A SYMBIOTIC RELATIONSHIP BETWEEN THE BLASTOCYSTIC RING TROPHOECTODERM AND UTERINE EPITHELIUM AT THE BEGINNING OF IMPLANTATION IN RAT

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SUMMARY: Two major fundamental questions are suggested to clarify understanding of cell signalling between trophoblast and uterine epithelium.

1. Is there a pseudosymbiotic recognition reducible to simplify the sum of different implant cell (trophoblast), to receipt cell (uterine epithelium)?

2. Are the recognition signals responsible for these mutually compatible friend cell functions? Are these restricted periods of cellular invasion completely devoid of correlation between the two different types of cells involved in 'compatible interactions'? The studies in pseudosymbiotic systems between implantive tissues, which recognize and control the substance transfer as well as morphogenesis are only different aspects suggested by an interactive phenomenon.

The cytological features of trophoblast cells suggest that the blastocystic ring cells consist of different trophoblastic types according which they have been structurally organized. Probably the functional specialization of trophoblast cells perform: i) supporting, preventing and feeding functions; ii) signalization, polarization, depolarization functions between blastocyst and uterine epithelium; iii) immunological, accepting or rejecting and secretory functions.

According to the ultrastructural evidences, the blastocyst trophoblast cells, showing different features, suggest that structural changes occur in relation to their functions. We believe that the development of these functional properties, during the pre-implantation stage of blastocyst, will be protected as a glaze for new investigations. The trophoblastic cells in different regions of the ring can not only maintain different cytoplasmic contents of small ions and substantial molecules but they can also use the same molecules for different purposes.

Key Words: Blastocyst, trophoblast, implantation, pregnancy.

INTRODUCTION

It is generally accepted that the implantation morphologically consists of the establishment of contacts between the trophoblast cells of blastocystic ring and uterine epithelium. During this blasto-uterine interaction, blastocyst undergoes a series of physiological and developmental changes after formation of the blastocystic ring prior to contact with the uterine epithelium; many factors play specific roles in this stage of implantation.

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Formation and differentiation of the blastocystic ring, its ultrastructural and cytochemical aspects have been extensively studied by many authors in various species; in human (1-5), in primates (6-16), in rabbits (17-20), in rats and mice (21-33).

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 (20,34).

A modern direction in the molecular studies of the implantation involves the role of essential elements of the inflammatory process (34-43). The mutual recognition of the implanting embryo and uterus has primary importance in the first stages of implantation. It probably demands that uterine factors firstly 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.

Basic ultrastructural studies on the implantation in rats have been made (24-26,44) and given evidences that the critical step of blastocyst attachment to the uterine epithelium, chorioallantoic placenta formation including cell death, extracellular matrix and new vessels formation and decidual reactions take place on the 5th day after fertilization (15,24,44-46).

The obscurity about cellular identification of blastocystic ring trophoblast cells leads us to the assumption that their structural differentiations in the rat are not uniform. They should have some differences in their properties according to the functional performance during the initiation of blasto-uterine interaction. A series of studies (32,33,47-50) discussed the role of microfilaments for the development with regard to differentiation of the dependent change in cell polarity and possibly signalling among blastomers during preimplantation period in rodents.

MATERIALS AND METHODS

Sexually mature rats were kept in normal animal laboratory conditions and fed with prefabricate feeds. For every two female albino rats, whose oestrus were determined by a technician, one male was placed into the same cage in the afternoon.

