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## Ultrastructural Observations on Connection Complexes Between the Mural Trophoblast Cells of Blastocyst in the Rat

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**Abstract:** It is generally accepted that implantation morphologically consists of the establishment of a contact between the blastocyst and the uterine tissues. During this contact and interaction process, many physiological, biochemical and ultrastructural factors are involved. The aim of this study was to describe the ultrastructure of connection complexes between mural trophoblast cells in the blastocystic ring in the rat. Implantation sites obtained from rats on day 5 of pregnancy were studied ultrastructurally.

Connected complexes were observed between mural trophoblast cells in different three types: a) dense long line connection; b) desmosome-like structures; c) regular suture-like interdigitation resembling parallel railings. According to the ultrastructural evidence, the trophoblast cells, exhibiting different features, suggest structural changes according to their functions.

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

### Sıçanda Blastosist Mural Trofoblast Hücrelerin Bağlantı Kompleksleri Üzerinde Ultrastrüktürel Gözlemler

**Özet:** İmplantasyon, genelde, blastosist ile uterus dokuları arasında morfolojik bir temasın kurulması olarak kabul edilir. Bu temas ve etkileşme esnasında, birçok fizyolojik, biyokimyasal ve ultrastrüktürel faktörler gerçekleşir. Bu çalışmanın amacı, sıçan blastosistinde mural trofoblast hücreleri arasındaki bağlantı komplekslerinin ince yapılarını incelemektir. İmplantasyon bölgeleri, gebeliğin 5. gününde olan sıçanlardan elde edildi.

Mural trofoblast hücreleri arasında; a) yoğun bir çizgi halinde, b) dezmozom benzeri yapılar şeklinde, c) birbirine paralel, parmaklıklar gibi düzenli ve sütür benzeri sitoplazmik katlantıların oluşturduğu yapılar olmak üzere üç tip bağlantı kompleksi gözlemlendi. Ultrastrüktürel verilere göre, farklı özellikler gösteren blastosistin mural trofoblast hücreleri, fonksiyona bağlı olarak, yapısal değişiklikler gösterir. İmplantasyon öncesi evredeki bu fonksiyonel özelliğin, yeni araştırmalar için bir sır olarak korunduğu inancındayız.

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

### Introduction

It is generally accepted that implantation morphologically consists of the establishment of contacts between the trophoblast cells of the blastocystic ring and uterine epithelium.

The formation and differentiation of the blastocystic ring, and its ultrastructural and cytochemical aspects have been extensively studied by many authors, and in various species; in

human early placentation and trophoblastic invasion (1–3), in primate varieties (4–12), in rabbits (13–17), and in rats and mice (18–25).

Due to the variation of implantation mechanisms in different species of mammals it is very difficult to describe the stages of blastocystic ring formation, preimplantation, epithelial penetration and related biochemical events (14, 17, 26).

Basic ultrastructural studies on implantation in rats have been carried out (19, 27) indicating that the critical step of blastocyst attachment to the uterine epithelium, chorioallantoic placenta formation including cell death, extracellular matrix and new vessel formation, and decidual reactions take place on the 5th day after fertilisation (23, 28).

The obscurity about cellular identification of blastocystic ring trophoblast cells leads us to the assumption that their structural differentiation in the rat are not uniform. They should have some differences in their properties according to the functional performance during the initiation of the blasto–uterine interaction. A series of studies (24, 30) discussed the role of microfilaments for the development with regard to differentiation dependent change in cell polarity and possible signalling between blastomers during the preimplantation period in rodents.

Because every blastocystic ring cell undergoes a series of interactional changes prior to implantation, it is generally possible to make an error in their cytological identifications. We estimated that it is possible to change cellular communications during their differentiation according to the cell functions during the preimplantation period. The purpose of this study was to investigate the cellular connections between the mural trophoblast of blastocyst and their diversification during the initial stage of implantation in the rat.

