were resuspended in echistatin (1 µM) and added to egg microdrops at time = 0. Eggs were recovered from the sperm suspension every 20 min, washed, stained with Acridine Orange and scored as whole mounts for numbers of adherent and penetrating spermatozoa. When specific adhesion of fibroblasts to fibronectin and vitronectin-coated microwells was measured, addition of echistatin caused almost 100% inhibition of binding to each matrix protein at 1 µM (Bronson et al., 1995).

**Table IV. Disintegrins that do not inhibit fibroblast adhesion to fibronectin fail to inhibit sperm–oolemmal binding**

<table>
<thead>
<tr>
<th>Disintegrin*</th>
<th>Mean no. adherent spermatoza**</th>
<th>Percentage inhibition of fibroblast adhesion***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decorsin</td>
<td>34.5</td>
<td>20</td>
</tr>
<tr>
<td>Ornatin</td>
<td>27.4</td>
<td>15</td>
</tr>
<tr>
<td>Kistrin</td>
<td>38.2</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>32.6</td>
<td>10</td>
</tr>
</tbody>
</table>

*Disintegrins were tested at 7.5–10 µM during gamete co-incubation. The activity of each disintegrin to block α6β3 was confirmed through its ability to inhibit platelet aggregation, confirming its activity. **Zona-free hamster eggs (10–20) were scored for the number of spermatozoa adhering to their oolemmas 90 min after addition of capacitated human spermatozoa. ***Adhesion of fibroblasts to fibronectin-coated microwells was measured colorimetrically and expressed as a percentage of that in the absence of disintegrins. The control consisted of C-8287, a non-RGD-containing peptide.

Our results studying the effects of disintegrins on gamete interactions suggest that other receptor–ligand interactions independent of RGD occur between spermatozoa and eggs leading to gamete adhesion and membrane fusion. Several other receptors that normally play a role in cell-to-cell adhesion and signalling within the immune system have been identified on the oolemma of mammalian eggs that could be involved in gamete interactions. These include Fcy receptors for immunoglobulin (Ig) G immunoglobulins (Bronson et al., 1990b, 1992), as well as the complement receptors C1cr (Fusi et al., 1991) and CR3 (Anderson et al., 1993). Several complement regulatory proteins have been detected on sperma- tozoa, and one of these, CD46, is present on the inner acrosomal membrane and appears to be expressed following the acrosome reaction (Cervoni et al., 1993; Taylor et al., 1994). It has been proposed that the complement component C3b could act as a bridge between acrosome-reacted spermatozoa (binding CD 46) and the oolemma (binding CR3), in this manner playing a role in sperm adhesion (Anderson et al., 1993). Mori et al. (1991, 1992) have detected the presence of a CD4-like molecule on the oolemma of mouse eggs by immunocytochemistry, and a class II-like major histocompatibility molecule (MHC) located on the sperm head (Mori et al., 1990). The nucleotide sequence of CD4 cDNA synthesized from murine eggs by reverse transcriptase-PCR was identical to that of T lymphocytes.

Sperm–oolemmal adhesion and fusion could also involve the sperm glycoprotein fertilin (Myles, 1993). Fertilin is an integral membrane heterodimer initially identified through the use of monoclonal antisperm antibodies raised against guinea pig spermatozoa (Primakoff et al., 1987) that has been shown to play a role in gamete interactions. Antibodies raised against fertilin block fertilization in the guinea pig. The gene for fertilin has been sequenced, and it appears to be conserved between species. The N-terminal region of the fertilin β chain possesses a 90 amino acid disintegrin-like domain (Blobel et al., 1992). Of note, the α chain contains a fusion peptide sequence, similar to that noted in viral fusion proteins. Peptides derived from the disintegrin domain of fertilin β, when coupled to fluorescent covaspheres, bind to the guinea pig egg plasma membrane. These peptides also inhibit sperm–egg binding (Almeida et al., 1995).

