

Structure of the Definitive Placenta of the Tenrec, *Echinops telfairi*

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Until recently, tenrecs were classified with insectivores in the order Lipotyphla, but nucleotide sequence data suggest they have closer affinities with a group of African mammals called Afrotheria. The placenta of *Echinops* has not been described and no studies involving electron microscopy of the placenta of any species of tenrec have been published. We used light and transmission electron microscopy to examine fixed placentae of embryos ranging from 25–66 mm in length. The placental disk is situated in the antimesometrial portion of the bicornuate uterus. The greater part of the disk consists of a labyrinth underlain by a spongy zone. The interhaemal barrier is unusual in that the trophoblastic component is a single layer of cytotrophoblast. These trophoblast cells have thick areas especially near the nuclei and extensive thin flanges but only occasionally have membrane-closed pore regions. The luminal surface has isolated patches of microvilli, and pinocytotic vesicles are numerous both apically and basally. In the centre of the placental disk is an elaborately folded haemophagous region. The primary folds have allantoic endoderm at one surface and columnar cytotrophoblast at the other. These trophoblast cells have numerous lipid droplets and vesicles, and often contain large yellow pigment crystalloids. The labyrinthine zone ends abruptly at the margins of the placental disk. However, the endoderm and connective tissue of the allantois and a layer of cytotrophoblast extend beyond the placental disk as a paraplacental region. Some of these distinctive features of *Echinops* placenta are shared with individual afrotherians, but no significant characteristic of definitive placentation is shared by all the Afrotheria.

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INTRODUCTION

The spiny tenrecs or Tenrecinae include the ‘Madagascar hedgehogs,’ *Setifer* and *Echinops*, which resemble the true hedgehogs in several dental features and in the spiny pelage [1]. Indeed, tenrecs usually are grouped with other insectivores on the basis of their dental structure, small brain case and poorly differentiated brain [1–3]. They exhibit a number of conserved character states, including the presence of a cloaca, nondescending testes in males and variable body temperature [4]. Apart from the otter shrews of western and central equatorial Africa, most tenrecs are found on Madagascar, where they have undergone extensive adaptive radiation, probably from two founder populations [5].

Tenrecs loom large in the current debate on mammalian phylogenetics. Based on analysis of the nucleotide sequences of genes, it has been proposed that extant mammals can be divided into four superorders. One of these, named Afrotheria, is thought to have emerged in Africa during the Cretaceous period. It comprises elephants, sea cows (manatees and dugong), hyraxes, elephant shrews, golden moles, tenrecs and the aardvark [6–8]. Although this hypothesis is supported by several types of molecular evidence, there is not a single

morphological character (synapomorphy) that would unite the tenrecs with other members of the Afrotheria [9,10]. Indeed, studies of bones, teeth and some soft tissue characters provide stronger support for the classical grouping of tenrecs with insectivores [11]. Fetal membrane characters have earlier been used as a guide to phylogeny [12,13] and Carter [14] pointed out that consideration of a number of features of placentation might throw light on these controversial phylogenetic relationships.

Some studies of the placenta of *Setifer* [15], *Hemicentetes* [16,17] and *Tenrec* [18] have been published, but the placenta of *Echinops* has not been studied and no studies involving electron microscopy of the placenta of any of these species has appeared. *Echinops telfairi* is a small Madagascar hedgehog tenrec which has been maintained successfully in a colony in Munich. The gestation period of the females in this colony is 50–60 days, and although as many as 8 or 9 embryos may be conceived, an average of 3–4 young is born once a year [19]. The present study was initiated to determine the structural features of the placenta of this genus.

MATERIALS AND METHODS

The colony of Madagascan lesser (pygmy) hedgehog tenrec, *E. telfairi*, was housed and maintained as described previously

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[19]. Three animals in the latter part of gestation were used for the present study. The animals, weighing between 140 and 155 g, were anaesthetized with tribromoethanol (1.0 ml/100 g, i.p.). One animal was perfused transcidentally with saline, followed by a phosphate-buffered solution (0.05 M) of 1 per cent paraformaldehyde and 2.5 per cent glutaraldehyde; subsequently the fetuses (length 25–27 mm) were removed and the placentae postfixed in the same solution. In the other two animals the fetuses (length 57–59 and 56–66 mm, respectively) were first removed through small incisions in the uterine wall (in a few cases the gestational sac was left intact). Subsequently the animals were perfused through the heart with saline followed by a solution of 4 per cent buffered paraformaldehyde. The uterus was removed and the placentae were hemisected and in some cases quartered. The resultant pieces were placed in part in 4 per cent paraformaldehyde, in part in 2 per cent paraformaldehyde–2 per cent glutaraldehyde fixative.

Subsequently sections from a total of 13 placentae were prepared and examined for light microscopy, of which seven were also examined by transmission electron microscopy (TEM). The placentae used for light microscopy were embedded in paraffin and sectioned at 5 μm . The slides were stained with haematoxylin and eosin or underwent antigen retrieval and were immunostained for cytokeratin to identify epithelial cells and trophoblasts. Tissue sections were incubated with a mouse monoclonal antibody for wide spectrum screening of cytokeratins (Clone AE1/AE3, Zymed, San Francisco, CA, USA). Immunostaining was identified by the avidin–biotin–peroxidase technique with diaminobenzidine as the chromogen. Tissues that were prepared in glutaraldehyde-containing fixative were postfixed in 2 per cent osmium tetroxide, dehydrated and embedded in Araldite epoxy resin. Approximately 1 μm sections were stained with Azure B and examined by light microscopy, after which selected regions were thin-sectioned and examined by TEM.

RESULTS

The chorioallantoic placenta of *Echinops* is of the discoid, labyrinthine and haemochorial type. There are prominent accessory structures, a central haemophagous zone and a peripheral paraplacenta. The placental disks are irregularly circular structures (Figure 1A) situated roughly antimesometrially along both horns of the bicornuate uterus. The greater part of each disk consists of the labyrinth overlain by allantoic endoderm and underlain by a spongy zone (Figure 1B). In the centre of the disk is an elaborately folded haemophagous zone. The spongy zone reaches the myometrium in some places; in others there is a thin layer of endometrial connective tissue and an occasional uterine gland. In only a few areas beneath the haemophagous zone can any uterine luminal epithelium be found.

Labyrinthine zone

The fetal surface of the placental disk faces the allantoic cavity. The allantoic endodermal cells overlying the disk are cuboidal with short microvilli extending into the allantoic cavity (Figure 2). Their lateral borders beneath the short apical junctional complexes are often elaborately interfolded, and lesser infoldings are seen on the basal surface adjacent to a thick basal lamina.

The cord vessels branch before reaching the surface of the placental disk (Figure 1A), and a variety of sizes of allantoic vessels are found within the connective tissue beneath the endoderm (Figure 3A). Some of these vessels extend well into the labyrinthine zone at right angles to the free surface (Figure 3A). Smaller capillaries also extend in this fashion into the labyrinthine zone, accompanied by smaller amounts of connective tissue.

Maternal channels whose endothelium has been replaced by a thin layer of cytotrophoblast roughly parallel the fetal vessels. They communicate with large, similarly lined spaces just beneath the fetal surface of the placental disk (Figure 3A). Within the labyrinth (Figure 3B) the maternal channels tend to be more irregular in shape than the fetal capillaries, are larger, and are clearly anastomotic, especially near the spongy zone. Under the allantoic surface the channels emerge from the large spaces by multiple openings into the labyrinth. The cytotrophoblast layer lining the maternal channels is cytokeratin-positive (Figure 3C).

A thick basal lamina initially underlies the trophoblast cells that form the maternal channels (Figure 4A). A thinner basal lamina underlies the fetal vessels, which are often separated from the trophoblast by a small amount of connective tissue, especially near the fetal surface. The trophoblast cells of the channels are more irregular in thickness than are the endothelial cells of the fetal capillaries (Figure 4B). The individual trophoblast cells forming the maternal channels have thick areas especially near the nucleus and thin flanges that have occasional areas of membrane-closed pores; the surface has isolated patches of microvilli. The patches of microvilli are most frequently in clefts, but not exclusively. They have no visible glycocalyx and bear no special relationship to any of the underlying organelles. There are occasionally but not always caveolae associated with them. The cytoplasm of the trophoblast cells contains short strands of granular endoplasmic reticulum and numerous pinocytotic vesicles both apically and basally. Where trophoblast cells abut one another apical junctional complexes and occasional desmosomes may be found. It is not clear whether the junctional complexes contain tight junctions in addition to adhesion areas.

The fetal capillaries are unremarkable, with thin areas but no pores and fewer pinocytotic vesicles than the trophoblast. In many places, where capillaries approximate maternal channels, the thin basal lamina of the fetal endothelium and thicker basal lamina of the trophoblast tend to be slightly separated. In other regions there are a few collagen fibres interposed, an occasional perivascular cell and even an occasional fibroblast.

