The placenta and foetal membranes of the lesser yellow house bat, *Scotophilus borbonicus* (E. Geoffroy, 1803) (Chiroptera: Vespertilionidae)

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A discoidal hemodichorial placenta is present in *Scotophilus borbonicus*. Evidence is presented which suggests that histotrophic nutrition, in addition to hemotrophic nutrition, occurs. The foetal trophospongium phagocytoses cell debris and glandular secretions derived from the maternal endometrium (decidua basalis). This cell debris forms a detritus zone between the decidua basalis and trophospongium. The existing concept of the basal lamina (intrasyncytial membrane) of the hemodichorial placenta is described in a modified form.

'n Diskoidale hemodikoriale plasenta is teenwoordig in *Scotophilus borbonicus*. Bevindinge word voorgelê wat aandui dat histotrofe voeding, bykomstig tot hemotrofe voeding, plaasvind. Die fetale trophospongium fagositeer seldebris en klierafskeidings afkomstig van die endometrium (decidua basalis). Die seldebris vorm 'n detritussone tussen die trophospongium en die decidua basalis. Die bestaande konsep van die basale lamina (intrasinsitiale membraan) van die hemodikoriale plasenta word in 'n gewysigde vorm beskryf.

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Histologically the microchiropteran placenta has been described as hemodichorial (Enders & Wimsatt 1968), i.e. the endothelium of the maternal blood vessels within the placental disc has been lost and is replaced by flattened processes of the syntrophoblast. However, the maternal endothelial basal lamina is retained and through it the processes of the syntrophoblast extend to line the maternal blood vessels. Futhermore, the syntrophoblast as well as the cytotrophoblast are retained as continuous layers, hence the name dichorial. The above-mentioned endothelial basal lamina is now known as the intrasyncytial membrane. This type of arrangement has been shown to exist in *Myotis lucifugus* (Enders & Wimsatt 1968), *Desmodus rotundus* (Bjorkman & Wimsatt 1968) and *Thyroptera tricolor* (Wimsatt & Enders 1980).

The relationship that exists between fetal and maternal capillaries in the placenta has received much attention. In bats the intimacy of this relationship, as determined by membrane thickness, varies between families.

In the Molossidae, foetal capillaries invade the chorionic epithelium to produce a closer relationship with the maternal capillaries; whereas no such invasion takes place in the Vespertilionidae (Wimsatt & Enders 1980).

The object of this investigation was to elucidate the placental morphology of the lesser yellow house bat *Scotophilus borbonicus*.

The taxonomy of the genus *Scotophilus* has been the subject of considerable debate, and the species we studied would key out as *S. leucogaster* in Hayman & Hill (1971). The trivial names *viridis* and *borbonicus* have also been used for the population we studied (Schlitter, Rautenbach & Wolhuter 1980; Rautenbach 1982; Smithers 1983; Fenton, Brigham, Mills & Rautenbach 1985; Fenton & Rautenbach 1986). However, pending a taxonomic revision of this genus, we choose to follow Meester, Rautenbach, Dippenaar & Baker (1986) in referring to this species as *S. borbonicus*.

Materials and methods

Bats were collected in the Pafuri area of the northern Kruger National Park, on a monthly basis during 1984, using two macro-mistnets (Rautenbach 1985). The trapped bats (average of 4 females per month) were kept overnight and killed with ether the following morning. The genital tracts of the females were dissected out. Uteri for light microscopy were placed on glass squares, flattened and tied down with gauze. They were then fixed by immersion in 10% phosphate buffered formalin. After a minimum of 4 days fixation the organs were dehydrated in graded alcohols, cleared in xylol, embedded in paraffin wax (Histosec, Merck) and serially sectioned, at 5 μm, using a rotary microtome. The sections were stained with the standard Mayer's haematoxylin and eosin technique. Some sections of the uteri of pregnant animals were stained according to the sulphuric acid toluidine blue method of Sulkin (1955), to demonstrate the basal laminae in the placenta.

