Ultrastructure of human blastocyst–endometrial interactions in vitro

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The interactions of seven human blastocysts with cultured endometrial cells were investigated by light microscopy and transmission electron microscopy. Trophoblastic–endometrial contact was observed at the lateral border of endometrial epithelial cells where trophoblast and endometrial epithelial cells shared apical junctional complexes and desmosomes. The first sign of penetration was invasion of a trophoblastic cytoplasmic protrusion between endometrial epithelial cells. In broad contact areas, lateral displacement of endometrial epithelial cells and formation of a peripheral pseudostratified epithelium were observed. When trophoblastic cells were interposed fully among endometrial epithelial cells, they formed a penetration cone and appeared to dislodge endometrial epithelial cells from the stromal compartment. A single penetration cone only was found in each specimen. Endometrial or trophoblastic degeneration was not observed. Formation of multinucleate (> three nuclei per cell) trophoblast cells was not observed, but many cells displayed areas with abrupt disappearance of well-defined plasma membranes, which is indicative of syncytium formation. In this study, adhesion and penetration occurred at the same time. The human blastocysts penetrated the endometrial surface epithelium by intrusive penetration. Epithelial penetration was achieved primarily by cellular syncytiotrophoblast-like cells and the first indications of syncytium formation were observed simultaneously with penetration of the epithelium.

Penetration of the uterine surface epithelium also varies among species. Comparative studies by Schlafke and Enders (1975) on animal models have distinguished three types of penetration: (i) displacement implantation, which is characterized by apoptosis of the uterine epithelium before invasion into the stroma starts (Parr et al., 1987), and is observed in mice (Potts, 1968) and rats (Enders and Schlafke, 1967; Welsh and Enders, 1991); (ii) fusion implantation, in which syncytiotrophoblasts fuse with maternal epithelial cells during penetration, and is found in rabbits (Falck Larsen, 1961; Enders and Schlafke, 1971); and (iii) intrusive implantation, in which trophoblastic cytoplasmic extensions penetrate between apparently intact epithelial cells, and which occurs in ferrets (Enders and Schlafke, 1972), rhesus monkeys (Reinius et al., 1973; Enders et al., 1983) and marmoset monkeys (Moore et al., 1985; Enders and Lopata, 1999).

In humans, the processes of adhesion and penetration have not been elucidated. The only report of attachment and penetration seems to be two photographs taken by light microscopy (Shettles, 1960), which may indicate intrusive implantation (O’Rahilly, 1973; for review see Lopata, 1996).

Notable differences, even between closely related species, indicate that great care should be taken when animals are used as models for humans, although individual phases might display similar mechanisms. Therefore, we developed a three-dimensional cell culture model of the human
endometrium (Bentin-Ley et al., 1994) in which the endome-
trial epithelial cells seem to preserve their morphology
compared with luteal phase biopsies (Bentin-Ley et al., 1995).
The model has been used for studies of human blastocyst
attachment, and the first seven sites of blastocyst attachment
obtained were used for investigation by transmission
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### Materials and Methods

#### Material for endometrial biopsy

The biopsies were obtained from women of proven fer-
tility who were undergoing regular menstruation and were
applying for tubal sterilization. All patients used barrier
methods for contraception. The biopsies were obtained by
curettage from the upper part of the endometrial cavity at
cycle days LH+5 to LH+7. Morphological dating was in
accordance with occurrence of the urinary LH peak, and
oestradiol and progesterone concentrations were normal for
the actual cycle day.

