Foetal membranes and placentation in the vespertilionid bat, *Scotophilus heathi* (Horsefield)

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Abstract. The arrangement and the structure of the foetal membranes of *Scotophilus heathi* at full term are described. The yolk sac has collapsed resulting in the reduction of the lumen into streak-like spaces. The splanchnopleure is highly folded and the mesodermal cells are enormously hypertrophied and vacuolated. These changes give the yolk sac a gland-like appearance. A small abembryonic segment of the yolk sac remains trilaminar and lies freely in the persistent uterine lumen on the mesometrial side. The parietal layer of chorion with hypertrophied trophoblastic cells forms an accessory syndesmochorial placenta by coming into intimate contact with the uterine endometrium on the lateral sides of the uterus. The chorio-allantoic placenta is discoidal, antimesometrial, labyrinthine and haemomonochorial with only syncytiotrophoblast lining maternal vascular channels. A PAS-positive membrane is a permanent component of the placental barrier, and this lies embedded in a cytoplasmic lamina of syncytiotrophoblast. The PAS-positive membrane is porous.

Keywords. Foetal membranes; placentation; haemomonochorial; *Scotophilus heathi*.

1. Introduction

Reviews of earlier literature on the embryology of bats (Wimsatt 1944, 1945, 1958; Gopalakrishna 1949, 1950a, 1958) reveal that most of the earlier studies on the embryology of Microchiroptera were made on a few species of Vespertilionidae, and only superficial observations were made on the development of one or two species of each of the following families: Megadermatidae (Gopalakrishna 1950b, 1958), Rhinolophidae (Branca 1927; Van der Sprenkel 1932), Hipposideridae (Van der Sprenkel 1932), Phyllostomatidae (Hamlett 1934, 1935; Wislocki and Fawcett 1941) and Molossidae (Sanson 1932; Hamlett 1934; Mossman 1937). During the past three decades, detailed studies have been made on the embryology of one or two members each of the families Rhinopomatidae (Srivastava 1952; Gopalakrishna 1958), Emballonuridae (Gopalakrishna 1958; Wimsatt and Gopalakrishna, 1958), Noctilionidae (Anderson and Wimsatt 1963), Rhinolophidae (Gopalakrishna and Bhiwgade 1974; Bhiwgade 1976), Hipposideridae (Gopalakrishna 1958; Gopalakrishna and Moghe 1960), Desmodontidae (Wimsatt 1954, 1958; Bjorkman and Wimsatt 1968), Phyllostomatidae (Wimsatt 1958; Fleming 1971; Rasweiler IV, 1972, 1974; Bonilla and Rasweiler IV 1974; Bodley 1974; Bleir 1975) and Molossidae (Wimsatt 1958; Stephens 1962, 1969; Stephens and Easterbrook 1968, 1969, 1971; Stephens and
Although morphologically the Microchiroptera appears to be an homogeneous group, there are wide differences among them in embryological characteristics.

There are considerable differences in the interpretation of the finer morphology of the placenta even among the vespertilionids whose embryology has been studied in more detail than the embryology of the members of the other families. Whereas most authors have described the placenta of vespertilionid bats as haemochorial (Duval 1894, 1895, 1896; Nolf 1896; Grosser 1927; Kempermann 1929; Ramaswami 1933; Wimsatt 1945; Gopalakrishna 1958; Enders and Wimsatt 1968) a few authors describe it as endotheliochorial (Branca 1927; Gerard 1928). Vacek (1959) described the placenta of Myotis myotis as being of a transitional condition between the syndesmochorial and haemochorial types.

Among the Indian vespertilionids the details of development of the foetal membranes have been studied in only two species, Vesperugo leisleri (Ramaswami 1933) and Scotophilus temmincki (Gopalakrishna 1949, 1950a) and the definitive structure and arrangement of the foetal membranes have been studied in Pipistrellus minimus (Gopalakrishna and Karim 1973).

