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Placentation in the Hottentot Golden Mole, *Amblysomus hottentotus* (Afrosoricida: Chrysochloridae)

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ABSTRACT

The placentation of the Hottentot golden mole *(Amblysomus hottentotus)* has been examined using light and electron microscopy and lectin histochemistry of nine specimens at both mid and late gestation. The placentae were lobulated towards the allantoic surface and the lobules contained roughly parallel arrays of labyrinthine structures converging on a central spongy zone. At mid gestation, the arrays were composed of an inner cellular and outer syncytial trophoblast layer, the inner layer enclosing scant connective tissue and fetal capillaries. Maternal blood spaces coursed through the outer trophoblast and were lined by trophoblastic microvilli; the blood spaces were narrow in mid gestation but enlarged near term, while the inner trophoblast layer became thinner and seemed to be syncytial. These features were confirmed by transmission electron microscopy. The microvillous surfaces and dispersed cytoplasmic particles were heavily glycosylated, as shown by lectin histochemistry, and exhibited changes with maturation, particularly a loss in *N*-acetyl glucosamine oligomers bound by *Phytolacca americana* lectin on the microvilli lining the maternal blood spaces and outer trophoblast particles. A substantial yolk sac was present both in mid and late gestation stages. It was clearly unattached to the uterus in the later stages. These morphological features are discussed in relation to the phylogenetic position of *Amblysomus* with respect to other members of Afrosoricida and Afrotheria.

1. Introduction

The golden moles are small, burrowing insectivores found in Sub-Saharan Africa. They are highly specialized for their subterranean way of life. Although bearing a superficial resemblance to the talpid moles, they differ from them in several ways, including the way in which they construct their burrows. The modified forepaws are positioned close beneath the body and loosen the soil by a pick-like action, whilst the leathery snout is used to shift loose soil and to compact the walls of the burrow [1]. Localisation of prey may be aided by seismic signals amplified by the bones of the middle ear, which are greatly hypertrophied in some species [2]. Their common name reflects the fact that the fur has an iridescent sheen.

In common with other small, insectivorous mammals, golden moles retain a number of primitive morphological characters. However, because they are highly specialized for their subterranean life style, they also have many derived traits. They have been included together with shrews, moles, hedgehogs, tenrecs and solenodons in the order Lipotyphla, but their taxonomic position is controversial and they have even been assigned ordinal status [3]. More recently studies based on molecular phylogenetics have rejected the Lipotyphla as a monophyletic group and proposed a new order the Afrosoricida comprising the tenrecs and the golden moles [4]. The molecular evidence strongly supports inclusion of Afrosoricida with five other orders in the superordinal clade Afrotheria [5,6]. There is little morphological support for this association, prompting us to suggest a re-assessment of placentation in tenrecs and golden moles [7,8].

The family Chrysochloridae comprises 20 species in nine genera [9,10] and two subfamilies are recognized [11]. Something is known of placentation in two species of the subfamily Chrysochlorinae, Grant's golden mole *Eremitalpa granti* [12–14] and the Cape golden mole *Chrysochloris asiatica* [15]. The Hottentot golden mole *Amblysomus hottentotus* belongs to Amblysominae, the second subfamily. Although it is a common species, its reproduction has been studied only recently [16] and nothing is known of its placentation. Nor has the placenta of any golden mole been studied at the ultrastructural level. We have undertaken to correct these

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deficiencies and to contrast placentation in *Amblysomus* with that of other members of Tenrecoidea [17–20] and Afrotheria [21].

2. Materials and methods

Hottentot golden moles, *Amblysomus hottentotus*, were collected as part of a control program to maintain the fairways on a golf course at KwaZulu Natal, South Africa (30°56′S, 30°18′E). Reproductive tracts that had been fixed in 10% buffered formalin were processed to 70% alcohol and shipped to Davis, California. The specimens selected fell into two groups: five mid gestation (fetuses 13–18 mm crown-rump length) and four late gestation (fetuses varying from 27 to 36 mm crown-rump length).

The uteri were opened and selected regions of the placenta were removed for further processing. Slices through the placental disc were either embedded in paraffin or placed in glutaraldehyde-formaldehyde fixative before a buffer rinse and postfixation in 1% osmium tetroxide. Other specimens were processed without osmication and, like the osmicated specimens, embedded in Araldite epoxy resin.

Non-osmicated plastic embedded specimens were sectioned at 0.75 μm and stained with a panel of 24 biotinylated lectins, followed by an avidin-peroxidase revealing system as previously described [22,23]. Sections were assessed using a semiquantitative ranking system where staining intensity was allocated a grade from 0 (negative) to 4 (intense staining).

Sections of both the postosmicated and non-osmicated plastic embedded tissues were stained with Azure B for light microscopic examination. Thin sections of osmicated material from three animals were examined by transmission electron microscopy.

Paraffin sections were stained with haematoxylin and eosin; in addition two specimens were examined by the Perl's method to indicate ferric iron.

3. Results

The disc of the chorioallantoic placenta was circular, clearly lobulated towards the allantoic surface (Fig. 1A), and situated either laterally or in a mid-lateral to antimesometrial position. The fetuses in later stages were oriented mesometrially to antimesometrially, and the chorioallantoic disc was pressed tightly against the abdomen. The allantoic vessels ramified conspicuously on the surface of the disc.

3.1. Light microscopic examination

The marginal lobules of the placental disc overlapped the surface of the endometrium except for the central region where there was a short pedicel through which maternal arteries and veins communicated with the placenta (Fig. 1B). Maternal blood lacunae lying just beneath the chorionic surface surrounded each placental lobule (Figs. 1C,D). These lacunae were variable in size and clearly anastomotic. They were lined by a double layer of trophoblast and gave off multiple branches to the underlying maternal blood spaces of the labyrinth (Figs. 1D and 2A).