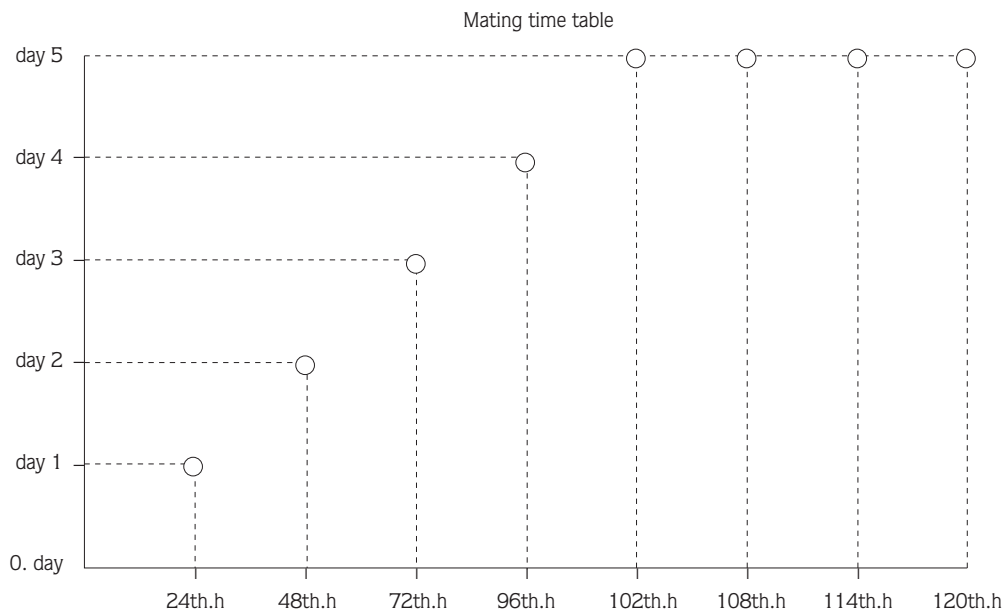
## **Materials and Methods**

Sexually mature rats were kept in normal animal laboratory conditions and were fed with prefabricated feeds. For every two female albino rats, whose oestrus smears determined, one male was placed in the afternoon.

To observe the mating time, the animal laboratory was slightly illuminated and a small hidden camera was suitably placed and for identification the females were marked with green and red dyes. Although at night the animals were watched by the camera and so that the copulation time could be determined, the following 24 h and 48 h were considered the first day and the second day of the pregnancy (Table 1). After 12 hours their vaginal smears were examined. When sperms were found, and they were isolated until the appropriate stage. 8 of 12 animals showed positive spermium presence. 96 h after the mating, beginning on day 5 of pregnancy, the animals were anaesthetised and then 1% Evens bule solution was injected via the femoral vein and after 15–20 minutes, the abdominal aorta was clamped near the uterine region and beneath the clamp Karnowsky's fixative solution (31) was injected into the abdominal aorta while the animal was anastheized.

In addition to this process, the same fixative was also injected into the uterine horns via the entry of the uterus under low pressure (4–5 ml/min). Blastocyst implantive areas were collected

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 mating the embryos were referred to as day 1. At the end of fourth day (96th h), beginning the fifth day of pregnancy, blastocysts were serially collected from animals every six hours (at 96th, 102nd, 108th, 114th and 120th h).



every 6 h systematically (Table 1). For the late stage of blastocysts at 102nd h, 108th h, 114th h, and 120th 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 and 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 pH 7.3, for one hour. Tissue specimens were then dehydrated in a graded series of alcohols, and finally passed through propylene oxide, and embedded in Araldite epoxy resin. Semithin and thin sections were taken with a Nova ultratome. Semithin sections were stained with toluidine blue for light microscopic examinations. Thin sections were stained with uranyl acetate and lead citrate and examined with a Jeol 100 C and a Philips 300 TEM.

## Results

### Orientation of the blastocystic ring in the uterine lumen

Blastocyst situated in the uterine crypt showed bulging trophoblast cells possessing irregular cytoplasm protrusions and many small projections of various lengths. Trophoblastic cells were

close to the surface of the uterine epithelium and caused the occurrence of uterine lumen. Uterine epithelial cells in the implantation chamber and along the lumen were covered with abundant cytoplasmic bulbous but not microvilli.

The observations of this study on the general orientation of the blastocyst on day 5 of pregnancy in the rat uterus crypt were consistent with those of previously published (18). It was observed that the blastocysts on day 5 at 102 h of pregnancy were situated in the antimesometrial region of the uterine lumen. They lost their coverings (13), and were slightly elongated prior to contact with the endometrial epithelium. They were similar to an elliptical ring. Uterine luminal epithelium was present completely in both the mesometrial and antimesometrial region of the blastocyst (Fig. 1a, b). The blastocysts were encompassed by an elongated crypt epithelium. The uterine epithelium cells displayed local exo- or endo-cytosis where they were in contact the trophoblastic cells that were not observed to be phagocytosing uterine cells during this preimplantation period.

The embryonic pole of the blastocyst, was smaller in volume than the polar abembryonic region. As the blastocystic period advanced, especially at the late stages of this preimplantation period at same time, the blastocysts were flattened in the abnormal plane to the embryonic abembryonic axis (Fig. 1a). Some of the trophoblast cells of abembryonic pole were rounded but the majority of them were flattened and constituted a communication between the rounded cells.