Placental material collected during October only was available for electron microscopy. Pieces of placental material were cut into 1-mm^3 blocks and fixed in cold (4°C) 4% glutaraldehyde prepared in 0,2 mol dm^{-3} sodium cacodylate buffer pH 7,3 (Mercer & Birbeck 1972).

After 4 days the specimens were post-fixed for 1 h at room temperature in 1% osmium tetroxide made up in the cacodylate buffer, rinsed twice in the same buffer, dehydrated through a graded ethanol series, cleared in

propylene oxide, and embedded in Polarbed 812 epoxy resin. The blocks were polymerized for 48 h at 60°C.

Semi-thin sections (1 μm) were cut with glass knives on a Reichert OmU4 ultra-microtome and stained with toluidine blue (1% toluidine blue in a 1% borax solution) (Nunn 1970) for conventional light microscopy and the determination of appropriate areas for thin sectioning. Thin sections (80–90 nm) were prepared using glass and diamond knives, picked up on 200 or 300 mesh copper grids, and stained for 30 min in a saturated aqueous solution of uranyl acetate (Watson 1958) and for 4 min in a 0,2% lead citrate solution (Reynolds 1963). The stained sections were examined in a Philips 301 transmission electron microscope.

Results

S. borbonicus has an antimesometrial discoidal, deciduate and hemodichorial placenta (Figure 1).

Macroscopically the fully formed placental disc has a concavo-convex shape with the concave part facing the fetus. The diameter of the disc in late pregnancy is approximately 15 mm and is thickest in the middle (2–3 mm), tapering towards the periphery. The extraplacental part of the chorion is a smooth surfaced spherical structure and is not attached to any part of the endometrium (Figure 1).

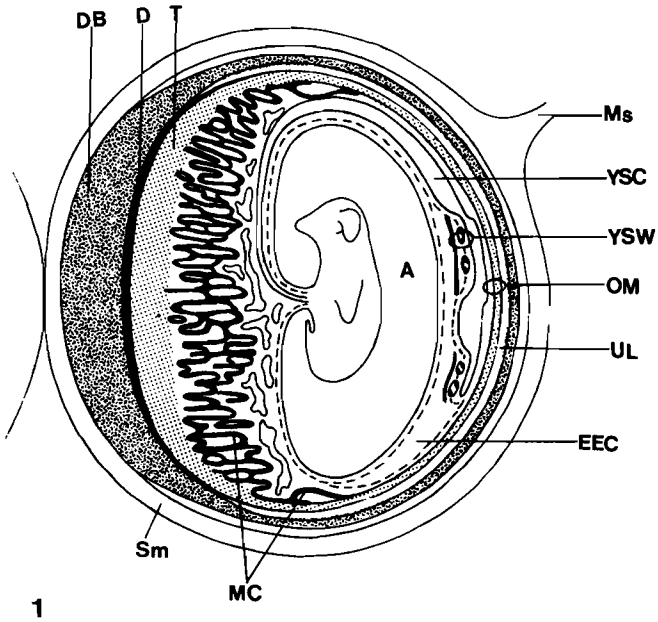

Figure 1 Diagrammatic representation of the foetal membranes and discoidal placenta of *Scotophilus borbonicus* shortly after formation, (×40). A – amniotic cavity; D – detritus zone; DB – decidua basalis; EEC – extra-embryonic coelom; MC – maternal channel; Ms – mesometrium; OM – fused yolk sac endoderm, non-vascularized mesoderm and chorionic ectoderm (omphaloplacenta); T – trophospongium; Sm – myometrium; UL – uterine lumen; YSC – yolk sac cavity; YSW – vascularized splanchnic mesoderm with cuboidal mesothelium lining the extra-embryonic coelom and cuboidal endodermal epithelium lining the yolk sac cavity.

Formation and light microscopic structure of the discoidal allantochorionic placenta

On completion of cleavage a typical blastocyst is formed consisting of an outer shell of cuboidal trophoblast cells and an inner cell mass. Even before this stage is reached the blastocyst is situated in an antimesometrial attachment chamber formed by the endometrium. The embryonic pole of the blastocyst lies against the endometrial epithelium at the deepest part of the chamber. The embryonic pole contains the inner cell mass and opposed to it in the endometrium is the site of the future discoidal allantochorionic placenta.