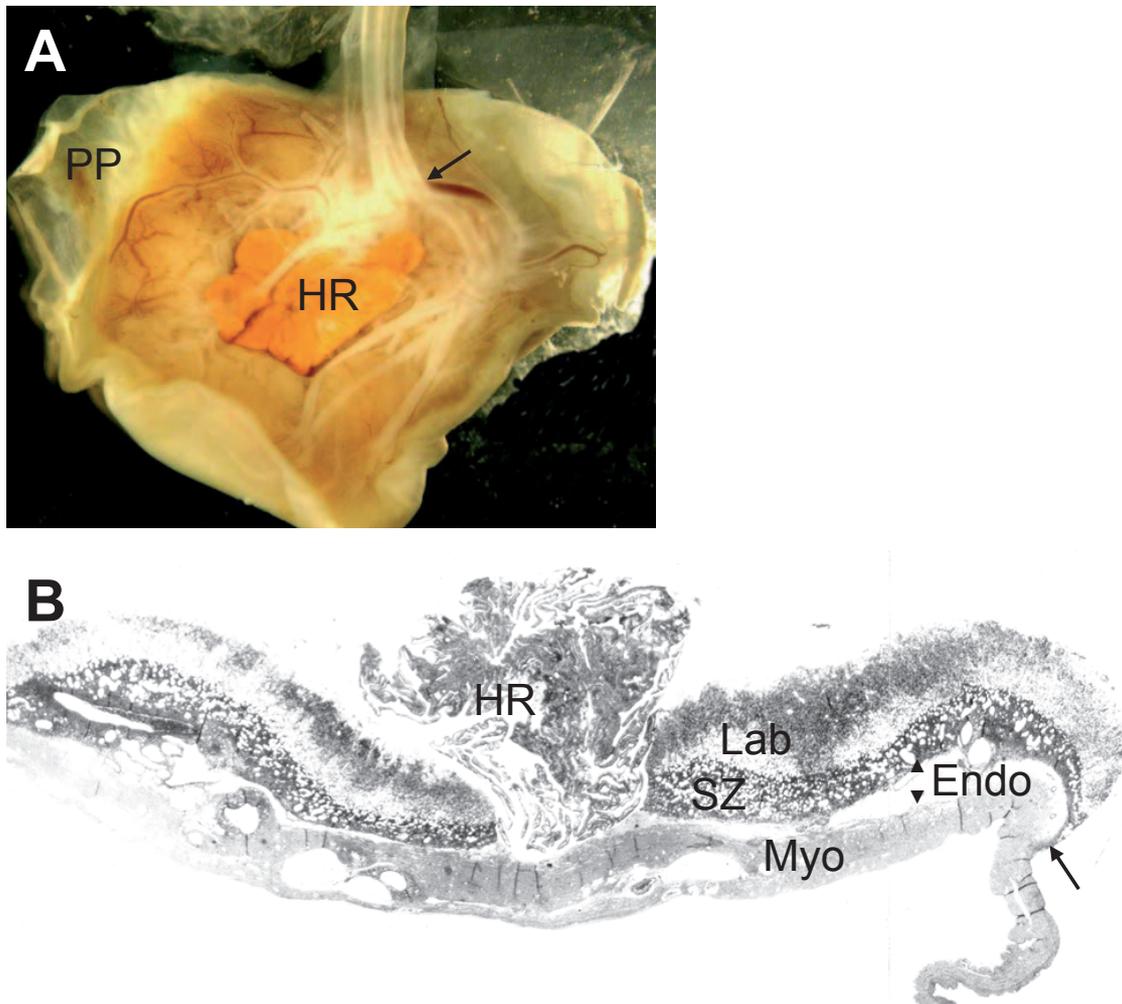


Figure 1. (A) Placental disk removed from the uterus. The circular disk is surrounded by the thinner paraplacental region (PP). Note the haemophagous region (HR) in the centre of the disk. The allantoic vessels branch above the level of the disk (arrow). CRL (crown–rump length) 26 mm. $\times 5$. (B) Section through the placental disk. The folded haemophagous region is in the centre. The labyrinth (Lab) is underlain by the spongy zone (SZ). The amount of endometrium (Endo) overlying the myometrium (Myo) is variable. At the edge of the disc, allantoic endoderm and connective tissue and cytotrophoblast continue to form a thin paraplacenta (arrow). The apparent differences in density within the labyrinth are due to variable amounts of retained blood. CRL 58 mm. $\times 12$.

Large cells with granules and/or vacuoles are found within the fetal connective tissue of the labyrinth, especially near the allantoic side of the labyrinth (Figure 5A). These cells, which appear to be fetal macrophages, are most obvious in the youngest specimen. They are somewhat similar in location and shape to the Hofbauer cell seen in many placentae and commonly abut other cells, which appear to be fibroblasts. While the fetal capillaries show no differences as the spongy zone is approached, the maternal channels in this location show considerable anastomosis (Figure 5B).

Trophoblast spongy zone

The fetal connective tissue ends abruptly at the junction between the labyrinth and the spongy zone (Figure 5C). The spongy zone consists of a thick pad of polygonal cytotrophoblast cells of varying sizes perforated by channels containing maternal blood. The channels are lined by squamous cells but

it is difficult to determine the origin of these cells at the different levels of the spongy zone. However, most of the flattened lining cells have cytokeratin staining similar to that of cytotrophoblast cells lining the maternal blood spaces within the labyrinth (Figure 5D). There is considerable heterogeneity within the spongy zone, and mitotic figures are more abundant throughout this zone than in the labyrinth. Multinucleate cells, although not abundant, are scattered near the maternal side of the spongy zone. They do not constitute a complete layer. Darker smaller trophoblast cells tend to surround the thinner cells lining some of the maternal channels. These dark cells are more numerous towards the labyrinthine side of the spongy zone than the uterine side (Figure 5C). At the margin of the placental disk the spongy zone is covered by allantoic endoderm and a somewhat thicker layer of stromal connective tissue than is found over the labyrinth.

Typically, trophoblast cells of the spongy zone have abundant granular endoplasmic reticulum with variable amounts of