Embryonic pole cells exhibited an irregular roundish shape. Three cell types were identified: hypoblast (presumptive endoderm), polar trophoblast cells and embryoblasts made up the inner cells mass (Fig. 1a, c). Embryoblast cells were bigger than the peripheral trophoblastic origin cells. Inner cells mass were surrounded by hypoblast and polar trophoblast cell lines.

#### **Mural trophoblast cells**

They had different shapes and structures, but no usual definitive microvilli on the external of interal free surface; with only very irregular and short cytoplasmic projections (Fig. 1a, 2, 3). Some trophoblast cells were high activated nucleate and at certain stages preimplantation many underwent mitosis.

The cytoplasm of some trophoblast cells of the blastocystic ring contained extensive deposits of lipid droplets and a moderate amount of rough endoplasmic reticulum and Golgi bodies were usually present (Fig. 2b). The trophoblastic cells contained mitochondria in two shapes. One type consisted of spheroids with a few mitochondria inner membrans. The other type had a more conventional appearance. Some of the dense mitochondria with few cisternae had diminished in number (Fig. 2a).

The cytoskeletal contents or fine fibrous material varied in amount, was dispersed through the cytoplasm and was more sparsely distributed.

#### **Connection complexes between mural trophoblast cells**

Many distinct junction complexes were observed at the apical and at the lateral cell boundaries of trophoblast cells. These complexes consisted of a region of close apposition