The placental lobules demonstrated an orderly arrangement of roughly parallel arrays of labyrinthine structures converging centrally on the spongy zones (Fig. 1C). Maternal blood entered irregular branched maternal blood spaces of the labyrinth from the large blood lacunae situated directly under the allantois (Fig. 1D). The maternal blood spaces were lined by darkly stained syncytial trophoblast which constituted the outer layer of trophoblast and by a more lightly stained inner layer of trophoblast abutting the scant fetal connective tissue and thin-walled fetal capillaries (Figs. 2C,D). Thus the chorioallantoic placenta should be classified as haemodichorial as judged by the interhaemal areas of the labyrinth. A number of dark granules were found in relationship to some of the larger maternal channels within the placental disc and scattered in portions of the labyrinth. The granules that demonstrated ferric iron using the Perl's method were situated only in the inner trophoblast layer. The more superficial granules associated with larger maternal channels were not reactive with the Perl's method.

Fetal veins and arteries were situated in the allantoic mesoderm overlying the disc, with smaller branches situated between the large marginal maternal blood spaces within the disc (Fig. 1D). Occasionally larger fetal vessels could be seen within the labyrinth. The outer layer of syncytial trophoblast of the labyrinth was continuous with syncytial trophoblast forming the spongy zone,

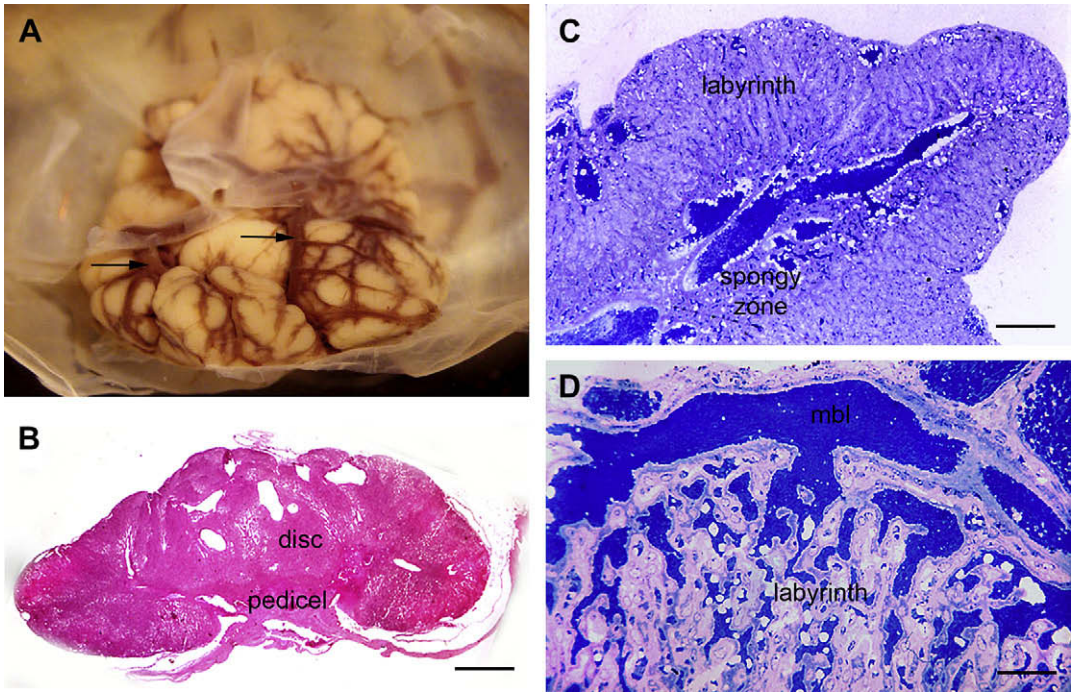

Fig. 1. A. Surface view of the intact placental disc. Note the lobulation of the placenta and the ramification of allantoic vessels on the surface of the disc (arrows). B. Cross-section through a late gestation placenta. Note the pedicel in the center and the overlapping of the marginal lobules of the disc. C. Section of a marginal lobule, showing the radial arrangement of the labyrinth and the convergence of maternal blood spaces within the spongy trophoblast. D. Edge of the placental disc, showing a large maternal blood lacuna (mbl) feeding branches into the labyrinth below. Scale bars = 1.2 mm (B), 295 μm (C), 54 μm (D).

