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Some Ultrastructural Observations on Cellular Interaction Between Trophectoderm and Uterine Epithelium During Preimplantation in Rat

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Abstract: The blastocystic trophoblast cells consist of different types of cells according to their structural architecture and their functional specialisation: a) supporting, preventing and feeding functions; b) signalization, polarisation, and depolarisation functions between blastocyst and uterine; c) immunological acceptance or rejection and secreting functions. The aim of this study was to investigate the ultrastructure of cellular interaction between mural trophoblast/trophectoderm cells of blastocyst and uterine epithelium in rat. Tissue samples obtained from rats on day 5 of pregnancy were studied ultarstructurally. In contrast to the polar trophoblast cells exhibiting degeneration according to implantataion progress, some mural trophoblast cells play an important role in the cellular interaction taking place between the uterine epithelium and blastocyst. The ultrastructural evidence, that structural diversification in blastocystic ring change according to the implantation period and these ultrastructural properties, suggests that the functional diversification is very important for implantation.

Key Words: Blastocyst, Trophoblast, Implantation, Pregnancy, Rat.

Sıçanda İmplantasyon Öncesi Evrede Trofektoderm ile Uterus Epiteli Arasında Hücresel Etkileşme Üzerinde Bazı Ultrastrüktürel Gözlemler

Özet: Blastosisti oluşturan trofoblast hücreler, yapısal ve fonksiyonal özelliklerine göre farklı tip hücrelerden oluşur ve onların fonksiyonları; a) destekleme, koruma ve besleme; b) uterus epiteli ile blastosist arasında signalleşme, polarlaşma ve polarlaşma özelliğini kaybetme; c) immunolojik kabul veya ret cevabı ile sekresyon yaptıkları ileri sürülür. Bu çalışmanın amacı, sıçanda blastosist trofoblastı ile uterus epiteli arasında meydana gelen hücresel etkileşmeyi araştırmaktır. Gebeliğin 5. gününde olan sıçanlardan elde edilen doku parçaları, ultrastrüktürel olarak çalışıldı. Polar trofoblast hücreler, implantasyon sürecine bağlı olarak bir dejenerasyon göstermesine karşın, bazı invaziv/saldırgan trofoblastlar, uterus epiteli ile blastosist arasında gerçekleşen hücresel etkileşmede önemli rol oynarlar. Bu ultrastrüktürel bulguların gösterdiği fonksiyonal çeşitlilik, blastosist implantasyonu için çok önemli olduğunu göstermektedir.

Anahtar Sözcükler: blastosist, trofoblast, implantasyon, gebelik, sıçan

Introduction

İmplantation consists of the establishment of physical and biochemical contacts between the mural trophoblast cells of the blastocyst and uterine epithelium. Blastocysts undergo a series of physiological and developmental changes prior to contact with the uterine epithelium; many factors play a role in different directions during this stage of implantation.

Some implantation mechanisms in different species of mammals show very specific features according to species during the stages of blastocystic ring formation, implantation and related biochemical events (1, 2).

The ultrastructural and cytochemical aspects of blastocysts have been extensively studied by many authors in various species; in human and nonhuman primate varieties (3-17), and in rodents (2, 18-31).

Recent molecular studies of implantation have examined the role of essential elements of the inflammatory process (1, 32-37). The mutual recognition of the implating embryo and uterus is of primary importance in the first stages of implantation. It probably requires that uterine factors first establish a tight contact with the embryo. It also accounts for the appropriate distribution of cytokines, substrates and adhesion molecules in the uterus and embryo.

According to our previous studies, the identification of the trophectoderm cells leads to the assumption that their structural differentiation in the rat is not uniform (28). They should have some differences in properties according to functional performance during the initiation of the blasto-uterine interaction. Some studies (28, 29, 38, 39) have discussed the role of cell skeletal fibers in development with regard to differentiation dependent

change in cell polarity and possible signalling between blastomers during the preimplantation period in rodents.

The purpose of this study was to describe the ultrastructure of cellular interaction between mural trophoblast/trophectoderm cells of the blastocyst and uterine epithelium during the initial stage of implantation in rat.

Materials and Methods

Sexually mature rats were kept in normal animal laboratory conditions and were fed with prefabricated feeds. During the afternoon, one male rat was put together with every two female albino rats, which had been subjected to oestrus smears.

To determine the exact time of mating, the laboratory was slightly illuminated and a small hidden camera was installed. For identification, the females were marked with green and red dyes. The animals were observed by means of the camera so that the copulation time could be defined, and the following 24 h and 48 h periods were considered the first day and second day of pregnancy respectively. After 12 hours, vaginal smears were examined. When sperms were found, they were isolated until the appropriate stage. Eight of the 12 animals showed positive spermium presence. 96 h after mating, beginning on day 5 of pregnancy, the animals were anaesthetised and 1% Evens blue solution was injected via the femoral vein. After 15-20 minutes, abdominal aorta was clamped near the uterine region and beneath the clamp Karnowsky's fixative solution (40) was injected into the abdominal aorta.

In addition to this process, the same fixative was also injected into the uterine horns via the entrance of the uterus under low pressure (4-5 ml/min). Blastocyst implantive areas were collected systematically every 6 h (Table 1). During the late stage of the blastocysts, at 102 h, 108 h, 114 h, and 120 h in the same gestation period, the procedures mentioned above were repeated.

Fixed pieces of perfused uteri exhibiting blue reaction areas were removed and placed in the same fresh fixative. Individual implantation sites were trimmed under a dissection microscope. After fixation, the tissues specimens were rinsed overnight in 0.1M phosphate buffer, then post-fixed in 1% Osmium tetroxide, in the same phosphate buffer at pH 7.3 for one hour. The tissue specimens were then dehydrated in a graded series of alcohol, passed through propylene oxide, and embedded in Araldyte epoxy resin. Semithin and thin sections were taken with a Nova ultratome. The semithin sections were stained with uranyl acetate and lead citrate and examined with a Jeol 100 C and a Philips 300 TEM.