Most viral fusion proteins contain a fusion peptide, a stretch of polar amino acids, distinct from the signal sequence and trans-membrane domain, which is conserved within virus families (White, 1990). This domain is capable of being modelled as an asymmetric α helix, in which most of the bulky hydrophobic residues are on one face. It has been hypothesized that this hydrophobic face may mediate virus–cell membrane interactions that lead to fusion. Recently, a peptide representing the putative fusion domain of fertilin α has been synthesized and its interaction with model lipid membranes studied (Muga et al., 1994). The synthetic peptide was able to induce fusion of large unilamellar vesicles. Myles et al. (1993) have proposed that sperm binding to the oolemma via fertilin β could lead to a conformational change in the fertilin alpha subunit revealing its hydrophobic fusion peptide. Indirect evidence suggests that the binding of fertilin β on spermatozoa to the oolemma may be mediated by the α6β1 integrin (Almeida et al., 1995). As previously noted, a function-blocking anti-α6 monoclonal antibody blocked the adhesion of mouse spermatozoa to mouse eggs. Mouse spermatozoa also bind to a murine F-9 cell line, which is known to express the α6β1 receptor. Binding of spermatozoa to an F-9 variant that did not express α6β1 was diminished. In addition, anti-α6 antibody inhibited sperm binding to F-9 cells, while a second anti-α6 antibody that was not function-blocking did not. While the α6β1 integrin classically has been shown to bind laminin, the presence of laminin could not be detected by immunofluoro-
was binding to another sperm-associated moiety, perhaps fertilin.

While fertilin was initially identified and its gene cloned in guinea pigs, there is increasing evidence that this moiety is but one of a family of related mammalian cysteine-rich proteins that contain metalloproteinase-like and disintegrin-like domains. Members of this family have been identified in monkey (*Macaca fuscata*), epididymis (Perry *et al.*, 1992) and human testis (Perry *et al.*, 1994), suggesting its wider role in reproduction. Fertilin appears to be a prototype of a widely distributed family of membrane proteins that possess an integrin ligand domain with a disintegrin motif (Wolfsberg *et al.*, 1995).

During the process of gamete membrane fusion and sperm incorporation, the oocyte becomes activated. The induction of intracellular calcium transients has been documented within mammalian oocytes following sperm–egg fusion (Miyazaki, 1991; Kline and Kline, 1992). Cortical granules are released which liberate substances that alter the oolemmal surface and the zona pellucida (Lee *et al.*, 1988) and that play a role in the prevention of polyspermy. These events lead to the reactivation of meiosis, the release of the second polar body, and the initiation of the meiotic cycle. There are two schools of thought regarding the mechanism of this phenomenon (Whitaker and Swann, 1993; Swan and Ozil, 1994). Signal transduction through oolemmal receptors has been documented to play a role in the induction of calcium transients and activation of invertebrate eggs (Jaffe, 1990). It has been suggested that this process, mediated by G proteins and inositol trisphosphate, also occurs in mammalian eggs (Swann *et al.*, 1989; Miyazaki, 1991; Miyazaki *et al.*, 1992). Evidence has accumulated in the hamster in support of this hypothesis. Oolemmal integrins could play a role in this process, given their known role in signal transduction. Fcγ receptors have also been shown to play a role in signalling during the process of phagocytosis by macrophages (Suzuki, 1983; Young *et al.*, 1994). It has been proposed that microaggregates of macrophage Fcγ receptors may lead to allosteric changes that activate membrane phospholipase A2, in turn activating the adenyl cyclase system. Alternatively, evidence has been presented that the spermatozoon carries with it into the ooplasm, at the time of gamete fusion, an as yet undefined moiety which triggers the generation of calcium transients and subsequent egg activation (Swann, 1990).