#### Preparation of cell cultures

Endometrial cell cultures, imitating the architecture of the
normal endometrium, were prepared as described by Bentin-
Ley et al. (1994) with minor modifications (Bentin-Ley et al.,
1999). In brief, the biopsies were minced with a scalpel and
separated by incubation with pancreatin–trypsin (10 ml of
1 × trypsin–EDTA solution (Gibco) supplemented with
0.05 g pancreatin ml⁻¹ (Sigma, Vallensbæk)) and collagenase
(150 iu ml⁻¹; Sigma). All cells were filtered through an 80 µm
mesh cell strainer (Falcon; Becton Dickinson, Brøndby) that
allowed single stromal cells to pass through, whereas most
glands were restrained in the filter. The stromal cells were
embedded in a collagen containing gel (Vitrogen 100; In
Vitro, Fredensborg), a thin layer of murine basement mem-
brane material (Matrigel; Becton Dickinson) was applied on
top of the gel and epithelial glands were seeded on top of the
artificial basement membrane.

The culture medium used was alpha modification of
Eagle’s medium: 100 ml was supplemented with 2000 iu
penicillin–streptomycin (Life Technologies, Roskilde), 0.2 ml
l-glutamine (200 mmol l⁻¹, Life Technologies), 4 ml
Amniomax® (Life Technologies), 5 ml fetal calf serum (FCS;
In Vitro), 0.5 g BSA (Behring, Redovre), 10 µg retinoic acid and
700–850 nmol progesterone l⁻¹. The FCS provided oestrogen
in sufficient amounts (0.25–0.30 nmol l⁻¹). All cultures were
placed in an incubator (Forma Scientific) at 37°C with 5%
CO₂ in air and the culture medium was changed at 2 day
intervals. After 5 days of culture, the epithelial layer was
confluent and ready for studies of attachment. These studies
were performed at Herlev University Hospital, Denmark
and Sahlgrenska University Hospital, Sweden. Cell cultures
for attachment studies were transported from Denmark to
Sweden in a transport incubator (K-systems; Henning
Knudsen Engineering, Birkerød).

#### Embryo culture and attachment studies

Surplus embryos from IVF treatment were obtained from
Sahlgrenska University Hospital, Herlev University Hospital,
Bredstrup Hospital and the Triangle Fertility Clinic in
Copenhagen. The embryos were cultured in S2 medium®
(Sandinavian IVF Science Ltd, Gothenburg) to the expanded
or hatching blastocyst stage. The blastocysts were placed on
the endometrial cell cultures and examined for attachment at
24 h intervals through a Zeiss stereomicroscope (Zeiss,
Göttingen). It was easy to identify blastocysts, as the cell
cultures were translucent. Attachment of the blastocysts was
checked by gentle shaking of the culture dish. One cell
culture (specimen number 1) was fixed approximately 24 h
after attachment was observed, whereas the other six
specimens were fixed approximately 48 h after attachment.

The study was approved by the local ethical committees in
Sweden and Denmark. All patients, both donors of endo-
metrium and embryos, gave their informed consent.

#### Control embryos

The influence of the different matrices on trophoblast
differentiation and syncytium formation was investigated
by culturing three embryos under different circumstances.
Two embryos fastened to Matrigel®-coated culture inserts
and were fixed approximately 48 h after attachment was
observed. The third embryo adhered on the collagen gel
(Vitrogen 100). All control embryos were cultured in the
same culture medium as was used for studies of attachment.

#### Preparation for light microscopy and transmission electron
microscopy

The cell cultures and control embryos were fixed in 2.5%
(v/v) glutaraldehyde in a sodium cacodylate buffer (0.1 mol
l⁻¹ (pH 7.2); Merck, Darmstadt) diluted with 20% (v/v)
distilled water for 24 h at 5°C. After rinsing in sodium cacodylate buffer (0.1 mol l–1 (pH 7.4)) for 4 × 15 min, the cultures were fixed with 1% osmium tetroxide in sodium cacodylate buffer (0.1 mol l–1) for 1 h at room temperature and the sites of blastocyst attachment were cut out with razor blades. Afterwards, the specimens were taken through a series of graded ethanol and acetone before they were embedded in Epon. The attached blastocysts and single embryos were cut in serial sections (3 μm thickness), between 45 and 110 sections per specimen, and each section was stained with toluidine blue for light microscopy. All sections were photographed with an Olympus light microscope before selected sections were re-embedded in Epon and cut into ultrathin sections for transmission electron microscopy (TEM) (Hyttel and Holm, 1987). At least ten ultrathin sections were studied from each block. The sections selected represented the first contact between blastocyst and endometrium, the area with penetration and the most advanced stage of penetration. Between one and five serial sections were studied by TEM for each blastocyst (Table 1).