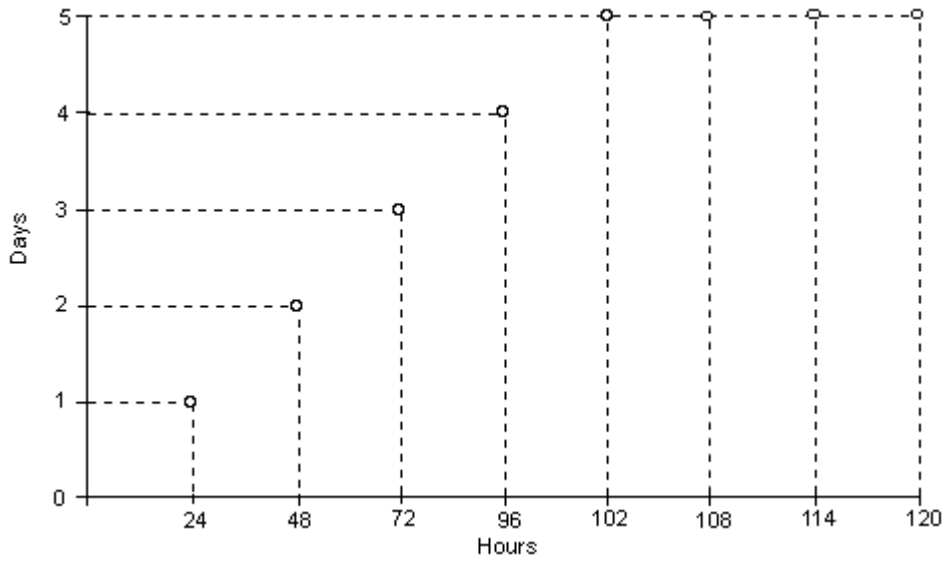
To observe the mating time, the animal laboratory was slightly illuminated and a small hidden camera was suitably placed. Identification of the female was accomplished by marking it with green and red dyes. During the night, animals were watched by the camera so that the copulation time was defined. The following 24 and 48 hours were naturally the first and the second days of the pregnancy. After 12 hours, their vaginal smears were examined. When sperms were found, they are isolated until the appropriate stage. In 8 of 12 animals, spermium was observed. 96 hours after the mating, beginning on day 5 of pregnancy, animals were anaesthetized and than 1% Evan's blue solution was injected via the femoral vein and after 15-20 minutes, abdominal aorta was clamped above and below the uterine region. Karnowsky's fixative solution (51) was then injected into the abdominal aorta while the animal was anaesthetized.

In addition to this process, same fixative solution was also injected into the uterine horns via the entry of uterus under a low pressure (4-5 ml/min). Blastocyst implantive areas were collected for every 6 hours systematically (Mating Time Table). The procedures mentioned above were repeated for later stage of blastocysts at 102nd, 108th, 114th and 120th hours of the gestation period.

Fixed pieces of perfused uterus exhibiting the 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 tissue specimens were rinsed overnight in 0.1M phosphate buffer, than post fixed in 1% Osmium tetroxide, in same phosphate buffer pH 7.3, for one hour. Tissue specimens were then dehydrated in a graded series of alcohol, finally passed through propylene oxide, embedded in Araldyte epoxy resin semithin and thin sections were taken with a Nova ultra tome. Semithin sections were stained with toulidine blue for light microscopic examinations. Thin sections were stained with uranyl acetate and led citrate. They were examined with a Jeol 100°C and a Philips 300 TEM.

Quantitative measurements were made by a micrometric scale attached to the microscope ocular. Analyses were made morphometrically on serial sections taken from blastocyst on the 5th day of pregnancy. Six sections were examined for every parameter and for each phase of gestation indicated in the Mating Time Table. The results obtained from technically unsatisfactory reproductions however were not included to the final evaluation.

The following parameters were utilized for this presentation: i) The cell numbers of the blastocystic ring, ii) the length of the blastocyst between embryonic and abembryonic poles, iii) the width of the blastocyst, iv) the width and length of the embryonic pole, v) the real number of embryonic cells and



Mating time table: Standardized 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 referred to as day 1. At the end of the 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).

their nuclei, vi) thickening of the uterine epithelium at the bottom and lateral walls of the cavity which has decidual reaction, vii) thickening of the uterine epithelium which has no decidual reaction on lateral walls.

These points are indicated in Figure 5. The quantitative results were evaluated by student-t test.

RESULTS

Orientation of blastocystic ring in uterine lumen

The blastocysts situated in uterine crept showed bulging trophoblast cells possessing irregular cytoplasmic protrusions and many small projections of various lengths. Trophoblastic cells close to the surface of the uterine epithelium and the affection of the uterine lumen to occur. Uterine epithelial cells in implantation chamber and along the lumen were covered with abundant cytoplasmic bulbous formations but not with microvilli (Figures 1A-B).

The observations of this study on the general orientation of the blastocyst on day 5 of pregnancy in the rat uterus crypt confirm the results of the previously published communications (21). It was observed that blastocysts on day 5 at 102nd hour of pregnancy are situated in the anti mesometrial region of the uterine lumen. They have lost their coverings (17), and are slightly elongated prior to the contact with endometrial epithelium. They are similar to an elliptical brewing ring. Uterine luminal epithelium was present completely both mesometrial and anti mesometrial regions to the blastocyst and reveal structural differences on the surface which are observed at Figures 1A-B. The blastocysts were encompassed by an elongated crypt epithelium. The uterine epithelium cells displayed local exo-or endo-cytosis where they contacted the trophoblastic cells that were not actually phagocytosing uterine cells at this pre-implantation period (Figures 2A,3A).