Results

The topography of blastocystic ring in uterine lumen

Blastocysts situated in the uterine crypt showed bulging trophoblast cells possessing irregular cytoplasm protrusions and many small projections of various lengths. Trophoblastic cells close to the surface of the uterine epithelium and affect of the uterine lumen to occur. Uterine epithelial cells in the implantation chamber and along the lumen were covered with abundant cytoplasmic bulbous but not mirovilli (Fig. 5, 7A, B).

The observations made in this study on the general are in agreement with orientation of the blastocyst on day 5 of pregnancy in the rat uterus crypt are in agreement

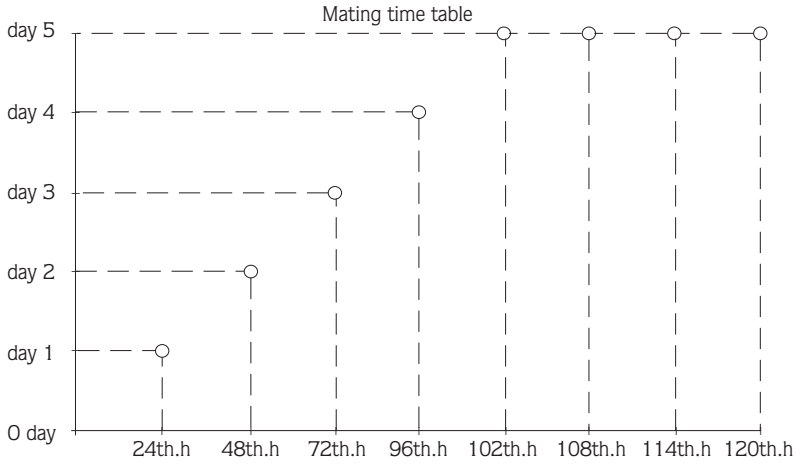


Table 1. Standardised embryonic age according to mating (post coitus, p.c.). The time of mating is defined as day 0. Twenty four hours after p.c. the embryos were refereed to as day 1. At the end of fourth day (96 th h), beginning the fifith day of pregnancy, blastocysts were serially collected from animals every six hours (at 96 th, 102 nd, 108 th, 114 th and 120 th h.

with those of a previous study (21). It was observed that the blastocysts on day 5 and at 102 h of pregnancy were located in the antimesometrial region of the uterine lumen. They had lost their coverings (18), and were slightly elongated prior to contact with the endometrial epithelium. They were similar to an elliptical ring. The uterine luminal epithelium was present complete with both mesometrial and antimesometrial region to the blacstocyst (Fig. 1). The blastocytsts were encompassed by an elongated crypt epithelium. The uterine epithelium cells displayed local exo-or endo-cytosis where they were in contact with the trophoblastic cells that were not observed to be phagocytosing uterine cells in this preinplantation period.

Embryonic pole trophoblasts of the blastocyst, in terms of volume, were smaller than the polar abembryonic region cells. As the blastocystic period went on, especially in the late stages of this preimplantation period in same time, the blastocysts were flattened in an abnormal plane to the embryonic abembryonic axis. Some of the trophoblast cells of the abembryonic pole were rounded but more of them were flattened and constituted a communication between the rounded cells (Fig. 1).

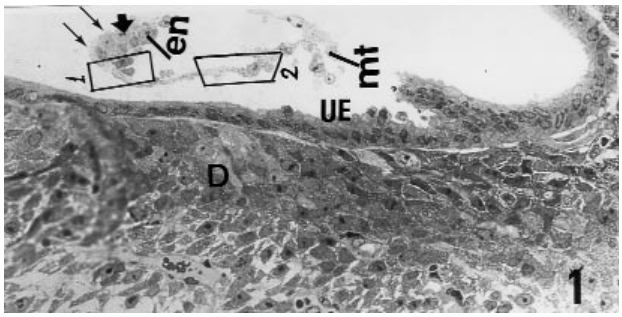


Figure 1. The micrographic resreseptation of the blastocystic ring during the initial stage of implantation. Orientation of blastocyst in uterine epithelium (UE) was noted with a light micrograph. Blastocyst from a rat recovered on day 5 at 102 nd h (from the mating) of pregnancy. Embryonic and abembryonic poles of blastocyst are appeared. Inner cell mass cells (with single arrow) surrounded by polar trophoblast (with double fine arrows) (out line) and presumptive endodrem cells (en) (inner line). Several mural trophoblast cell (mt) types showing different contents and association with each other are seen in blastocystic ring. D=decidua. x625.