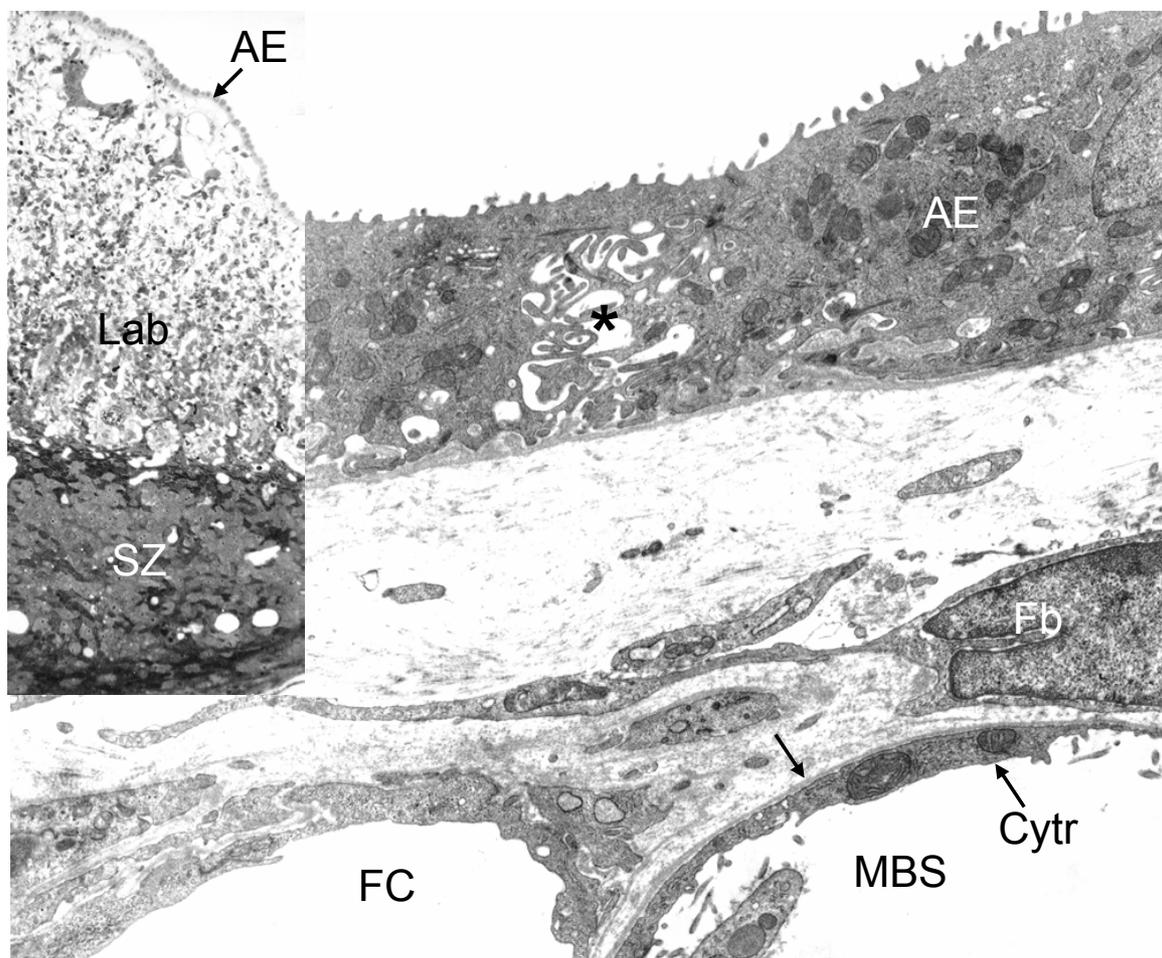


Figure 2. Allantoic side of the labyrinth. Note the interdigitated region (*) between the two cells of the allantoic endoderm (AE). A fetal capillary (FC) and maternal blood space (MBS) underlie a region of fetal connective tissue, including a fibroblast (Fb). Note the distinct basal lamina (arrow) beneath the trophoblast (Cytr) that delimits the maternal blood space. CRL 58 mm. $\times 11\,500$. Inset: a section through the placental disk to show the position of the allantoic endoderm in relation to the labyrinth (Lab) and spongy zone (SZ). CRL 58 mm. $\times 100$.

flocculent internal content (Figure 6). Although the cells are roughly polygonal they often have numerous projections, especially near the maternal surface. They share numerous adhesion junctions, especially desmosomes, and frequently have flocculent extracellular matrix between them but no collagen fibrils. Patches of intermediate filaments are often seen in the cytoplasmic projections, and membrane bound granular structures sometimes with heterogeneous content, which are probably lysosomes, are also numerous.

The uterine tissues underlying the spongy zone vary considerably. In places where the endometrium is thick normal stromal cells may be found. In other places some necrosis and numerous macrophages are present. The cytotrophoblast of the spongy zone often interrupts endometrial glands (Figure 7A) and in places is directly adjacent to glands (Figure 8) or to the myometrial layer. Where maternal arteries extend into the spongy zone their circumference is interrupted by cytotrophoblast cells which also extend into both the adjacent vessel walls and more rarely into the vessel lumen (Figure 9A). The smooth muscle cells of arteries in the underlying endometrium

tend to have unusually large deposits of glycogen (Figure 9A–B). Occasional arteries below the spongy zone within maternal tissue show luminal invasion by cytotrophoblast either alone or in addition to invasion of the walls of these vessels (Figure 7B–C). Veins in the endometrium are not invaded by cytotrophoblast and veins at the junction with the spongy zone are interrupted in a fashion similar to that of the glands.

Directional flow of blood within the placenta

The maternal blood supply originates in the endometrium as large arteries surrounded by smooth muscle containing large amounts of glycogen. As the arteries come to the base of the spongy zone, trophoblast cells tend to intrude into the walls. As they proceed into the spongy zone the vessel wall is completely replaced by cytotrophoblast, with loss of the endothelial cells and smooth muscle (Figure 10B–C). The cytotrophoblast cells nearest the lumen of the artery tend to be

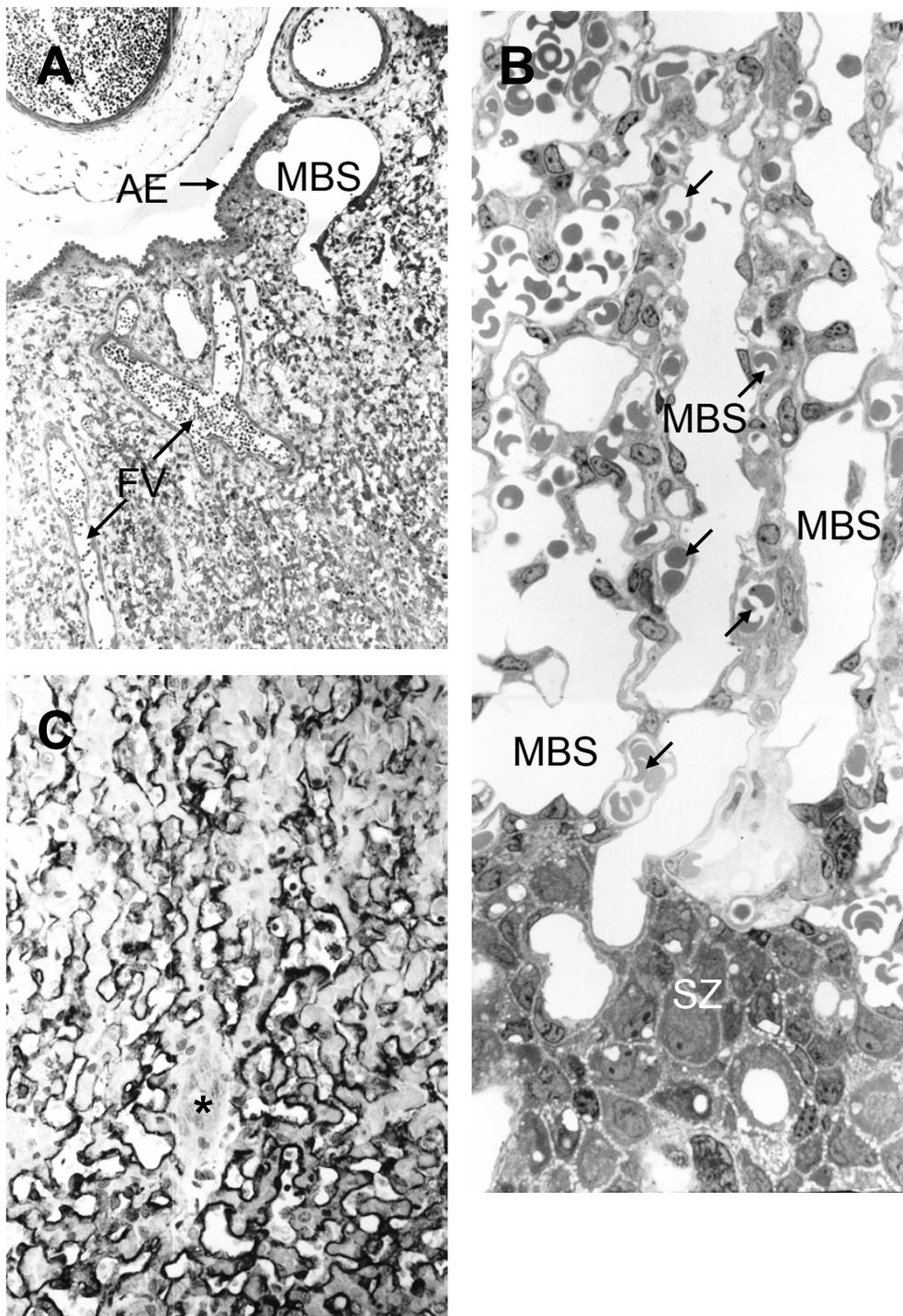


Figure 3. (A) Maternal blood spaces (MBS) and fetal vessels (FV) that carry blood into the labyrinth are seen near the surface of the labyrinth beneath the allantoic endoderm (AE). A large allantoic vessel appears in the upper left. CRL 26 mm. $\times 100$. (B) Junction of the labyrinth with the spongy zone (SZ). The numerous fetal capillaries (arrows) are confined to the connective tissue septae between the maternal blood spaces (MBS). CRL 58 mm. $\times 740$. (C) Cyokeratin stains the trophoblast lining the maternal blood spaces but not the capillaries or arteriole (*) constituting the fetal vascular component of the labyrinth. CRL 63 mm. $\times 270$.

smaller and more elongated than those of the surrounding spongy zone. The more distal cytotrophoblast is similar to the rest of the spongy zone trophoblast. These arterial channels pass into the labyrinth where they expand in diameter and

their walls thin to only a couple of layers of trophoblast cells, with a bit of connective tissue between the trophoblast and adjacent labyrinth channels. When the major channels reach the fetal side of the labyrinth they extend laterally into a series