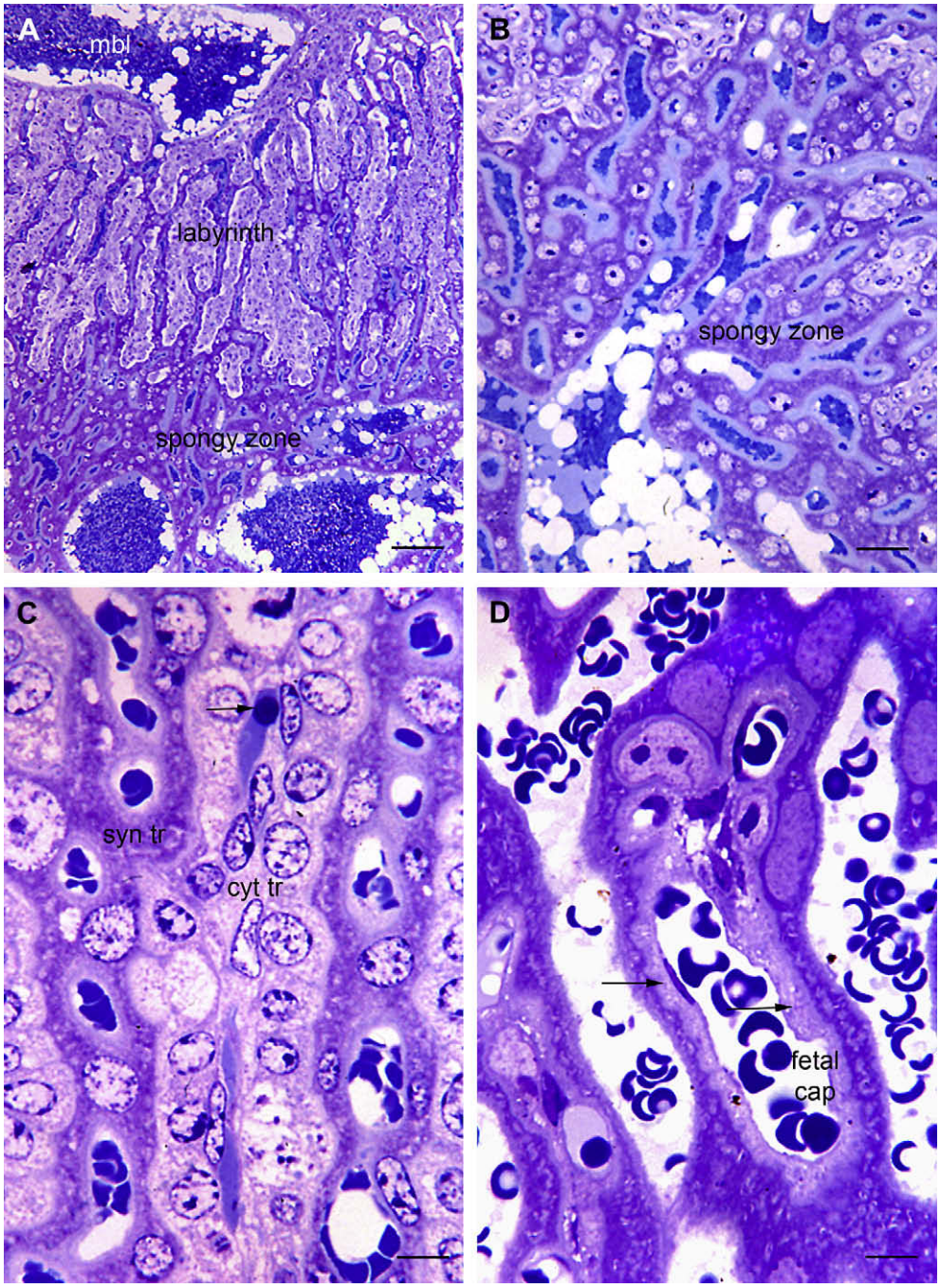

Fig. 2. A. A maternal blood lacuna (mbl) feeds blood into the labyrinth, situated above the spongy zone. Note the robust inner layer of trophoblast in this mid gestation placenta. B. Blood channels lined by syncytial trophoblast coalesce within the spongy trophoblast zone. C. Labyrinth in mid gestation showing dark syncytial trophoblast (syn tr) lining maternal blood spaces. The syncytial trophoblast is underlain by large cuboidal inner trophoblast cells (cyt tr). Note the nucleated erythrocyte in the fetal capillary (arrow). D. Labyrinth in late gestation. The inner layer of trophoblast (arrows) is much thinner, but the labyrinth remains haemodichorial. The fetal erythrocyes are slightly larger than maternal erythrocytes. Scale bars = 80 μm (A), 35 μm (B), 7.2 μm (C, D).

where maternal blood spaces coalesced into large channels (Fig. 2B), constituting the apex of each cone-shaped lobule. From here the maternal blood spaces coalesced into even larger channels before entering the veins of the pedicel. The blood spaces within the spongy zone were anastomotic and the lining syncytial trophoblast, although similar in staining to the outer trophoblast layer of the labyrinth, was thicker than that layer.

There was a marked difference between mid and late gestation placentae. The inner layer of trophoblast was cuboidal and relatively thick in the mid gestation placentae and was thin although continuous in the later placentae (Figs. 2C,D).

3.2. Electron microscopic examination

Despite the extraction of materials caused by the long fixation in formalin and sojourn in 70% alcohol, some information could be obtained from transmission electron microscopy. The outer layer of trophoblast was clearly syncytial, with very extensive granular

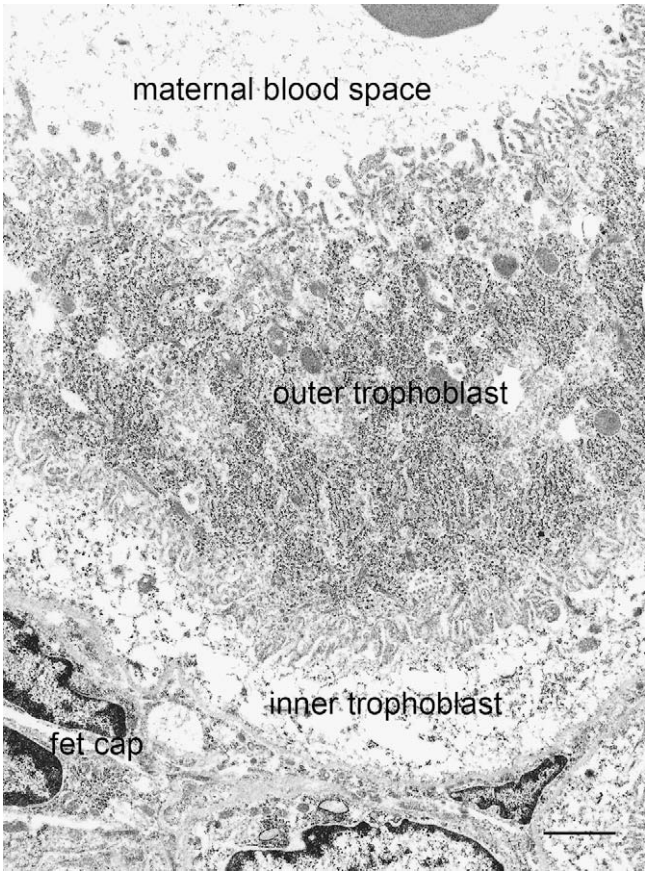

Fig. 3. Electron micrograph of a late gestation placental labyrinth. The microvilli are abundant on the surface of the maternal blood spaces and there is interdigitation of microvilli between the outer trophoblast and the pale inner trophoblast. Note the abundant rough endoplasmic reticulum in the outer trophoblast layer. Fetal capillary (fet cap). Scale bar = 1.3 μm.