2. Materials and methods

The present report is based on the examination of five pregnant specimens of Scotophilus heathi collected during the second and third weeks of April, 1976 at Bangalore (one specimen) and Cochin (four specimens). The specimens were killed by chloroform and the pregnant uteri were fixed in alcoholic Bouin’s fluid. After fixation for 24 hr, the uteri were transferred to 70% ethanol.

Each specimen carried a full term foetus in each cornu of the bicornuate uterus. In one case the two uterine cornua with the foetuses were kept intact and dehydrated slowly by passing through graded ethanol, cleared in xylol, embedded in paraffin and sectioned transversely from one end to the other at a thickness of 10 μ. In other cases the two uterine cornua were separated, and the foetuses were completely removed before further processing the uterus. In one of these specimens the two uterine cornua without the foetuses were processed as usual and sectioned at 8 to 10 μ in thickness. In the rest of the specimens the placental disc was cut away and sectioned at thicknesses varying between 5 to 8 μ. For routine histological study the sections were stained with Ehrlich’s haematoxylin and counterstained with eosin. Some sections from each series were stained with periodic acid-Schiff procedure (Pearse 1968), some without and some after prior salivary digestion. A few slides in each series were also stained by Heidenhain’s azan technique and a few slides by Mallory triple staining procedure.

3. Observations

3.1. Arrangement of the foetal membranes

The general disposition of the foetal membranes in Scotophilus heathi at full term is indicated in figure 1. The chorion is in contact with the uterine wall on all the
sides except on the mesometrial side, where a small region of the chorion hangs freely in the persistent uterine lumen into which remnants of uterine glands open by wide mouths. The yolk sac lies in the exocoelom in the form of a collapsed bag with intensely folded walls. The major part of the yolk sac wall lies on the mesometrial side. A small part of it, however, occurs on the lateral side adjacent to the placental disc. This is evidently the part of the yolk sac splanchnopleure which has been pulled along with the umbilical cord towards the placental disc as the foetus changes its orientation and comes to lie with its ventral side facing the placental disc during the final stages of gestation. The amnion is a thin bilaminar membrane closely adhering to the body of the foetus except in the region of the cranial flexure of the foetus where
a small fluid-filled amniotic cavity intervenes between the body of the foetus and the amnion. The placenta is the most conspicuous structure in stained sections and occurs as a circular, concavo-convex disc on the antimesometrial side of the uterus. It is 10 to 12 mm in diameter and about 3 mm in thickness at the centre of the disc, and gets progressively thin towards the margin of the disc.

3.2. The yolk sac

The yolk sac splanchnopleure (figure 2:1) is richly vascularized except for a small abembryonic area, where the trilaminar omphalopleure persists freely in the uterine lumen. The original lumen of the yolk sac is reduced to narrow streak-like spaces lined by flat or cuboidal endodermal cells (figure 2:3). The yolk sac splanchnopleure is highly folded, and the mesodermal layer grows out into numerous villi-like projections into the exocoelom thereby further accentuating the folded appearance of the splanchnopleure (figure 2:2). The mesodermal cells are enormously hypertrophied and contain centrally placed vesicular nuclei, and vacuolated cytoplasm (figure 2:4). The entire structure, thus, attains a gland-like appearance. The mesodermal cells contain abundant amounts of both saliva-resistant and saliva-digestible PAS-positive material. The PAS-positive substance occurs as evenly distributed, finely granular material generally located near the distal regions of the cells.

3.3. Chorion

The parietal layer of chorion, (figure 2:5) which is closely apposed to the lateral wall of the uterus, consists of an outer layer of trophoblast cells containing large vesicular nuclei and an inner layer of extra-embryonic mesoderm. The trophoblast layer is composed of enormously hypertrophied cells (figure 2:6) each with a large centrally placed vesicular nucleus. The trophoblast layer is in intimate contact with the maternal endometrial tissue forming a synesmochorial placenta on the lateral sides of the uterus. The parietal chorion undercuts the placental disc at its margin and imperceptibly merges with the syncytiotrophoblastic shell on the maternal border of the chorio-allantoic placenta.