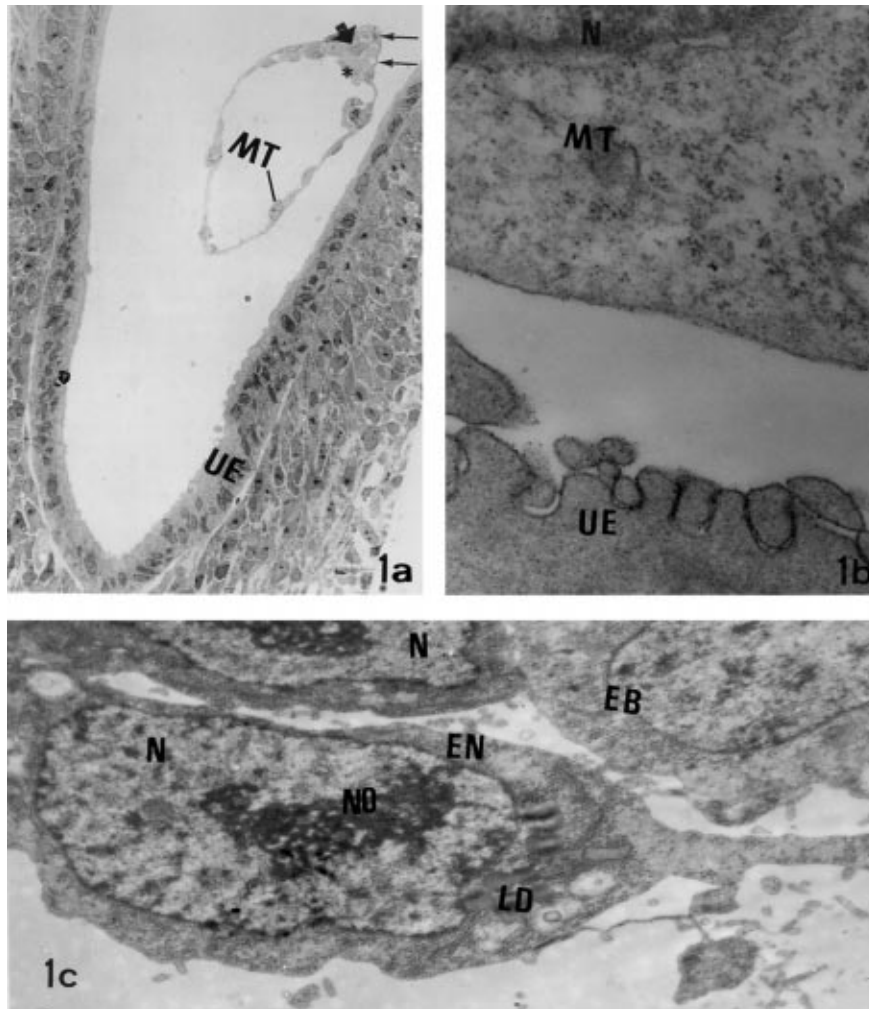


Figure 1. (a) The micrographic representation of the blastocystic ring during the initial stage of implantation. Orientation of blastocyst in uterine epithelium (UE) was noted with a light micrograph. Blastocyst taken from a rat on day 5 at the 102 nd h (after mating) of pregnancy. Embryonic and abembryonic poles of blastocyst are visible. Inner cells mass cells (with single arrow) surrounded by polar trophoblast (with double fine arrows) and presumptive endoderm cells (with asterisk). Several mural trophoblast cell (MT) types exhibiting different contents and associations with each other are seen in the blastocystic ring. (b) Structural differences on the surface of the uterine epithelium (UE) are seen before the blastocystic contact period. Some irregular cytoplasmic hills extending to the blastocystic ring are observed. (c) The region blastocystic neck connecting the mural trophoblast to inner cell mass cells. Presumptive endoderm (EN) (hypoblast) cells with bizarre shaped well developed endoplasmic reticulum, different condensation areas of cytoplasm, polysomes, glycogen particles and lipid droplets (LD), and their associations with the ectoderm (epiblast) cells (EB) are seen. N=nucleus, No=nucleoli, LD=lipid droplet. a: (x 625); b: (x22.500).

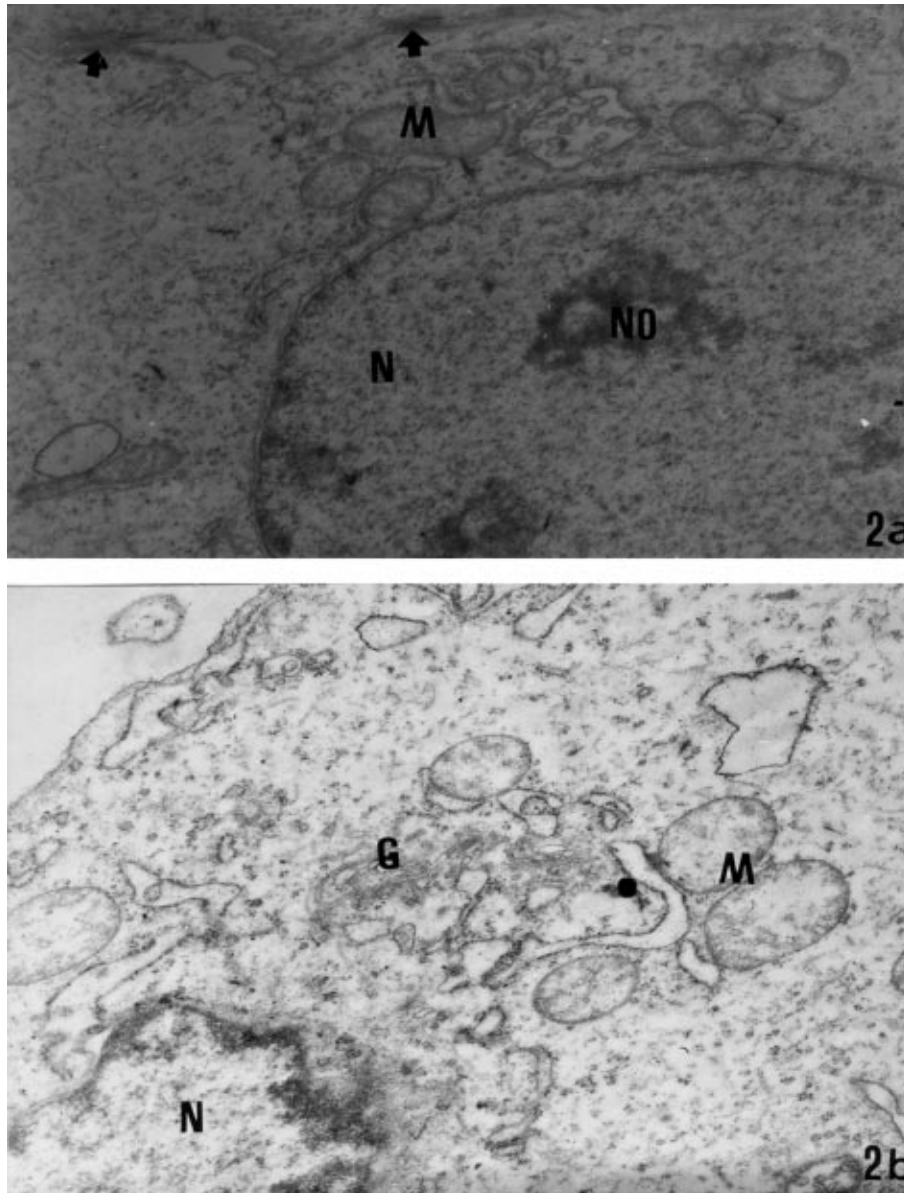


Figure 2. This figure shows mural trophoblast cells with a homogenous cytoplasm attached to each other by different connection complexes and junction adheres (a), and in the primitive junction (with arrows) exhibiting increased density in the adjacent cytoplasm in which a long line junction complex structure and regular shaped elliptical eucromatic nucleus (N), nucleoli (No) mitochondria (M), Golgi regions (G) (b) are seen. a and b: (x 17.750).

suggesting a tight connection structure between the cell membranes of the cytoplasm. Occasionally, individual junction complexes, desmosome-like structures were seen in the junction communication line at certain intervals. A lot of projections into the intercellular areas constructed a regular or irregular interdigitation connection complex found between blastocystic ring cells.