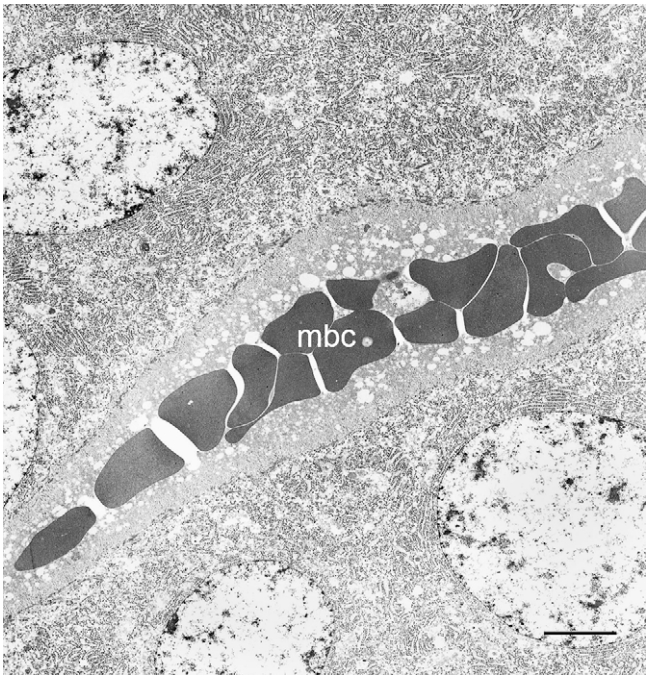

Fig. 4. Maternal blood channel in the spongy trophoblast. The syncytial trophoblast shows abundant rER, similar to the outer trophoblast layer of the labyrinth with which it is confluent. Scale bar = 2 μm.

endoplasmic reticulum (ER) (Fig. 3). The surface facing maternal blood spaces had numerous microvilli, and there was interdigitation of microvilli at the border between the outer and inner layers of trophoblast. Trophoblast in the spongy zone resembled the outer layer of trophoblast both in surface microvilli and in abundance of granular ER (Fig. 4). Small areas without granular ER could often be found near the middle of the outer layer (Fig. 3). Evidence of the presence of intercellular membranes was seen in the inner layer of trophoblast in the younger placentae (not shown). This layer both in younger and in older placentae was extensively extracted, showed little ER and, like the outer layer, had a scattering of small mitochondria. In the older specimens the inner layer was extremely thin in places but was never discontinuous. Much of this layer in the later specimens seems to be syncytial (see the section on lectins).

3.3. Results of lectin staining

Application of a battery of lectins revealed significant differences in glycosylation of components of the labyrinth of mid and late gestation placentae (Table 1, Fig. 5).

3.3.1. Mid gestation placentae

The apical microvillous membrane of the outer trophoblast layer, facing the maternal blood space, was highly glycosylated and bound ePHA (Fig. 5A), ALA, VVA, DSA, STA, WFA (Fig. 5E), SNA-1 and PAA (Fig. 5G) most strongly. The cytoplasmic particles of this layer also bound several lectins. The microvillous interface between the inner and outer layers of trophoblast and the lateral walls of the trophoblast cells of the inner layer bound some lectins intensely, e.g. ePHA (Fig. 5A), ALA, BSA-1B_4 (Fig. 5C), DSA, STA, LEA, WFA (Fig. 5E) and PAA (Fig. 5G). All other lectins bound to some extent. The inner cytotrophoblast cells tended to stain more heavily than the outer syncytial layer.

3.3.2. Late gestation placentae

The inner trophoblast layer had lost any indication of cellularity in that no staining of lateral cell membranes could be found in late placentae. There was also an increase in glycosylation, shown particularly with the binding of PSA, SBA, PAA (Fig. 5H) and WGA in this layer. The apical microvillous membrane of the outer syncytiotrophoblast layer showed stronger binding of PSA and loss of VVA and PAA staining (Fig. 5H). Staining with ePHA and WFA was similar in mid and late gestation placentae (Figs. 5A,B,E,F). The striking contrast in the absence of staining of the surface microvillous region of the outer trophoblast with BSA-1B_4 compared to the strong staining of the interface microvilli with the same lectin was also the same in mid and late gestation placentae (Figs. 5C,D). The outer trophoblast showed some minor changes in the binding of cytoplasmic particles and a reduction in their number. This could be seen best with ALA, MPA, LEA, WFA, SNA-1 and PAA. The microvillous interface between trophoblast layers showed some loss in lectin binding, especially with MPA, ECA and SNA-1, but other lectins were unaffected. The inner trophoblast layer was markedly thinner with increased glycosylation, especially with PSA, SBA, and WGA.

3.4. Pedicel, yolk sac and paraplacenta

The pedicel constitutes the junction of endometrial tissue with the placental disc. In some regions, endometrial tissue directly abutted the spongy trophoblast. In other areas there was a junctional zone with extravasated erythrocytes, occasional irregular

Table 1
Lectin staining of golden mole placenta at mid and late stages of gestation.

Lectin	Outer trophoblast MVM		Outer trophoblast cytoplasm/particles		Interface MVM		Inner trophoblast cytoplasm		Lateral cell walls
	Mid	Late	Mid	Late	Mid	Late	Mid	Late	Mid
CONA	2	3	3–4	2–3	2–3	2	2–3	3–4	2–3
PSA	1–2	4	1/3+	2/3++	1	2	1	2+++	1
ePHA	4	4	1/3+	1/4++	3–4	3	2	2++	3
PHA	1	1	1	0–1	1	0	1–2	0–2	1
ALA	4	3	1/4++	1/3+	4	3–4	1–2	2–3	3
MPA	1–2	1	1–2/2++	0	1–2	0	1–2	1–2	1
VVA	2–3	1	1	0	1–2	1–3	1	1–2	1
BSA-1B_4	0	0	0	0	4	4	2	1–2	2–3
DSA	4	4	1/4++	1/3++	4	4	2	2–3	3
STA	3	4	2/3+++	1/2++	4	4	3	3	3–4
LEA	2	2	1/2++	0	4	4	2–3	3	3–4
HPA	1	0	1	0	3	2	2	1–2++	2
ECA	1–2	0	1	0	2	0	1	1–2 Basal	1
SBA	1	0	1	0	3	2–3	1	2–3	1–2
WFA	4	3–4	1/3+++	1	4	4	2–3	3–4	3
SNA-1	2–3	3–4	2/3++	0–1	3	1–2	2	1–2	3
PAA	4	0	1/2++	0	4	4	2–3	3–4	3
WGA	1	2	1	1/2+	3	4	1–2	2–4	1–2

Staining intensity from 1 (weak) to 4 (intense). Particle density from + (sparse) to +++ (abundant). MVM, microvillous membrane. LTA, UEA-1, AAA, DBA, AHA, MAA are not shown as weak or negative.

patches of syncytial trophoblast with clustered nuclei, occasional mononucleated cells and some cell debris. Within the pedicel, the placental artery branched while still in the endometrium, forming several branches that dilated before entering the substance of the placenta (Fig. 6A). Although a few large mononucleated cells were found in the outer portions of the tunica media of the maternal arteries within the pedicel, the vessels showed little modification while within the endometrium. As the vessels reached the junctional zone they were dilated and showed loss of the endothelium as well as smooth muscle, before entering the double-lined channels within the placental disc that led to the superficial maternal blood spaces (Fig. 6A). A few giant cells with one to several nuclei were found in the junctional zone, endometrium (Fig. 6B) and in the muscularis; these cells were more abundant in the younger specimens (not shown).