Embryonic pole of the blastocyst, volumetrically, is smaller than the polar abembryonic region. As blastocystic period advanced, especially at the late stages of this pre-implantation period the blastocysts are flattened in an abnormal plane to the embryonic abembryonic axis. Some of the trophoblast cells of abembryonic pole are rounded but more of them are flattened and constitute a communication position between the rounded cells (Figure 1A). The nearby embryonic pole cells show an irregular roundish shape. Three cell

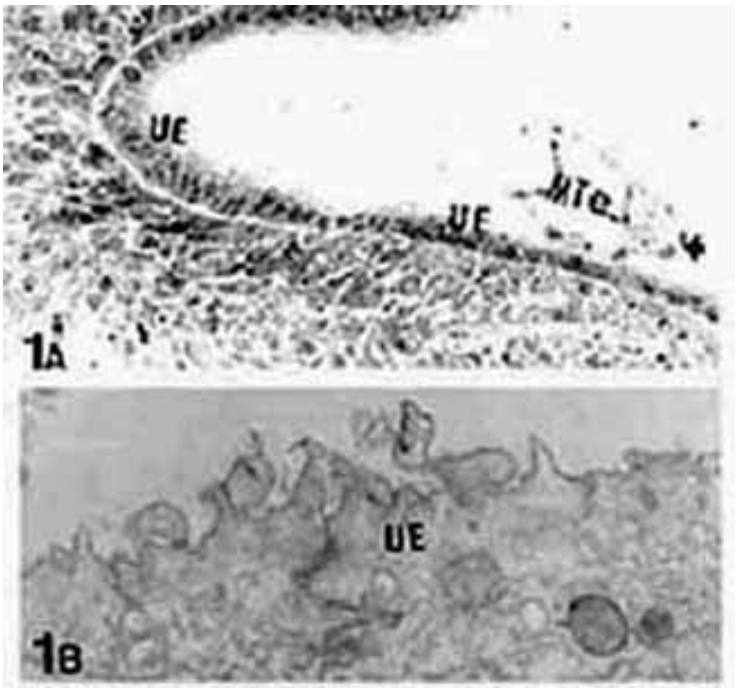


Figure 1: (A) The micrographic representation of the blastocystic ring during the initial stage of implantation. Orientation of blastocyst in uterine epithelium (UE) was noted with a light micrograph. Blastocyst from a rat recovered on day 5 at 102nd h (from the mating) of pregnancy. Embryonic and abembryonic poles of blastocyst are seen as well as the inner cell mass cells (with double arrows) surrounded by polar trophoblast (with single arrow) (out line) and presumptive endoderm cells (e) (inner line). Several mural trophoblast cell types (MT) showing different contents and association with each other are seen in blastocystic ring. (B) Structural differences on the surface of uterine epithelium (UE) are seen before blastocystic contact period. Some irregular cytoplasmic hills extending to the blastocystic ring are observed. A:(x 625); B:(x 22.500).

types are identified there; hipoblasts (presumptive endoderm), polar trophoblast cells and embryoblasts constructing the inner cells mass. Embryoblast cells are bigger than the peripheral trophoblastic origin cells. Inner cell mass is surrounded by hipoblast and polar trophoblast cell lines (Figure 1A).

Cytological features of mural trophoblast cells

Trophoblasts have different shapes and structures, but have not any usual definitive microvilli on external or internal free surface. They have only very irregular and short cytoplasmic projections. Some trophoblast cells have highly activated nuclei and at certain stages of pre-implantation many undergo mitosis.

The cytoplasm of some trophoblast cells of blastocystic ring contains extensive deposits of lipid droplets (Figure 2B) and moderate amount of rouge endoplasmic reticulum and Golgi bodies.

The trophoblastic cells contain mitochondria in two shapes. One type consisted of spheroids with a few mitochondria inner membranes. The other type has a more conventional appearance. On the other hand, the dense mitochondria with few cisternae had diminished in number.

The cytoskeletal contents or fine fibrous materials varied in amount dispersed through the cytoplasm.