Cellular connected complexes were observed between trophoblastic cells in different three types: a) dense long line connection (Fig. 4a); b) desmosome-like structures (Fig. 3b, 4b); c) regular suture-like interdigitation resembling parallel railings (Fig. 4c). Many interdigitation complexes were observed between connecting and other trophoblast cells.

Junction complexes between trophoblast cell of the blastocystic ring had an occasional increased density of the cytoplasmic side of apposed and welded together cell membranes. Junction complexes at apical intercellular borders had associated intermediate filaments; these connected complexes were subtended by completely formed desmosome-like structures (Fig. 3b). Interdigitations exhibiting regular and also irregular arrangement with pits placed in their deep regions were distinctive and common among the interior intercellular border (Fig. 3b, 4b).

The first connection associated with the trophoblast and uterine epithelium was observed between two apical plasma membranes of a canalculated trophoblast cell of the blastocystic ring and a uterine epithelium cell by cytoplasmic protrusions forming a fusion event. Special connection complexes formed at these first contact areas. Both of the apical membranes were fused and welded to each other without any structural elements. After the contact events, some cellular debris was observed in the implantive space between the abembryonic pole and uterine lumen.

## Discussion

In our studies we described electron microscopically the trophoblastic cells with different shapes and structures and the diversification of blastocyst cells as elliptical or rounded, flattened trophoblast cells, lipid droplet-rich cells, homogenous and canalculated cells. We also found many distinct junction complexes at the apical and lateral boundaries of trophoblast cells.

We have previously shown that during preimplantation in rats, trophoblast cells exhibited different structural features according to their functional differences (24, 25). Apart from the dense long line connection complexes and desmosome-like structures between trophoblast cells, interestingly, many interdigitation-like complexes were observed. In contrast to previous descriptions (32), our results suggest that these complexes are not wider or narrower than each other, they are regularly arranged in parallel with regular areas and in regular thickness (25). Connected complexes are formed between the cells at early stages of embryogenesis and communication persists in most tissues throughout the development (33). The trophoblastic cells joined by specific junctions share their metabolites, inorganic ions which results in the coordination of cellular activities and a consequent elimination of differences between cells (34).