**A multiple receptor model of mammalian fertilization**

A heterogeneous group of receptors then exists on eggs and their ligands on spermatozoa that appear to be involved in the molecular events in fertilization. Many of these same receptors are present on leukocytes, where they appear to play a role in mediating the adherence of circulating white blood cells to endothelium. A model has been proposed in which specific receptors, each members of a larger family, serve a specific function in this process (Butcher, 1991; Springer, 1994).

Although we have not proven that similar receptors, which exist on gametes, serve similar functions, this is a useful working hypothesis for further experimentation. A different set of specific receptors would then be involved in each of the events whereby the spermatozoon tethers to the oolemma (docking-selectins), binds tightly (anchorage-integrins), and whereby the plasmalemma of the equatorial segment fuses with the oolemma (fusion-fertilins) and the rostral portion of the spermatozoan head is incorporated by the oocyte (phagocytosis-integrins, C’R, and FcγR). The recent discovery of CD 15, a ligand for P-selectin on spermatozoa (D’Cruzz *et al.*, 1994), and the detection of P-selectin on human spermatozoa and human eggs that have been penetrated by spermatozoa reinforces this model (Fusi *et al.*, 1995b).

The selectins are a family of three proteins that mediate adhesive interactions between leukocytes and the endothelium and platelets in the vascular compartment (Springer, 1994). They are large glycoproteins that function as lectin-like receptors by virtue of C-type lectin domains at their amino terminal ends (Rosen and Bartozzi, 1994). The counter-receptors or ligands for the selectins consist of specific sialylated glycoconjugates. Ligands for P-selectin are present on neutrophils, monocytes and some subsets of lymphocytes. The recruitment of leukocytes into inflammatory sites requires the presentation of ligands with selectin-specific epitopes. These contain extracellular domains with a mucin organization containing sialic/threonine-rich regions that are densely substituted with O-linked carbohydrate chains. These mucin domains are thought to be highly extended and rigid structures. For example, the P-selectin ligand PSGL-1 is predicted to extend over 50 nm from the cell surface, which compares with 40 nm for P-selectin and <20 nm for a typical integrin (Springer, 1994).

The selectins and their ligands, extending out from the cell surface, would be well-structured to play a role in tethering of the spermatozoon to the egg surface. The integrins, which appear to play a role in oolemmal adhesion as well, would be expected to provide the tight binding required for the anchorage of a motile cell such as the spermatozoon. They have been assigned this role in leukocyte–endothelial interactions. Given their ability to transduce signals across the plasma membrane of non-reproductive cells, it is not unreasonable to propose that they may also serve a similar role during fertilization. The Fcγ receptors, which also exist on the egg surface (Bronson *et al.*, 1990b, 1992), have also been shown to play roles in signal transduction during phagocytosis by macrophages (Suzuki, 1983; Young *et al.*, 1994). Finally, the fertilins might play roles in both gamete adhesion, through their disintegrin domain, as well as in gamete membrane fusion, given their possession of a viral fusion protein-like domain. The recent discovery of a fertilin-like molecule secreted by the human epididymis (Perry *et al.*, 1992) supports their possible role in human fertilization.

**Endometrial integrins: their possible roles in embryo implantation and in the pathogenesis of endometriosis**

Approximately 10% of hospitalizations for gynaecological care are associated with a diagnosis of endometriosis (Haney, 1987; Mahood and Templeton, 1991). Although there is evidence that genetic, mechanical, hormonal, paracrine/autocrine, and immunological factors all influence the development and progression of this disease, the aetiology of endometriosis remains obscure. It is believed to result from the adhesion, implantation...
and growth of endometrial cells following their transport into the pelvis either via retrograde menstruation or lymphatic deportation. Viable endometrial cells are present in the menstrual effluent, which could be transported to ectopic sites via the Fallopian tubes (Sampson, 1927). Abundant lymphatic channels have also been demonstrated between the uterus and the ovary. As they have been shown to contain endometrial fragments, extrauterine growth of endometriosis could arise, in some women, from the transport of endometrial fragments via the pelvic lymphatics.

