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Ultrastructural investigations at varied stages of development in the interhaemal membrane of Thyropteridae bat, *Tylonycteris pachypus*

Jyotsna A. Mahaley[1], Shantaj M. Deshbhratar[2], Sonali R. Raut[2] and D. A. Bhiwgade[2]

[1]Department of Zoology, Vartak College, Vasai road (W), Dist- Palghar
[2]Department of Zoology, Bhavan's H. S. Somani College, Chowpatty, Mumbai- 07

ABSTRACT

Present investigations of the interhaemal membrane of thyropterid bat, Tylonycteris pachypus, at an electron microscopic level through the different stages of development have been observed. At the early stages of gestation, distinct maternal endothelium is distinctly observed along with compactly arranged cytotrophoblastic layers followed by syncytiotrophoblast. At this stage of development, cytotrophoblastic layer bears spongy rough endoplasmic reticulum and well developed concentrically arranged Golgi Complexes in few numbers. The maternal endothelium is absent at mid and term stage of placenta. The enclosed lacunae of the maternal blood space comes in direct contact with syncytiotrophoblast i.e. ectoplasmic layer followed by discontinuous intrasyncytial lamina in the syncytiotrophoblast. The syncytiotrophoblast appears spongy due to the presence of modified vesiculated rough endoplasmic reticulum. The cytotrophoblastic basal lamina displays modified podocytic specialization with few desmosomal connections. Glycogen rosettes at the periphery are commonly seen in both the trophoblastic layers. At places there is a considerable attenutation of both layers into thin phalanges, bringing the maternal blood space and foetal capillary closer thereby minimising the thickness of the interhaemal barrier. Absence of maternal endothelium and presence of both the trophoblastic layers at term designates the placenta to be haemodichorial that has been holistically discussed in these contents.

Key words: Placenta, Tylonycteris pachypus, Trophoblast, Intrasyncytial lamina, Haemodichorial.

INTRODUCTION

The bat forms one of the largest group of living mammals including 18 families and 180 genera. Their unique morphological adaptation suited to their aerial mode of life and peculiar posture are unmatched by any other mammal [21].Bats are a highly successful, widely distributed group, with considerable variation in placental structure [18].

It is generally recognised that the chorioallantoic placental barrier displays a variable structural organization in different sps. of bats at different stages of pregnancy[3][7] [10].These differences might reflect important interspecific variations in the histophysiology of the organs. During the last three decades much attention has been given to the ultrastructure of the vascular relationships of foetal and maternal tissues during gestation [3] [6] [5][12].Electron microscopy has helped the placentologist in resolving the no. of cellular layers [1] [9] and the fate of maternal endothelial cells present in the barrier [3] [13].The technique indicates that active transport mechanism exists in this barrier. The ultrastructure of these layers clearly confirms the physiological evidence that the placental labyrinth is an organ of very high metabolic activity in synthesis as well as transport [4] [8].

In view of the above ideas it is obvious that placental morphology and physiology offer challenging field for research with more modern methods. Till date, the fine structural studies on the discoidal placenta have been investigated and published in 12 out of 18 families belonging to both the suborders of Chiroptera.

The present study has been undertaken at electron microscopic level in order to find out the cytological information available on placental structure. The flat-headed bat *Tylonycteris pachypus* belongs to the family Thyropteridae [14] Microchiroptera. It is very unique bat because it shares the peculiar characteristics of a coarse syncytium with the Pteropid bat- Megachiroptera, *Rousettus leschenaultia* [15] & Cynopterus gangeticus [18] and podocytic modifications of the basal lamina which is found in man, macaque and ungulate placenta [4] [11] and Cynopterus sphinx gangeticus & Scotophilus heathii [18] .

According to Enders and Wimsatt (1968) the cellular transformations takes place during implantation and tendency for reduction in thickness of layers between the foetal and maternal circulation during gestation. The endotheliodichorial condition lasts till late limb bud stage following with the complete disappearance of the maternal endothelium along with the transformation of interstitial membrane into the intrasyncytial lamina and progressive development of ectoplasmic layer at term stage leads to the establishment of a haemodichorial placenta.

