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Comparative aspects of trophoblast development and placentation

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Abstract

Based on the number of tissues separating maternal from fetal blood, placentas are classified as epitheliochorial, endotheliochorial or hemochorial. We review the occurrence of these placental types in the various orders of eutherian mammals within the framework of the four superorders identified by the techniques of molecular phylogenetics. The superorder Afrotheria diversified in ancient Africa and its living representatives include elephants, sea cows, hyraxes, aardvark, elephant shrews and tenrecs. Xenarthra, comprising armadillos, anteaters and sloths, diversified in South America. All placentas examined from members of these two oldest superorders are either endotheliochorial or hemochorial. The superorder Euarchontoglires includes two sister groups, Glires and Euarchonta. The former comprises rodents and lagomorphs, which typically have hemochorial placentas. The most primitive members of Euarchonta, the tree shrews, have endotheliochorial placentation. Flying lemurs and all higher primates have hemochorial placentas. However, the lemurs and lorises are exceptional among primates in having epitheliochorial placentation. Laurasiatheria, the last superorder to arise, includes several orders with epitheliochorial placentation. These comprise whales, camels, pigs, ruminants, horses and pangolins. In contrast, nearly all carnivores have endotheliochorial placentation, whilst bats have endotheliochorial or hemochorial placentas. Also included in Laurasiatheria are a number of insectivores that have many conserved morphological characters; none of these has epitheliochorial placentation. Consideration of placental type in relation to the findings of molecular phylogenetics suggests that the likely path of evolution in Afrotheria was from endotheliochorial to hemochorial placentation. This is also a likely scenario for Xenarthra and the bats. We argue that a definitive epitheliochorial placenta is a secondary specialization and that it evolved twice, once in the Laurasiatheria and once in the lemurs and lorises.

Introduction

Early in mammalian development, the conceptus differentiates into an inner cell mass and an outer sphere of cells, the trophoblast. The latter contributes solely to the extra-embryonic membranes. The tissues of the embryo are all derived from the inner cell mass as are the amnion and major components of the yolk sac and allantois. The yolk sac endoderm may soon become apposed to the trophoblast, but this two layered structure (the bilaminar omphalopleure) is not regarded as a true placenta. Efficient maternal-fetal exchange first becomes possible when mesoderm is interposed between the endoderm and trophoblast, carrying with it branches of the vitelline vessels. Together these tissues form the choriovitelline or yolk sac placenta (trilaminar omphalopleure). Somewhat later, the allantois grows into the exocoelom and expands until it comes into contact with the trophoblast. The chorioallantoic placenta is formed of trophoblast and allantois and is vascularized by the allantoic or umbilical vessels. In some mammals, the allantois displaces the yolk sac from the trophoblast, so that yolk sac placentation is succeeded by chorioallantoic placentation (Figure 1). Some mammals, including man, form only a chorioallantoic placenta. In still others, notably rodents and lagomorphs, the yolk sac placenta persists until term. There is then partial or complete inversion of the germ layers such that yolk sac endoderm forms a maternal-facing absorptive epithelium (inverted yolk sac placenta).

In marsupials, the separation of embryonic and trophoblastic areas of the blastocyst is less distinct than in eutherians [1]. Most marsupials form only a yolk sac placenta, although bandicoots do have an additional chorioallantoic placenta. Nevertheless, it has been argued that the allantochorion is an ancestral character present in the stem species of marsupials and that the yolk sac became predominant during evolution of the marsupial placenta [2]. Extra-embryonic membranes participate in maternal-fetal exchange in many non-mammalian vertebrates, too, as detailed in several recent publications [3-5]. However, the present review will be restricted to placentation in eutherian mammals.

The interhemal area

The trophoblast sometimes but not always invades the uterine wall displacing and/or replacing one or more layers of tissue. Grosser's classification of placentas [6], based on the number of tissues separating maternal from fetal blood, has had an enormous influence [7]. According to whether the trophoblast is apposed to uterine epithelium, the endothelium of maternal vessels, or directly to maternal blood, placentas are classified as, respectively, epitheliochorial, endotheliochorial or hemochorial. Nevertheless, once placentas could be studied at the ultrastructural level, it became apparent that this classification is of limited functional significance, since extensive thinning of the tissues reduces the diffusion distance between fetal and maternal blood even in epitheliochorial placentas [8]. At the same time, studies of placental ultrastructural made it clear that there were many variations in the structure of the interhemal area (Table 1).