A substantial yolk sac was present both in mid and late gestation stages. The yolk sac was clearly unattached to the uterus in the later stages. The endodermal epithelium in the mid gestation specimens was tall columnar and more cuboidal in later stages (Figs. 7A,B). Although vascularized, the yolk sac capillaries were well spaced. There was no elaboration of the surface of the yolk sac into villi, even proximal to the embryo.

The paraplacenta was closely applied to a very thin layer of uterine epithelium. The smooth surface of the paraplacenta had a low cuboidal layer of trophoblast, a prominent basal lamina, and several thin layers of connective tissue covered by a squamous mesothelium (Fig. 7C). A few large vessels were associated with the interface of the paraplacenta, and there were also small capillaries within the connective tissue layers. There were no villous regions or other elaborated areas.

4. Discussion

Placentation in the Hottentot golden mole *Amblysomus* is similar to that of Grant's golden mole *Eremitalpa* as described by Gabie [13,14] and the Cape golden mole *Chrysochloris* as described by De Lange [15], although the authors of these papers had only mid gestation and no late gestation placentae. All three show the lobulation of the placental disc, a central flattened pedicel, and a peripheral labyrinthine zone draining to a central spongy (ectoplacental) zone. De Lange [15] showed clearly the circulation of maternal blood entering the large peripheral blood spaces and returning through the labyrinth and spongy zone to the pedicel. In addition he showed a cuboidal inner layer of cytotrophoblast beneath an outer syncytial trophoblast (plasmoditrophoblast). A possible difference between the two subfamilies of golden moles is the extent to which trophoblast invades the uterine arteries. In *Eremitalpa*, masses of syncytial trophoblast occur in the maternal vessels beneath the placenta. They were originally described as endothelial in origin [12–14] although elsewhere we have argued this interpretation is erroneous [8]. In *Amblysomus* there is remodeling of the arteries as they enter the junctional zone but there is no sign of intravascular trophoblast at the stage examined. It would be useful to see earlier stages of placentation in *Amblysomus* since in other respects placentation is closely similar between the three species of golden mole studied. Similarity to other members of the Afrosoricida, on the other hand, is much more tenuous.

Both *Amblysomus* and *Echinops* [17,18], for example, have an allantois, yolk sac, labyrinth and spongy zone. The labyrinth, however, is haemodichorial in the golden mole with a syncytial outer layer, and cellular haemomonochorial in *Echinops* [17]. The spongy zone also is syncytial in the golden mole, and cellular in *Echinops*. The placenta of the otter shrew *Micropotamogale* differs even more from that of *Echinops* in that it is endotheliochorial [19]. Furthermore both *Micropotamogale* and *Echinops* have haemophagous regions, which *Amblysomus* does not.

In *Echinops* [18] and *Microgale* [20], as in many other mammals [24], a yolk sac placenta is formed but it is succeeded by the chorioallantoic placenta and the yolk sac is dislodged from the uterine wall by expansion of the exocoelom. The diagram and synoptic table on golden moles in Mossman's monograph [25] leave the impression that the yolk sac placenta persists to term. We did not find this to be the case in *Amblysomus* and note that Mossman's collection contains only mid gestation specimens of *Eremitalpa* supplied by van der Horst (UWZM 20483). It seems likely, therefore, that golden moles do not differ from tenrecs in this respect.

4.1. Glycosylation patterns

The microvillous surface of the outer trophoblast both in the labyrinth and spongy zone is the layer in direct contact with maternal blood. It is consequently important in carrying a battery of