MATERIALS AND METHODS

The collections of these bats were done periodically from the caves, forts and trees along the hilly regions of Trichur, Kerala situated in South India. The bats were collected from early November to last week of April during 1993-94 to represent the different stages of embryonic development.

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 40^{o}C. 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 10minutes in Reynolds Lead Citrate. Sections were examined and photographed under JEM JEOL 100S Electron Microscope [17].

Observations-
Early Limb bud Stage

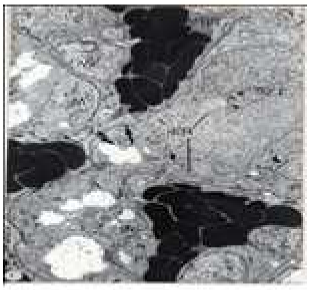

Fig. 1

X 3500 A continuous layer of interstitial membrane

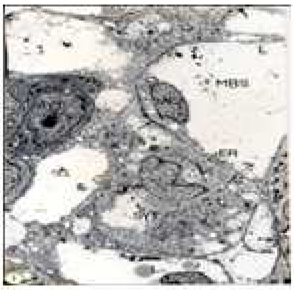

Fig.2

X 3500 Maternal blood space with distinct maternal Endothelium

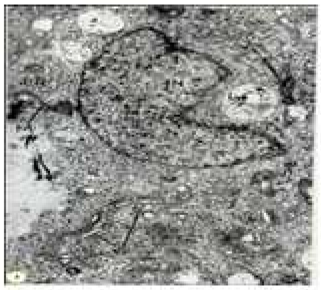

Fig.3

X 10000 Dense bodies, coated vesicles and lysosomes around the nuclear edge

Late Limb bud stage:

Fig.4

X 2500 Maternal blood space is surrounded by maternal endothelium

Fig.5

X 7000 Discontinuous interstitial membrane at regular intervals

Full Term Stage:

Fig.6

X 5000 Fetal capillary is very much closer to the maternal blood capillary

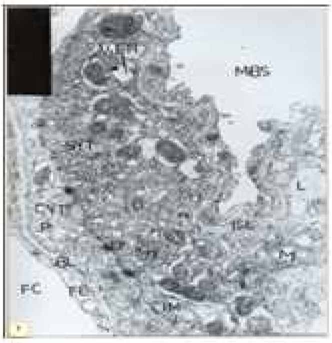

Fig.7

X 13000 Hypertrophied mitochondria surrounded by vesiculated rough endoplasmic reticulum

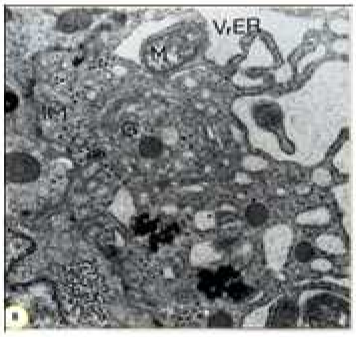

Fig.8

X 25000 Rough endoplasmic reticulum shows sac-like appearance.

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. ➔– Interstitial membrane
25. – Interdigitating folds
26. ICS – Intracellular space
27- Free floating cells
28. IT – Intrasyncytial tract
29. B-Cyt – Giant cells / Binucleate cytotrophs
30. ► – Podocytic modifications of interdigitating membrane

Early limb bud stage- Maternal vasculature is well developed which is limited by a unicellular layer of endothelial cells with darkly stained distinct nuclei and nucleoli. The maternal endothelial cells based on continuous homogenous acellular layer referred to as the interstitial membrane. The cytoplasm is characterised by a no. of electron dense mitochondria and granular endoplasmic reticulum. Interstitial membrane is uniform in thickness with interdigitating process. The syncytiotrophoblastic layer is extremely electron dense with irregularly shaped nuclei embedded in a common ground matrix. Syncytiotrophoblast is followed by well-defined cytotrophoblast. Cytotrophoblast shows well developed concentrically arranged Golgi complexes around the nucleus and distinct rough endoplasmic reticulum. The basal lamina is continuous acellular double membrane layer running along the proximal length of the cytotrophoblast.