3.4. The chorio-allantoic placenta

The discoidal chorio-allantoic placenta is composed of a complex, three dimentional network of placental tubules (figure 3:7) inside which circulates maternal blood. Foetal mesenchyme and capillaries lie in the meshes of the placental network. The placental tubules are completely devoid of endothelial lining and are lined by a single layer of syncytiotrophoblast in which large nuclei occur scattered irregularly (figure 3:8). Sections stained by the PAS procedure exhibit the presence of a scarlet stained membrane lying on the maternal surface of the placental tubules (figure 3:9). This membrane takes a bluish-green stain in Heidenhain's azan and Mallory triple staining procedures. The thickness of the PAS-positive membrane seems to be directly related to the calibre of the placental tubules. Hence, in the larger placental tubules near the foetal border of the placenta the membrane is
Figure 2. (1-6)  (See captions in p. 126)
Figure 3. (7-12) (See captions in p. 126)
prominent, whereas it is very thin in the small placental tubules within the deeper regions of the placenta. A careful examination reveals that this membrane appears to be perforated by innumerable number of apertures. Hence, in sectional views the membrane appears to be discontinuous (figure 3:10). The exact nature of this membrane is not known. It may be composed of partly by the persistent basement membrane of the original maternal endothelium of the maternal capillary, but mostly it appears to be the product of the trophoblast. A thin cosinophilic cytoplasmic layer is visible on the maternal border of the PAS-positive membrane in sections washed with eosin after PAS. The discontinuity of the membrane and the presence of the cytoplasm on its maternal border in the placental tubules would suggest that the cytoplasm of the syncytiotrophoblast might have passed through the pores of the PAS-positive membrane to form a continuous lining on the inner surface of the tubules. Since there is a single layer of trophoblast, and since there is a direct contact of maternal blood with the trophoblast, the placenta should be considered as haemomonochorial.

3.5. Utero-placental junction

The maternal border of the placenta has a thick shell of syncytiotrophoblast (figure 3:11) which is pierced in several places by maternal blood vessels entering the placenta. The syncytiotrophoblastic shell is bordered on its maternal surface by a layer of loose endometrial tissue with large vacuolated cells. This is apparently the line of separation of the placenta during parturition.

3.6. Maternal vascularization of the placenta

Six to eight large maternal blood vessels pierce the syncytiotrophoblastic shell and pass towards the foetal border of the placenta. These vessels give rise to one or two branches each before reaching the foetal border, and these branches also travel towards the foetal border of the placenta. At the foetal border of the placenta these vessels give rise to numerous smaller vessels which radiate towards the margin of the placenta. There is no endothelial lining to any of these vessels. Each radial vessel opens into a large number of placental tubules by narrow ostia along its course. Maternal blood is returned through the placental tubules into large pools of blood on the foetal side of the syncytiotrophoblastic shell at the utero-placental junction. Uterine veins drain off the blood from these pools.

3.7. Foetal vascularization of the placenta

Foetal blood is brought to the placenta by two large allantoic arteries which break into several branches on the foetal border of the placental disc. Each of these branches give rise to numerous smaller branches which enter the placental complex into the meshes of the network of the placental tubules. After circulating in the placenta within fine capillaries in the meshes of the placental labyrinth foetal blood is returned by an umbilical vein.

3.8. The umbilical cord

Sections of the umbilical cord reveal the presence of five blood vessels—two allantoic arteries, a vitelline artery, an allantoic vein and a vitelline vein. A narrow endo-
dermal allantoic duct is present in the proximal segment of the cord (figure 3:12) but is absent from the distal segment.

