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Comparative evaluation of ultrastructural modifications & changes in the interhaemal membrane of the short-nosed fruit bat, *Cynopterus sphinx gangeticus* during early (trilaminar, neural groove) & full-term stages of development

Deshbhratar Shantaj M.[1], Mahaley Jyotsna A.[2], Raut Sonali R.[1], Hile Vijay K.[3] and Bhiwgade Dayanand A.

[1]*Research Laboratory, Dept. of Zoology, Bharatiya Vidya Bhavan's H. Somani College, Mumbai*
[2]*Department of Zoology, Vartak College, Vasai*
[3]*Department Botany, Bharatiya Vidya Bhavan's H. Somani College, Mumbai*

ABSTRACT

The present investigation deals with the holistic information & knowledge of the interhaemal membrane of the megachiropteran bat, Cynopterus sphinx gangeticus during early (trilaminar, neural groove) & full-term stages of development. The morphology and histology of the definitive placenta of Pteropodidae bat, Cynopterus comprised of a discoidal, symmetrical and labyrinthine with haemodichorial condition. The orientation of the embryonic mass of the blastocyst was mesometrial and the attachment of the blastocyst being circumferential along with superficial attachment. The ultra-structural studies on the interhaemal membrane reflected that, initially the placenta was endotheliochorial in origin with the presence of maternal endothelium that persisted till the neural groove stage. The maternal endothelium was well-developed with large irregularly shaped blood lacunae which beared a unicellular layer of endothelial cells and later eroded & the maternal blood space came in direct confluence with syncytiotrophoblast. Free-floating amoeboid-shaped cells were also noted in the Maternal Blood Space. The interstitial membrane became highly inconspicuous by the subsequent developing stage. Most uniquely the intra-syncytial lamina was absent and later noticed only at few sites as a double-layered discontinuous membrane with homogenous maternal inside during the full-term stage. As such the conventional history of conversion of the interstitial membrane into intra-syncytial lamina did not hold true in this case. Occasional "Blebbing" or ballooning of the intra-syncytial lamina was also noted. The cytotrophoblast immensely vacuolated and sometimes it was such greatly attenuated at sites that it resembled a haemomonochorial condition with the presence of only syncytiotrophoblast. Certain binucleate cytotrophs or Giant cells were also observed. The confirmative absence of maternal endothelium and the presence of syncytio & cytotrophoblast at full-term stage resolved that the placenta is haemodichorial and thus the ambiguity of it being haemomonochorial or haemodichorial is finally resolved.

Keywords: Maternal Endothelium, Maternal Blood Space, Syncytiotrophoblast, Cytotrophoblast, Cytotrophs, Blebbing

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INTRODUCTION

The sub-order Megachiroptera includes a single family, Pteropodidae; of the 42 genera and 166 species of this family [20], only the placentae of Rousettus leschenaulti [18] and Pteropus giganteus [23]have been examined ultra-structurally. Light microscopic studies were focused on Cynopterus sphinx, C. marginattus [2] [10]

[23] [13] [14] and Pteropus giganteus [1][2][23][12][13][14]. Early trophoblastic development in three other Cynopterine species has been reported in the evaluation of the same at the light microscopic and electron microscopic level.

The term placenta of Pteropus & Cynopterus has been described as being endotheliochorial at the light microscopic level by Gopalakrishna & Karim, [13], whereas according to the more recent findings of Karim & Bhatnagar [23] it was hemochorial in Pteropus, while the earlier stages of placentation i.e. until midgestation in Rousettus leschenaultia had consistently been considered to be endotheliochorial by Gopalakrishna & Karim [14] but Bhiwgade [26] reported that the placenta of Rousettus leschenaulti as very unique in being hemochorial from the very beginning of functional placentation with the notable exception of the early trilaminar blastocyst stage. In the past, such discrepancies at the light microscopic level were settled by reappraisal of the same at the electron microscopic level, which was then assessed as being final[27].