The list in Table 1 by no means exhausts the known number of placental types. Many placentas have specialized areas concerned with histiotrophic nutrition, that is, absorption of secretions from the uterine glands or phagocytosis of cellular debris. A large number have hemophagous regions in which maternal erythrocytes are taken up as a source of iron for the fetus. There are often large areas that seem to be concerned more with protein synthesis than materno-fetal exchange, as they are not penetrated by fetal blood vessels; examples are the spongy zones of rodent and hyrax placentas. Not surprisingly, there are many variations in placental shape, from the diffuse and cotyledonary forms in hoofed mammals through the circumferential ("zonary") placenta of carnivores to the familiar discoid form of the human or mouse placenta.

Phylogenetic perspectives

Clearly each placental type is able to support a successful pregnancy. Nevertheless, students of placental structure have continued to ask why there is so much variability and how the placenta evolved. The debate started with Huxley and Owen in the nineteenth century (reviewed by Pijnenborg and Vercruysse [9]), continued with Wislocki [10] and Portmann [11], and culminated with the publications of Mossman [12,13] and Luckett [14]. The success of molecular phylogenetics in identifying the relations between the orders of mammals allows us to view placental structure from a new perspective [15,16]. It appears that all extant eutherian mammals can be classed in four superorders. These have been named Afrotheria, Xenarthra, Euarchontoglires and Laurasiatheria [17,18]. Accordingly, the approach taken here will be to review placentation in each superorder in turn.

Placentation in the Afrotheria

This superorder was proposed following analysis of DNA sequence data. It includes six orders of mammals. Three of these, elephants, sea cows and hyraxes, were already considered to be closely related. Inclusion of the elephant shrews and the aardvark is not controversial. There has, however, been some debate about the sixth order, Tenrecomorpha or Afrosoricida, which comprises the golden moles and tenrecs. This is in part because analysis of morphological characters fails to reveal a single synapomorphy in support of Afrotheria [19].

Placentation in the African elephant has been well studied [20-23]. The placenta is of the circumferential type, forming a broad equatorial band, but is attached to the uterus by only a narrow hilus. There is now information on the fine structure of the interhemal area [23]. It is endotheliochorial with the maternal blood vessels closely invested by cellular trophoblast. The basement membrane of the maternal endothelial cells is thickened, but is pierced by narrow processes from the trophoblast cells. On the fetal side, these are deeply indented by fetal capillaries, reducing the diffusion distance between maternal and fetal blood (Figure 2A). Beneath the labyrinth is a zone of cellular trophoblast that is actively phagocytic [20]. Of particular note is the persistence of a large allantoic sac divided into four compartments by septa that carry branches of the umbilical vessels.

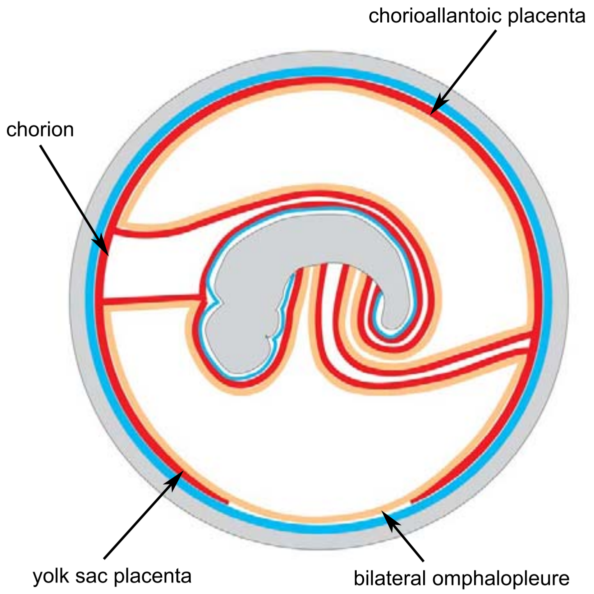

Figure 1
Eutherian fetal membranes. The embryo is enclosed in the amnion. Trophoblast (blue) and mesoderm (red) form the chorion. Trophoblast and yolk sac endoderm (yellow) together constitute a bilaminar omphalopleure. A choriovitelline or yolk sac placenta is then formed by interposition of mesoderm containing fetal blood vessels. Later the allantois expands into the exocoelom and the allantoic and chorionic mesoderm fuse to form a chorioallantoic placenta. The allantois will continue to expand into the exocoelom and eventually displace the yolk sac. The full sequence of events is seen most clearly in species with epitheliochorial placentation, such as horse and pig, although this cartoon is based on the rock hyrax (reference [82]).