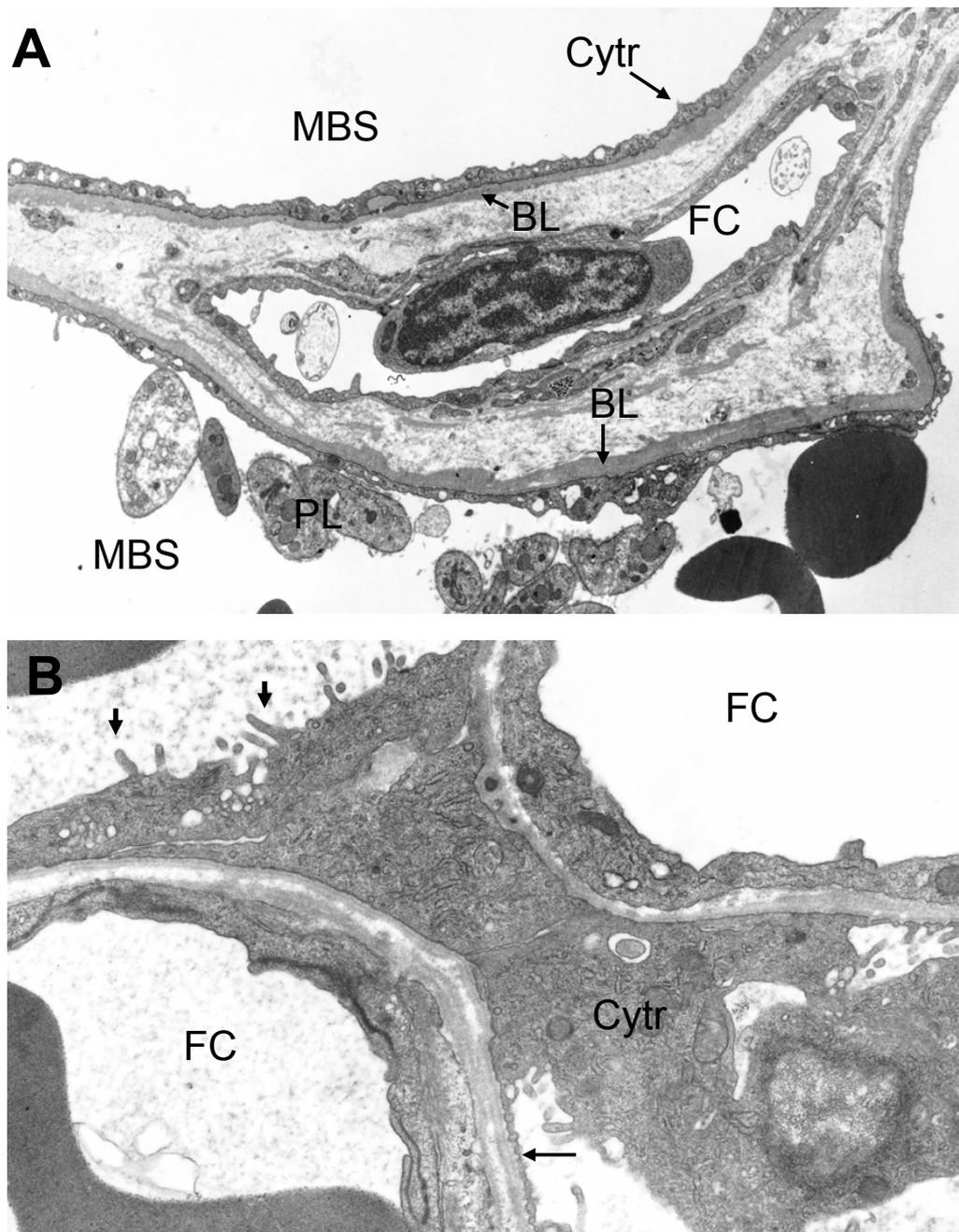


Figure 4. (A) A fetal capillary (FC) is situated in the connective tissue between two maternal blood spaces (MBS). Note the robust basal lamina (BL) beneath the trophoblast (Cytr) lining maternal blood spaces. CRL 58 mm. $\times 8900$. (B) Interhaemal area of the labyrinth. Parts of three cytotrophoblast cells (Cytr) are interposed between two fetal capillaries (FC). Note the microvilli (short arrows) projecting into the maternal blood space at upper left and the pore region (arrow) in the thinnest flange of trophoblast. PL, platelets. CRL 58 mm. $\times 15\,000$.

of pooled maternal blood spaces which have multiple openings into the labyrinth. Maternal blood entering the multiple irregular channels subsequently passes down towards the spongy zone, between columns and septae of connective tissue containing fetal vessels. Near the border with the spongy zone there are numerous anastomoses of the maternal channels but no large spaces as at the fetal surface. The pathway back through the spongy zone is less direct than that in the

labyrinth, consisting of small branching channels, then larger confluences towards the maternal junction (Figure 10D). The channels eventually connect to dilated but otherwise normal veins in the endometrium.

The fetal blood arrives from many arteries that branch close to the fetal surface of the disk (Figure 3A). Then a number of small uniform arteries from the allantoic connective tissue extend towards the spongy zone. The branches of these small

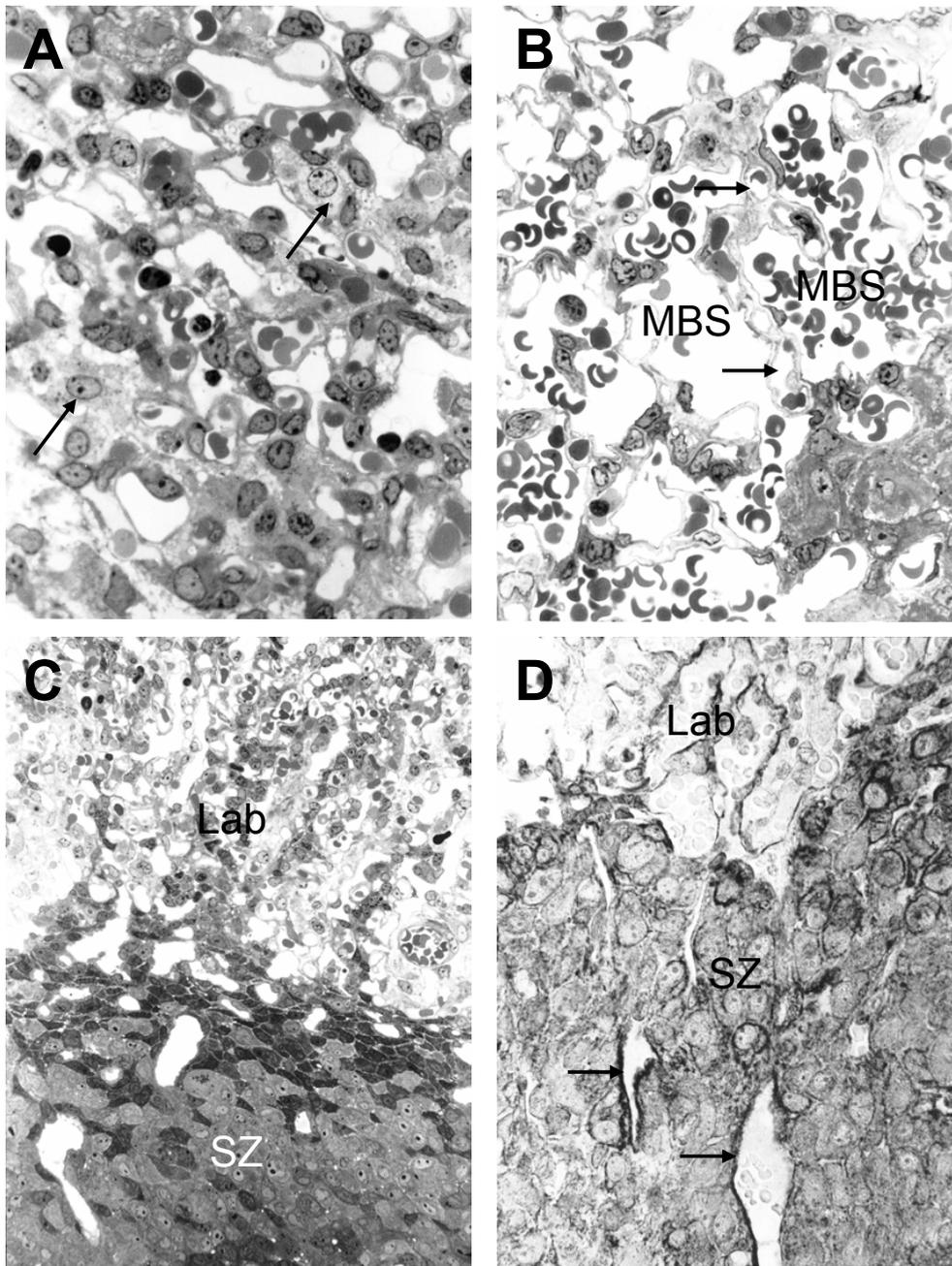


Figure 5. (A) Fetal macrophages (arrows) are found in the connective tissue of the labyrinth. CRL 26 mm. $\times 740$. (B) The maternal blood spaces (MBS) are larger and more irregular than the fetal capillaries (arrows). Note the anastomotic nature of the maternal blood space on the right just above a few spongy zone trophoblast cells. CRL 58 mm. $\times 740$. (C) Some heterogeneity in trophoblast cell size is seen at the junction of the labyrinth (Lab) and spongy zone (SZ). Note that the darker trophoblast cells found near the border and adjacent to maternal channels are somewhat smaller than the more numerous pale trophoblast cells. CRL 26 mm. $\times 300$. (D) The cytotrophoblast cells at the labyrinth–spongy zone junction stain somewhat variably with cytokeratin antibodies. Note that the thin trophoblast (arrows) lining the maternal blood spaces is more strongly cytokeratin-positive. CRL 63 mm. $\times 470$.

arteries wander back in a more convoluted fashion between maternal blood spaces to return to the fetal surface.