Ultrathin sections were contrasted with uranyl acetate and lead citrate and viewed in a Philips EM 208 transmission electron microscope. This method meant that the same sections could be observed by both light microscopy and TEM.

Results

In total, 67 human blastocysts were used for studies of attachment (Fig. 1). Attachment was observed between 16 and 42 h after onset of coculture. Only seven of the 36 attached blastocysts were used for the present study. Three blastocysts were used for scanning electron microscopy. The other 26 attached blastocysts were kept for future immunohistochemical studies.

The findings of light microscopy are shown (Table 1). In specimen 3 the epithelial cell layer was absent underneath the blastocyst. An empty space was observed between the trophoblastic and stromal cells. Therefore, blastocyst–endometrial interactions could not be studied in this specimen.

One blastocyst (specimen 1) adhered with only a single trophoblastic cell to the apical epithelial surface (Fig. 2a). All other blastocysts showed a trophoblastic–endometrial interface with adhesion formation to endometrial epithelial cells peripherally. In two attachment sites (specimens 2 and 4), a cavity-like area was found more centrally (Fig. 2b,c). In specimens 4 and 6, a broad trophoblastic–endometrial interface where trophoblastic cells seemingly displaced the endometrial epithelial cells laterally was observed (Fig. 2c,d), giving an impression of a double-layered endometrial epithelium. Generally, endometrial epithelial cells seemed to flatten towards the centre of the attachment area where intrusion of trophoblast between endometrial epithelial cells was present.

When trophoblast cells were fully positioned between endometrial epithelial cells (specimens 5 and 7) (Fig. 2e,f) they formed a penetration cone composed of cells belonging to the inner cell mass. Serial sections demonstrated only a single penetration cone at each attachment site. Trophoblastic cells were present along lateral and basal plasma membranes of endometrial epithelial cells, thereby separating single cells from the underlying stromal compartment (Fig. 2f). No contact with apical endometrial epithelial plasma membranes was observed. The inner cell mass was composed of numerous cells, almost filling the blastocoel in embryo specimens 4–7.

The embryo that attached to the culture insert membrane had a small blastocyst cavity, which was surrounded by a single layer of trophoblast cells. It was not possible to distinguish between trophoblastic cells and cells belonging to the inner cell mass.

Light microscopy of non-attached blastocysts

In the three embryos that did not attach to the epithelium, the blastocyst cavity was well defined and a few cells at one pole comprised the inner cell mass. An ill-defined layer on the surface of two blastocysts gave suspicion of a thin layer of zona pellucida. This layer was not visible under the stereomicroscope.
This blastocyst presented a well-defined blastocyst cavity adhered to the collagen gel was also buried into the matrix, many irregular cytoplasmic protrusions. The blastocyst that totally in both of these embryos. The trophoblast displayed invading into the matrix. The blastocyst cavity was collapsed area without contact between trophoblast and endometrial epithelium is present (arrow). At the periphery, the trophoblast adheres to the endometrial cell culture and formation of a trophoblastic penetration cone is shown (arrows). The penetration cone is occupied by cells from the inner cell mass that almost fill the blastocyst cavity. (f) Light micrograph of penetration phase in specimen 7. In this section, the trophoblastic cells have penetrated the epithelial layer completely and several trophoblastic cells have contact with the stromal compartment (arrowhead). One endometrial epithelial cell at the penetration margin seems to have become dislodged from the stromal compartment by a penetrating trophoblast cell (arrow). Scale bars represent 50 μm.