All these features are apparent in the placenta and fetal membranes of the manatee [24], rock hyrax (Figure 2C) [25] and aardvark [26,27]. However, whilst the aardvark and possibly the manatee [13] have endotheliochorial placentation, the rock hyrax placenta is hemomonochorial. The single layer of trophoblast in the interhemal barrier is cellular not syncytial [28]. One could thus envisage how this arrangement could be derived from an endotheliochorial placenta such as that of the African elephant.

Elephant shrews have a discoid placenta. It is hemochorial and the trophoblast in the barrier may be syncytial [29], although this needs to be re-examined. Elephant shrews do share the large, four-lobed allantoic sac with other afrotherians [30,31]. Golden moles [32] and tenrecs [33] also tend to have discoid, hemochorial placentas. In elephant shrews and tenrecs, there is an additional circumferential area or paraplacenta [31,33]. We examined the interhemal area of the Madagascar lesser hedgehog tenrec and found that it comprised a single layer of cellular trophoblast similar to the rock hyrax (Figure 2B) [33]. In the otter shrews, the only tenrecs found on mainland Africa, the situation is more complex. There appears to be a circumferential, endotheliochorial placenta (Enders, Carter and Vogel, unpublished data). The giant otter shrew has the four-lobed allantoic sac found in all the other orders of Afrotheria [34].

Many of the placentas of afrotherians have hemophagous regions (Table 2). These are very prominent in the tenrecs [33], found at the margins of the placenta in elephants and aardvark [20,26], and distributed across the surface of the placenta sea cows [24]. Hemophagous regions are absent in hyraxes, elephant shrews and golden moles, all of which have hemochorial placentation.

Nothing is known about early development in sea cows, but an extensive choriovitelline placenta is formed in all other orders of Afrotheria. Following establishment of the chorioallantoic placenta, however, the yolk sac is very much reduced.

Based on current knowledge of placentation in the extant species, it seems possible that the common ancestor of the Afrotheria had the following features. (1) A circumferential, endotheliochorial placenta as in the elephant, manatee, aardvark and otter shrews. Loss of maternal endothelium would lead to the hemochorial condition seen in rock hyraxes, elephant shrews and some tenrecs. The formation in elephant shrews and tenrecs of a discoid placenta with a more or less extensive paraplacenta would also be a derived state. (2) A prominent hemophagous organ as in otter shrews and tenrecs, retained in elephants, aardvark and manatee, but lost in rock hyraxes and elephant shrews. (3) A four-lobed allantoic sac, retained in otter shrews and all other Afrotheria, but seemingly lost in some members of Tenrecomorpha. (4) A short cord sending four leashes of vessels to the placenta.

Placentation in Xenarthra

This superorder is thought to have evolved in South America during the geographical isolation of this subcontinent in the Cretaceous period. The close association of armadillos with American anteaters and sloths is apparent from analyses of morphological characters and is strongly supported by molecular phylogenetics [35]. In all three orders, the embryo develops within a simplex uterus. Placentation has been most thoroughly examined in some species of armadillo. In the nine-banded armadillo the placental villi enter a series of maternal venous sinuses in

Table 1: Variations in the trophoblast comprising the interhemal area of the chorioallantoic placenta.

Placental Type	Trophoblast Component	Examples
Hemomonochorial	Syncytial trophoblast, villous	Armadillo; higher primates
	Syncytial trophoblast, labyrinthine	Most hystricomorph rodents (e.g. guinea pig, capybara); sciuromorph rodents (squirrels)
	Cellular trophoblast	Hyraxes; spiny tenrecs (e.g *Echinops*); mollosid bats (late gestation); some myomorph rodents (*Zapus*, *Jaculus*)
Hemodichorial	Syncytial (maternal facing) and cellular trophoblast	Lagomorphs; vespertilionid bats
Hemotrichorial	Cellular trophoblast (maternal facing) and two layers of syncytial trophoblast	Most myomorph rodents (e.g. rat, mouse)
Endotheliochorial	Syncytial trophoblast (fenestrated)	Sloths; shrews (insectivores)
	Syncytial trophoblast (nonfenestrated)	Carnivores and pinnipeds
	Cellular trophoblast	Elephants; some sciuromorph rodents (*Dipodomys*, *Microdipodops*); some bats (*Natalus*, *Saccopteryx*)
Epitheliochorial	Cellular trophoblast only	Lower primates; pangolin; some artiodactyls (e.g. pig)
	Cellular trophoblast with trophoblastic girdle cells	Equids (perissodactyls, e.g. horse)
Synepitheliochorial	Cellular trophoblast; fusion of binucleate trophoblastic cells with uterine epithelium	Ruminants (artiodactyls, e.g. sheep, cow)