Diversification of mural trophoblast cells

The diversification of cell types forming the abembryonic pole and lateral region of blastocystic ring is identifiable as folllows:

a) Elliptical or rounded cell type are situated

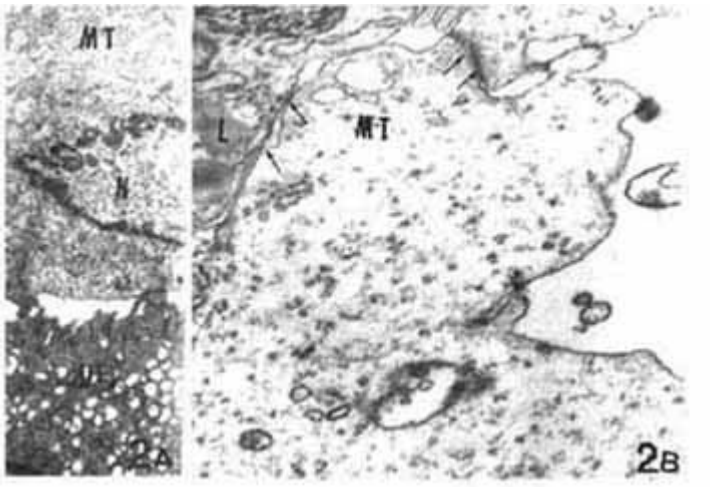


Figure 2: (A) A mural trophoblast cell (MT) established a contact point with uterine epithelium (UE) is seen. This figure is showing first communtication physically between blastocyst and uterine epithelium. (B) Two mural trophoblast cells, having a homogenous cytoplasm attached to each other by different connection complexes and junction adheres (with arrows) and in the primitive junction showing increased density in the adjacent cytoplasm in which along, line junction complex structure and have regular shape of lipid droplets is seen. A:(x 12.500), B:(x 17.750).

between two flattened cells and connected to each other laterally or in other positions by very long junction complexes (Figure 1A). Their nuclei are euchromatic, rounded and centrally situated. In these cells large cytoplasm areas are seen which are limited by a unit membrane, filled with some materials showing different condensations, for example regularly shaped tubules or multivesicular structures, electron dense homogenous materials, and irregularly shaped nonfibrillar plaques (Figures 3B, C). Some of them contain long-stick-like structures of homogenous substances. In these same cells very large vacuoles containing different materials have been observed. Mitochondria, with very few cristae, are also observed in different shapes. Endoplasmic reticulum clusters and Golgi complexes are also present.

b) Flattened trophoblast cells reveal evidence of communication between other trophoblast cells. Their cytological features are not very clear but within the cytoplasm many large and small vesicles, dense bodies and elongated mitochondria are observed. Flattened cells have, generally, a highly condensed cytoplasm with complexity of contents. Interestingly the first basal lamina is observed beneath them. These very flattened trophoblast cells are connected with the embryonic pole cells. Similarly, definite basal lamina showing a very fine dense line beneath the endoderm cells is also observed. Endodermal cells eventually form the Reichert's membrane while the basal lamina is originally formed by only the trophoblasts.

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

d) Homogenous cell types, having a homogenous cytoplasm, are devoid of cytoplasmic organelles. These are especially observed at the late stage on day 5, at 114th and 120th hours after the mating.

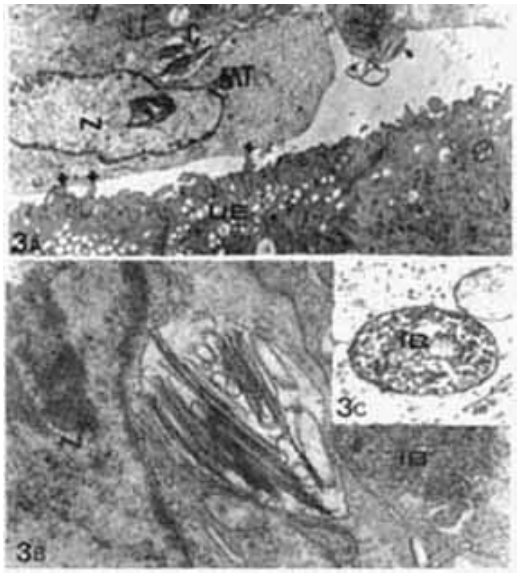


Figure 3: (A) Mural trophoblast cells (MT) situated opposite to the uterine epithelium (UE) are seen continuously. Every trophoblast cell is showing an effort for contacting at least some points with uterine epithelium. In this figure, blastocystic ring cell is attached to the lumine epithelium at different points. The membrane adherence are occurred between blastocystic ring cell and uterine epithelium (with arrows) are showing typical regions of attachment points on preimplantation period on day 5, at 114 th h after mating of pregnancy. Very interesting tubule-canalliculi complex (C) structures surrounded by a membrane borderline are observed at supra nuclear region of the connecting mural trophoblast cell and its high magnification is seen in figure 3B. Inclusion bodies (IB) are generally observed as dense aggregates of granules, membranes and vesicles in different forming conditions in trophoblast cells and its high magnification is seen in figures 3B and C. A:(x 12.500); B:(x 40.000); C:(x 22.000).