This arrangement of the major vessels results in an overall pattern of countercurrent flow. However, there are cross current components at every level, resulting from the irregular nature of the maternal blood spaces and convoluted pathways of the fetal vessels within the labyrinth.

Haemophagous region

The haemophagous region is a multiply folded region in the centre of the placental disk (Figure 11A). The primary folds have allantoic endoderm at one surface and columnar cytotrophoblast at the other (Figure 11B). Connective tissue and allantoic vessels are interposed. Secondary folds consisting

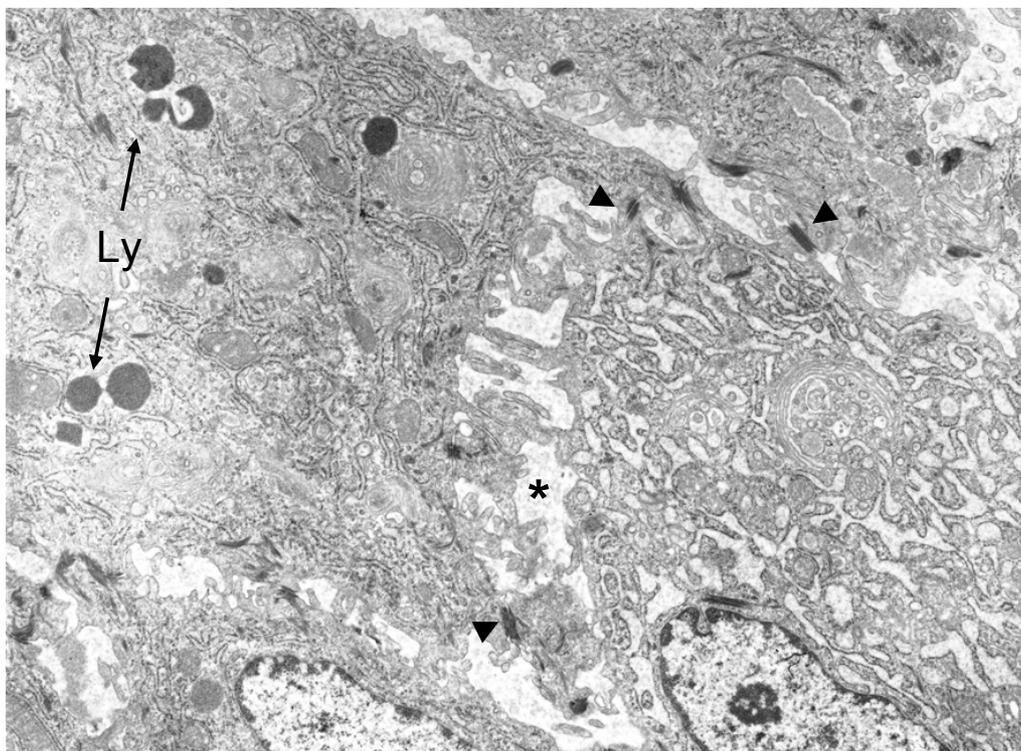


Figure 6. Trophoblast cells in the spongy zone have abundant granular endoplasmic reticulum which is sometimes dilated. They are joined by adhesion junctions (arrowheads) and have a flocculent material but no collagen fibrils in the intercellular spaces (*). Ly, lysosomal structures. CRL 63 mm. $\times 11\,500$

simply of trophoblast epithelium and underlying connective tissue and vessels increase the complexity of folding in this region (Figure 11B). The allantoic endoderm cells are cuboidal, do not exhibit as much lateral interfolding in this region as they do over the labyrinth, and generally lack any unusual inclusions (Figure 12). The trophoblast cells on the other hand are tall columnar, have numerous lipid droplets, a number of small granules and vesicles (Figure 12), and often contain large yellow pigment crystalloids. The crystalloids in these cells give an overall appearance of orange to the haemophagous region.

Except for a small area of one sample (Figure 11C), there were rather few phagocytosed erythrocytes at this definitive stage of placental development. The luminal area between secondary folds was usually clean but in patches had some debris including crystalloids but did not have many unmodified erythrocytes. The crystalloids in this region gave a positive Gmelin reaction for haematoidin.

The primary folds of the haemophagous region reflect from the chorioallantoic placenta around the margin of the central depression. They vary in level of attachment from the labyrinthine zone to the junction between the labyrinthine and spongy zone. In some areas the depression under the haemophagous region appears denuded of uterine luminal epithelium; in other regions a small amount of luminal epithelium remains.

Paraplacenta

The labyrinthine zone ends abruptly at the margins of the placental disk. However, the endoderm and connective tissue of the allantois and a layer of cytotrophoblast cells extend beyond the placental disk as a paraplacental region (Figure 13). Consequently the paraplacental region consists of a layer of low cuboidal endodermal cells, connective tissue, including a few allantoic vessels, and varying numbers of cytotrophoblast cells (Figure 13A). The latter have a similar structure to the cytotrophoblast cells of the spongy zone, as they lie along a thick basal lamina (Figure 13C). In some areas where the paraplacenta abuts endometrial glands, extensive processes from the trophoblast cells are present towards the glandular epithelium. In many areas the cytotrophoblast cells adjacent to maternal connective tissue have irregular processes with flocculent extracellular matrix similar to that in the spongy zone. The underlying maternal connective tissue is unmodified showing no evidence of inflammation or immune response. The number of trophoblast cells and the thickness of the region vary considerably (Figure 13 B, D) and in dissected specimens this region was extensively folded, probably due to muscle contraction. Although the paraplacenta extends around the entire circumference of the uterus, on the mesometrial side it is quite thin and in a few places short patches of uterine luminal epithelium are found beneath the paraplacental

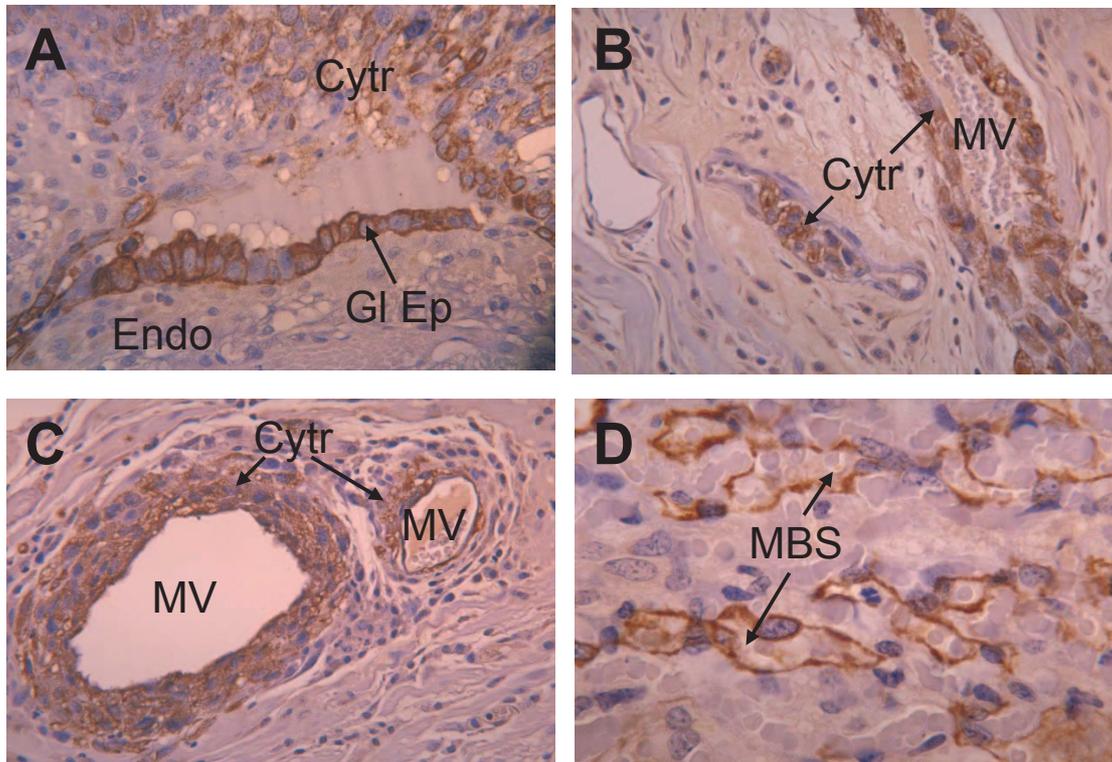


Figure 7. Cytokeratin-stained sections. (A) The cytotrophoblast (Cytr) of the spongy zone interrupts a uterine gland. GI Ep, uterine gland epithelium; Endo, endometrium. CRL 58 mm. $\times 300$. (B–C) Cytotrophoblast invasion of the walls and lumen of maternal vessels (MV) in the endometrium. CRL 63 mm. $\times 180$. (D) Trophoblast lining the maternal blood spaces (MBS) within the labyrinth is strongly cytotrophoblast-positive. CRL 63 mm. $\times 450$.

trophoblast. In such areas the paraplacenta consists of one or two layers of elongated trophoblast cells overlain by a thin region of connective tissue and allantoic endoderm.