the fundic region of the uterus [36,37]. The villi then spread within the sinuses in the body of the uterus, and the villi that were initially in the fundic region degenerate. Thus the placenta starts as discoid, then appears to be zonary. However the allantoic vessels of the four quadruplets vascularize different areas of the zonary band. The placental villi are similar to those of primates in having an outer layer of syncytial trophoblast in contact with maternal blood (Figure 3B); they differ however in having the cytotrophoblast confined to the growing tips of the villi rather than as a Langhans layer. Little is known about placentation in anteaters except that they have a hemochorial placenta and greatly enlarged fetal mesenchymal cells in the villi in late pregnancy, as do the armadillos. As pointed out by Benirschke [38], it is difficult to compare the anteaters to other xenarthrans because of the absence of fine structural studies of this group.

The sloth placenta differs in several respects from that of anteaters and armadillos. It is diffuse in early stages but later becomes discoid or even double discoid. The exchange area is labyrinthine, not villous, and the interhemal area is endotheliochorial (Figure 3A). It consists of hypertrophied maternal endothelial cells; a small amount of extracellular material and spindle-shaped cells of presumed maternal origin; a layer of syncytial trophoblast that although thick is fenestrated; and the fetal capillary endothelium. In addition, the fetal connective tissue contains hypertrophied mesenchymal cells similar to those in the armadillo and anteater, but also found in shrews and tree shrews; these cells are characterized by abundant granular endoplasmic reticulum [39].

In contrast to the afrotherians, xenarthrans have only a rudimentary allantoic sac. However, the armadillos have large inverted yolk sacs that persist well into gestation. Little is known about yolk sac placentation in anteaters. It is doubtful that sloths have a choriovitelline placenta since in late gestation the yolk sac is small and rudimentary.

Since the hemochorial placenta of the armadillo is not preceded by an endotheliochorial condition and the placenta of armadillos and anteaters differs from that of the sloths, it is not clear which might be considered more derived. It is notable, however, that epitheliochorial placentation is not encountered in Xenarthra or Afrotheria, the superorders considered closest to the root of the eutherian tree [17].

Placentation in Euarchontoglires

The Superorder Euarchontoglires or Superprimates comprises two sister groups. Glires unites rodents and lagomorphs (rabbits and pikas). The Euarchonta include three orders: primates, flying lemurs and tree shrews

Placentation in Glires

The rodent order is so rich in species that only a small sample of placentas has been described [40]. However, the placentas of rodents and lagomorphs tend to conform to a basic pattern. They are discoid to spherical in shape, the exchange area is labyrinthine and hemochorial, and there is a well developed spongy zone. An inverted or partially inverted yolk sac placenta supports the early development of the embryo and is retained until term.

There are three principal types of interhemal area in Glires [41-43]. Hystricomorph rodents, such as the guinea pig