The general trend during placental development may be that a typical endothelial – chorial placenta develops first and subsequently changed into a hemochorial condition[31][35]. According to the Enders and Wimsatt, [7], the cellular transformations that take place during implantation and the general tendency for reduction in thickness of the layers interposed between the fetal and maternal circulation during gestations combine to make observations of the relationship between the fetal and maternal elements difficult to resolve in many of the placental types. The present investigations of the interhemal membrane of the Mega-chiropteran Pteropoididae bat, Cynopterus sphinx gangeticus have been undertaken at electron microscopic level in order to extend and elaborate the cytological information and an attempt to reveal the structural and cellular changes during the different stages of development.

MATERIALS AND METHODS

The collections of these bats was done periodically from the caves, forts and trees along the hilly regions of Trichur, Kerala situated in South India, commencing from the first day of implantation until the term development during the period November 1994 – April 2002.

The pregnant females were brought to the laboratory in live condition in finely meshed metallic wire cages. The cages were covered with slightly wet gunny bags to regulate the temperature of the surroundings. The cages were placed in dark room and the food comprising ripe fruit, insects, etc. and water was provided at regular intervals. The animals were dissected open under ether anaesthesia and the placental tissues were removed and immediately placed in fixative and processed further for electron microscopic studies.

The placental tissues were removed and fixed with 3% Glutaraldehyde for 3 hours. They were washed thrice in 0.1M Cacodylate buffer and post fixed in 1% Osmium tetroxide for 2 hours at 4^0C. The tissues were rapidly dehydrated in graded alcohols and Propylene Oxide and embedded in Araldite resins. Three blocks of each tissue were sectioned with glass knife. Ultrathin sections were cut on 2000S Ultra-microtome from selected areas. The sections were mounted on 400µ mesh copper grids and stained for 20 minutes in 10% Uranyl Acetate and for 10 minutes in Reynolds Lead Citrate. Sections were examined and photographed under JEM JEOL 100S Electron Microscope[28].

RESULTS

Maternal Blood Space: During the trilaminar and neural groove stage the cytoplasm was smooth without any microvillus or podocytic vesicles or protrusions. The underlying lacunae present beneath the free surface at term, imparted a percolated appearance to the same.[Fig. 1,2,3,6,8,& 9]

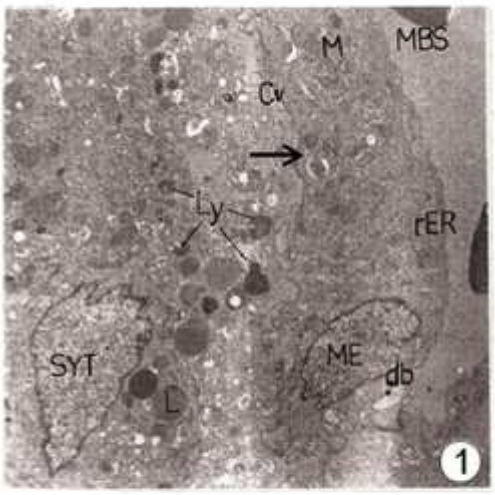
Maternal Endothelium: The maternal endothelium was well developed with large irregularly shaped blood lacunae and lasted till the neural groove stage. The ME showed numerous mitochondria, lysosomes, dense bodies, etc. and rested on an inconspicuous interstitial membrane.[Fig. 1,2,3,4]

Interstitial Membrane: It was untraceable, but a discontinuous intrasyncytial lamina was noted only at few sites during full-term stage. The ectoplasmic layer was also not evident.[Fig.1,4,6,7,9]

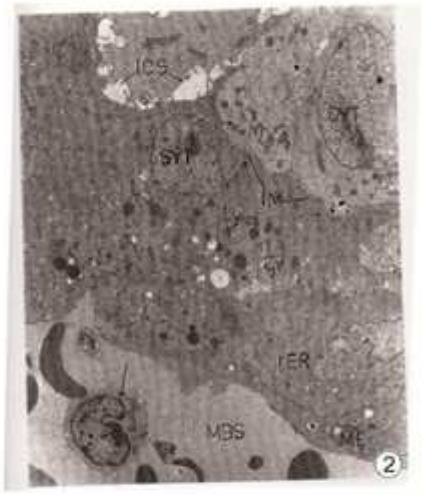
Syncytiotrophoblast: It was extremely electron dense much more than cytotrophoblast and bore mitochondria, lipids, r-ER and other cellular inclusions like lysosomes, glycogen granules, coated vesicles, polyribosomes, etc. Very peculiar desmosomal connections, intra syncytial tracts & intrasyncytial desmosomes were noted and thus the syncytiotrophoblast was referred to as 'coarse', 'interlobular' or spongy. Fenestrations or lacunae formation imparted an almost "lace-like" filigreed appearance.[Fig. 1to 9]