While there is no simple answer to the question why ectopic endometrium grows at extraterine sites, logic suggests several alternative mechanisms. The amount of endometrial tissue transported to the peritoneal cavity may be increased in women who develop endometriosis. The eutopic endometrium present within the uterine cavity may be more adhesive in these women than those without disease. Alternatively, the endometrium may be more responsive to normal growth factors, or these factors may be present at a higher concentration within the peritoneal cavity of women with endometriosis. Endometrium of women with endometriosis may be more resistant to natural immunoregulatory mechanisms that normally inhibit growth of ectopic endometrium or these mechanisms may be impaired (Hill, 1993). Indeed, there is likely to be no one aetiology of endometriosis, which may represent the end stage of several diverse processes. The balance of growth promoting and inhibiting forces, varying between individual women, would then determine the likelihood of the establishment of endometriosis.

A common theme in its pathogenesis, however, is the initial adhesion of viable endometrial cells derived from within the uterus upon peritoneal surfaces, an event that can be likened to the implantation of the embryo during nidation. Embryo implantation occurs ~5–6 days after ovulation in humans, as determined by examination of hysterecmy specimens obtained at known post-ovulatory dates in the luteal phase of the menstrual cycle (Hertig et al., 1956) and confirmed more recently in donor oocyte cycles in which fertilized eggs were transferred to the uterus of recipients at known times after ovulation (Berg and Navot, 1992).

The presence of integrin subunits on human endometrium has been assessed throughout the menstrual cycle using immunohistochemistry (Tabibzadeh, 1992; Lessey et al., 1992, 1994a; Bischof et al., 1993) While several integrin chains were constitutively expressed by endometrial epithelium, αβ3 and αβ1 exhibited changes related to the menstrual cycle. Phasic epithelial expression of α1, α4, αv, and β3 chains was observed by Lessey et al. (1992). The α1 chain was seen during days 15–28 of the luteal phase, α4 appeared only in the mid-luteal phase (days 14–24). The αβ3 integrin appeared first in endometrial epithelium on post-ovulatory days 5–6 (days 19–20 of the menstrual cycle) and was then expressed throughout the luteal phase. The co-expression of the α1, α4, and β3 integrin chains appeared to frame the implantation window (Figure 7). As absent β3 expression has been observed in endometrial biopsies from women with luteal phase defects (Lessey et al., 1992) and in women with unexplained infertility (Lessey et al., 1995), these investigators have proposed that

![Figure 7](https://academic.oup.com/molehr/article-abstract/2/3/153/1056280)
underwent endometrial biopsy before laparoscopy also revealed that nearly all women with defects in β3 expression had mild or minimal pelvic endometriosis. Hence, the failure to detect this integrin chain on late luteal endometrial biopsies might serve as a marker that predicts the presence of endometriosis.

Could the altered expression of integrins by eutopic endometrium in certain women promote its adherence to peritoneum, in this manner allowing a ‘beachhead’ of endometrial cells within the refluxed menstrual effluent to be established in an ectopic location? Circulating anti-endometrial IgG and IgA antibodies, an increased number of peritoneal macrophages and lymphocytes, and an increased peritoneal fluid concentration of interleukin-1 (IL-1) and tumour necrosis factor (TNF)-α have also been reported in women with endometriosis (Olive et al., 1985; Hill et al., 1988; Halme, 1989) supporting a role of the immune system in this disease. Preliminary evidence suggests that these cytokines, which are secreted by activated macrophages, might play a role in promoting the initial attachment of endometrial cells deported through the Fallopian tubes to the peritoneum (Zhang et al., 1993). When normal eutopic endometrial stromal cells were labelled with chromium-51, and the proportion of cells adhering to human peritoneal mesothelial monolayers was determined by γ counting, TNF-α, but not IL-1, increased their adhesion. Recent evidence indicates that integrins can be up-regulated or activated by cytokines (Nathan and Sporn, 1991; Miller and Krangel, 1992; Oppenheim et al., 1991), suggesting that promotion of implantation of ectopic endometrium might be mediated by cytokine-altered expression of endometrial integrins.