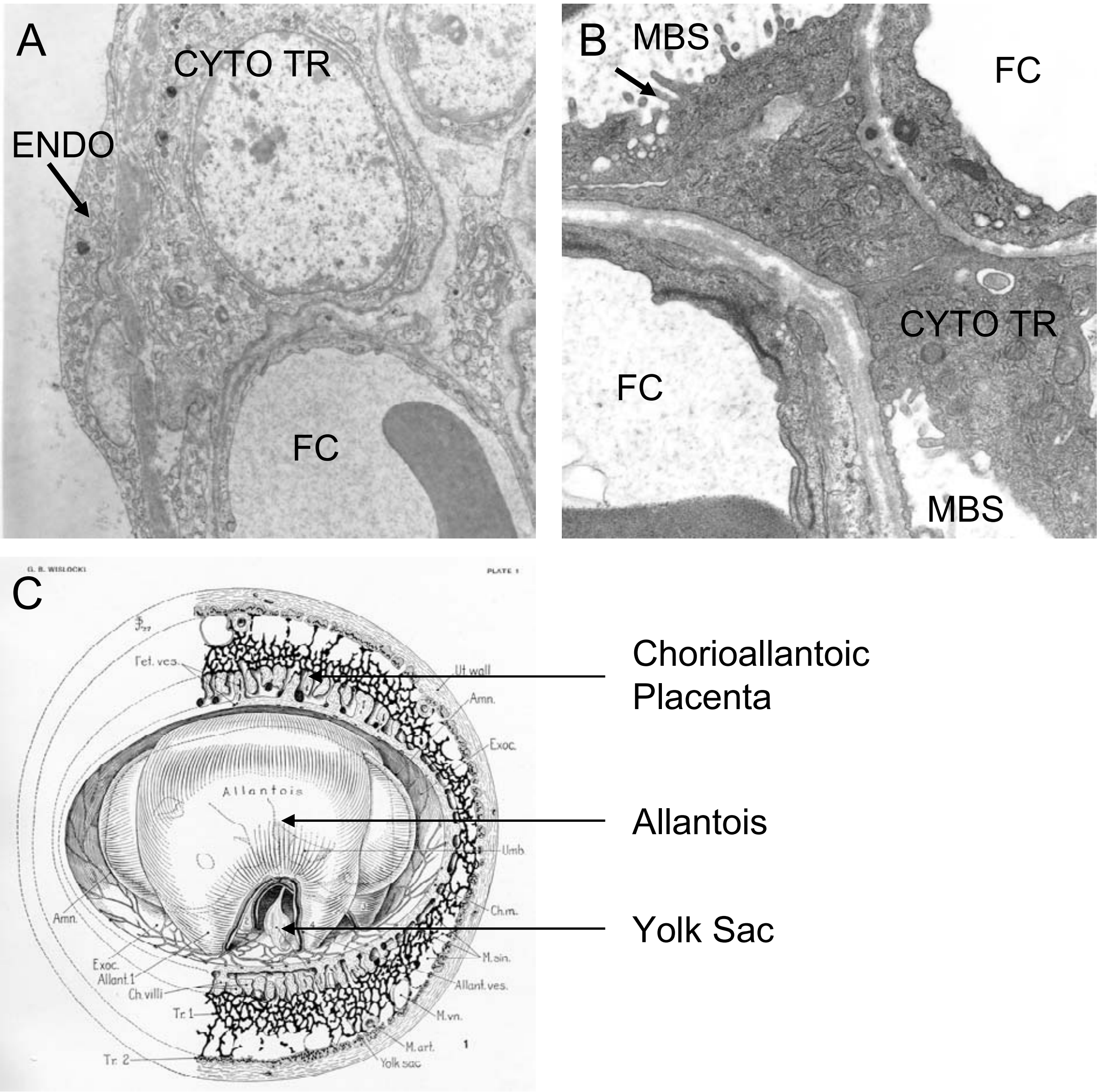

Figure 2
Placentation in Afrotheria. (A) Endotheliochorial placenta of the African elephant, *Loxodonta africana*. The cellular trophoblast (CYTO TR) is deeply indented by the fetal capillary (FC), which is separated by <3 μm from the endothelium of the maternal capillary (ENDO). Reprinted from reference [23] with permission ©Elsevier 2003. (B). Hemochorial placenta of the lesser Madagascar hedgehog tenrec, *Echinops telfairi*. Maternal blood spaces (MBS) are lined by cellular trophoblast (CYTO TR) with microvilli (arrow). Reprinted from reference [33] with permission ©Elsevier 2003. (C) Fetal membranes in the rock hyrax *Procavia capensis*. The large allantoic sac is divided into four lobes by septa that carry four sets of allantoic vessels to the zonary placenta. The yolk sac is greatly reduced in late gestation. Reprinted from reference [83] with permission from the Carnegie Institution of Washington.

Table 2: Morphological evidence of iron transfer: hemophagous zones.