Electron Micrographs of Cynopterus sphinx gangeticus – Early Developments

Trilaminar Blastocyst



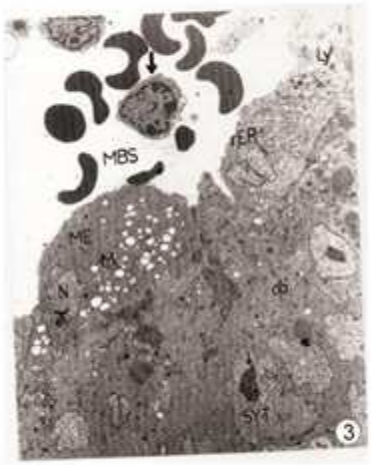
1. X8000 ME rests on interstitial membrane (inconspicuous)



2. X2500 Two trophoblastic layer (SYT & CYT) free floating cells & IM showing foldings

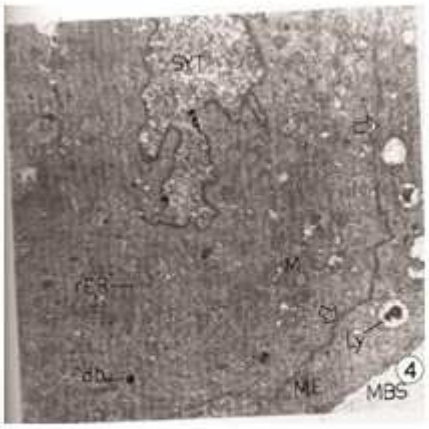
Presence of cell organelles like mitochondria , lysosomes, RER, lipids, etc.

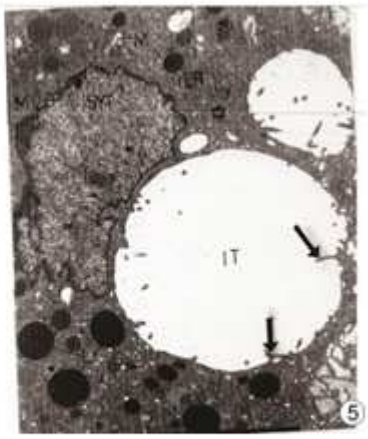
Neural Groove stage



3. X 2000 ME with distorted mitochondrial cisternae and free floating cell

NEURAL GROOVE STAGE



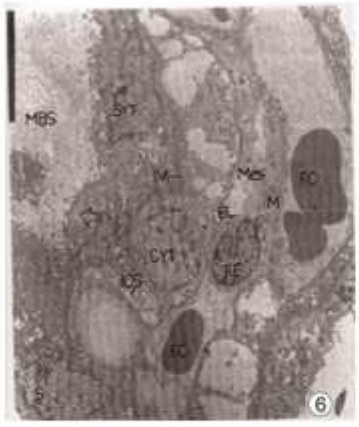


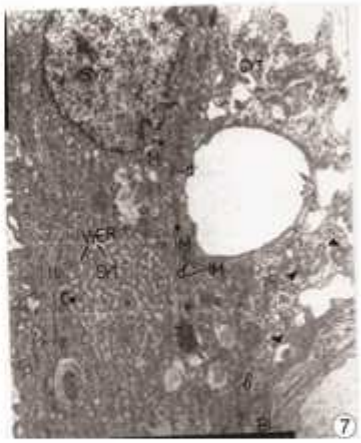
4. X10000 SYT with rER, db and prominent ME

5. X15000 SYT with IT,lipids

Presence of cell organelles like Golgi, MVB, db, rER, Nucleolus etc.