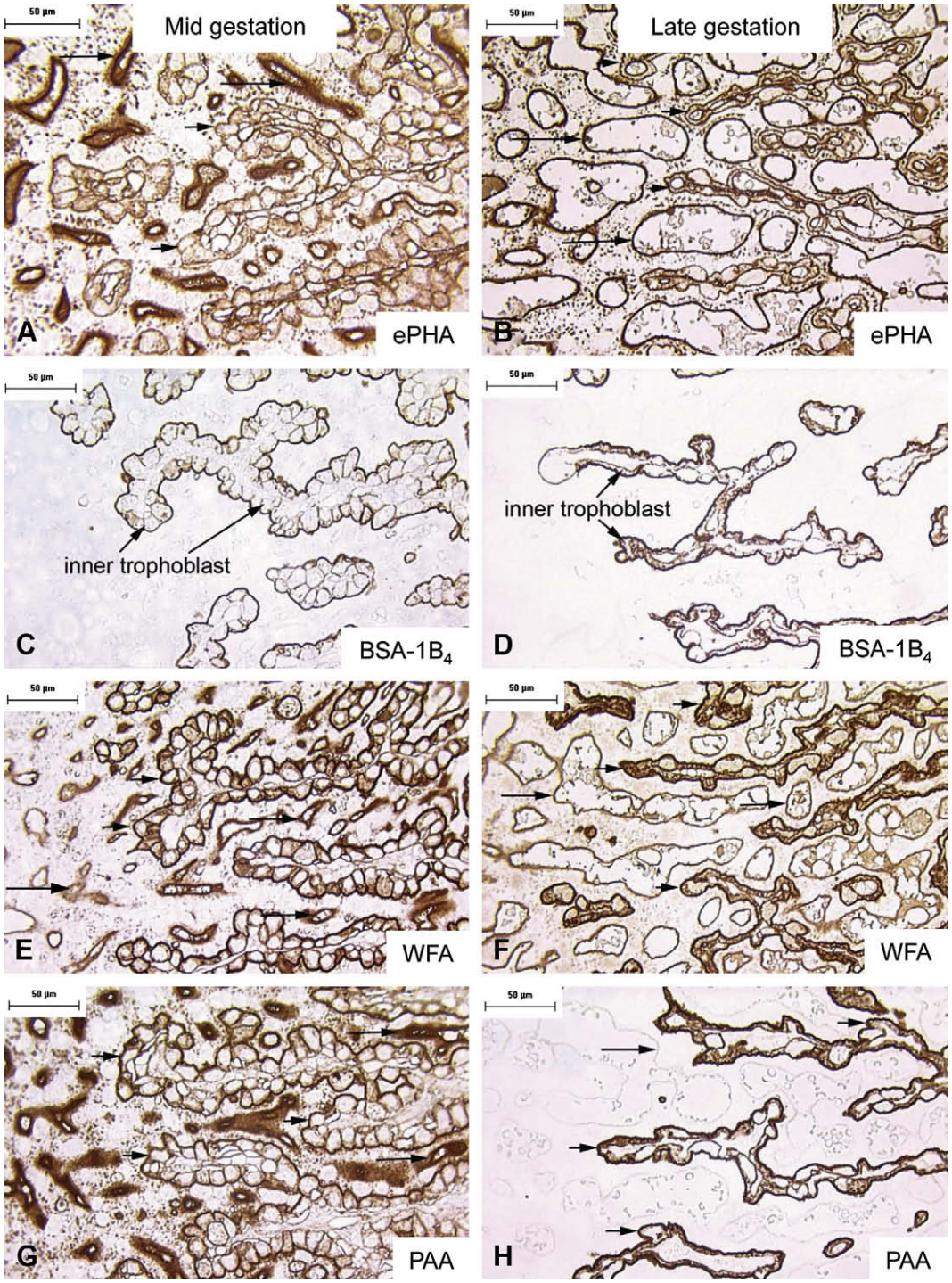

Fig. 5. A and B: *Phaseolus vulgaris* (erythroagglutinin, ePHA) lectin. A. At mid gestation, there is intense staining of the microvilli of the outer trophoblast adjoining the maternal blood space (long arrows), and a strong reaction at the microvillous interface between inner and outer trophoblast (short arrows). Lateral walls of the inner layer are stained as well as numerous particles in the outer trophoblast while staining is more diffuse in the cellular layer. B. By late gestation, the microvilli adjoining the enlarged maternal blood spaces still stain strongly, as does the interface between inner and outer trophoblast, the inner layer having become syncytial and much thinner. Particulate structures are prominent throughout both trophoblast layers. C and D: *Bandeiraea simplicifolia* (BSA-$1B_4$) lectin. C. At mid gestation, only the microvillous interface between the inner and outer trophoblast, and inner lateral walls bind this lectin. D. By late gestation, the inner layer has become syncytial with slightly stronger, irregular cytoplasmic staining. E and F: *Wisteria floribunda* (WFA) lectin. E. At mid gestation, the microvillous interface between inner and outer trophoblast binds this lectin most strongly (short arrows) while the microvilli lining the maternal blood space (long arrows) stain less strongly in the spongy layer. Inner trophoblast lateral walls and cytoplasm stain as well as numerous particles. F. The pattern of staining does not change significantly in late gestation apart from some loss on the microvilli lining the maternal blood spaces. G and H: *Phytolacca americana* (PAA) lectin. G. In mid gestation there is heavy staining of the microvilli adjoining the maternal blood space (long arrows) and at the interface of inner and outer trophoblast (short arrows), with lateral wall and diffuse cytoplasmic staining of the inner trophoblast. Numerous cytoplasmic particles bind this lectin at this stage. H. By late pregnancy, there is no staining of microvilli around the maternal blood spaces or of cytoplasmic particles. The intertrophoblast microvilli still stain strongly while the inner trophoblast has irregular cytoplasmic staining.

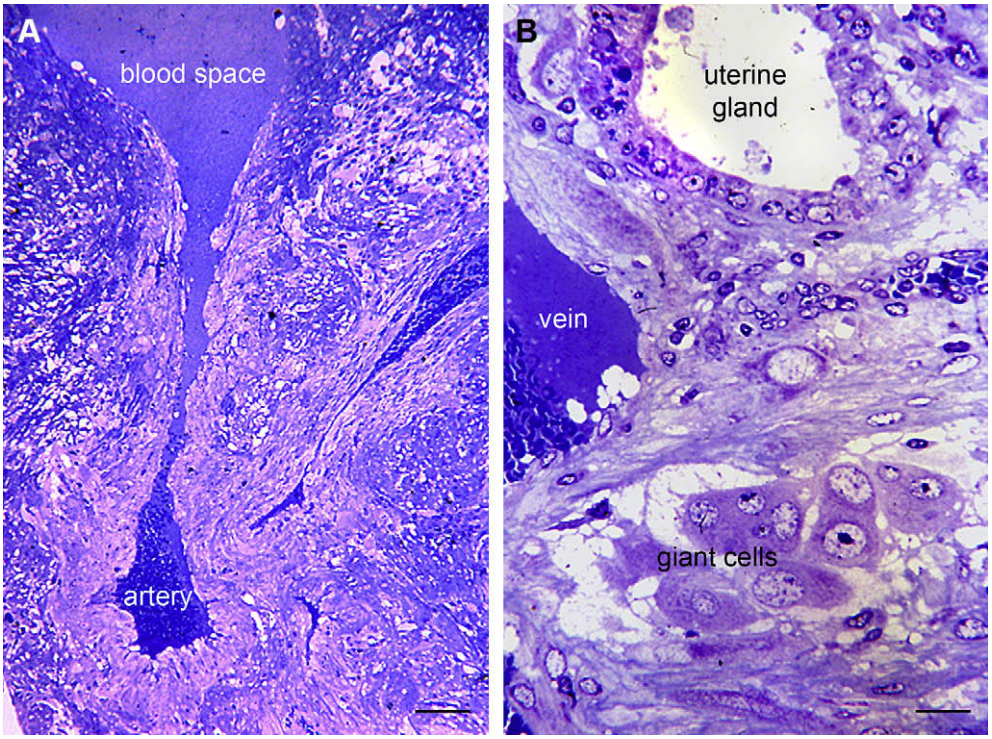

Fig. 6. A. A major placental artery branches within the pedicel. Note the relatively unmodified wall of the artery below, and its expansion into a blood space above. The loss of smooth muscle and endothelial lining of the artery occurs at the transition to the expanded region that is within the labyrinthine portion of the disc. B. Endometrium below the placenta in mid gestation. In addition to the vein and gland, some giant cells are present within the endometrium. Scale bars = 80 μm (A), 22 μm (B).