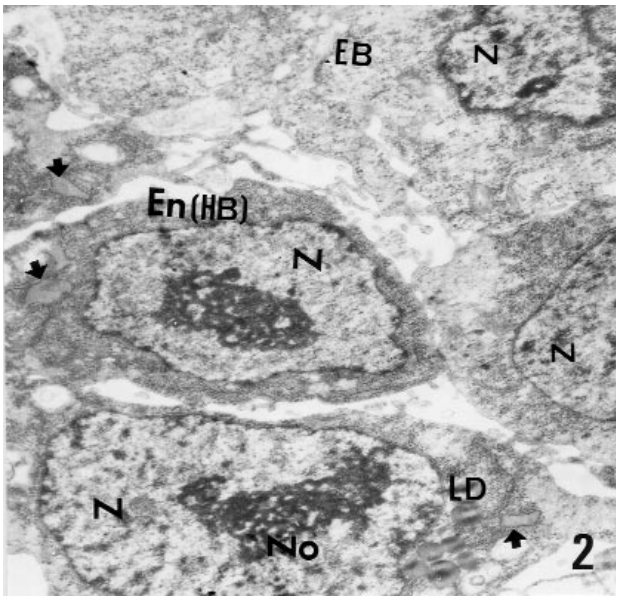


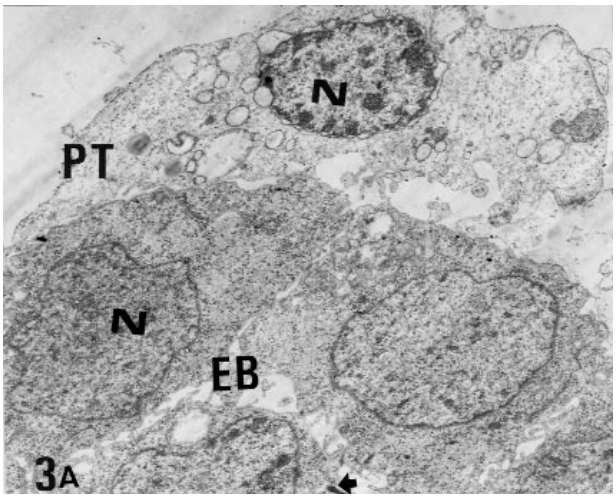
Figure 2. The ultrastructural appearance of the region of blastocystic neck (indicated with rectangle by number 1 in figure 1) connecting the mural trophoblast to embryonic pole. Presumptive endoderm cells (hipoblast) (En-HB) connected to each other and to epiblast (EB) by tight junctions and with bizarre in shaped including well developed granular endoplasmic deticulum (with single arrows), different condensation areas of cytoplasm, polysomes and glycogen particles are seen. N=nucleus, No=nucleoli, LD=lipid droplet. x10.000.

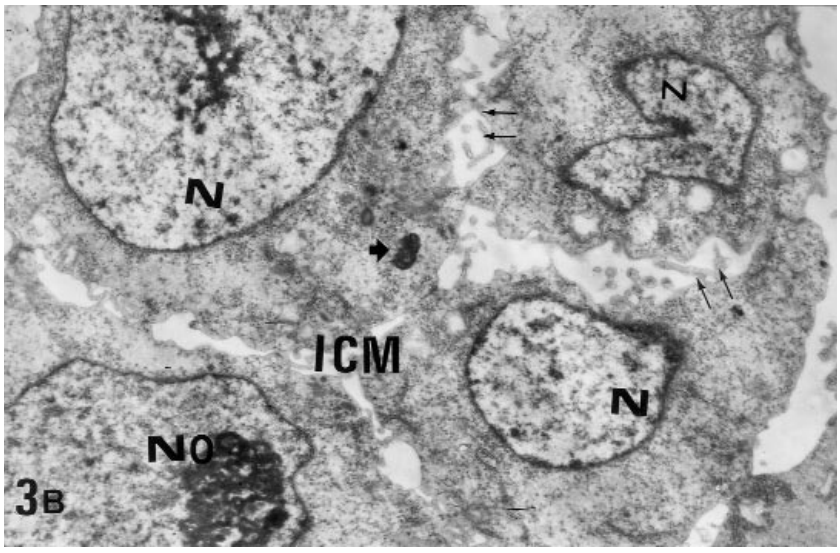
The embryonic pole cells had an irregular roundish shape. Three cell types were identified there; hipoblast presumptive endoderm), polar trophoblast cells and embyoblasts comprising the inner cell mass (Fig. 2, 3A, B, C). The embryoblast cells were bigger than the peripheral trophoblastic origin cells. The inner cell mass was surrounded by hipoblast and polar trophoblast cell lines (Fig. 3A).

Diversification of trophectoderm cells

These had different shapes and structures, but did not have any usual definitive microvilli on the external or internal free surfaces, with only very irregular and short cytoplasmic projections. Some trophoblast cells were high activated nucleate and at certain stages pre-implantation many under mitosis.

The cytoplasm of some trophoblast cels in the blastocystic ring contained extensive deposits of lipid droplets and a moderate amount of rough endoplasmic reticulum and Golgi bodies were usually present (Fig. 4A, 5A).





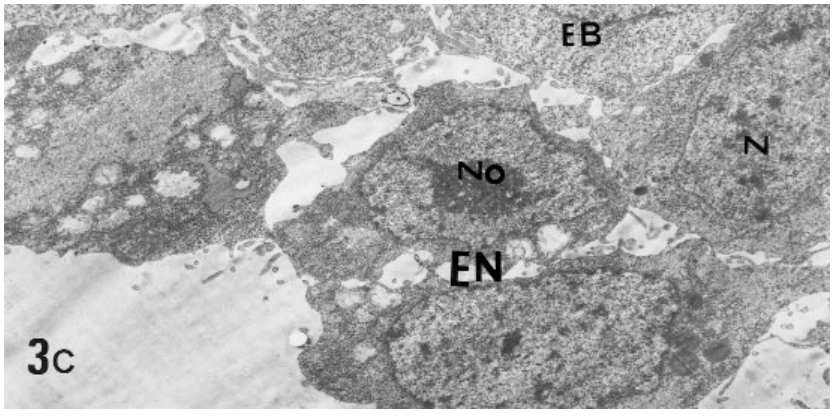


Figure 3. Several inner cell mass cells (ICM; embryoblasts, EB), polar trophoblast cells (PT) and endodrem cells (EN; hipoblasts) from the embryonic pole are illustrated in this figures. Day 5 on 102nd h blastocyst the embryonic pole including some rounds embryoblasts cells (EB), inner cell mass cells with high magnification (B), associated with other polar cells, underline by the polar trophoblast (PT) (A) and endoderm (EN) (C). Polar trophoblast (PT) overlying embryoblasts of in blastocystic ring showing a lightly cytoplasm and lack of organelles and coated pits and vacuoles are celarly seen (A). A embryoblast cell, associated with both brother embryoblast and polar trophoblast cells (A and B), with elliptical shaped euchromatic nucleus (N) showing definite nucleolus (No), and distinctive cellular debris, inclusion bodies (with single arrows), the presence of both intercellular spaces (with double arrows) resembling a labyrinth and irregular cytoplasmic projections into spaces are seen. A: x7.000; B; x19.000; C: x7.000.