Full Term Development





6. X2500 CYT being vacuolated with ICS at sites. SYT with intrasyncytial blebbing

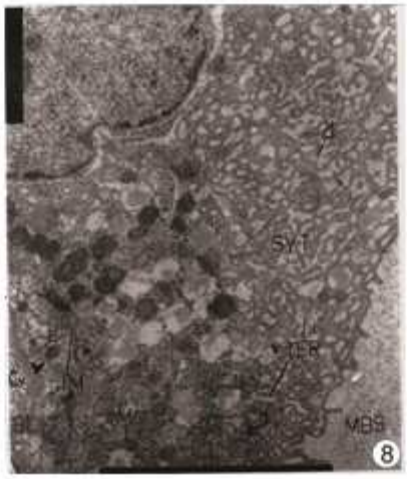
7. X8000 Porous and spongy SYT with VrER with discontinuous IL& BL with podocytic modifications

Interdigitating membrane: It is distinct double membrane structure that ran along the two trophoblastic layers and was encountered from trilaminar blastocyst stage.

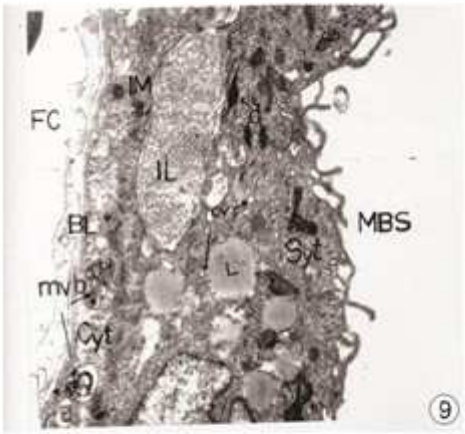
The interdigitating membrane bore brush-like fibrillar elements along its border that imparted fuzzy appearance.[Fig. 6,9]

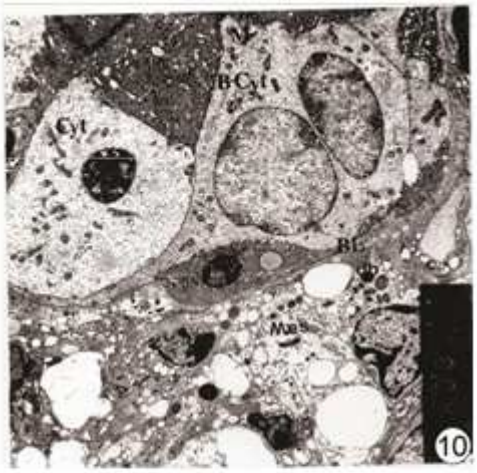
Cytotrophoblast: The cytotrophoblast projected compartmentalization with well- defined membrane limits that enclosed the surrounding cytoplasm and clearly distinguished them. A pecularity inherent was the continuity of intratubular canal system right from digitating membrane up to the basal lamina at term stage, which also bore few desmosomes. A few bi-nucleated Giant cells or cytotrophs were also sighted.[Fig. 1,2,3,6,7,9,10]

FULL TERM STAGE



8. X8000 Extremely porous SYT with intrasyncytial Desmosomes. IM shows loops .





9. X16500 SYT with ILshowing matrix, thin CYT with MVB, BL, etc.

10. X5000 IM and BL prominent CYT, shows Giant cells.

Presence of cell organelles like mitochondria, rER, vrER , dense bodies, Golgi, MVB, lipids, coated vesicle, B Cyt.(Giant cell)

Basal lamina: The cytotrophoblast rested on a distinct double membrane viz. basal lamina bearing a homogenous electron dense matrix within their limits. It appeared constant, even-width along its entire length with few podocytic modifications.[Fig.6,7,8,9,10]

Foetal endothelium, Foetal capillaries: Foetal capillaries were edged out by the basal lamina and foetal endothelium . FC bore large nucleus and were embedded in mesenchyme.[Fig. 6,9,10]

DISCUSSION

Every layer of the interhemal membrane had well defined activities/function, but most important being the enhancement of foetal survival through transport of various substances in both directions [28]. In addition, the morphological modifications such as coarse syncytium and indentations were measures for improving transplacental efficiency and foetal survival[22][32].