Ultrastructure of human blastocyst–endometrial interactions

Endometrial epithelial cells lateral to the attachment area were polarized and had a few, short and branching microvilli at the apical plasma membrane (Fig. 4). Close apposition of endometrial and trophoblastic apical plasma membranes and interdigitating microvilli was not observed in the most peripheral sections of all attachment sites, although low power micrographs gave a false impression of trophoblastic cells stretching out on the apical surfaces of endometrial epithelial cells (Fig. 5a). Higher magnification of these contact areas showed that the trophoblast shared apical junctional complexes and desmosomes with the endometrial epithelial cells (Fig. 5b), thereby separating the endometrial epithelial cells at the lateral borders (see Alberts et al., 1994 for definition of different plasma membrane domains). When the contact area was broad (specimens 4 and 6), displacement and accumulation of endometrial epithelial cells towards the periphery of the contact area was observed. The first signs of penetration, not identifiable by light microscopy, were observed as intrusion of a trophoblastic process between the lateral surfaces of two adjacent endometrial epithelial cells (specimen 1). As well as an apical junctional complex (Fig. 6), the lateral plasma membranes of trophoblastic and endometrial epithelial cells showed interdigitations and several desmosomes (specimen 7). At the centre of the attachment area, a trophoblastic protrusion was observed between two endometrial epithelial cells (specimen 4), which formed a contact with the stromal compartment. Filaments and a few vesicles dominated the cytoplasm of the trophoblastic protrusion; organelles were not present. The intercellular spaces between epithelial cells were more pronounced in this area. No contacts were present between trophoblast cells and the apical endometrial epithelial plasma membranes.

When penetration was more obvious (specimens 5 and 7), a trophoblastic shell reached the stromal compartment and was occupied with cells from the inner cell mass (Fig. 7). In Fig. 2. (a) Light micrograph of specimen 1, demonstrating adhesion of a human blastocyst to the endometrial cell culture. During the preparation procedure, the blastocyst was torn off the culture, leaving a single trophoblastic cell on the endometrial epithelial surface. Serial sections demonstrated no other areas of attachment. (b) Light micrograph of specimen 2. This blastocyst adheres to the lateral plasma membranes of endometrial epithelial cells in two areas. To the right, the endometrial epithelial cell cracked during the preparation procedure, whereas the slit present between the attachment areas is a true cavity. Trophoblastic cells on the blastocyst surface do not adhere to apical plasma membranes of the endometrial epithelial cells. (c) Light micrograph of specimen 4, showing a broad contact area between trophoblast and endometrial epithelium. The height of the endometrial epithelial cells decreases towards the centre of the contact area where a slit-like area without contact between trophoblast and endometrial epithelium is present (arrow). At the periphery, the trophoblast adheres to the endometrium and the endometrial epithelial cells lie in two or three layers (arrowhead). This blastocyst adheres with the embryonic pole. (d) Light micrograph of penetration in specimen 5. The trophoblast adheres to the endometrial cell culture and formation of a trophoblastic penetration cone is shown (arrows). The penetration cone is occupied by cells from the inner cell mass that almost fill the blastocyst cavity. (f) Light micrograph of penetration phase in specimen 7. In this section, the trophoblastic cells have penetrated the epithelial layer completely and several trophoblastic cells have contact with the stromal compartment (arrowhead). One endometrial epithelial cell at the penetration margin seems to have become dislodged from the stromal compartment by a penetrating trophoblast cell (arrow). Scale bars represent 60 μm.
specimen 7, several trophoblastic cells were in contact with the stromal compartment (Fig. 8). The trophoblastic cells were in contact with the basal compartment of adjacent endometrial epithelial cells, thereby separating the epithelium from the stromal compartment. Centrally, trophoblastic cells showed ectoplasmic protrusions that were directed deeper into the collagen gel and contained numerous filaments. Generally, epithelial and trophoblastic cells showed no signs of degeneration, whereas some necrotic cells were observed in the inner cell mass.