The trophoblacstic cells contained mitochondria in two shapes. One type consisted of spheriods with a few mitochondria inner membranes. The other type had a more conventional appearance. The number of dense mitochondria with few cisternae had decreased. The cytoskeletal contents or fine fibrous material which varied in quantity throughout the cytoplasm was more sparsely distributed. The diversification of the cell types forming the abembryonic pole and lateral region of blastocystic ring was determined as follows:

a) Elliptical or rounded cell type, situated between two flattened cells and connected to each other laterally or in other positions by very long junction complexes (Fig. 1, 4A). Their nuclei were rounded and centrally situated, showing euchromatic feature. Large cytoplasm areas which are limited by a unit membrane, filled with some materials showing different condensation, in regular shaped tubules or multivesicular structures, electron-dense homogenous materials, and irregularly shaped nonfibrillar plaques. Some of them were long-stick-like structures containing homogenous substances. Very large vacuoles, containing different materials, were observed. Mitochondria, showing very few cristae, were observed in different shapes. Endoplasmic reticulum clusters and Golgi complex present (Fig. 4A).

b) Flattened trophoblast cells constituted a means of communication between other trophoblast cells. Their cytological features were not very clear but many large and small vesicles, dense bodies, and elongated mitochondria were observed. Interestingly, the formation of the first basal lamina was observed beneath these very flattened trophoblast cells connected with the embryonic pole cells (Fig. 1). Flattened cells generally had very dense cytoplasm with a variety of contents (Fig. 4B). Similarly, definite basal lamina showing very fine dense lines beneath the endodrem cells were also observed. Endodermal cells eventually form Reichert's membrane, the basal lamina originally formed by only trophoblast (38).