**Integrins and the regulation of trophoblast outgrowth during placentation**

While the common theme of dynamic regulation of integrin expression at the time of nidation repeats itself during placentation, a more complex set of interactions occurs among invading trophoblast, extracellular matrix proteins and metalloproteinases secreted by the cytotrophoblast cells themselves, as well as matrix proteins derived from the stromal decidua cells, cytokines and other growth factors produced by granulated lymphocytes of the decidua (King and Loke, 1990; Bulmer et al., 1991; Wegmann et al., 1993). The phenotype of cytotrophoblasts *in vitro* has been shown to vary depending upon the matrix proteins on which they are cultured, and both integrin expression, and their secretion of collagenases appears to be altered by the binding of specific integrins to different extracellular matrix proteins.

That decidualized endometrium played a role in limiting and regulating the outgrowth of trophoderm was graphically demonstrated in the pioneering experiments of Kirby (1970). When murine ectoplacental cone was transferred into the uterus of cycling, non-pregnant mice, the trophoblast that developed deeply invaded the muscular wall of the uterus, even destroying the myometrium. That integrins could play a role in the regulation of trophoderm outgrowth has been suggested by the recent observations that while certain integrins are expressed in pre-implantation mouse embryos continuously throughout the peri-implantation period, others are developmentally regulated (Sutherland et al., 1993). Messenger RNA for α2, α6 and α7 was first detected in the late blastocyst, coincident with the development of the ability of these embryos to attach to endometrial epithelium. α4β1 and α2β1 form receptors for laminin, and it is known that laminin expression in decidualized endometrium is up-regulated in response to the implanting embryo. In vitro studies of trophoblast outgrowth on laminin fragment substrates have also suggested that these developmentally regulated integrins play a role in implantation. Experiments performed studying in vitro trophoblast attachment and outgrowth on defined substrates have been a useful model for embryo implantation *in vivo*. Blastocyst attachment and outgrowth on fibronectin can be inhibited by RGD-containing peptides (Armont et al., 1993; Sutherland et al., 1988). Laminin supports the in vitro outgrowth of trophoblasts, but appears to interact with the trophectoderm via a galactose-binding lectin (Armont, 1991). Trophoblast migration on entactin (Yelian et al., 1993) and collagen is also sensitive to RGD-mediated inhibition. Kimber et al. (1993) have presented evidence, in the mouse, that glycoproteins containing terminally fucosylated Gaβ3GlcNAc terminal sugar groups are synthesized by uterine epithelium, under oestrogen stimulus, and act as a ligand for an as yet undefined blastocyst receptor. They have proposed that an 'implantation-adhesion' cascade similar to that noted earlier to occur during leukocyte-endothelial interactions could exist at the level of the trophectoderm—endometrial epithelium as well.

After the attachment and penetration of the uterine epithelium by the blastocyst, mononuclear trophectoderm cells fuse to form syncytiotrophoblasts that invade the endometrial stroma. In humans, as in mice, regulated trophoblast invasion of the uterus is a prerequisite for successful placental development. Once the placenta villi are formed, cytotrophoblast cells at the tips of some anchoring villi (which contact the uterine wall) proliferate outwards to form cell columns from which individual cells migrate into decidua tissue. The process whereby these cells acquire an invasive phenotype is poorly understood but appears to involve interactions between the invading cytotrophoblast, the extracellular matrix of the endometrial stroma, and different cell populations within the decidua. In theory, defects in this process, which may alter the depth of penetration of cytotrophoblast, could lead to placental pathologies. Evidence is accumulating that insufficient invasion of the decidua and maternal arterioles by cytotrophoblasts could play a role in pre-eclampsia and fetal growth retardation, while unrestricted invasion might result in placenta accreta (Redman, 1991).