The inner cell mass consisted of four morphologically different cell types. One cell population comprised tightly packed polygonal cells with large lipid inclusions and dilated rough endoplasmic reticulum. A second cell population revealed a well-developed secretory apparatus, including rough endoplasmic reticulum, Golgi and many secretory vesicles. Many collagen fibrils were present in this area. The third population consisted of spindle-formed cells that encircled cavities containing necrotic cells. These cells were attached to each other by desmosomes and were encircled by a basal lamina. The last cell population was composed of spindle-like cells that covered the surface of the inner cell mass and the basal lamina of the mural trophoblast.

**Syncytium formation**

Formation of double-nucleated trophoblast cells was observed occasionally in specimens 4–7 (Fig. 9a) but not in specimens 1–3. Abrupt disappearance of well-defined plasma membranes occurred and was often associated with interdigitation of the membranes, leaving isolated segments of plasma membrane (Fig. 9b) or isolated desmosomes in the cytoplasm (Fig. 9c). Prominent filaments, cut at various directions, were...
also present. Syncytium formation was not observed in embryos that adhered to the culture insert membrane. Embryos that did not adhere to the endometrial cell culture also showed isolated segments of plasma membrane in the cytoplasm and double-nucleated trophoblastic cells. One of the two embryos that adhered to the Matrigel® layer was necrotic. Multinucleated cells were not observed in the other embryo that adhered to the Matrigel® layer.

Trophoblastic cells presented irregular surfaces dominated by cytoplasmic protrusions containing filaments in the blastocyst that attached to the collagen gel. Inside the trophoblastic shell and underneath the inner cell mass, two cells displayed large electron dense and very irregular nuclei with large nucleoli. One of these cells contained two nuclei (Fig. 10). The cytoplasm was dominated by heavily dilated rough endoplasmic reticulum and there was an intracellular cavity with microvilli towards the lumen. The two cells formed desmosomes with trophoblastic cells.

Discussion

The results of this study question whether ‘adhesion’, defined as close apposition of apical plasma membranes (Schlafke and Enders, 1975; Enders, 1976 (animal models)), is necessary for initiation of implantation in humans. In this context, it is necessary to give a definition of the apical domain of the plasma membrane: the apical domain faces
the lumen and has specialized features, such as cilia or microvilli. The apical domain is demarcated from the basolateral domain by a ring of tight junctions (Alberts et al., 1994).

In the attachment sites investigated in the present study, neither apposition of apical plasma membranes or extensive interdigitation of microvilli were observed. The first morphological sign of attachment was observed at the ring of tight junctions in endometrial epithelial cells, which demarcate the apical plasma membrane from the lateral compartment (Alberts et al., 1994). The apical plasma membranes of two adjacent endometrial epithelial cells were observed displaced from each other, thereby, like opening a zipper, giving access to the trophoblastic protrusions along the lateral plasma membranes. It appears that formation of junctions at the lateral compartments of endometrial epithelial cells provides sufficient stability for the trophoblast to induce cell separation.

In the in vitro system used in the present study, an intrusive type of endometrial epithelial penetration by the human blastocyst was observed, which was characterized according to animal studies by Schlafke and Enders (1975): (i) penetration without extensive destruction or alteration of adjacent epithelial cells; (ii) sharing of uterine apical junctional complexes between trophoblastic and endometrial epithelial cells; and (iii) sharing of desmosomes between intruding trophoblastic cells and the lateral cell margins of uterine epithelial cells. All of these morphological features were demonstrated in this model. Disappearance of plasma membranes between trophoblastic and endometrial epithelial cells, illustrating fusion penetration, or necrotic epithelial cells next to the trophoblast, indicating displacement penetration, were not observed. The excessive growth of the inner cell mass was due to endodermal cells, which were characterized by large lipid inclusions and a dilated rough endoplasmic reticulum, and extraembryonic mesodermal cells with well-developed secretory apparatus. Furthermore, endothelial-like cells were present, forming capillary-like formations and cells resembling visceral and parietal endoderm (A. E. Enders, personal communication). Earlier attempts to culture human blastocysts in vitro gave the impression of degeneration of the inner cell mass.