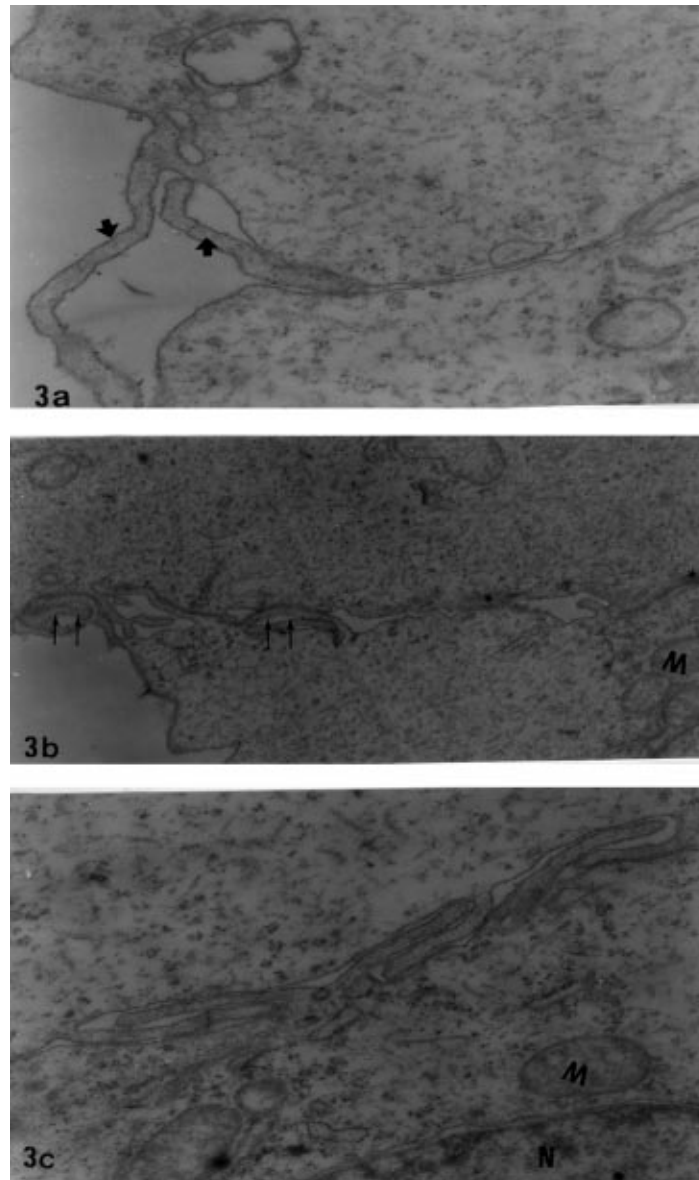


Figure 3. The formation of regular suture-like interdigitation resembling parallel railings between two mural trophoblast cells are seen step by step: (a) beginning formation by cytoplasmic projections (with single arrows), (b) developing step with cytoplasmic projections association (with double arrows) and junctional formation (with asterisk), (c) mature step with regular railing-like connection complexes. Cellular connections may change due to the implantation of the blastocyst and play an important role in blastocystic movement. N=nucleus, M=mitochondrion. a: (x35.000), b: (x17.750), c: (x35.000).

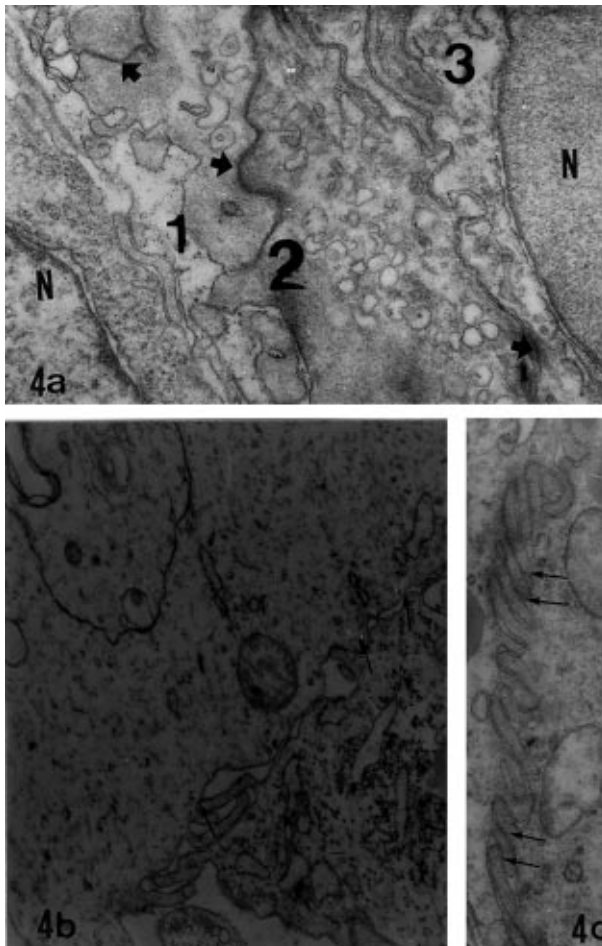


Figure 4. Section from a part of the blastocystic ring during the late stage of preimplantation period, at the 114th h, on day 5 of pregnancy is observed. Some mural trophoblast cells (1, 2, 3), have homogenous cytoplasm joined and connected with each other by three different junction complexes which are dense long line junctions (a) (with thick arrows), junction adheres (b) (with fine arrows) and cytoplasmic protrous (c) (with double arrows). In this rat blastocystic ring evidence of polarity of mural trophoblast cells is seen in the primitive junction exhibiting increased density in the adjacent cytoplasm with a long line junction complex structure (a). N=nucleus. a and c: (x20.750); b: (x17.750).

Junctional communication between trophoblast cells in the blastocystic ring results in the coordination of cellular activities and a uniform tissue phenotype. Cells in different regions of a tissue can be subjected to different homeostatic pressures. So different activities are more likely to be associated with cells in different conditions (35, 36). The trophoblastic cells in different regions of the ring may not only maintain different cytoplasmic contents of ions and other substances, but they may also use the same molecules for different purposes.

Analysis of the patterns of junction communication (26, 33, 34, 37, 38) in these structures have shown that: (i) cells in the lateral and abembryonic pole regions of the blastocystic ring communicate freely with each other, (ii) trophoblast cells immediately above these regions short-out intercommunication blastomers according to their respective differentiation density;

(iii) the differentiated trophoblast cells in the various poles of the blastocyst (39) either remain well coupled within their intercellular areas or become poorly coupled.