During the process of placentation, two differentiation pathways of cytotrophoblast development exist, which give rise to cell populations that are morphologically and functionally distinct. Floating villi do not contact the uterine wall and possess polarized epithelial monolayers of cytotrophoblasts, anchored to a basement membrane, that differentiate by fusing to form a syncytial layer. Anchoring villi, in contrast, contain cytotrophoblastic stem cells that either fuse to form a syncytium, or break through the syncytium and form multilayered columns of non-polarized cells, giving rise to highly invasive cytotrophoblasts. These cell columns of the anchoring villi physically connect the embryo to the uterine wall.

Burrows et al. (1993) have presented evidence that the
relative expression of laminin and fibronectin integrin receptors on cytotrophoblast cells determines their morphology and behaviour during placentation. Immunohistology of first trimester human placenta revealed α5 and β1 integrin chains on cells of the cytotrophoblast columns nearest the villi, but a distinct reduction of staining intensity for β1 at the distal end of the columns. The pattern of staining of α5 was in complete contrast to β1, where these chains were not expressed by villous cytotrophoblast, but began to appear on cells of trophoblast columns. While very little laminin can be demonstrated in proliferative phase endometrium, it appears in significant amounts during the luteal phase, and appears to be synthesized by the decidual stromal cells at a time coinciding with the implantation period (Loke et al., 1989). As invasive cytotrophoblast cells possess α5β1 laminin receptors, their interaction with decidual laminin could play an important role in trophoblast outgrowth.

Other investigators as well have presented evidence that down-regulation of the integrin α5β1 (a receptor for laminin) occurs, with reciprocal up-regulation of α5β1 (a fibronectin receptor) in the cytotrophoblasts of the distal cell columns (Damsky et al., 1992; Bischof et al., 1993). While cells derived from cytotrophoblast cell columns of human first trimester placentae adhere to both laminin and fibronectin (Loke et al., 1989; Loke, 1990; Burrows et al., 1993), their morphology differs on the two matrix proteins. Given evidence that integrins act as signalling receptors, it would be expected that the binding of α5β1 to decidual laminin by cytotrophoblasts would transmit a different signal from that induced through α5β1. Evidence has accumulated that extravillous trophoblast secretes an alternatively spliced form of fibronectin (Feinberg et al., 1991) under cyclic AMP regulation, and that decidual cells produce, in a peri-cellular distribution, laminin as well as fibronectin. Decidual tissue then is infiltrated by a complex mixture of laminin and fibronectin, which could provide signals to invading cytotrophoblast through the mediation of integrins. In theory, the correct balance of growth promoting and inhibiting signals provided by these extracellular matrix proteins would be important for the regulation of trophoblast outgrowth.

Damsky et al. (1992) have compared the presence of integrins and extracellular matrix ligands on cytotrophoblasts in vilii, cell columns and from placental bed biopsies following elective pregnancy terminations, obtaining similar results. Two patterns of integrin switching were observed in normal pregnancy. One transition took place in the cell columns of the anchoring viliius and was characterized by a down-regulation of α5β1 and an up-regulation first of α5β1, as had been observed by Burrows et al. (1993), and later of α5β1 within the uterine wall. The second transition occurred as a function of gestational age: α5 was expressed by cytotrophoblast cells in all locations from the second trimester onwards. At term, placental bed cytotrophoblast expressed the α5 but not the β1 integrin chain.