4. Discussion

Although the general topography of the foetal membranes of Scotophilus heathi resembles that of other vespertilionid bats so far studied, there appears to be considerable differences in the details of the histological structures of the various membranes. The collapse of the yolk sac splanchnopleure with the resulting reduction of the lumen of the yolk sac into streak-like spaces, the intense folding of its walls and the enormous hypertrophy of the mesodermal cells recalls the conditions of the yolk sac in the primitive Microchiroptera such as the Emballonuridae and Rhinopomatidae rather than the yolk sac described in other vespertilionid bats. Perhaps the earlier authors did not pay attention to the peculiar changes undergone by the yolk sac in other vespertilionids. In Scotophilus heathi, as in Emballonuridae and Rhinopomatidae (Gopalakrishna 1958; Srivastava 1952), the yolk sac attains a gland-like appearance. Only a small area of the yolk sac wall on the abembryonic side remains as a trilaminar membrane without hypertrophy of its cellular elements.

The close apposition of the parietal layer of the chorion to the lateral wall of the uterus establishes an accessory syndesmochorial placental relationship. The fact that the trophoblast cells of this part of the chorion are enormously hypertrophied recalls the condition of trophoblastic giant cells. Apparently this is an important structure through which physiological exchange can occur between the foetus and the mother. The occurrence of an accessory syndesmochorial placenta has been so far reported only in one other vespertilionid bat, Myotis myotis (Vacek 1959).

As in all the other vespertilionid bats so far studied, the chorio-allantoic placenta in Scotophilus heathi is discoidal, antimesometrial and labyrinthine. However, this differs from the placenta of several other vespertilionid bats in its finer morphology. The ripe placenta in Scotophilus heathi is haemomonochorial and has only the syncytiotrophoblastic layer. A PAS-positive, discontinuous interstitial membrane lies embedded in an eosinophilic lamina which is evidently the cytoplasm of the syncytiotrophoblast which has percolated through the pores in the interstitial membrane and has come into direct contact with the maternal blood. Such an interpretation of the placental barrier has been also given by Bjorkman and Wimsatt (1968) in another vespertilionid bat, Myotis lucifugus lucifugus.

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Captions for figures 2 and 3

Figure 2. (1-6) 1. Part of the collapsed yolk sac splanchnopleure to show the outgrowths of the mesodermal layer in the form of villi. Note the narrow yolk sac lumen (arrow) and blood vessels (arrow head) (Haematoxylin-eosin) x 60. 2. Part of the highly folded yolk sac wall and narrow lumen (arrow). Note a large blood vessel (arrow head) (PAS and Weigert haematoxylin.) x 60. 3. Part of figure 1 magnified to show the flat endodermal cells bordering the yolk sac lumen and hypertrophied mesodermal cells x 450. 4. Part of figure 2 magnified to show the accumulation of PAS-positive material near the distal regions of the mesodermal cells. x 450. 5. Part of the lateral wall of the uterus to show the parietal layer of the chorion (arrow) in contact with the uterine endometrium. A few artificial spaces have, however, formed between the chorion and the endometrium in some places. x 100. 6. Part of figure 5 enlarged to show the hypertrophied trophoblastic cells of the parietal chorion. x 360.

Figure 3. (7-12) 7. Part of the chorio-allantoic placenta to indicate its labyrinthine nature. A large radial maternal blood channel at the bottom of the figure is at the foetal border of the placenta. Numerous tortuous placental tubules form the bulk of the placenta. x 80. 8. A part of the placental labyrinth enlarged to show a few placental tubules filled with maternal blood. Note a few scattered nuclei of the syncytiotrophoblast. x 450. 9. Part of the placenta (PAS staining) to show the presence of a distinct PAS-positive membrane lining the inner surface of the placental tubule. x 600. 10. Part of the wall of the placental tubule (PAS staining) to show the discontinuous nature of the PAS-positive membrane. x 960. 11. Utero-placental junction. Note the continuous syncytiotrophoblastic shell (arrow) separating the maternal endometrium with vacuolated cells (left side of the shell) and the placental border of the shell (right side of the shell) with pools of maternal blood. x 180. 12. Section of the proximal segment of the umbilical cord. Note the presence of a narrow, allantoic duct (arrow) in addition to five blood vessels. x 32.