According to the results of our studies, connected complexes between the blastocystic ring cells are of three different types, they possess no structural similarities, but may exhibit certain physiological size exclusion limits. Furthermore, it appears that these three different cell-to-cell communication systems (26, 32, 37, 40) are involved in the molecular and biochemical coordination of trophoblastic cells within the specialised blastocyst.

Trophoblastic cells in first stage adhere to the uterine epithelium at some points by means of very special "connection complexes" resembling dense long lines and in the second stage trophoblast cells degrade the uterine epithelium and penetrate the epithelial basement lamina (27). During this stage, apart from 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 be established between the trophoblast and uterine cells.

Cell to cell interactions between the trophoblast and uterine epithelium may take place throughout three stages, (i) recognition and adhesion, (ii) engulfment and establishment, and (iii) control.

In conclusion, the cytological features of trophoblast cells suggested that the blastocystic ring cells consist of different trophoblastic types according to their structural architecture. Owing to these features, it is possible that they are divided into functional groups.

### **Acknowledgement**

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### **References**

1. Lopada AD, Kohlman D and Johnson I. Fine structure of normal and abnormal human embryos developed in culture. In: Beier HM and Lindner HR, editors. *Biological Basis and Clinical Applications. Fertilisation of the Human Egg in vitro*. Springer Verlag, New York 89–210, 1983.
2. Pijnenborg R. Trophoblast invasion and placentation in the human: morphological aspects. In: H–W Denker and JD Aplin, editors. *Trophoblast Research Vol. 4* Plenum Publishing Corporation 33–47, 1990.
3. King A and Loke YW. Uterine large granular lymphocytes: a possible role in embryonic implantation? *Am J Obstet Gynecol* 162: 308–310, 1990.
4. Enders AC and Schlafke S. Differentiation of blastocyst of the Rhesus monkey. *Am J Anat* 162: 1–21, 1981.
5. Enders AC, Hendrickx AG and Schlafke S. Implantation in the Rhesus monkey: initial penetration of endometrium. *Am J Anat* 167: 275–298, 1983.

6. Enders AC, Welsh AO and Schlafke S. Implantation in the Rhesus monkey: endometrial responses. *Am J Anat* 173: 147–169, 1985.
7. Denker H–W, Enders AC and Schlafke S. Bizarre hypertrophy of vascular endothelial cells in rhesus monkey endometrium: experimental induction and electron microscopical characteristics. *Verhd Anat Geslf* 79: 545–548, 1985.
8. Hearn JP. The embryo–maternal dialogue during early pregnancy in primates. *J Reprod Fertil* 76: 809–819, 1986.
9. Owiti GEO, Cukierski M, Tarara RP, Enders AC and Hendrickx AG. Early placentation in the African Green monkey (*Cercopithecus aethiops*). *Acta Anat* 127: 184–194, 1986.
10. Tarar R, Enders AC, Hendrickx AG. Early implantation and embryonic development of the baboon: stage 5, 6 and 7. *Anat Embryol* 176: 267–275, 1984.
11. Enders AC and Schlafke S. Implantation in nonhuman primates and in the human. In: *Comparative Primate Biology*. Vol. 3: Reproduction and Development. New York, Alan R Liss 291–310, 1989.
12. Enders AC. Current topic: structural responses of the primate endometrium to implantation. *Placenta* 12: 309–325, 1991.
13. Denker H–W and Gerdes H–J. The dynamic structure of rabbit blastocyst coverings. I. Transformation during regular preimplantation development. *Anat Embryol* 157: 15–34, 1979.
14. Denker H–W. Trophoblast–endometrial interactions at embryo implantation: a cell biological paradox. *Trophoblast Research* 4: 3–29, 1990.
15. Lieser R and Denker H–W. The dynamic structure of rabbit blastocyst coverings. II. Ultrastructural evidence for a role of the trophoblast in neozona formation. *Anat Embryol* 179: 129–134, 1988.
16. Marx M, Winterhager E and Denker H–W. Penetration of the basal lamina by processes of the uterine epithelial cells during implantation in the rabbit. In: H–W Denker and JD Aplin, editors. *Trophoblast Research Vol 4*, Plenum Publishing Corporation 417–430, 1990.
17. Denker HW Cell Biological view of embryo implantation (Review). *T J Med Sciences Suppl* 1–12, 1995.
18. Enders AC and Schlafke S. A morphological analysis of early implantation stages in the rat. *Am J Anat* 120: 185–226, 1967.
19. Schlafke S and Enders AC. Cellular basis of interaction between trophoblast and uterus at implantation. *Biol Reprod* 12: 41–65, 1975.
20. Welsh A O and Enders AC. Occlusion and reformation of the rat uterine lumen during pregnancy. *Am J Anat* 167: 463–477, 1983.
21. Fleming TP, Warren PD, Chisholm JC and Johnson MH. Tropoectodermal processes regulate in expression of the inner cells mass of the mouse expanding blastocyst. *J Embryol Exp Morph* 84: 63–90, 1984.
22. Demir R and Üstünel. Distribution of some enzymes in implantation site of pregnant rats. *Placenta* 10 (5): 457–458, 1989.
23. Demir R, Üstünel I, Demir N. Light and electron microscopical observations on cellular interactions during initial stages of implantation and trophoblastic invasion in rats. *Placenta* 10 (5): 464–465, 1989.