The primary event in the formation of the placenta is the proliferation of the trophoblastic cells of the embryonic pole to form the trophoblastic syncytium with its characteristic invasive properties. The trophoblast differentiates in two directions:

(i) proximally in the placenta to form a syn- and cytotrophoblast which will encase the maternal blood vessels thereby forming a region known as the zona intima, and

(ii) more distally in the placenta and directly opposed to the endometrium to form a multi-layered zone of trophoblastic cells, the trophospongium (Figure 1).

Concomitantly with the above two changes a decidua basalis develops (Figure 1).

Formation of the syn- and cytotrophoblast

The trophoblastic syncytium at first erodes the endometrial epithelium and its underlying connective tissue leaving the maternal vessels interdigitating with branched finger-like trophoblastic processes. Allantoic (splanchnic) mesoderm and its associated umbilical vessels invade these trophoblastic processes, establishing them as true chorionic villi. The maternal vessels between them are encased in trophoblastic syncytial cells now known as the syntrophoblast. Proximal to the latter another layer of cells, the cytotrophoblast, is established. It is seen as a continuous epithelial layer of flat to cuboidal cells with slightly basophilic cytoplasm, definite cell membranes and nuclei with indistinct nucleoli. The syntrophoblastic cells, on the other hand, are recognized by the absence of intercellular cell membranes and the presence of prominent vesicular nuclei with distinct nucleoli.

During the process of encapsulation of the maternal vessels by the syntrophoblast, the endothelium of the vessels is presumably eroded by syntrophoblastic processes which penetrate through pores in the basal lamina. This basal lamina can be observed as a Periodic Acid Schiff-positive membrane which is even more conspicuous after staining with the sulphuric acid-toluidine blue method of Sulkin (Figure 2). The processes which expand on the inside of the vessels form the new lining for the vessels. The latter are now known as the maternal channels (Figures 2 & 3). They form a labyrinthine network of channels which is referred to as the zona intima, forming the deepest portion of the placenta, i.e. nearest to the embryo (Figures 1 & 3).

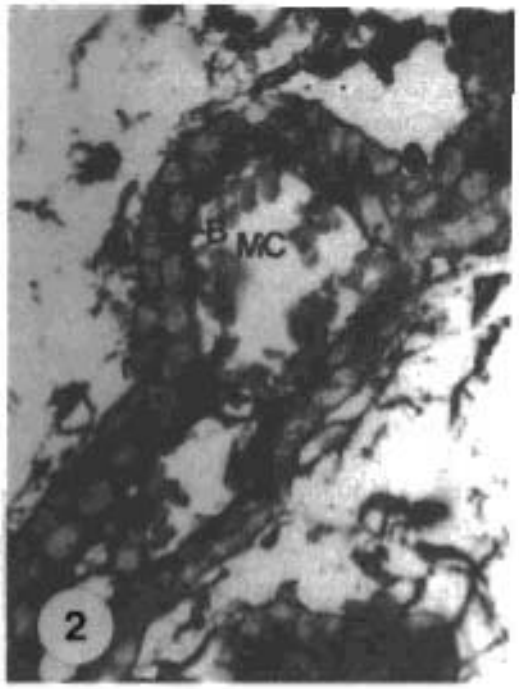

Figure 2 The zona intima of the placenta of *Scotophilus borbonicus* stained with the sulphuric acid-toluidine blue method (Sulkin 1955) to demonstrate the basal lamina (B) within the maternal channel (MC). (×400).