Placental Type	Species	Hemophagous zone
Synepitheliochorial	Sheep, goat	Base of fetal villi
Endotheliochorial	Dog and cat	Marginal
	Mustelids	Central
	Sac wing bat	Base of fetal villi
	Elephant	Lateral area of placental band
	Shrew	Trophoblastic curtain
Hemochorial	Tenrecs	Central
	Otter shrews	Central off labyrinth
	Hyena	Junctional zone

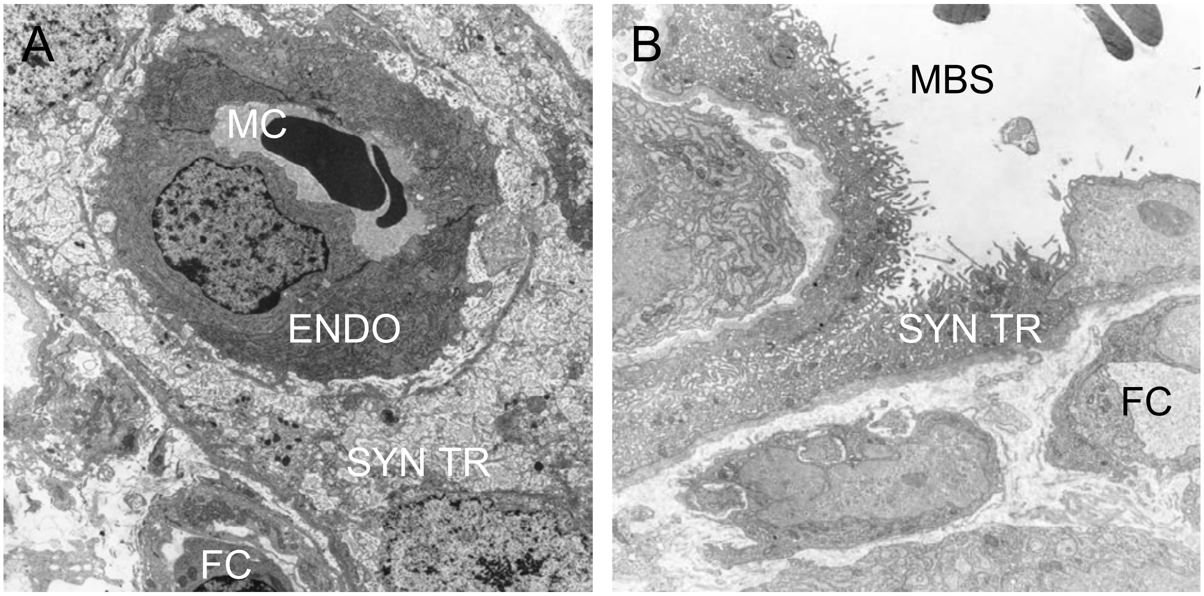

Figure 3
Placentation in Xenarthra. (A) Endotheliochorial placenta of the three-toed sloth, *Bradypus tridactylus*. The interhemal membrane comprises the enlarged maternal endothelial cells (ENDO), syncytial trophoblast (SYN TR) and the endothelium of the fetal capillary (FC). (B) Villous, hemomonochorial placenta of the nine-banded armadillo, *Dasypus novemcinctus*. The maternal blood space (MBS) is separated from the fetal capillary (FC) by syncytiotrophoblast (SYN TR).

and capybara, have hemomonochorial placentas with a single layer of syncytial trophoblast in the interhemal barrier. So do the sciuromorph rodents or squirrels (Figure 4B). In lagomorphs the placenta is hemodichorial with an outer layer of syncytial and inner layer of cellular trophoblast. In most myomorph rodents the interhemal area is hemotrichorial (Figure 4A). Although the outer layer is cellular, it contains large pores and the inner layers of syncytial trophoblast are thought to constitute the placental barrier [44].

There are some notable exceptions. The kangaroo rats *Dipodomys* and *Microdipodops* are sciuromorph rodents, but in this family (Heteromyidae) the interhemal area is endotheliochorial and contains cellular trophoblast (Figure 4D) [45]. In two families of myomorph rodents, jerboas (Dipodidae) and jumping mice (Zapodidae), there is a reduction in the trophoblast layers and the maternal blood channels are lined by trophoblastic giant cells (Figure 4C) [46].