Late limb bud stage- Maternal endothelium is not distinctly seen and is more irregularly shaped. Interstitial membrane begins to undergo attenuation at sites with progressive advanced ingestation. The maternal endothelial cells based on continuous homogenous acellular layer referred to as the interstitial membrane. The cytoplasm is characterised by a no. of electron dense mitochondria and granular endoplasmic reticulum. The syncytiotrophoblast layer is extremely electron dense with irregularly shaped nuclei embedded in a common ground matrix. Syncytiotrophoblast is followed by well-defined cytotrophoblast and the basal lamina.

Full term stage :-The maternal endothelium is absent in the full term pregnancy, instead the enclosed irregularly shaped lacunae of the maternal blood space, comes in direct contact with the cytoplasm of the syncytiotrophoblast i.e. ectoplasmic layer. This layer is formed with the loss of the maternal endothelium. The most striking change is the thinning of the ectoplasmic layer with few microvillus projections. Coated vesicle, dense bodies are seen in the ectoplasmic layer. Most distinct feature is the conversion of interstitial membrane to intrasyncytial lamina. The ectoplasmic layer and the intrasyncytial lamina are constant features in the placental structure of this bat.

Syncytiotrophoblast is quite thinner as compared to that of the limb bud stages. The nuclei are large and irregular in shape with darkly stained nucleoli. It resemblance a coarse syncytium due to the presence of vesiculated rough endoplasmic reticulum. Term placenta shows the presence of clusters of electron dense darkly stained glycogen bodies and glycogen rosettes are seen towards the cytotrophoblast. This is the unique feature of this placenta.

Interdigitating membrane is present throughout the development and runs along the stretch of the two trophoblastic layers. At term stage it is thrown into convoluted folds and separates the two trophoblastic layers. The cytotrophoblast shows the presence of podocytic protrusions. This feature is absent in earlier stages of development.
Foetal capillaries and maternal blood capillaries are distinctly observed at term stage. They are very close to trophoblastic layers serving to bring foetal vasculature in to a much closer proximity with that of maternal circulation. Absence of maternal endothelium and presence of both trophoblastic layer at term, designates the placenta to be haemodichorial.

DISCUSSION

- Every layer of the interhemal membrane had well defined activities/functions, but most important being the enhancement of foetal survival through transport of various substances in both directions. In addition, the morphological modifications such as coarse syncytium and indentations were measures for improving transplacental efficiency and foetal survival.
- The maternal blood space represented by large bays of lacunae that were lined by irregular layer of cytoplasm and the microvillus profile of luminous syncytio trophoblastic surface area had edged the MBS, considered to be morphological modification for increased surface area. The free floating cells around the MBS did not really account for the endotheliochorial condition[20].
- The maternal endothelium had not only being restricted to performing active reabsorption but along with mitochondria, r-ER and Golgi it was believed to be associated with protein synthesis.
- The interstitial membrane comprised of an acellular homogenous content and supported the maternal endothelium apart from the possible immunological isolation role of foetal and maternal components.
- 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, the intrasyncytial lamina also contributed to the nutritional requirement o the developing foetus.
- 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 intercellular transport within the ground matrix, in addition to the draining of maternal blood from placenta. The intrasyncytial desmosomes prevented the syncytium from collapsing.
- The interdigitating membranes was formed at the confluence of the basal portion of the syncytiotrophoblast and the apices of underlying cytotrophoblast that probably lent a structural support to the framework.
- The presence of various cell organelles showed active transport and protein synthesis. 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, while the irregularity of plasma membrane allowed an increased area of exchange between trophoblastic cell and the space utilised for elimination of secretary products. The basal lamina served to separate the trophoblastic complement of the interhemal barrier from the foetal side and the modifications/infolding helped to reduce diffusion distance and time and as such increased the bi-directional transportation and exchange efficiency between maternal and foetal blood circulation [16].
- The indentation of foetal capillaries facilitated and enhanced foetal/neonatal survival.

CONCLUSION

Confirmative presence of the two trophoblastic layers i.e. cytotrophoblast and syncytiotrophoblast and the complete absence of maternal endothelium at term stage, designates the placenta to be haemodichorial.

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