Homogenous trophoblast cells in which the storage materials have a less conspicious form a very fine granular composition and are devoid of organelles. The presence of similar material in baboon blastocysts has been considered as protein deposition areas (49). Some parts of cytoplasm of mural trophoblast cells and presumptive endoderm are occupied with similar homogenous storage materials. These areas of cytoplasm are largely devoid of organelles.

e) Chanalicculed cells showed abundant tubulovesicular mass limited by main border membrane and great complexity of internal organization, and well developed system of smooth surfaced tubule-chanaliculi structures (Figures 3A, B). These structural characters are especially observed only in these cells, contacting with uterine epithelium, which have only very thick and short cytoplasm projections but not microvilli and in neighbouring cells. The parts of cytoplasm apposed to the uterine epithelium are devoid of organelles.

Inclusion bodies are generally distributed in different cell types, especially in rounded and flattened cells during this period (Figures 3A, C). All types of trophoblast cells forming blastocystic ring have an extensive endocytic complex including coated pits situated deep of the cytoplasmic protrous on the surface and vacuoles, absorption tubules and multivesicular bodies isolated by a border membrane line in cytoplasm. The multivesicular bodies are striking in the mural trophoblast cells of the rat blastocyst and they were seen in three different forms; forming, maturing and denser. These evidences suggest that of the presence of formed phagozomic and digestive materials may pass the trophoblast without lysosomal activities. Because we have not succeeded to observe the lysosomes in cytoplasm.

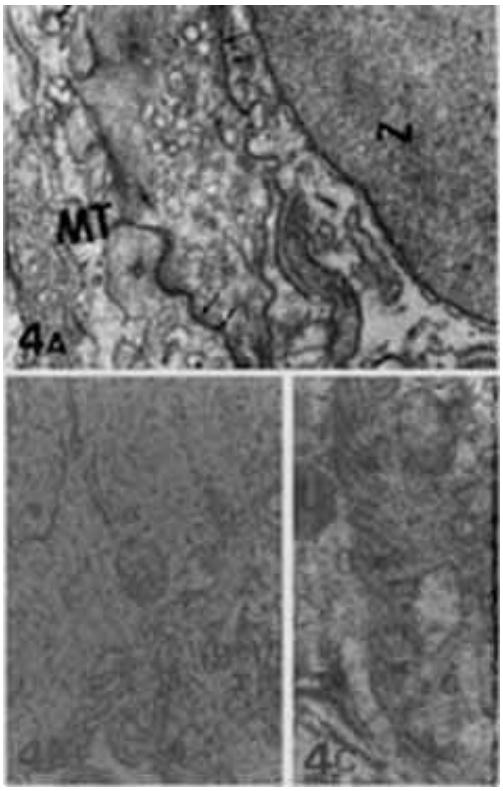


Figure 4: Cellular connected complexes between mural trophoblast cells (MT) in three different types are seen; dense long line connection (with double arrows) (A); desmosom-like structures (with single arrow) (B); regular sture-like interdigitation resembling a railing in situated parallel with each other (with asteriks) (C); many interdigitation complexes are observed between contacting and other trophoblast cells. Nucleus (N), granular endoplasmic reticulum (ger), mitochondrium (M), lipid droplets (L), A:(x 22.500); B:(x 12.500); C:(x 25.000).

Connection complexes between mural trophoblast cells

Many distinct junction complexes are observed at the apical and the lateral cell boundaries of trophoblast cells. These complexes consist of a region in close apposition suggesting tight connection structure between the cell membranes of the cytoplasm. Occasionally individual junction complexes, desmosome-like structures are seen in junction communication line by certain intervals. A number of projections into the intercellular areas constructed a regular or irregular interdigitating connection complexes are found between blastocystic ring cells.

Cellular connecting complexes were observed between trophoblastic cells in three different types: a) dense long line connections (Figures 2B, 3A, 4A); b) desmosome-like structures (Figures 2B, 4B); c) regular structures-like interdigitations resembling a railing in parallel conditions with each other (Figure 4C). Many interdigitation complexes have been observed between contacting and other trophoblast cells.