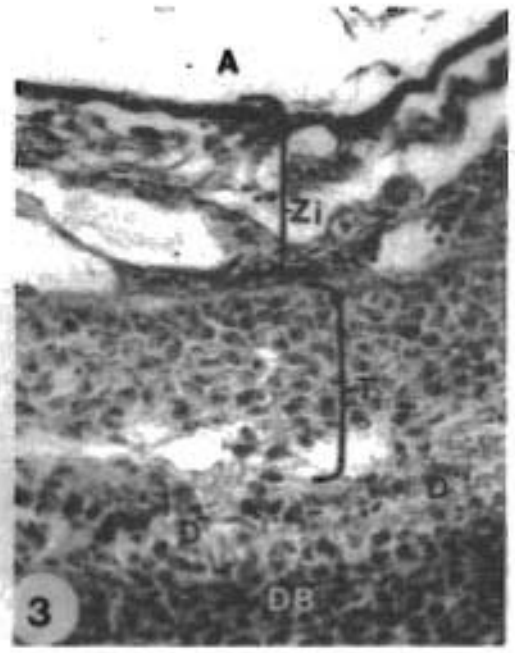

Figure 3 A section of the zones of the placenta of *Scotophilus borconicus* shortly after establishment, (×200). A – amniotic cavity; Zi – zona intima; T – trophospongium; D – detritus zone; DB – decidua basalis.

Formation of the trophospongium and detritus zone

During the process of interdigitation of chorionic villi and maternal vessels described above, the distal tips of the chorionic villi are covered by a cytotrophoblast resting on its own basal lamina. The tip is compressed so that a double basal lamina with intervening mesoderm comes to separate the two cytotrophoblastic layers. The more distal layer of cytotrophoblast forms a stratified layer of polygonal cells invading the superficial layers of the endometrium whereas the more proximal one forms a syntrophoblast which encases the maternal vessels. The double layer of basal laminae and cytotrophoblast forms a distinct layer separating the zona intima from the stratified layer. The latter is not a syntrophoblast because cell membranes are distinct (desmosomes are present) and it thus represents a multilayered chorionic epithelium, the trophospongium. Its cells reveal extreme vacuolation (Figure 3).

The trophoblastic zone is initially responsible for erosion of the endometrial epithelium and its stroma. It presumably stimulates the epithelium and stroma to undergo nuclear pycnosis, karyorrhexis and extreme cytoplasmic eosinophilia before being desquamated. This process results in a detritus zone being formed between the trophospongium and the endometrium. This detritus zone consists of cell debris and glandular secretions (Figure 3).

Changes in the endometrium

Once erosion of the endometrial epithelium and superficial stroma has taken place, the remaining stromal fibroblasts are presumably stimulated to undergo massive proliferation forming a cellular layer of endometrial connective tissue, the decidua basalis (Figures 1 & 3). This layer is denser in appearance than the trophospongium. Between the two layers the detritus zone is present (Figure 3).

Formation of and changes in the yolk sac

During blastocyst formation, endodermal cells differentiate from part of the inner cell mass and spread out to line the trophoblast beyond the inner cell mass.

The bilaminar yolk sac is complete when the lining has been fully formed. Its wall is a bilaminar omphalopleur.

Mesoderm grows between the ectoderm (trophoblast) and endoderm of the yolk sac. In the embryonic half this mesoderm is much thicker than in the abembryonic half where it is seen as a single layer of squamous cells. Neither vitelline blood vessels nor blood cells have yet been formed, thus at this early stage most of the yolk sac wall is avascular trilaminar omphalopleur.

Soon after the implantation process is initiated the vitelline blood vessels are seen to arise in the splanchnic mesoderm of the yolk sac, initially as blood islands which then coalesce to form blood vessels. This vascularization of the yolk sac starts in the embryonic region of the yolk sac and progresses towards the abembryonic pole. A small area in the abembryonic region, the omphaloplacenta remains in an avascular three-layered condition to term (Figure 1).

With the vascularization of the yolk sac, marked changes occur in the endoderm and mesoderm. The endodermal lining on the embryonal side undergoes marked hypertrophy which results in its cells becoming