In a subsequent study (Zhou et al., 1993), placental bed biopsies were obtained at Caesarean section from pregnancies complicated by pre-eclampsia, stained with anti-integrin antibodies, and compared with those from normal pregnancies. The results showed that the previously described integrin switching by invasive cytotrophoblast was abnormal in pre-eclampsia. Trophoblast expression of integrins was altered in pre-eclampsia in that α5β1 was strongly expressed by cytotrophoblast within the placental beds of pre-eclamptic patients, instead of becoming down-regulated. These cells also failed to up-regulate the expression of α5β1, which is normally expressed by placental bed cytotrophoblast throughout pregnancy, while α5β1 expression was not altered. Trophoblast expression of extracellular matrix molecules was not altered in pre-eclampsia. Zhou et al. also confirmed previous observations (Khong et al., 1986; Redman, 1991) that abnormally shallow invasion of the decidua and maternal arterioles by trophoblast is associated with pre-eclampsia. They speculated that, as function-blocking antibodies to α5 inhibited in-vitro cytotrophoblast outgrowth, while antibodies to α5 inhibited invasion, it was likely that cytotrophoblast interaction with fibronectin constrains via α5β1 its growth whereas interactions with laminin via α5β1 facilitate it (Librach et al., 1991, 1993) Flow cytometric analysis of integrin expression by cytotrophoblasts isolated from first trimester human placenta has also revealed, consistent with this hypothesis, that exogenous TGF-β up-regulated their expression of α5β1 and reduced their migratory ability in vitro on plastic culture dishes (Irving and Lala, 1995). In these experiments, however, anti-α5 and β1 function-blocking antibodies inhibited cell migration, indicating its dependence on the expression of α5β1.

Bischof et al. (1995) have provided evidence that integrin-mediated signal transduction plays a role in the expression of an invasive phenotype by cytotrophoblasts through their varied secretion of metalloproteinases. They studied the differential expression of integrins on human cytotrophoblast cells isolated from first trimester pregnancy terminations. A technique using an antibody (W6/32) directed against a common framework epitope of class I HLA molecules, coupled with magnetic particles, was utilized to fractionate cells positive versus negative for HLA-G, a trophoblast-specific histocompatibility antigen expressed by extravillous but not villous cytotrophoblast (Kovats et al., 1990; Chumbley et al., 1993). These cells were further characterized, utilizing specific anti-integrin antibodies, in a similar manner, for their integrin expression. The purity of the cell fractions was confirmed by immunocytochemistry, and their secretion of gelatinases studied by zymography and degradation of radiolabelled collagen type IV, as well as the secretion of fibronectin and human chorionic gonadotrophin (HCG). The cytotrophoblast population that was HLA-G positive (extravillous) and expressed the α5β1 integrin subunit secreted higher levels of gelatinase and lower levels of fibronectin than cells that expressed the α5 subunit. The expression of this latter integrin subunit, once they reach the placental bed, appears to be associated with cessation of an invasive phenotype by the cytotrophoblast.

Summary: the future recapitulates the past
Two clear themes emerge from this review. The integrins play a role in all processes important for human reproduction. However, they appear to be only one of several families of receptors that serve specific functions in these reproductive processes. The economies of nature would lead one to expect
that similar receptors would be involved in common processes within diverse tissues. The players, so to speak, would be the same within the reproductive system as in other areas of the body. It appears that not only are the same families of receptors involved in such diverse reproductive processes as nidation and fertilization, but that these are the same receptors that play significant roles in inflammatory and immunological processes.

The participation of specific receptors in fertilization, nidation, and placentation raises the possibility of discovering naturally occurring defects in receptor structure and function that could play a role in impaired reproductive performance. Conversely, these same receptors might provide the locus for development of a new generation of contraceptives, through the use of specific anti-receptor antibodies or chemically modified ligands that would impair receptor function. The commonality of receptor–ligand interactions in reproductive and non-reproductive processes is both a blessing and a curse. It will no doubt help to advance the field of knowledge in human reproduction. At the same time, the challenge will be to target the new contraceptive bullets to the reproductive tract, given the ubiquitous nature of their receptors.

Acknowledgement
We thank Dr James Gailit for his review of the manuscript.

References
Feinberg, R.F. and Kliman, H.J. (1993) Tropho-uronectin (TUN): a unique oncofetal fibronectin deposited in the extracellular matrix of the tropho-
R.A. Bronson and F.M. Fusi


Received on July 18, 1995; accepted on November 22, 1995