Fig. 5. (b) For legend see page 343.
This finding illustrates the difficulties of normal embryonic stem cell differentiation in vitro. The ultrastructural appearance of human blastocyst penetration in vivo has not been described previously. The results from the present in vitro studies seem to confirm earlier observations of human trophoblastic intrusion by light microscopy (Shettles, 1960; reviewed by O’Rahilly, 1973).

As the cell cultures were checked for attachment sites only once a day, the exact start of attachment could not be established for each blastocyst. Therefore, time-dependent differences between attachment sites could not be investigated. Variations in time before attachment was observed probably depended on differences in blastocyst development, as some blastocysts were expanded and others were fully hatched.

The morphologies of the different attachment sites indicated that adhesion and penetration occur at the same time by initial lysis of existing inter-epithelial apical junctional complexes and desmosomes, followed by regeneration of these junctions with participation of trophoblast cells. Previous scanning electron microscope studies also demonstrated a confluent surface at the endometrial–trophoblastic interface (Bentin-Ley et al., 1999). The present study did not confirm the impression of former scanning electron microscope studies that human blastocysts attach directly to apical plasma membranes of pinopode-presenting endometrial epithelial cells (Bentin-Ley et al., 1999). As sections give much better information on this

Fig. 6. Transmission electron micrograph of specimen 7, illustrating an apical junctional complex and desmosomes between the penetrating trophoblast cell (T) and endometrial epithelial cells (E) (for orientation, see Fig. 5a). No cytoplasmic specializations are present in the endometrial epithelium towards the trophoblastic cell. The apical plasma membranes of endometrial epithelial cells are not in contact with the trophoblast cell. Scale bar represents 600 nm.
aspect, the results of scanning electron microscopy of blastocyst attachment to apical epithelial plasma membranes must be revised. However, pinopode-presenting endometrial epithelial cells are present at the blastocyst–endometrial interface.

Access to the stromal compartment is achieved by pushing the epithelial cells aside, giving an impression of endometrial epithelial displacement and accumulation peripheral to the penetration site. At a later stage, epithelial cells may be lifted off the artificial basement membrane by a penetrating trophoblastic cell. In an in vivo implantation site on day 7 after ovulation, trophoblastic cells displaced stromal cells at the basal surface of the luminal epithelium (Carnegie Institution, specimen number 8020; Falck Larsen, 1974). The nuclei of the endometrial epithelial cells lie very close in two or three layers, which might indicate peripheral stacking of cells (Falck Larsen, 1974). A stratified endometrial epithelium in the immediate area of the implantation site has also been observed in rhesus monkeys (Enders et al., 1983).

In the present study, the trophoblastic cells at the penetration cone displayed a morphology similar to that of human syncytiotrophoblast, characterized by irregular nuclei often with more than one nucleolus, a well-developed rough endoplasmic reticulum and Golgi complexes (Knoth and Larsen, 1972). Lopata et al. (1995) studied invasion of marmoset blastocysts on Matrigel®. The area of deepest penetration was composed mostly of cellular trophoblast that was undergoing transition to syncytial cytotrophoblast, characterized by segments of broken plasma membranes associated with bundles of microfilaments; these observations were also observed in human blastocysts in the present study.