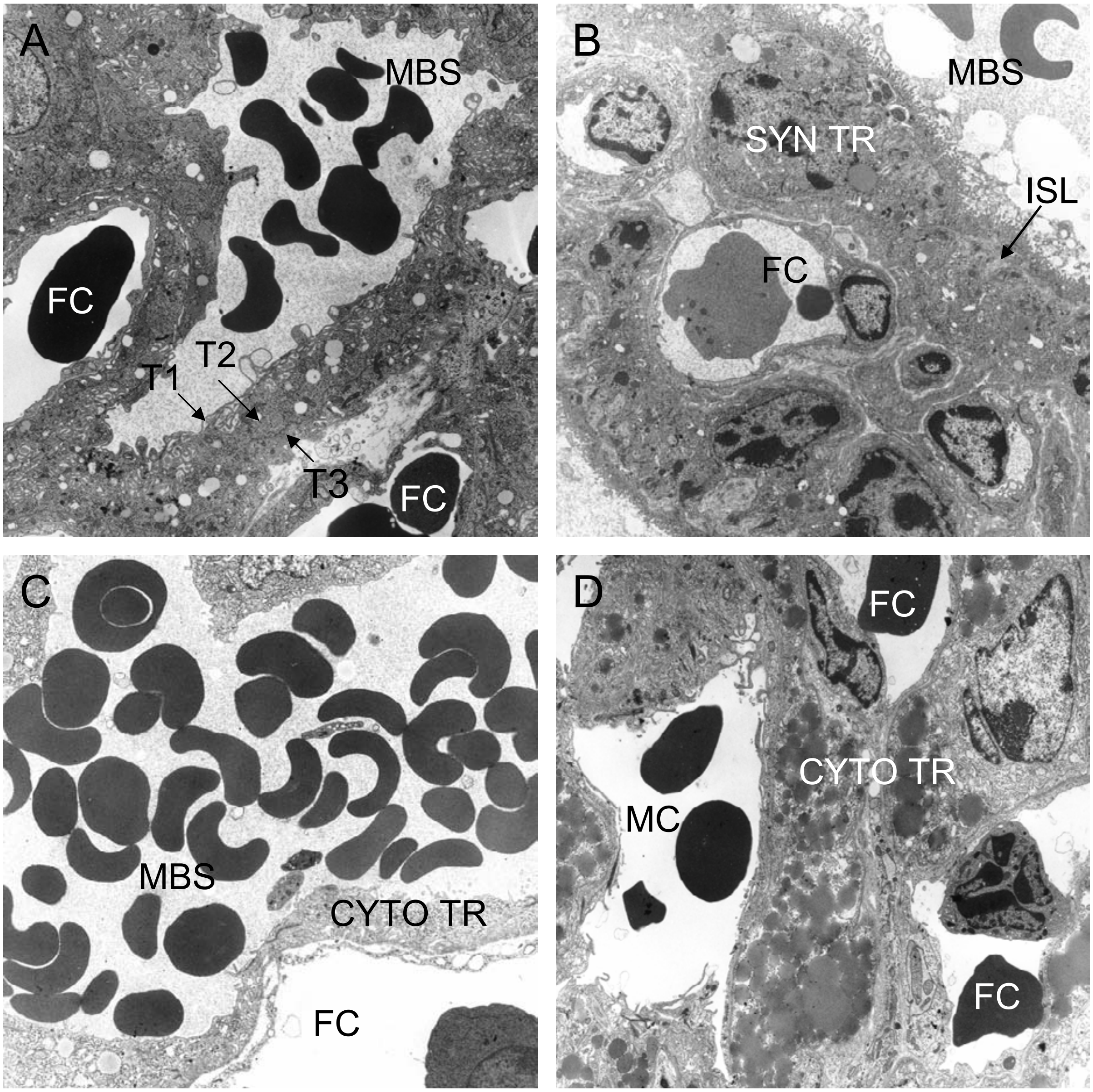

Figure 4
Placentation in rodents (Glires). (A) Hemotrichorial placenta of the lemming, *Lemmus lemmus*. One layer of cellular trophoblast (T1) and two layers of syncytiotrophoblast (T2–T3) are interposed between the maternal blood space (MBS) and fetal capillaries (FC). (B) Hemomonochorial placenta of the golden-mantled ground squirrel, *Spermophilus lateralis*. The syncytiotrophoblast (SYN TR) has intrasyncytial bays (ISL). (C) Hemomonochorial placenta of a jumping mouse, *Zapus sp*. The interhemal membrane includes a single layer of giant trophoblast cells (CYTO TR). (D) Endotheliochorial placenta of a kangaroo rat, *Dipodomys sp*. The interhemal membrane comprises the endothelium of the maternal capillary (MC), cellular trophoblast (CYTO TR) with lipid inclusions and the endothelium of the fetal capillary (FC).