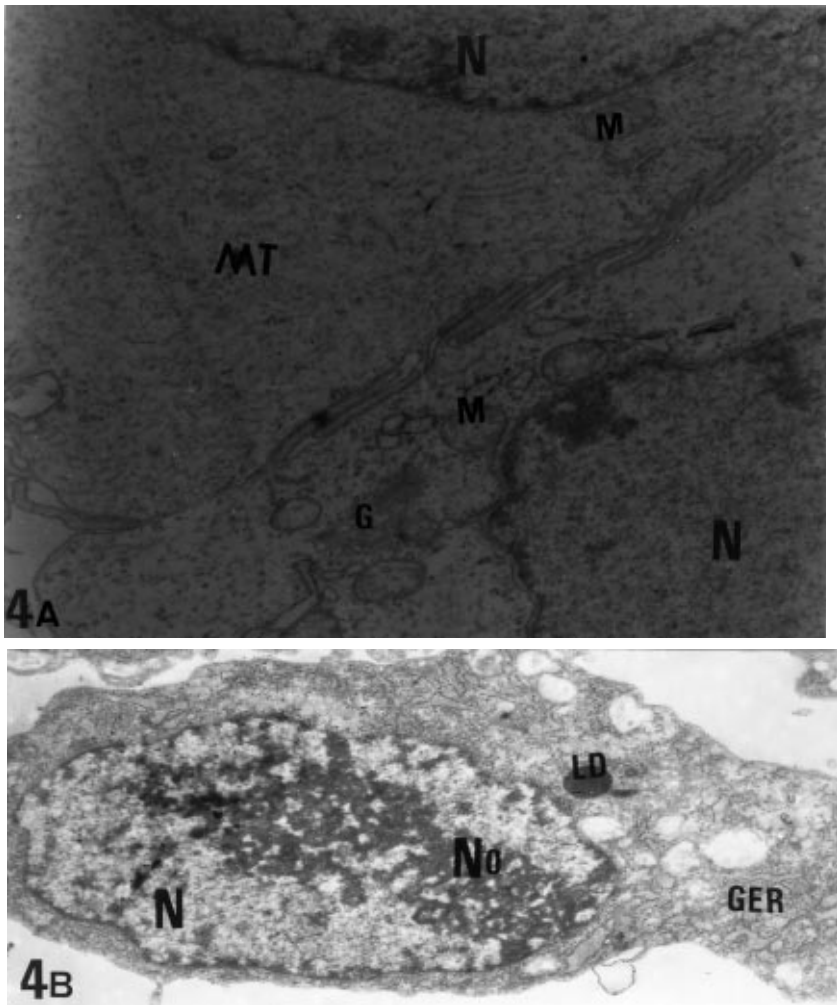


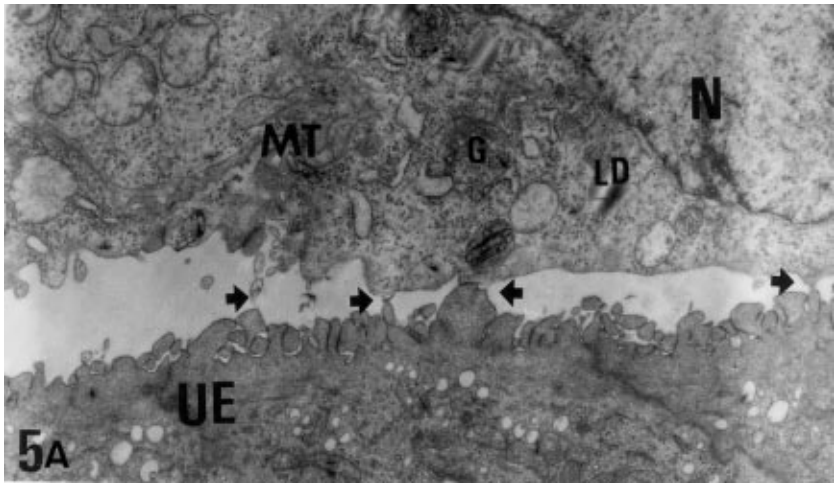
Figure 4. The ultrastructure of different types mural trophoblasts (MT) are seen in this figure indicated with rectangle in figure 1 by number 2. Two mural trophoblast have regular homogenous cytoplasm with elliptical euchromatic nuclei and connected to each other by regular interdigitation similar a parallel railing-like connection are seen (A). Flattened a mural trophoblast with condensation by irregular cytoplasmic protrudes, situated between two elliptical cells, having many ribosomes and endoplasmic reticulum clusters, lipid droplet (LD) and local accumulations of glycogen particles are seen (B). These mural trophoblast cells showing different shape and structures are observed with an activation in lateral region of blastocystic ring (see figure 1). N=nucleus, No=nucleoli, M=mitochondrion, G=Golgi region, GER=granular endoplasmic reticulum. A: x17.750; B: x10.000.

c) Lipid dorplets rich cells, a lot of lipid doplets were found in some of the abembryonic pole trophoblastic cells (lipid-rich cells). Occasionally several of the lipid droplets showed a topographical relation to each other, forming groups and also joining with multivesicular areas. Some lipid droplets were exported from the cytoplasm to outside or inside borderline of the blastocystic ring. This cell type also showed other normal cellular features.

d) Homogenous cell types, showing homogenous cytoplasm, showed exhibited a lack of cytoplasmic organelles. These were observed especially during the late stage of pregnancy, on day 5, 114 and 120 h after mating.

Homogenous trophoblast cells were observed in which the storage materials had a less clear from, devoid of organelles and with a very fine granular composition. It has been suggested that similar material in baboon blastocysts was protein formation materials (38). Some parts of the cytoplasm of mural trophoblast cells (Fig. 4A, 6A) and presumptive endodrem (Fig. 2, 3C) were filled with similar homogenous storage materials. These areas of cytoplasm were largely devoid of organelles.

e) Chanalicculed cells, showed abundant tubulo-vesicular mass limited by a main border membrane with great complexity of internal orgasition and well developed system of smooth-surfaced tubule-chanaliculi structures



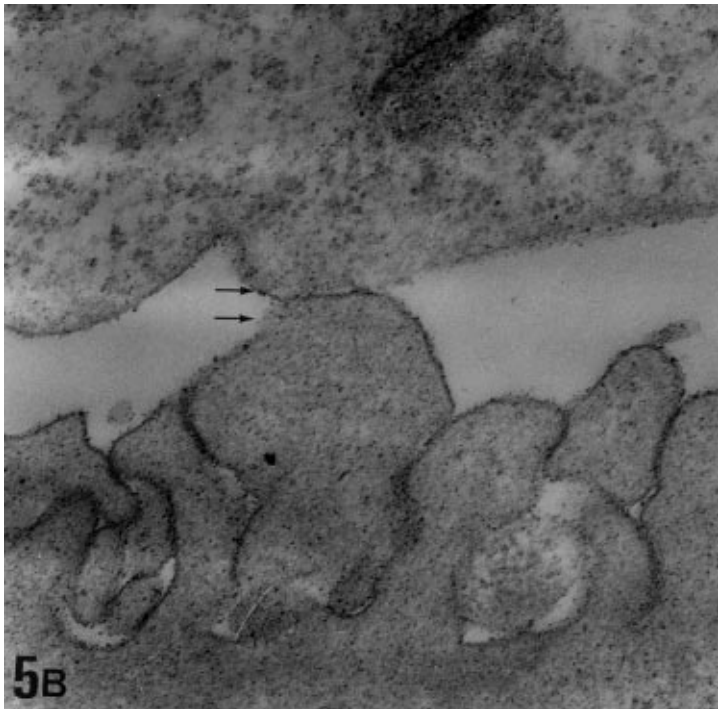


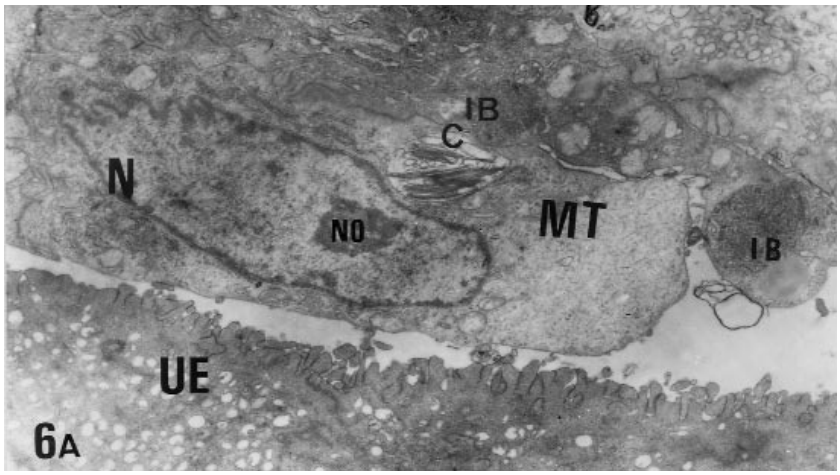
Figure 5. Mural trophoblast cells (MT) situated opposite to the uterine epithelium (UE) are seen continuously (A). One of these tropnoblast cells is showing an effort for contacting at least some points with uterine epithelium (with single arrows). These contact points are magnificated step by step owing to the cellular continuous and parallel section (B). The memsrane to membrane adherence are occurred between blastocystic ring cell and uterine epithelium (with double arrows) are showing typical regions of attachment points on preimplantation period on day 5, at 114th h after mating, of pregnancy. N=nucleus, G=golgi region, LD=lipid droplet. 5A: x12.500, 5B: x65.000.