The endodermal cells covering the paraplacental region have numerous short microvilli on their free surface but have less elaborate intercellular borders than are found over the labyrinth (Figure 13C). There is a thick and slightly irregular basal lamina beneath the endodermal epithelium. Cross-banded collagen fibrils are present in the connective tissue beneath these cells, and the fibroblasts in this connective tissue tend to be elongated with rather thin extensive processes. A thick basal lamina is found between the connective tissue and the trophoblast cells, and some basal lamina material seems to extend irregularly into the connective tissue. Since the paraplacenta is poorly vascularized by fetal vessels, exchanges from the maternal system to the fetal side would be through the allantoic endoderm to the allantoic cavity.

DISCUSSION

Two intriguing features of placentation in *Echinops* are the prominent haemophagous organ at the centre of the disk and the extensive paraplacenta. In these respects *Echinops* is closely similar to *Setifer setosus* [15]. Two other species of tenrec, *Hemicentetes semispinosus* [16,17] and *Tenrec ecaudatus* [18],

have placentae with the same basic plan, but their paraplacenta is more clearly adapted to histiotrophic nutrition. Thus in *Tenrec*, the uterine glands open into large sinuses within the paraplacenta, where the secretions of the uterine glands accumulate (Sekretblasen; [18]). In all Madagascar tenrecs so far examined, the placental disk comprises a labyrinth and a spongy zone. The ultrastructure of tenrec placenta has not been described previously. We have confirmed that the placenta is haemochorial and shown that the trophoblastic component in the interhaemal area is comprised of a single layer of cytotrophoblast.

Tenrecs have long been regarded as among the most primitive of living mammals and for this reason attracted the attention of Strahl [20] and Grosser [21], who were somewhat perplexed to find they had haemochorial placentation. Until recently, tenrecs were classified with other insectivores in the order Lipotyphla. Analysis of nucleotide sequence data, however, suggests that they have closer affinities with a clade of African mammals called the Afrotheria [22]. This conclusion is strengthened by recent analyses of insectivores and tenrecs that encompass a large number of relevant taxa [9,23]. Yet it remains controversial because analyses of teeth and bones and some soft tissue characters do not support Afrotheria and are more consistent with the classical view of tenrecs as insectivores [11,24,25]. In the following we highlight some of the features of placentation in *Echinops* and discuss them in relation to other afrotherians.

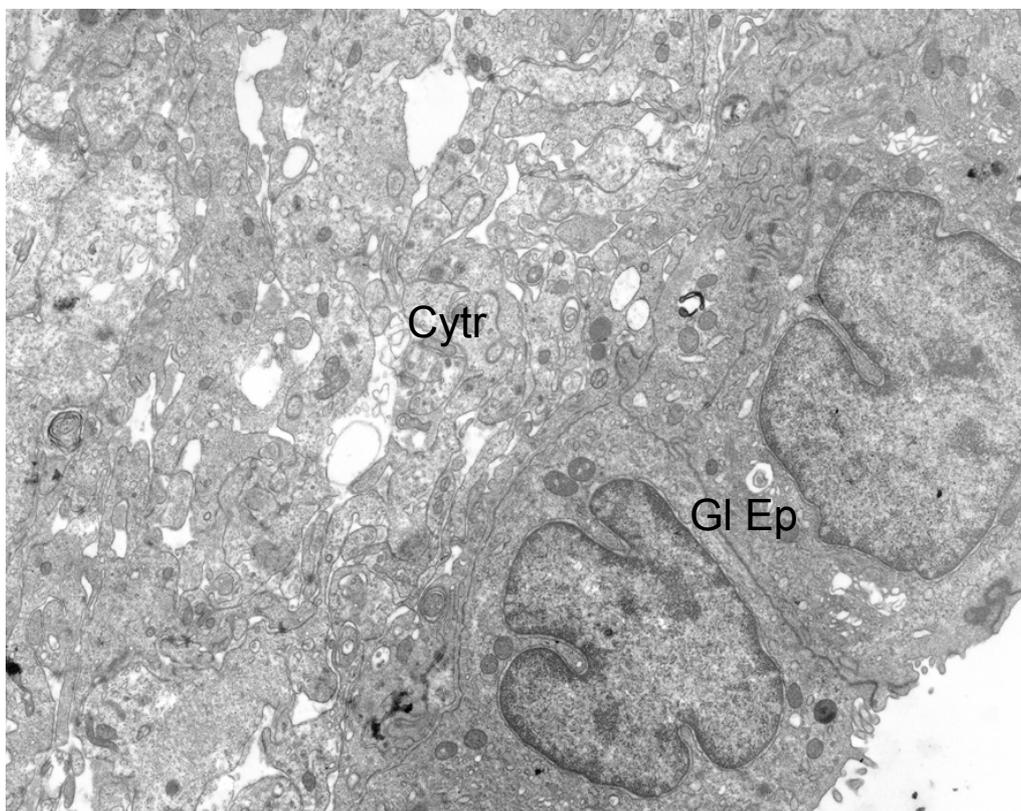


Figure 8. In the spongy zone, numerous projections of the trophoblast cells (Cytr) abut one another and endometrial gland epithelial cells (GI Ep). CRL 58 mm. $\times 11\,500$.

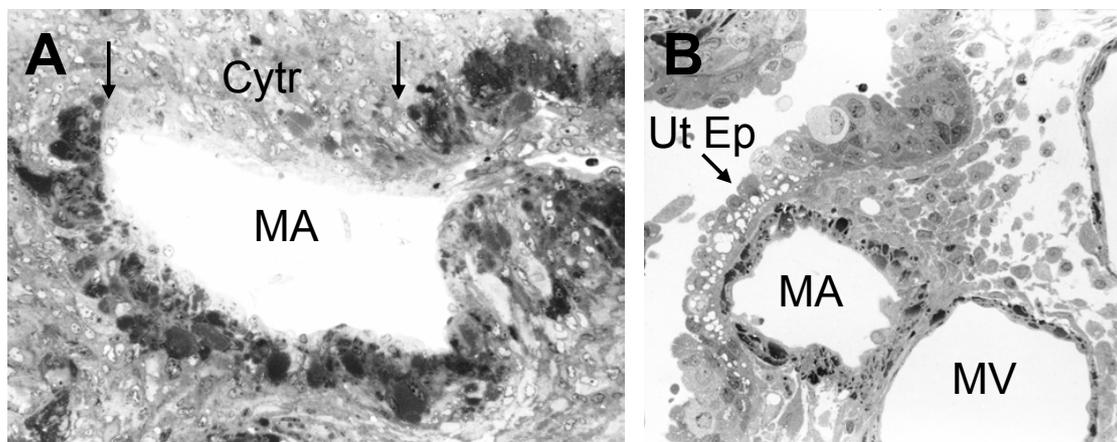


Figure 9. (A) A patch of cytotrophoblast (Cytr) has replaced all aspects of the wall of a maternal artery (MA) between the arrows. The enlarged smooth muscle cells of the artery appear dense due to the staining of the large amounts of glycogen within these cells. CRL 63 mm. $\times 300$. (B) Endometrium below the placental disk. The maternal artery (MA) and vein (MV) have smooth muscle cells with small amounts of glycogen (dark deposits). The residual uterine epithelium (Ut Ep) is appreciably modified. CRL 58 mm. $\times 300$.

The interhaemal barrier of *Echinops* is unusual in that the trophoblastic component is a single layer of cytotrophoblast. This occurs in one other afrotherian, the yellow-spotted hyrax, *Heterohyrax brucei* [26]. On the other hand, the single layer of trophoblast in the golden-rumped elephant shrew, *Rhynchocyon chrysopygus*, is syncytial in nature [27]. The ultrastructure of the interhaemal barrier has not been studied in other afrotherians, except for the African elephant, which

has cellular trophoblast in an endotheliochorial relationship [28]. Cellular haemomonochorial placentation does occur in some unrelated species [29]. Thus, in some molossid bats, the central area of the placenta near term is a labyrinth of cellular trophoblast, although with intrasyncytial bays [29,30]. The jumping mouse (*Zapus* sp.) and jerboa (*Jaculus* sp.) also have cellular haemomonochorial placentae in late gestation [31].