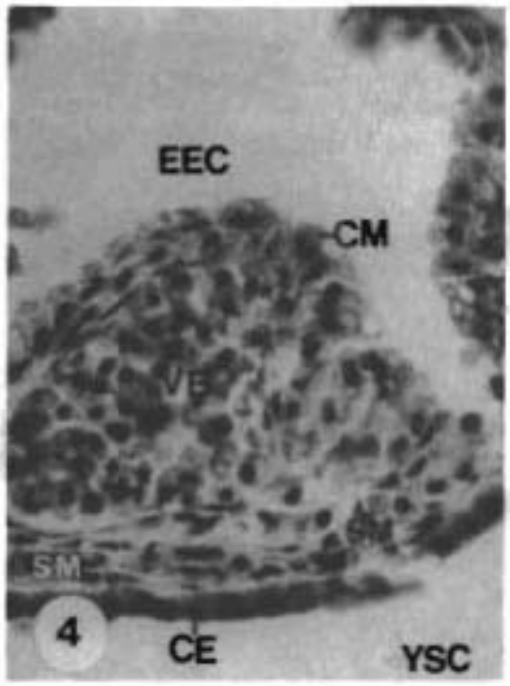

Figure 4 Photomicrograph of the vascularized embryonic yolk sac wall of *Scotophilus borbonicus* (see Figure 1). Note the extra-embryonic coelom (EEC) lined by cuboidal mesothelium (CM) containing vitelline blood vessels (VB) filled with nucleated foetal red blood cells. The cuboidal endodermal lining (CE) of the yolk sac cavity (YSC) and the splanchnic mesoderm (SM) are clearly shown, (×400).

cuboidal (Figure 4). This hypertrophy is always associated with the vitelline blood vessels. The cells lining the abembryonic side, which is the avascular part of the yolk sac, remain as an undifferentiated squamous epithelium.

The mesothelium facing the extra-embryonic coelom changes from the initial squamous epithelium to cuboidal epithelium where the individual cells are characterized by a bulging apex. The mesothelium and underlying mesodermal connective tissue are thrown into folds which do not incorporate the endodermal cells; consequently the surface area of the mesothelium is much larger than that of the endodermal lining (Figure 4).

As pregnancy proceeds the embryonic region of the yolk sac gradually invaginates with the result that the yolk sac cavity decreases in size. At term it is represented by a slitlike cavity in the abembryonic region which is still lined by cuboidal epithelium. A small thin area of endoderm, unsplit unvascularized mesoderm and ectoderm (a trilaminer yolk sac or omphalopleur) remains in the abembryonic region at term (Figure 1).

As the amnion enlarges with growth of the embryo, adherence of amniotic somatopleur and yolk sac splanchnopleur occurs in the regions where these two membranes abut on each other (Figure 1) thereby forming a single membrane, the vitello-amnion.

Amniogenesis

Blastocysts collected at the end of July show the initiation of amniogenesis while those collected at the end of August show a well-formed amnion. No body folds are present at this stage. The epithelium lining the amnion becomes covered on its outer surface by somatic mesoderm.

The amniotic membrane now consists of ectodermally derived squamous epithelium covered by somatic mesoderm. The latter rapidly adhere to the allantoic mesoderm to form the allanto-amnion (Figure 1).

Allantoic cavity

The allantoic cavity is represented by a short section of the urachus which extends for a short distance into the proximal part of the umbilical cord. It was lined by cuboidal epithelium in one specimen collected at the end of September. As the urachus was not encountered in any later stages of development of the umbilical cord, early regression can be assumed. However, the allantoic splanchnic mesoderm and associated umbilical vessels grow out from the hindgut to vascularize the extraembryonic membranes (chorion and amnion).

Umbilical cord

The umbilical cord is ca. 12 mm long, coiled and contains the five blood vessels, two umbilical arteries and one vein and one vitelline artery and vein. It is covered by squamous amniotic epithelium which is not provided with amniotic plaques. The supporting connective tissue, initially mucous connective tissue, gradually becomes more fibrous by deposition of reticular and collagen fibres.