24. Demir R, Demir N, Üstünel I and Erbençi T. Cellular identification in blastocystic ring at the beginnings of implantation in rats. *Placenta* 12 (4): 380, 1991.
25. Demir R, Demir AY, George K, Erbençi T, Üstünel I, Demir N. Fine structure of cellular connections between trophoblast cells of blastocyst and uterine epithelium in rats. In: Magies–Magies L, Rodriguez MI, Rios A, Arias JM, editors. *Electron microscopy 92*, Vol. 3; Biological sciences. Granada 829–830, 1992.
26. Finbow ME, Thompson P, Keen J, Jackson P, Eliopoulos E, Meoghe R. A structural analysis of the molecules involved in gap junctional communication. In: Robards AW, Jongsma H, Lucas WJ, Pitts J, Spray D, editors. *Parallel in Cell to Cell Junctions in Plants and Animals*. Springer Verlag, Heidelberg 136–148, 1990.
27. Schlafke S, Welsh AO and Enders AC Penetration of the basal lamina of the uterine luminal epithelium during implantation in the rat. *Anat Rec* 212: 47–56, 1985.
28. Welsh AO and Enders AC Chorioallantoic placenta formation in the rat: I. Luminal epithelial cell death and extracellular matrix modifications in mesometrial regions. *Am J Anat* (2): 215–231, 1992.
29. Edwards RG Physiological and molecular aspects of human implantation. *Human Reprod.* 10 (Suppl 2): 1–13, 1995.
30. Levy JB, Johnson MA, Goodall H and Maro B the timing compaction: control of a major developmental transition in mouse early embryogenesis. *J Embryol Exp Morphol* 95: 213–237, 1986.
31. Karnovski, MJ A formaldehyd–glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* 27: 137A, 1965.
32. Swenson KI, Jordon RJ, Bayer EC Paul DL Formation of gap junction by expression of connexins in *Xenopus* oocytes cell pairs. *Cell* 57: 145–155, 1989.
33. Pitts JD. Junctional communication: the role of communication compartments in complex multicellular organisms. In: Robards AW, Jongsma HH, Lucas WJ, Pitts J, Spray D, editors. *Parallels in Cell to Cell Junctions in Plants and Animals*. Springer Verlag, Heidelberg, 58–79, 1990.
34. Pitts JD, Finbow ME and Kam E. Junctional communication and cell differentiation. *Brit J Cancer* 58: 52–57, 1988.
35. Kam E and Pitts JD. Patterns of junctional communication in skin. *J Invest Dermatol* 87: 748–753, 1986.
36. Kam E and Pitts JD. Tissue specific regulation of junctional communication in the skin of fetuses homozygous for the repeated epilation mutation. *Development* 107: 923–929, 1989.
37. Serras F, van den biggelaar JAM Progressive restrictions in gap junctional communication during development. In: Robards AW, Jongsma H, Lucas WJ, Pitts J, Spray D, editors. *Parallels in Cell to Cell Junctions in Plants and Animals*. Springer Verlag, Heidelberg 85–98, 1990.
38. Enders AC Morphological manifestations of maturation of the blastocyst. In: Yoshinaga K and Mori T, editors. *Development of Preimplantation Embryos and Their Environment*. Alan R Liss, New York 211–223, 1989.
39. Demir R, Çelebi A, Demir R. Morphometric analysis of blastocyst dimensions at day 5 of pregnancy in rat. *Tr. J. Med. Sciences.* 20: 159–163, 1994.
40. Ducibella T, Albertini Df, Anderson E, Biggers JD The preimplantation in mammalian embryo: characterisation of intercellular junctions and their appearance during development. *Develop Biol* 45: 231–250, 1975.