enzymes and transporters for substance transfer across the placenta. Lectin staining showed some changes in this surface with maturation, mainly an increase in non-bisected bi/tri-antennary complex *N*-glycan (PSA) and α2,6-linked sialic acid (SNA); reduction in *N*-acetyl galactosamine/lactosamine (VVA, MPA and ECA); and complete loss of *N*-acetyl glucosamine/lactosamine bound by PAA. There was also a significant loss of α2,6-sialic acid, *N*-acetyl galactosamine and *N*-acetyl glucosamine oligomers (SNA-1, WFA, LEA, PAA) in outer trophoblast particles with increasing maturity, as well as some α1,6-linked fucosyl residues (ALA). Glycosylation of the late stage microvillous interface between the inner and outer trophoblast differed, however, in that the *N*-acetyl glucosamine oligomers bound by PAA were not lost, unlike the dramatic reduction seen at the microvillous interface with maternal blood. There was some loss of expression of *N*-acetyl galactosamine/lactosamine (MPA, ECA) and α2,6-linked sialic acid (SNA-1) while other glycans expressed on these microvilli remained mostly unchanged. The inner trophoblast layer exhibited an increase in non-bisected bi/tri-antennary complex *N*-glycan (PSA) as well as *N*-acetyl galactosamine (SBA) and glucosamine oligomers (WGA) with maturity, but other changes were of a minor degree. Sialic acid in α2,3-linkage, bound by MAA, was not found in the trophoblast at any stage, which is unusual for haemochorial placentae [23], while fucosyl residues in α1,2-linkage were also weak or absent, as shown by the lack of binding of LTA and UEA-1. The difference in glycosylation between the two microvillous surfaces may reflect altered functional requirements. One is adjacent to maternal blood and may therefore require higher levels of sialylation with increased negative charge to block adherence and/or immunorecognition by maternal lymphocytes, while the interface between the inner and outer trophoblast may involve mechanisms of interepithelial adhesion (selectins, galectins) and as such exhibit different properties.

An unusual attribute shared between *Amblysomus* and the tenrec *Echinops* is the presence of WFA- and VVA-binding *N*-acetyl galactosamine residues in the trophoblast, which is not seen in haemochorial placentae of non-tenrecoid mammals (human, armadillo, hyena, guinea pig) [23]. There are, however, important differences between tenrec and golden mole glycosylation in that strong staining with DBA in the cytotrophoblast of the labyrinth in *Echinops* is absent in *Amblysomus* and SBA, WGA, PAA and BSA-1B_4 staining, which is prominent in the *Amblysomus* labyrinth, is virtually absent in that of *Echinops*.

4.2. Phylogenetic aspects

The evidence linking golden moles to tenrecs rests largely on molecular data, particularly analysis of the nucleotide sequences of nuclear and mitochondrial genes [5,6]. Golden moles and tenrecs share some plesiomorphic (“primitive”) characters but golden moles in particular have many derived characters associated with their subterranean lifestyle that they do not share with tenrecs. Some years ago one of us suggested that it was worth examining the placentas and fetal membranes of these creatures in the hope of finding shared, derived characters that might support a common origin [7]. More recently, however, we were compelled to conclude we could form no coherent picture of placentation in Afrosoricida [8]. There are striking differences even between the subfamilies of tenrecs with the interhaemal barrier being endotheliochorial in otter shrews and haemochorial in Tenrecinae and Oryzorictinae. The placenta of *Amblysomus* closely resembles that of other golden moles and all are distinguished by a haemodichorial placenta of a type not yet found among the tenrecs. Arrangements for histotrophic nutrition [26] include haemophagous areas that are prominent in some tenrecs but have yet to be described in golden moles.

One feature of Afrosoricida that has been highlighted is division of the allantoic sac into four lobes as in *Potamogale* [19]. Although this is now accepted as a synapomorphy for Afrotheria [27–29], we were not able to demonstrate this feature in *Amblysomus*.