(Fig. 6A, B). These structural characters were observed only in these cells which were in contact with the uterine epithelium, which had only very thick and short cytoplasm projections but no microvilli in the cells near to it. The parts of the cytoplasm apposed to the uterine epithelium were devoid of organelles.

In general, inclusion bodies were distributed in the different cell types, especially in rounded and flattened cells during this period (Fig. 6A, B). All types of trophoblast cells forming the blastocystic ring had extensive endocytic complex including coated pits situated deep in the cytoplasmic bodies isolated by a border membrane line in cytoplasm. The multivescular bodies were striking in the mural trophoblast cells of the rat blastocyst and they were seen in three different forms: forming, maturing and dense. This evidence suggests that formed phagosomes and digestive materials may pass the trophoblast without lysosomal activities, as no lysosomes could be detected in the cytoplasm.

Embryonic pole cells

Three groups of cells were observed: embryoblast, inner cell mass, hipoblast and polar trophoblast cells. The embryoblasts were larger than the trophoblast cells and were surrounded by polar trophoblasts at the outer border line and by endoderm on the inner border line (Fig. 3A-C). They were roundish in shape, connected to each other by tight junction complexes forming on cytoplasmic projections forming labyrinth-like narrow spaces, were observed. They have big and rounded eucromatic nuclei situated centrally, and have distinctive nucleoli (Fig. 3B). These cells showed a few cisternae of granular endoplasmic reticulum and there was a large number of ribosomes, polysomes and mitochondria. Electron-dense inclusion bodies were observed in the cytoplasm. They had no basal lamina or cytoplasmic heterogeneity when compared with the endoderm and some mural trophoblast cells in which there was a patch of cytoplasm free of organelles. No desmosomes or gap



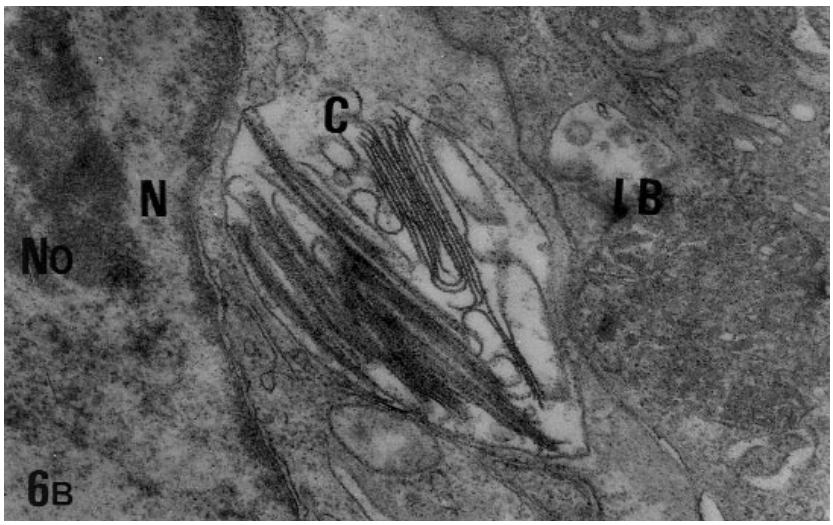


Figure 6. Attaching region of trophoblast cell (MT) to uterine epithelium (UE) is appearing in this figure. Structural differences on the surface and in the cells of uterine epithelium (UE) with no clearly cellular organelles, but some irregular cytoplasmic hills extending to the blastocystic ring, and lipid droplets, vacuoles are seen during blastocystic contact period (A). Very interesting tubulo-canalliculi complex structures surrounded by a membrane borderline (C) are observed at supranuclear region of the mural trophoblast cell. Inclusion bodies (IB) are generally observed as dense aggregates of granules, membranes and vesicles in different forming conditions in mural trophoblast cells (A) and its high magnification is seen in figure (B). N=nucleus, No=nucleoli. 6A: x12.500; 6B: x60.000.