The maternal blood spaces represented by large bays of lacunae were lined by irregular layer of cytoplasm and the microvillus profile of luminal syncytiotrophoblastic surface area that edged the MBS was a morphological modification for increased surface area[34][36]. The free-floating cells, although maternal in origin did not really account for the endotheliochorial condition [8].

The maternal endothelium had not only being restricted to performing active reabsorption[35] but along with mitochondria, r-ER and Golgi it was believed to be associated with protein synthesis [16] [17]. The interstitial membrane comprised an a cellular homogenous content and supported the maternal endothelium apart from the possible immunological isolation role of foetal and maternal components [5][7].

In addition to amplifying the apical plasmalemma of syncytiotrophoblast for absorption and secretory purposes selective filtration, structural support, establishment and maintenance of cell polarity and tissue differentiation [25][35], the intrasyncytial lamina also contributed to the nutritional requirement of the developing foetus [10]. The tubular r-ER enlarged and modified into vesiculated form that made the syncytium "coarse" and "spongy" thereby increasing the surface area of exchange of materials.

Further the syncytial modifications provided absorptive surface for improved efficiency of both intra and inter cellular transport within the ground matrix in addition to the draining of maternal blood from placenta[33][29]. The intrasyncytial desmosomes prevented the syncytium from collapsing [3][[15][19][21].

The interdigitating membrane was formed at the confluence of the basal portion of the syncytiotrophoblast and the apices of underlying cytotrophoblast which probably lent a structural support to the framework [15].

The presence of various cell organelles showed active transport and protein synthesis[18]. The cytotrophs maintained connections with each other via junctional complexes and desmosomes. The intracellular spaces seemed to offer a free pathway from the trophoblastic basement membrane to the syncytium [6], while the irregularity of plasma membrane allowed an increased area of exchange between trophoblastic cell and the space utilized for elimination of secretory products[28]. The Giant cells together with syncytiotrophoblast played an important role in selective phagocytic erosion of the maternal tissue essential for placental growth and steroid production [4] [21].

The basal lamina served to separate the trophoblastic complement of the interhemal barrier from the foetal side and the modifications/ infoldings helped to reduce diffusion distance & time and as such increased the bi-directional tranplacental transport and exchange efficiency between maternal and foetal blood circulation [27].
The indentation of foetal capillaries facilitated and enhanced foetal/ neonatal survival[9][24].

CONCLUSION

The presence of maternal endothelium up to the neural groove stage and its subsequent loss, with the presence of two trophoblastic layers i.e., syncytiotrophoblast and cytotrophoblast at term, confirmed the definitive placentation to have changed from endotheliochorial to haemodichorial condition. Further higher animals with haemochorial at term stage needs to be investigated at ultrastructural levels to confirm and resolve any ambiguities on the persistence of any endotheliochorial condition at the early trilaminar blastocyst or neural groove stage.

Abbreviations:

1. ME – Maternal Endothelium *2. SYT – Syncytiotrophoblast*
3. CYT – Cytotrophoblast *4. IM – Interdigitating membrane*
5. BL – Basal Lamina *6. FE – Foetal Endothelium*
7. FBV – Foetal Blood Vessel *8. MBS – Maternal Blood Space*
9. IL – Intrasyncytial Lamina *10. FC – Fetal Capillaries*
11. MVB – Multivesicular bodies *12. d – Desmosomal connections*
13. L – Lipids *14. Lys – Lysosomes*
15. Mes – Fetal mesenchyme *16. db – Dense bodies*
17. cv – Coated vesicles *18. G – Golgi bodies*
19. M – Mitochondria *20. N – Nucleus*
21. Jc – Junctional complex *22. r-ER – Rough Endoplasmic Reticulum*
23.v-ER – Vesiculated Endoplasmic reticulum *24. ► – Podocytic modifications of interdigitating membrane*
25. ➔– Interstitial membrane *26. – Interdigitating folds*
27. ICS – Intracellular space *28. - Free floating cells*
29. IT – Intrasyncytial tract *30. B-Cyt – Giant cells / Binucleate cytotrophs*

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