To date, it has not been shown when formation of syncytium starts in humans in vivo. In marmoset monkeys, single cells with morphology consistent with that of...
syncytiotrophoblast have been described (Smith et al., 1987), and it is questioned whether this trophoblast layer differentiates into syncytium before implantation (Moore et al., 1985). An early in vivo implantation site of rhesus monkey blastocyst, which has been examined by electron microscopy, failed to demonstrate syncytium formation (Reinius, 1973). Rhesus monkey blastocysts cultured in vitro on plastic present spontaneous syncytium formation at peri-implantation (Enders et al., 1989). In the present study, double nucleation, isolated desmosomes in the cytoplasm and disappearance of plasma membranes were observed in the blastocysts. Syncytiotrophoblastic cells, which are characterized by their dilated rough endoplasmic reticulum content and very irregular nuclei (giving an impression of multinucleation on several serial sections), were present in the embryo that adhered to collagen gel. Conflicting results have been published regarding syncytium formation in vitro. Yeger et al. (1989) found that culture on collagen gels had an inhibitory effect on syncytium formation in human cytotrophoblast cells. Kao et al. (1988) demonstrated that addition of FCS to the culture medium could induce spontaneous fusion of first trimester cytotrophoblast cells cultured on collagen gels. As the culture medium used in the present study contained FCS, absence of syncytium in the presence of serum could be regarded as an indication of a normal physiological event.

In conclusion, in this study of human blastocyst–endometrial interactions in vitro it is demonstrated that adhesion takes place at the lateral plasma membranes and may be part of the process of penetration. The trophoblast

Fig. 8. Transmission electron micrograph of the penetration area in blastocyst attachment site 7 (see also Fig. 2f). In this specimen, several trophoblastic cells (T) have penetrated through the endometrial epithelium (E) and started to invade into the stromal compartment. At the penetration margin, trophoblastic cells have also made contact with the basal plasma membrane of the epithelial cells (arrow), thereby partly removing them from the underlying stromal compartment. This is most clearly demonstrated at the left side, where part of the basal plasma membrane is in contact with the stroma (arrowhead), whereas a trophoblastic cell undermines the other part. At the border between these two areas, a constriction furrow is present (double arrow). Endometrial epithelial or trophoblastic cell degeneration is not seen but some cells belonging to the inner cell mass are necrotic. Scale bar represents 3 μm.
probably induces lysis of interepithelial apical junctional complexes and desmosomes. Regeneration of these junctions is achieved by participation of trophoblastic cells. Human blastocysts penetrate the endometrial surface epithelium by intrusive penetration. Trophoblast cells resembling cellular syncytiotrophoblast penetrate the epithelial layer and the first indications of syncytium formation occur simultaneously with epithelial penetration.

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**Fig. 9.** (a) Transmission electron micrograph of a double-nucleated trophoblastic cell (specimen 6). Serial sections did not demonstrate fusion of the two nuclei. This trophoblastic cell shows the presence of well-defined plasma membranes between cells. The apical plasma membrane forms many short microvilli. A thin layer of basal lamina is present at the basal plasma membrane facing the blastocoel. The cytoplasm contains Golgi complexes (G) and small vesicles. (b) Transmission electron micrograph of trophoblastic cells in a blastocyst that did not attach to the endometrial cell culture, demonstrating sudden disappearance of plasma membranes, leaving isolated parts of membranes and desmosomes in the cytoplasm. (c) Transmission electron micrograph of a trophoblastic cell in specimen 7, showing two isolated desmosomes in the cytoplasm (arrows), indicating fusion of plasma membranes. Scale bars represent 600 nm.

**Fig. 10.** Transmission electron micrograph of a human blastocyst that is adhering to a collagen gel. This ultramicrograph demonstrates a large multinucleated cell, indicative of syncytiotrophoblast. Serial sections demonstrated the presence of at least two different nuclei. One nucleus is very irregular with deep indentations and a large nucleolus, whereas the other nucleus is round and with no nucleolus present. The cytoplasm is dominated by dilated rough endoplasmic reticulum and two intracellular luminae are present also (asterisks). To the lower left, part of another very irregular nucleus is shown. Scale bar represents 2 μm.
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