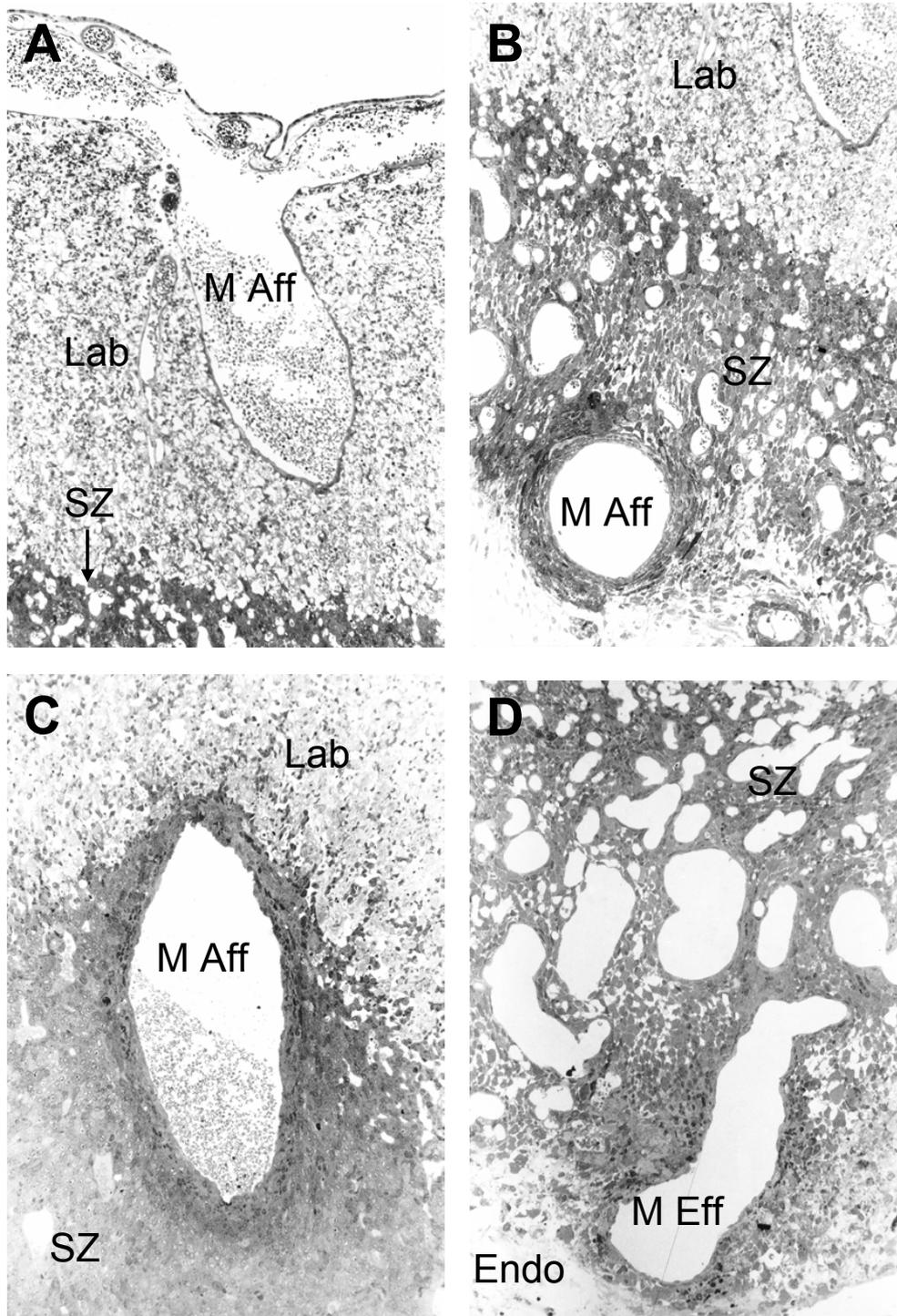


Figure 10. (A) A major maternal afferent channel in the labyrinth (M Aff) extends under the allantoic surface to the pooled maternal blood spaces that supply the channels of the labyrinth (Lab) which return maternal blood to the spongy zone (SZ). CRL 58 mm. $\times 100$. (B) The same major maternal channel appears in the upper right. The multiple efferent channels in the spongy zone are seen as is the base of a major afferent channel. CRL 58 mm. $\times 100$. (C) A major maternal afferent channel is seen at the border between the labyrinth and the spongy zone. CRL 63 mm. $\times 100$. (D) The channels in the spongy zone are relatively small near the labyrinth and increase in size as they approach the endometrial side, where they coalesce to form efferent channels (M Eff), lined by trophoblast, returning maternal blood to the endometrium (Endo). CRL 58 mm. $\times 100$.

In common with the placentae of many mammals, those of afrotherians all have a spongy zone of uncertain function. It has been suggested that it secretes hormones, but no

product has been identified. A recent study demonstrated that the placenta of the African elephant is entirely devoid of steroidogenic capacity and does not possess gonadotropic

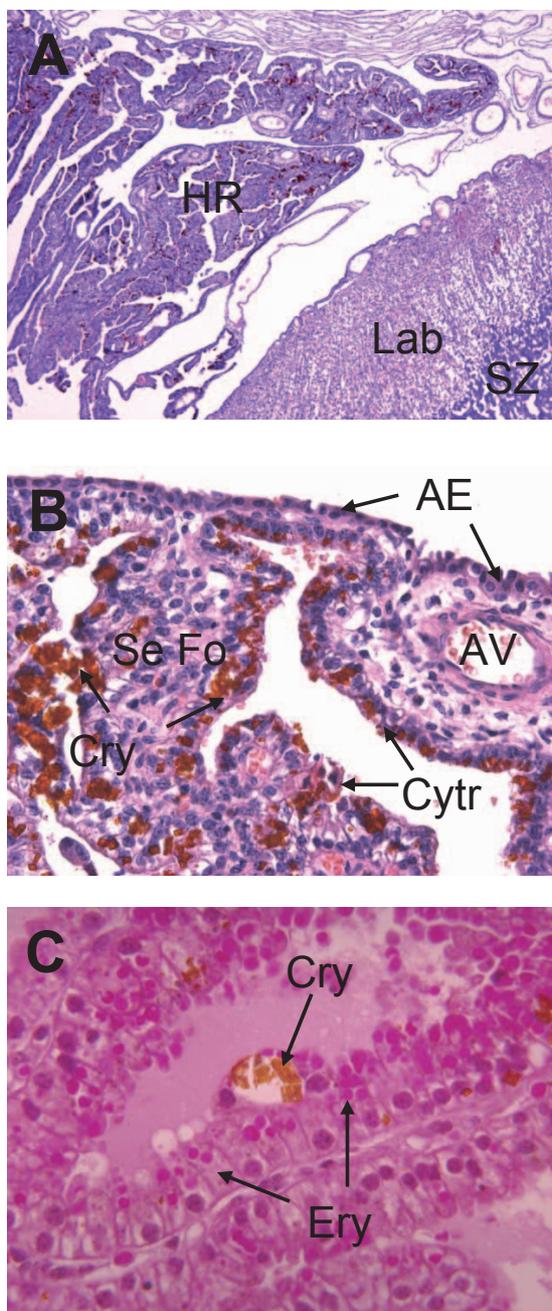


Figure 11. (A) The haemophagous region is multiply folded and found at the centre of the placental disk. Portions of the labyrinth (Lab) and spongy zone (SZ) are seen at lower right. CRL 58 mm. $\times 40$. (B) The primary folds of the haemophagous region have allantoic endoderm (AE) at one surface and columnar cytotrophoblast (Cytr) at the other with connective tissue and allantoic vessels (AV) interposed. Secondary folds (Se Fo) have trophoblast on both surfaces. The trophoblast cells contain orange crystals (Cry) of haematoidin. CRL 58 mm. $\times 400$. (C) Phagocytosed erythrocytes (Ery) can be found within the trophoblast cells as well as haematoidin crystals. CRL 26 mm. $\times 300$.

activity [32]. The placenta of the rock hyrax does not make progesterone [33].