Ultrastructure

In the zona intima the maternal blood channels are surrounded by the syntrophoblast. The maternal endothelium is lost but the endothelial basal lamina is retained (Figure 5). This basal lamina now occupies a space ca. 160 nm thick containing in its centre an electron dense line of ca. 75 nm wide, the lamina densa. The latter represents the lamina densa of the basal lamina of the maternal endothelium which has been doubled on itself. This doubling results in a thicker lamina densa with a lamina lucida on either side (Figure 6). This thickened basal lamina is referred to as the intrasyncytial membrane. Syntrophoblastic processes are pushed through the original endothelial basal lamina causing its doubling. The maternal blood vessels are referred to as maternal channels and are lined by syntrophoblastic processes (Figure 7). The latter are joined together by desmosomes and are provided with microvilli on the luminal side. These cytoplasmic processes contain few organelles, but do contain caveolae and multivesicular bodies. The major part of the syntrophoblastic syncytium is situated periferal to the intrasyncytial membrane, and contains the nuclei and organelles. The nuclei are irregularly shaped with prominent nucleoli and the granular endoplasmic reticulum is well developed. Numerous desmosomes

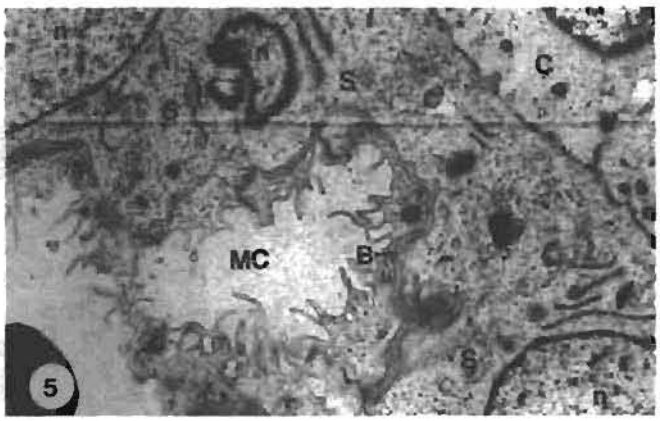

Figure 5 A low magnification electron micrograph of a placenta of *Scotophilus borbonicus* showing a maternal channel (MC) surrounded by the double layered basal lamina (intrasyncytial membrane; B) and syntrophoblast (S), and lined by syntrophoblastic processes with numerous microvilli. The cytotrophoblast (C) lies proximal to the syntrophoblast. Nuclei of both are seen (n), (×3 500).

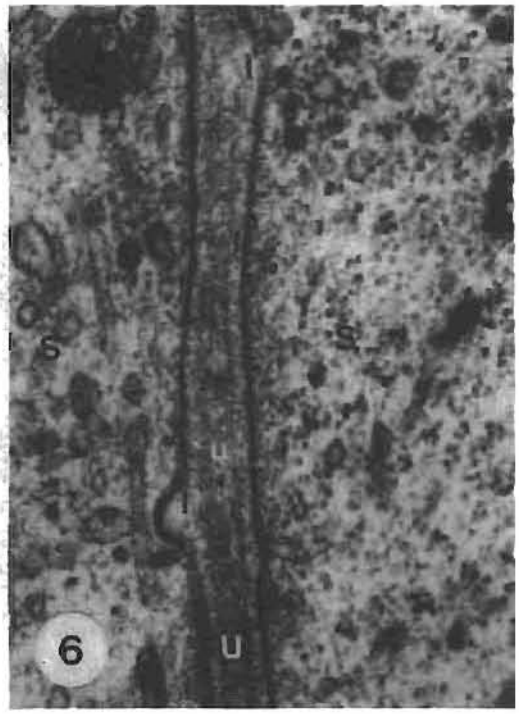

Figure 6 Electronmicrograph of the placenta of *Scotophilus borbonicus* illustrating a basal lamina [with a lamina lucida (I) and a lamina densa (u)] within the syntrophoblast (S), (×42 000).

connect the syntrophoblastic layer to a more proximally situated layer, the cytotrophoblast.

The cytotrophoblast is characterized by the irregularly shaped smaller nuclei with indistinct smaller nucleoli and less granular endoplasmic reticulum. The cytotrophoblast is more electron-lucent than the syntrophoblast (Figure 8).

The foetal blood vessels which are separated from the cytotrophoblast by fused splanchnic and somatic mesoderm, exhibit an endothelium resting on its own basal lamina. In the younger stages foetal blood vessels are easily identified by their nucleated erythrocytes.