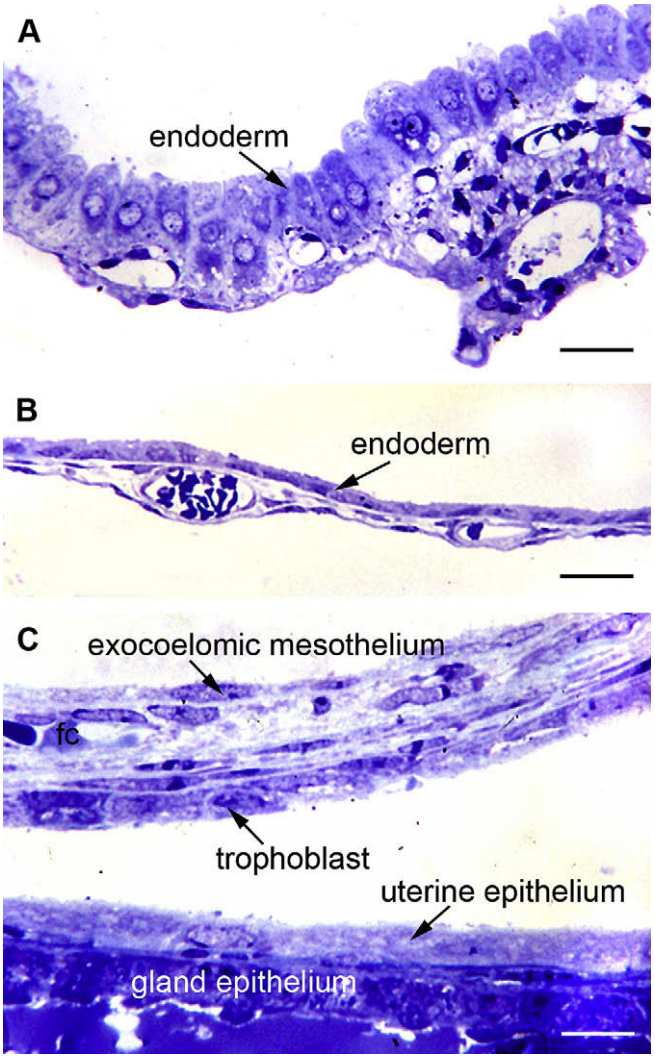

Fig. 7. A and B. Yolk sac from mid gestation (A) and late gestation (B). Note that although both yolk sacs are vascularized, the endodermal layer is more robust in the earlier yolk sac. C. Paraplacenta from mid gestation. Both the trophoblast of the paraplacenta and the epithelium of the uterus are simple cuboidal layers. The lumen of a gland appears at the bottom of the field. Scale bars = 34 μm (A, B), 13 μm (C).

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References

[1] Kuyper MA. The ecology of the golden mole *Amblysomus hottentotus*. Mammal Rev 1985;15:3–11.
[2] Mason MJ. Bone conduction and seismic sensitivity in golden moles (Chrysochloridae). J Zool (London) 2003;260:405–13.
[3] Symonds MR. Phylogeny and life histories of the "Insectivora": controversies and consequences. Biol Rev Camb Philos Soc 2005;80:93–128.
[4] Stanhope MJ, Waddell VG, Madsen O, de Jong W, Hedges SB, Cleven GC, et al. Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. Proc Natl Acad Sci U S A 1998;95:9967–72.
[5] Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, et al. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 2001;294:2348–51.
[6] Waddell PJ, Kishino H, Ota R. A phylogenetic foundation for comparative mammalian genomics. Genome Inform Ser Workshop Genome Inform 2001;12:141–54.
[7] Carter AM. Evolution of the placenta and fetal membranes seen in the light of molecular phylogenetics. Placenta 2001;22:800–7.
[8] Carter AM, Enders AC. Placentation in insectivoran grade mammals. Int J Dev Biol 2009 (in press).
[9] Bronner GN. Comparative hyoid morphology of nine chrysochlorid species (Mammalia: Chrysochloridae). Ann Transvaal Mus 1991;35:295–311.
[10] Bronner GN. Cytogenetic properties of 9 species of golden moles (Insectivora, Chrysochloridae). J Mammal 1995;76:957–71.
[11] Bronner GN, Jenkins PD. Order Afrosoricida. In: Wilson DE, Reeder DM, editors. Mammal species of the world. A Geographic and taxonomic reference. Baltimore: The Johns Hopkins University Press; 2005. p. 71–81.
[12] Van der Horst CJ. Some early embryological stages of the golden mole *Eremitalpa granti* (Broom). Special Publication of the Royal Society of South Africa: Robert Broom Commemorative Volume, pp. 225–234.
[13] Gabie V. The early embryology of *Eremitalpa granti* (Broom). J Morphol 1959;104:181–204.
[14] Gabie V. The placentation of *Eremitalpa granti* (Broom). J Morphol 1960;107:61–78.
[15] De Lange D. Contribution to knowledge of the placentation of the Cape golden mole (*Chrysochloris*). Bijd Dierkunde Amsterdam 1919;21:161–73.
[16] Schoeman S, Bennett NC, van der Merwe M, Schoeman AS. Aseasonal reproduction in the Hottentot golden mole, *Amblysomus hottentotus* (Afrosoricida: Chrysochloridae) from KwaZulu-Natal, South Africa. Afr Zool 2004;39:41–6.
[17] Carter AM, Blankenship TN, Künzle H, Enders AC. Structure of the definitive placenta of the tenrec, *Echinops telfairi*. Placenta 2004;25:218–32.
[18] Carter AM, Blankenship TN, Künzle H, Enders AC. Development of the haemophagous region and labyrinth of the placenta of the tenrec, *Echinops telfairi*. Placenta 2005;26:251–61.
[19] Carter AM, Blankenship TN, Enders AC, Vogel P. The fetal membranes of the otter shrews and a synapomorphy for Afrotheria. Placenta 2005;27:58–68.
[20] Enders AC, Blankenship TN, Goodman SM, Soarimalala V, Carter AM. Placental diversity in Malagasy tenrecs: placentation in shrew tenrecs (*Microgale* spp.), the mole-like rice tenrec (*Oryzorictes hova*) and the web-footed tenrec (*Limnogale mergulus*). Placenta 2007;28:748–59.
[21] Carter AM, Enders AC, Kunzle H, Oduor-Okelo D, Vogel P. Placentation in species of phylogenetic importance: the Afrotheria. Anim Reprod Sci 2004:82–3, 35–48.
[22] Jones CJP, Wooding FBP, Dantzer V, Leiser R, Stoddart RW. A lectin binding analysis of glycosylation patterns during development of the equine placenta. Placenta 1999;20:45–57.
[23] Jones CJP, Carter AM, Enders AC. Glycosylation at the fetomaternal interface in hemomonochorial placentae from five widely separated species of mammal: is there evidence for convergent evolution? Cells Tissues Organs 2006;185:269–84.
[24] Enders AC. Reasons for diversity of placental structure. Placenta 2009;30(Suppl A):S15–8.
[25] Mossman HW. Vertebrate fetal membranes: comparative ontogeny and morphology; evolution; phylogenetic significance; basic functions; research opportunities. Houndmills, Basingstoke, Hampshire and London: The Macmillan Press Ltd; 1989.
[26] Enders AC, Carter AM. Comparative placentation: some interesting modifications for histotrophic nutrition. Placenta 2006;27(Suppl A):S11–6.
[27] Mess A, Carter AM. Evolutionary transformations of fetal membrane characters in Eutheria with special reference to Afrotheria. J Exp Zoolog B Mol Dev Evol 2006;306:140–63.
[28] Seiffert ER. A new estimate of afrotherian phylogeny based on simultaneous analysis of genomic, morphological, and fossil evidence. BMC Evol Biol 2007;7:224.
[29] Asher RJ, Lehmann T. Dental eruption in afrotherian mammals. BMC Biol 2008;6:14.