Junction complexes between trophoblast cells of blastocystic ring had an occasional increased density on the cytoplasmic side of apposed and welded with cell membranes were found. Junction complexes at apical intercellular borders had associated intermediate filaments; these connected complexes were subtended by completely formed desmosome-like structures. Interdigitations showing regular and also irregular arrangement with pits placed their deep regions were distinctive and common among the interior intercellular borders were observed.

First connection associated with the trophoblast and uterine epithelium was observed between two apical plasma membranes of chanaliculed trophoblast cell of blastocystic ring and uterine epithelium cell by cytoplasmic protrudes forming a fusino event. Special connection complexes form at these first contact areas (Figures 2A, 3A). Both of two apical membranes are fused and welded which each other without any structural elements. After the contact events, some cellular debris were observed interween implantive space occur between abembryonic pole and uterine lumen.

Quantitative analysis

The uterine epithelium changes are observed at different points and in different gestational hours, between the 102nd and 114th hours (Figure 6). The blastocystic ring cells are increased in parallel to the gestation time between the 98th and 102nd hours (Figure 7). The sizes of both trophoblasts and embryoblasts decrease with division.

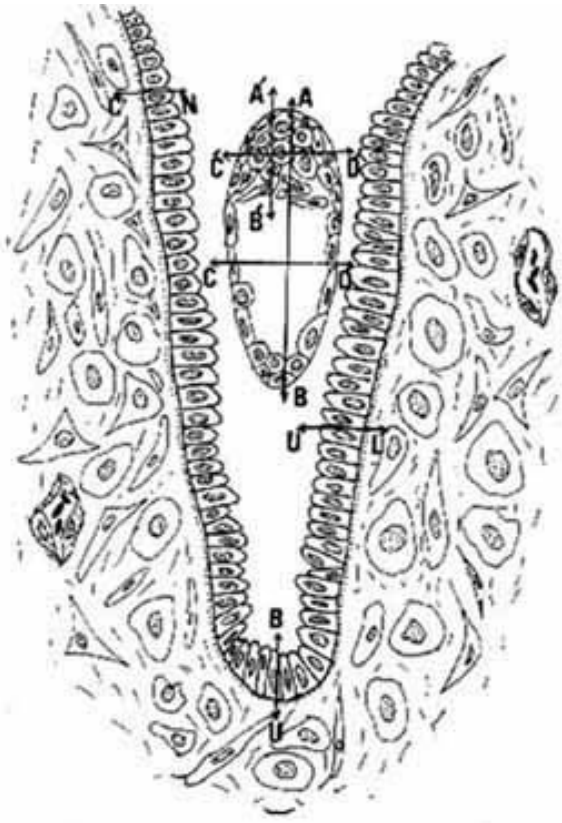


Figure 5: Blastocyst is situated in uterine lumen on day 5 of pregnancy. The measured points are indicated as follows. AB = the length of blastocystic ring; A'B' = the length of embryonic pole; CD = the width of blastocystic ring; C'D' = the width of embryonic pole; UB = uterine button (presence of decidualization); UL = uterine lateral walls (presence of decidualization); CN=control of uterine epithelium (absence of decidualization).

Cellular real values and nuclear real values of embryoblasts are also decreased (Figure 8). As a result of measuring the distances mentioned in Figure 5, the difference between the embryonic pole and the blastocystic ring becomes obvious (Figure 9).

DISCUSSION

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

We have previously shown that during the preimplantation in rats' trophoblast cells reveal different structural features in accordance with their functional differences (31-33). Except the dense long line connection complexes and desmosome-like structures between trophoblast cells, interestingly, many interdigitation-like complexes have been observed. In contrast to previous descriptions (52), our results suggest that these complexes with regular distances in between each very regularly arranged in parallel condition (32). Connecting complexes are formed between the cells at early stages of embryogenesis and communication persists in most tissues throughout the development (53). The trophoblastic cells joined by specific junctions share their contents of metabolites, inorganic ions, which result in the co-ordination of cellular activities and a consequent elimination of differences among cells (54). Junctional communication between tro-

phoblast cells in 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 (54,55). 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 (53,57,58) in these structures has 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 either remain well coupled within their intercellular areas or become poorly coupled.

According to the results of our studies, 'connecting complexes' between the blastocystic ring cells are three different types. They posses no structural similarities, but may exhibit certain exclusion limits in physiological functions. Furthermore, it appears that these three different cell-to-cell communication systems (52, 57,58) are involved in the molecular and biochemical co-ordination of trophoblastic cells within specialized blastocysts.

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.