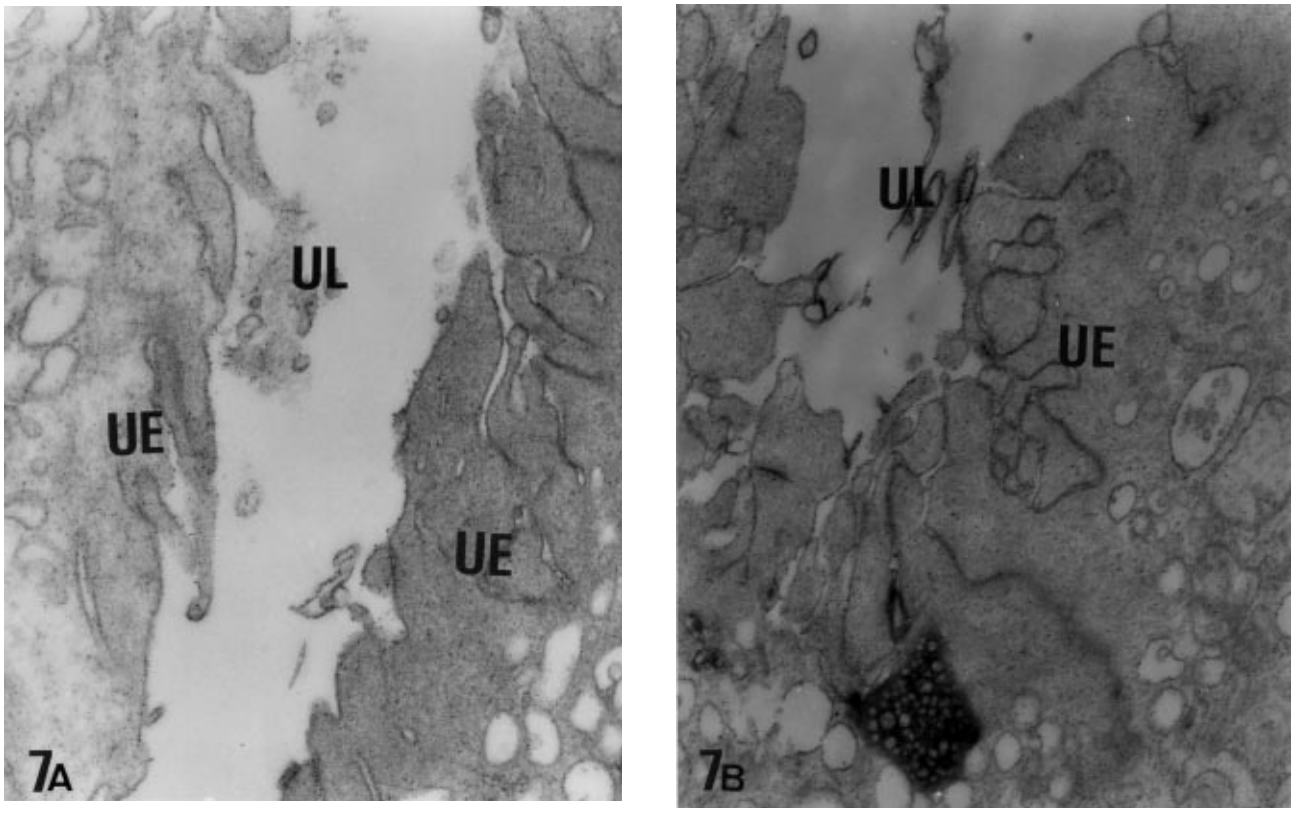


Figure 7. Contrary to the uterin epithelium cells presented in figure 6A, in this figure no any blastocystic attachment in uterine lumen. Irregular cytoplasmic hills on the surface of uterine epithelium (A) and formed a knob-like structure (with arrows), joined darker cytoplasmic region showing different condensation due to the functional activities (B) are seen. 7A: x45.000; 7B: x35.000.

junctions were found between the embryoblasts and polar trophoblasts, or and between the endoderm and other cells, but occasional increased density was observed on the cytoplasmic projection side of apposed cell membranes, like tight junctions (Fig. 2, 3A-C).

The embryoblast cells were surrounded by polar trophoblast and endoderm cells along the outside and inside line. Although the outlined polar trophoblast cells had light cytoplasm with poor organelles, the unlined ones, presumptive endodermal cells, had dark cytoplasm with well-developed endoplasmic reticulum and abundant riboprotein and a few glycogen particles (Fig. 2, 3C).

The polar trophoblast cells were located above the embryoblasts (inner cell mass cells-ICM) and were connected to them by tight junctions, commonly formed between two cytoplasmic projections, with lightly condensed cytoplasm and many coated pits, vesicles and moderately large vacuoles along the interior intercellular border of the cytoplasm (Fig. 3A-C). No basal lamina was observed beneath the polar trophoblast or between ICM cells and the trophoblast in this gestational stage. However, definitive basal lamina was observed beneath trophoblast cells sutiaten in the neck region of the blastocyst; between the lateral region and embryonic pole connection. It continued beneath th endoderm in a limited area and was then interrupted. Polar trophoblast cells were not found in the embryonic pole at 114 and 120 h of the gestation period. They were probably displaced from the surface of the ICM cells.

Endoderm formation was seen beneath the ICM cells and was connected to the ICM by presumptive junctions. They frequently occurred in rather bizarre shapes. They exhibited darkened cytoplasm compared to the ICM and polar and mural trophoblast cells, and were attached to the ceiling of the blastocyst cavity in an irregular arrangement. Many cytoplasmic projections on the surface extended in different directions, and cytoplasmic fragments were scattered in the area around the spaces.

Some striking features of the endoderm were: distinctive extended and flattened cytoplasmic portions, dilated endoplasmic reticulum sacs, the presence often heterogeneous cytoplasm with local accumulation of riboproteins particles, and a patch of cytoplasm areas in which there were none of these particles or other

organellse (Fig. 2, 3C). Endoderm cells were attached to ICM cells by tight complexes formed between two cytoplasmic protrusions of cells.

The basal lamina, which appeared to be granular and in a fine line, continued from the mural trophoblast to the endoderm, where it was exposed to interruption due to the endoderm cell compositions.

It has been suggested that the endoderm has a potential differentiation as a source of hemopoietic tissues. In contrast to the close connection into two states, visceral and parietal, were observed in advanced blastoystic ring cells at implantation. Beneath the presumptive endoderm cells, to further differentiate the parietal region, probably, was observed the connection neck of mural trophoblasts and embryonic pole cells, a very fine dense definite line (38).

Discussion

Cell-to-cell interactions between the trophoblasts and the uterine epithelium may take place through three stages: (i) recognition and adhesion, (ii) enfulfement and ebtablishment, and (iii) control.