Maternal arteries below the spongy zone of the placenta and within the myometrium of *Echinops* all showed unusually large glycogen deposits and many arteries showed both luminal and

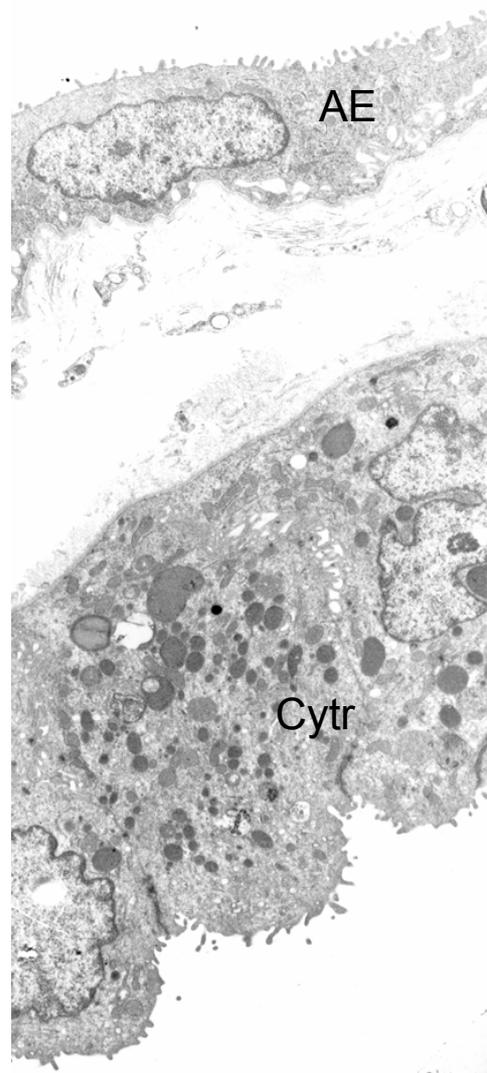


Figure 12. Section through a major fold of the haemophagous region. The allantoic endodermal cells (AE) are cuboidal. The columnar trophoblast cells (Cytr) have numerous granules, but most are devoid of phagocytosed erythrocytes at this stage. CRL 58 mm. $\times 8900$.

intramural invasion by cytotrophoblast. Maternal vessels are invested by a sheath of cytotrophoblast in rock hyraxes [34,35], manatee [36] and African elephant [37]. The tunica media is possibly transformed in the elephant shrew *Elephantulus* [38] and is invaded by giant cells in golden moles [39].

All Madagascar tenrecs thus far examined have a prominent, centrally situated haemophagous region [15,16,18]. There is no comparable structure in other afrotherians. The zonary placentae of the African elephant and armadillo have marginal haemophagous regions and, in the manatee, haemophagous regions are found over the entire fetal surface of the labyrinth rather than at the centre. The placentae of golden moles, elephant shrews and rock hyraxes seem not to have haemophagous regions. A haemophagous region of comparable complexity is, however, found in carnivores, with variable location including a central position in mustelids and raccoons

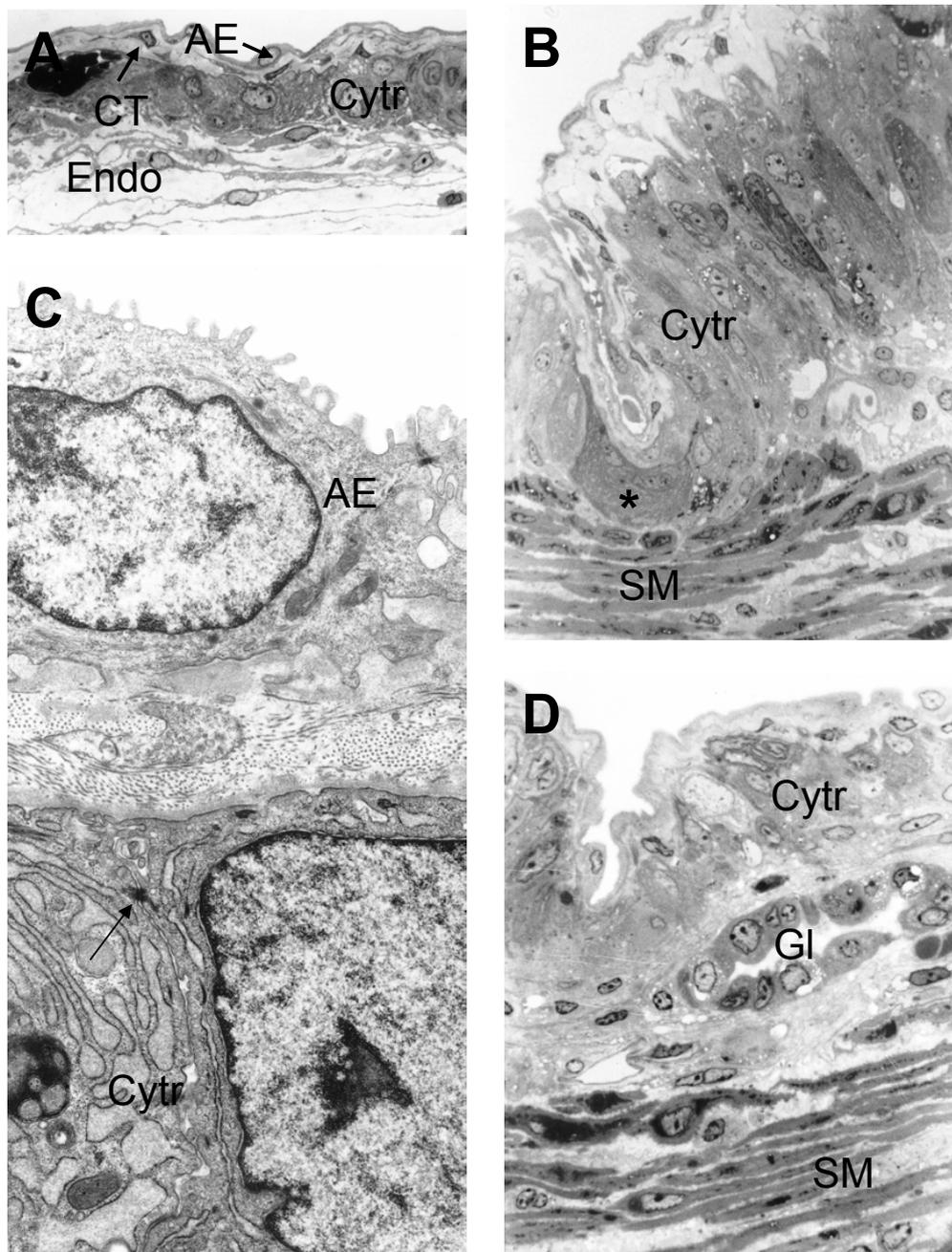


Figure 13. Paraplacental region. (A) A layer of connective tissue that includes fibroblasts (CT) is situated between the allantoic endoderm (AE) and the trophoblast (Cytr). The fibroblasts in endometrial connective tissue (Endo) are elongated. Note the allantoic vessel on the left. CRL 58 mm. $\times 740$. (B) An area where trophoblast of the paraplacenta reaches the myometrial smooth muscle (SM). Note the multinucleate trophoblast cell (*). CRL 58 mm. $\times 740$. (C) Allantoic endoderm, connective tissue and trophoblast of the paraplacental region. Note that the structure of the trophoblast cells in this region is similar to that of the spongy zone, including the desmosomal type adhesion junction (arrow). CRL 58 mm. $\times 15\,000$. (D) A region where the paraplacenta overlies a small uterine gland (Gl). CRL 58 mm. $\times 740$.

[40–42]. The haemophagous region of the definitive placenta of *Echinops* contained very few phagocytosed erythrocytes, suggesting that it plays a greater role earlier in development. The crystalline deposits are probably haematoidin, a breakdown product of haemoglobin as seen in the haemophagous region of the mink [43] and another tenrec, *S. setosus* [15].

An extensive paraplacenta is present in four of the Madagascar tenrecs as well as the otter shrew, *Potomagale velox*

[44]. In the paraplacenta of *Tenrec*, the secretions of the uterine glands accumulate in large sinuses or Sekretblasen [18]. An accessory placenta similar to the tenrec paraplacenta is found in the golden-rumped elephant shrew, *R. chrysopygus* [39]. These arrangements contrast to those in many other afrotherians. The uterine glands are reduced at an early stage in golden moles [45], the African elephant [46] and rock hyrax [47]. They are small and inactive in the manatee [36]. Instead,

histiotrophic nutrition is obtained by the phagocytic action of a basal zone of trophoblast in rock hyrax [35], manatee [36] and African elephant [46] and in at least one of the elephant shrews, *R. chrysopygus* [39].

In conclusion, *Echinops* possesses a cellular, haemomonochorial, chorioallantoic placenta and, in common with other Tenrecinae, obtains histiotrophic nutrition through a central haemophagous region and a paraplacenta. Some of these

distinctive features are shared with individual afrotherians. Thus, an interhemal barrier with a single layer of cytotrophoblast is found in a rock hyrax, and a paraplacenta is present in the otter shrews and elephant shrews. However, no significant feature of definitive placentation is shared by *Echinops* and all other Afrotheria. It remains to be seen whether or not the earlier developmental stages of the fetal membranes offer better support for their supposed phylogenetic affinities.

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