Trophoblastic cells in first stage adhere to the uterine epithelium on some points by means of very special 'connection complexes' resembling dense long-line and with each other, in second stage trophoblast cells degrade the uterine epithelium and penetrate the epithelial basement lamina (24). During this stage, except any digestion process, trophoblastic bridges establish a connection with the step in a dynamism of penetration. During this process, there is not only a cellular digestion, but a symbiotic relationship may be established between trophoblast and uterine cells.

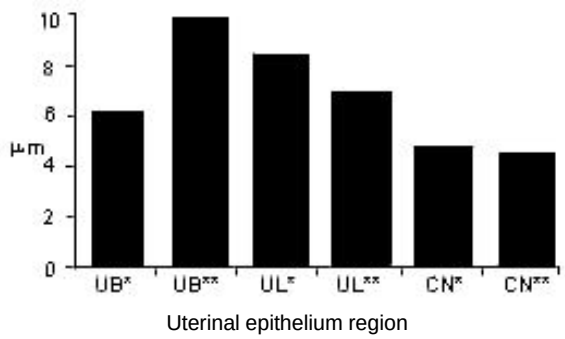


Figure 6: Comparison of uterine epithelium thickness between 102nd and 114th h of pregnancy on day 5. UB* = uterine button at 102nd h, UB** = uterine button at 114th h, UL* = uterine lateral walls at 102nd h, UL** = uterine lateral walls at 114th h, CN* = control at 102nd h, CN** = control at 114th h.

An interesting phenomenon of blastocystic ring development is the formation of the first contact points between the abembryonic pole and uterine epithelium. Some specialized trophoblast cells, having canalicular systems and cytoplasm devoid of organelles, attached appropriately in arrangement with blastocyst to uterine epithelium. These cells participating in the pre-implantation contact processes have no microvilli on their surface opposite to the uterine epithelium, the part of their cytoplasm associated with the contact points is devoid

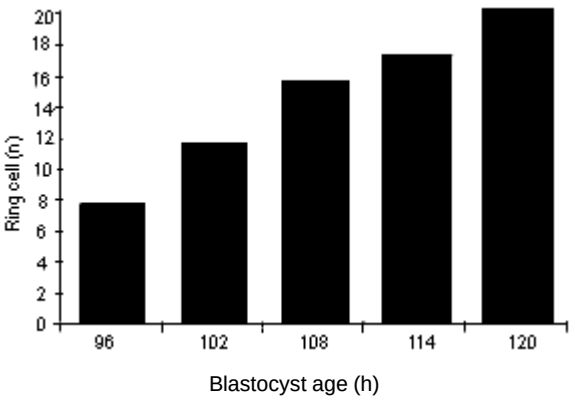


Figure 7: Comparison of the cell numbers in blastocystic ring between 96th and 120th h of pregnancy.

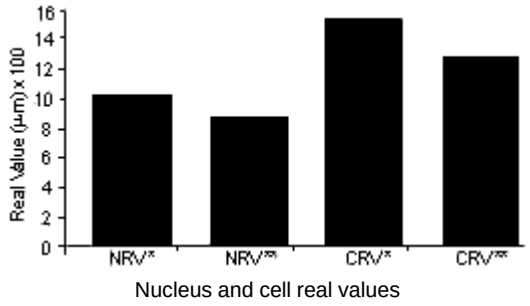


Figure 8: Comparison of cellular and nuclear real values of embryoblast cells between 102nd and 114th h of pregnancy. NRV* = nuclear real value at 102nd h, NRV** = nuclear real value at 114th h, CRV* = cellular real value at 102nd h, CRV** = cellular real value at 114th h.

of organelles, but interestingly they have very special tubule-chanalicular systems. First contact points and a mutualist relation, a pseudosymbiosis for a limited period, is established between the blastocyst and uterine epithelium by means of this specialized cell types.

We address two major questions fundamental in the understanding of cell signalling (59) between trophoblast and uterine epithelium: *(i)* is there a pseudosymbiotic recognition reducible to simply the sum of implant cell (trophoblast) and receipt cell (uterine epithelium), *(ii)* are the recognition signals responsible for these mutually friend cells' compatibility? Are these periods of invasive cellular actions and communication compatible interactions or are they completely lacking in correlation? Investigation of the phenomenon of

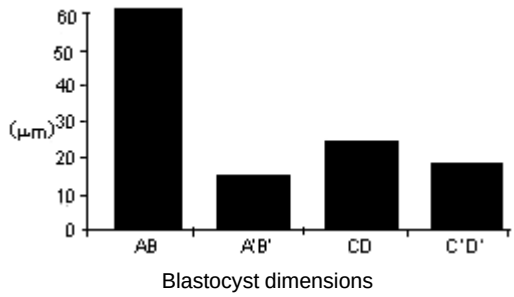


Figure 9: Blastocyst dimensions according to the measured points indicated in the figure 5, at 102nd h of pregnancy.

pseudosymbiont recognition and the establishment and maintenance of a pseudosymbiosis thus relates to a particularly complex experimental system that raises considerable complications in the laboratory work (59,60).