Figure 7 Electronmicrograph of the placenta of *Scotophilus borbonicus* illustrating a syntrophoblastic process (P) passing through the basal lamina (B). Numerous microvilli extend into the maternal channel from this process, (×36 600).

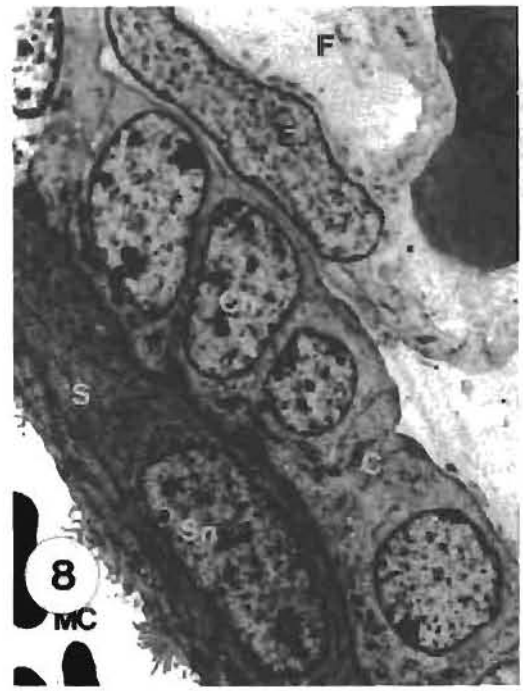

Figure 8 Electronmicrograph of the placenta of *Scotophilus borbonicus* illustrating a maternal channel (MC) with its syntrophoblast (S), syntrophoblast nucleus (Sn), cytotrophoblast (C), and cytotrophoblast nucleus (Cn). A foetal blood vessel (F) with an endothelial lining cell (E) is seen adjacent to the cytotrophoblast, (×8 100).

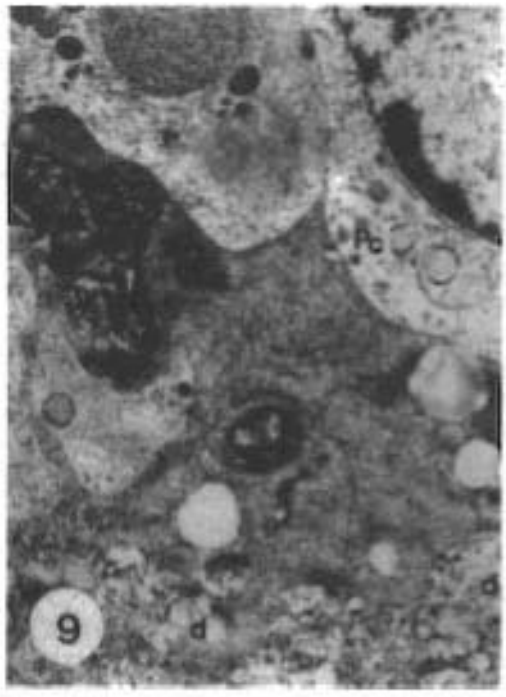

Figure 9 A dilated perinuclear cisterna (Pc), phagocytosed collagen fibrils (f) and detritus (d) are shown in this electronmicrograph of a cell of the trophospongium of the placenta of *Scotophilus borbonicus*, (×12 200).

The trophospongium exhibits a stratified cytotrophoblastic layer in which the cells have distinct cell membranes and prominent nuclei with distinct nucleoli. The cells have dilated perinuclear cisternae, which are confluent with dilated granular endoplasmic reticulum containing a granular substance. The cells adjacent to the detritus zone contain phagocytosed nuclear debris, bits of collagen fibres and numerous vacuoles. The general appearance of these cells is that of phagocytic cells (Figure 9).

The cells of the decidua basalis show a prominent dilated granular endoplasmic reticulum indicative of protein synthesis.

The detritus zone is characterized by cell debris and collagen fibrils.

Discussion

Allantochorionic placenta

The morphology of the placenta has, at least superficially, been described in all but four of the seventeen families recognized in the order Chiroptera (Wimsatt & Enders 1980). However, in the light of the findings of this investigation a review of the bat placental morphology is essential.