Trophoblastic cells in the first stage adhere to the uterine epithelium at some points by means of very special "connection complexes" resembling dense long-lines. In the second stage, trophoblast cells degrade the uterine epithelium and penerate the epithelial basement lamina (23). During this satge, not taking into account any digestion process, trophoblastic bridges establish a connection with the step in a dynamism of penetration. During this process, there is not only cellular digestion, but a symbiotic relationship may also be established between trophoblast and uterine cells.

An interesting phenomenon of blastocystic ring development is the formation of the first contact points between the abembryonic pole and uterine epithelium. Some specialised trophoblast cells, having canalicular systems and cytoplasm devoid of organelles, become attached in the arrangement of the blastocyst to the uterine epithelium. These cells involved in the preimplantation contact processes have no microcilli on their surface opposite to the uterine epithelium. The part of their cytoplasm connected to the contact points is devoid of organelles, and, interestingly, they also have very special tubule-chanalicular systems. First, contact points, and then a mutual relation, pseudosymbiosis for limited period, are established between the blastocyst and uterine epithelium by means of these specealised cell types.

We address two mawor questions fundamental to the understanding of cell signaling (41) between trophoblast and uterine epithelium: (i) is there a pseudosymbiotic recognition reducible to simply the sum of implant cells (trophoblasts) and receipt cells (uterine epithelium)? (iii) Are the recognition signals responsible for these mutual cells ' compatibility and the restricted periods of nivasive cell compatible interactions compelety lacking in correlation?

Investigation of the phenomenon of pseudosymbiont recognition and the establishment and maintenance of pseudosymbiosis thus relates to a particularly complex experimental system that creates considerable complications in laboratory work (41, 42).

The study of pseudosymbiotic systems between implantive tissues that recognise each other, control of subtance transfer and morphogenesis are only different aspects of an interactive phenomenon. According to the literature, the cell biology (41, 43) of pseudosymbiosis for host tissue could be better undestood by investigation of the structure and dynamics of the surface. Current assassments may be made of the molecular organisation throughout interaction during the initial stage of preimplantation (35, 36).

Understanding of the signalling between the partner tissues in the recognition of the existence of stimuli that are significant for the establishment of reciprocal messages (44). The significance of these interactions and their correlation with signalling mechanisms are poorly understood. Signalling between the blastocyst and its maternal tissue is generally presumed to be important for the successful establishment of pregnancy in mammals. In some species, cell-signalling depends on molecular signals and is well established, particularly for the ligand-receptor system presented by soluble molecules such as hormones which are signials received by specific information (1, 33, 35, 45, 46). It has been suggested that it shoud be possible to use some specific uterine protein production to characterise the signals from the embryo and it may be concluded that the blastocyst synthesises and releases different soluble factors during the preinplantation period (46, 47).

During the precontact phase of trophoblasts and the uterine epithelium on day 5 after fertilisation, some coated pits and very small vesicles were observed in the space between the two implantive members. These findings suggest that there is an information exchange between them.

According to extensive studies, it appears that the metabolic capacity of the blastocystic cells, like that of the uterine epithelium, is involved in the signalling barrier effect by metabolising or converting substances. Blastocyst cells may have an asymmetry in the location of enzymes, carriers and receptors on the maternal (outer) side and foetal (basal) side plasma membranes. The functional polarity of blastocyst cells is expressed in metabolism, nutrients and ions transport and most of the secretions carrying information for acceptance or rejection in the endometrium.

In every condition the interfaces of cells in contact with each other must be kept under constant control via stimuli that regulate compatibility and substantial exchanges. The cell surface glycoproteins, colloidal iron and cationized ferrite associated with reduction in surface negative charge have been examined in the effect of blastocyst on the initial stage of implantation (1, 18, 33, 37). The contact between the surfaces of both cell types may be an adherence-type junction (29, 48). We observed that the areas of fusion of trophoblast knobs with uterine epithelial cells were described in detail at the beginning of the contact face of implantation. These findings are in agreement with the SEM and TEM results reported by different authors (22, 48, 49, 50, 51).

Our findings indicate that there are fine structural alterations in the apical plasma membrane of uterine epithelial cells, the site of the first contact between the maternal and trophoblast cells of the blastocyst at he beginning of implantation in rat.

There have been studies of the hypertrophy of uterine epithelial cells in forming the uterine thickness. According to Enders and Schlafke (52) and Enders (17), "uterine plaque" in the rhesus monkey, is observed at the beginning of the pre-contact phase implantation in rat uteri. The thickness of the uterine epithelium changes due to the decidualisation regions and to the implantation process (53).

Before implantation, the blastocysts differentiated into mural and polar trophoblast cells, and the embryonic pole, including embryoblast, polar trophoblast and endodermal cells. Differentiation of the endoderm into visceral and parietal portions consisting of individual structures, stellate-like cells, with numerous cytoplasmic projections and filopodia were observed using scanning electron microscopy (28, 29, 54).

Finally, during the implantation of the blastocyst, two endodermal derivatives have described diverse conditions and different types of associated function. The probable functional specialisations of the trophoblast cells are: (i) supporting, perventing and feeding functions: (ii) signalization, polarisation-depolarisation functions between the blastocyst and uterine epithelium, (iii) immunological acceptance or rejection and secreting functions, (iv) current contact taking place between two implantive members with cytoplasmic membrane fusion, and (v) pseudosymbiosis, established between these two membrers for a limited period.

During observations of blastocyst differentiation in our studies, many pieces of cell debris with different contents and size were observed in some blastomers, especially in the mural trophoblast cells forming the blastocystic ring. Sometimes trophoblast cells near the uterine epithelium contained serial degenerative remainders. These ultrastructural findings suggest that it may be necessary for some cells to be dead for blastocystic differntiation.

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