The studying of pseudosymbiotic system between implantive tissues that recognize the control of substance transfer and morphogenesis are only different aspects suggested on an interactive phenomenon. According to the literature knowledge of the cell biology (59,61) of pseudosymbiosis for host tissue could be proceeded by investigation of the structure and dynamics of their surfaces and current assessments may be made of their molecular organization throughout the interaction during initial stage of pre-implantation (39,40).

Understanding of signalling between the partner tissues depends on recognition of the existence of stimuli that are significant for the establishment of reciprocal messages (61). The significance of these interactions and their correlation with signalling mechanisms are presently poorly understood. Signalling between the blastocyst and its maternal tissue is generally presumed to be important for 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 signals received by specific proteins or glicoprotein membrane receptors responsible for activating the signal information (34,36,39,41,61,64). It was suggested that it should be possible to use some specific uterine protein production to characterize the signals from the embryo and it may be concluded that the blastocyst synthesizes and releases some soluble different factors during pre-implantation period (64,65).

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

According to the extensive studies, it appeared that the metabolic capacity of the blastocystic cells and that of the uterine epithelium, participate to the removal of the signalling barrier which is effected by metabolizing or by converting substances by the two communicating cells. Briefly, blastocyst cells may have an asymmetry in the location of enzymes, carriers, and receptors on maternal (outer) side and foetal (basal) side plasma membranes. The functional polarity of blastocyst cells is expressed by the changes of cellular metabolism, and transport of nutrients and ions and most of its secretions, which carry information which may be accepted or rejected by the endometrium and vice versa under every condition. The interfaces of cells contacting with each other must be continuously kept under control via stimuli that regulate its compatibility and substantial exchanges. The cell surface glycoproteins, colloidal iron and cationized ferrite associated with the reduction in surface negative charge were discussed in the affect of blastocyst initial stage of implantation (17,34,36,41). The contact between the surface of both cell types may be via adherence type junction (32,33,65). We have observed and described in detail that the areas of fusion of trophoblast knobs with uterine epithelial cells at the beginning of the area of contact and through implantation. These findings were in agreement with the SEM and TEM results reported by different investigators (9,33,44,66-68).

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

Some findings on the hypertrophy of uterine epithelial cells to form the uterine thickness, according to Enders (16) and Schlafke (23) named 'uterine plaque' in the rhesus monkey, are also observed at the beginning of the pre-contact phase of implantation in rat uterus.

Before the implantation, the blastocysts differentiated into mural, polar trophoblast cells and embryonic pole including embryoblasts, polar trophoblasts and endodermal cells. Differentiation of endoderm into the visceral and parietal portions consists of individual structures, stellate-like cells with numerous cytoplasmic projections and filopodia were observed using scanning electron microscope (32). Finally, during the implantation of blastocyst, two endodermal derivatives have been described presenting with diverse characteristics and associated with different functions. During the observations of the blastocyst differentiation in our studies, many cell debris with different contents and various sizes were observed in some blastomers especially among the mural trophoblast cells forming blastocystic ring. Sometimes, trophoblast cells near the uterine epithelium contained serial degenerative remainders. These ultrastructural findings suggested that death may be necessary for some cells during blastocystic differentiation.

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.

Probably functional specialization of trophoblast cells are; *(i)* supporting, preventing and feeding functions; *(ii)* signalization, polarization-depolarization functions between blastocyst and uterine epithelium, *(iii)* immunological, accepting or rejecting and secreting functions, *(iv)* current contact taking place between two implantive members with cytoplasmic membrane fusion, *(v)* a pseudosymbiosis, established between these two members implantation for a limited period.

Of course, most of these interpretations need more studies to be clarified out. According to the ultrastructural evidences, the blastocyst trophoblast cells, showing different features, suggest structural formations according to their functions. We believe that the procedure of these functional properties, during the pre-implantation stage of blastocyst, will be protected as a mystery awaiting new investigations.

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