Primarily the placenta is constructed for active metabolic interchange between mother and foetus. The manner of nutrition of the developing foetus is both histotrophic and hemotrophic. The former occurs in the trophospongium and the latter in the zona intima (Mossman 1987).

In histotrophic nutrition the trophospongium is responsible for the phagocytosis of detritus, consisting of desquamated endometrial epithelial cells, endometrial stromal tissue and glandular secretions. In older foetuses this cellular debris mainly arises by the proliferation and desquamation of cells in the decidua basalis.

The fibroblasts of the decidua basalis are responsible for collagen and presumably ground substance formation, a fact which has not been noticed in the Chiroptera by previous workers. In this respect the chiropteran decidua corresponds to the human decidua which also secretes proteins (Kisalus, Wallace & Herr 1987). The active phagocytosis of the collagen fibres presumably forms a source of amino-acids for the fetus.

The trophoblastic cells of the trophospongium not only exhibit the characteristics of phagocytosis viz. a vacuolated cytoplasma as seen by the light microscope and a debris-filled cytoplasm by the electron microscope, but also all the features of a protein-secreting cell. These features are a dilated perinuclear cisterna and a dilated and prominent granular endoplasmic reticulum. The proteins are most probably enzymes for the breakdown of the debris in the detritus zone prior to phagocytosis, and for digestion of phagocytosed material.

This investigation has revealed that in the zona intima, the intrasyncytial space with its intrasyncytial lamina (Enders & Wimsatt 1968; Bjorkman & Wimsatt 1968; Wimsatt & Enders 1980) is the basal lamina of the original maternal endothelium which has been doubled on itself. The intrasyncytial space of the above authors is therefore homologous to the two laminae lucidae, and the intrasyncytical lamina is really a duplicated lamina densa (Martinez-Hernandes & Amenta 1983). This fact explains Enders & Wimsatt's (1968) finding that maternal vascularly injected *thorotrast* [thorium dioxide — used by Sinha (1967) to determine placental permeability] is found within the syncytium but not in the 'intrasyncytial space'. In this arrangement the syncytium is in direct contact with the maternal blood, but owing to the presence of this fused basal lamina within the syncytium, there must be some impediment to the flow of metabolites to the foetus. This type of placental structure is in contrast to the primate placenta where the chorionic villi more or less hang within the maternal blood spaces (Mossman 1987). In both, however, the foetal syncytium is in direct contact with maternal blood.

The persistence of the yolk sac up to term, and the hypertrophy of the endodermal and mesothelial cells are characteristic of all chiropteran placentae thus far described (Wimsatt & Enders 1980). After the foetal blood circulatory system and its constituents, as well as sexual differentiation (formation of primordial germ cells) have been established, the yolk sac may regress as is the case in ungulates (Gerneke 1985; Latshaw 1987). However, in their electron microscopical investigation of the yolk sac of *Myotis lucifugus lucifugus*, Enders, Wimsatt & King (1976) have shown that the hypertrophied endodermal cells are capable of synthetic and/or secretory activity. These cells all have a well developed Golgi apparatus and granular endoplasmic reticulum and therefore, in contrast to the yolk sac of ungulates, may have additional synthetic functions. The

hypertrophied mesothelial cells facing the extra-embryonic coelom were shown to be able to absorb proteins injected into the maternal vascular system (Enders *et al.* 1976). Although there is as yet no direct evidence that the absorbed protein is transported to the foetal blood circulation, the proximity of these cells to the vitelline blood vessels suggests that it may be possible. Specimens of the yolk sac were not available for electron microscopy in this investigation, but light microscopy has confirmed the hypertrophy of the endodermal and mesothelial cells and it can be assumed that the yolk sac plays an important metabolic role until parturition.

The amnion is formed as a schizamnion in members of the genus *Scotophilus* (Gopalakrishna & Karim 1979). This manner of formation is possibly an adaptation to the relative speed of the implantation process which takes about 14 days in *S. borbonicus*. With further development the amnion and allantochorion adhere and in this respect the placenta of *S. borbonicus* resembles that of the cloven-hoofed animals (Gerneke 1985; Latshaw 1987).

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