In most rodent placentas the labyrinth and spongy zone form distinct layers. In hystricomorph rodents, however, folding gives rise to a lobed placenta. The lobes are the exchange areas and they are separated by interlobular trophoblast that is the equivalent of a spongy zone. In addition, hystricomorphs have a unique structure of unknown function, the subplacenta [47].

Rodents represent the only order of eutherian mammals for which placentation has been subjected to a strict cladistic analysis. Mess [43] concluded that the stem pattern was labyrinthine and hemochorial with a single layer of syncytial trophoblast in the exchange area. She did not extend this analysis to include the lagomorphs. It is pertinent to note that, on this analysis, the mouse placenta represents an elaboration of the stem pattern. This has potential implications for discussion of structural and functional homologies between mouse and human placentas [48,49].

Placentation in Euarchonta

Ever since the seminal work of Hill [50], the evolution of placentation in primates has posed some interesting questions [51]. Clearly there is a striking difference between the lemurs and lorises (Strepsirhini) and other primates (Haplorhini). The former have epitheliochorial and the latter hemochorial placentation. The recognition that tree shrews were closely related to primates, which receives further support from molecular studies, adds a further layer of complexity, since tree shrews (Scandentia) have endotheliochorial placentas [52]. Finally Hill, who regarded hemochorial placentation as the derived state, opined that it had evolved separately in the tarsiers than in the monkeys and apes.

The colugos or flying lemurs (Dermoptera) have attracted less attention. Molecular phylogeneticists group them with primates and tree shrews, although the relations between the three orders are not well resolved [53]. Their placenta has been little studied. It is discoid and appears to contain labyrinthine and villous areas. The interhemal area is hemodichorial [13].

In contrast, the fetal membranes of tree shrews have been well studied [52,54] and were included in an early cladistic analysis by Luckett [55]. Tree shrews have a quite distinctive form of endotheliochorial placentation (Figure 5B), involving twin discoid attachments to special pads developed on the lining of the uterus. Conserved features of their fetal membranes include retention of a large allantoic vesicle and a large yolk sac [55].

Lemurs and lorises present a curious case in that they have diffuse, non-deciduate and epitheliochorial placentas (Figure 5A). The resemblance to placentation in pigs extends to the presence of thickened areas of trophoblast opposite the openings of the uterine glands [50], which parallel the areolae of the porcine placenta. There is a large allantoic vesicle, which is lobed as in the afrotherians. A temporary yolk sac placenta supports development of the early embryo.

Tarsiers have a discoid, hemochorial placenta. Except that it is labyrinthine rather than villous, the placenta is generally similar to that of monkeys and apes. The allantois is not vesicular, as in lemurs, but forms an almost solid connecting stalk of mesodermal tissue as in higher primates, a point to which Hill [50] attached particular importance.

There are strong resemblances in the early development of the platyrrhine or New World monkeys, the catarrhine monkeys of the Old World and the anthropoid apes. However, in platyrrhine monkeys, proliferation of the trophoblast continues until much later in gestation and connections persist between the villi, which form a trabecular network, not unlike that of the tarsier placenta. Only at a late stage of fetal development are branched villi found within a more or less continuous intervillous space. In catarrhine monkeys, on the other hand, arborescent chorionic villi are present from a very early stage, as they are in the human placenta. Although macaque and baboon implantation sites develop – sequentially – trophoblastic plate, lacunar and villous stages as do the anthropoid apes, in the former animals trophoblast taps maternal vessels early and the lacunae expand the placenta into the uterine lumen [56]. Macaques also form a secondary placenta on the opposite side of the uterus from the original implantation. In man and anthropoid apes the trophoblast proceeds further into the uterus and the abembryonic as well as the polar trophoblast forms lacunae, resulting in an interstitial placenta. In all of these animals the definitive placenta is discoidal with free and anchoring villi and an intervillous space perfused by maternal blood (Figure 5C).

Based on their diffuse, non-deciduate and epitheliochorial placentation, Hill [50] argued that the lemurs and lorises were a more primitive form of primate than the tarsiers. This argument went to the very heart of the current debate about parallelism in the evolution of the placenta. Many found it difficult to accept that rodents, insectivores and primates arose from a common stem with a diffuse type of placentation. Among the doubters was Wislocki [10], who advanced the view that hemochorial placentation was the more primitive type of placentation and that the epitheliochorial placenta of the lemurs was the result of a secondary simplification.

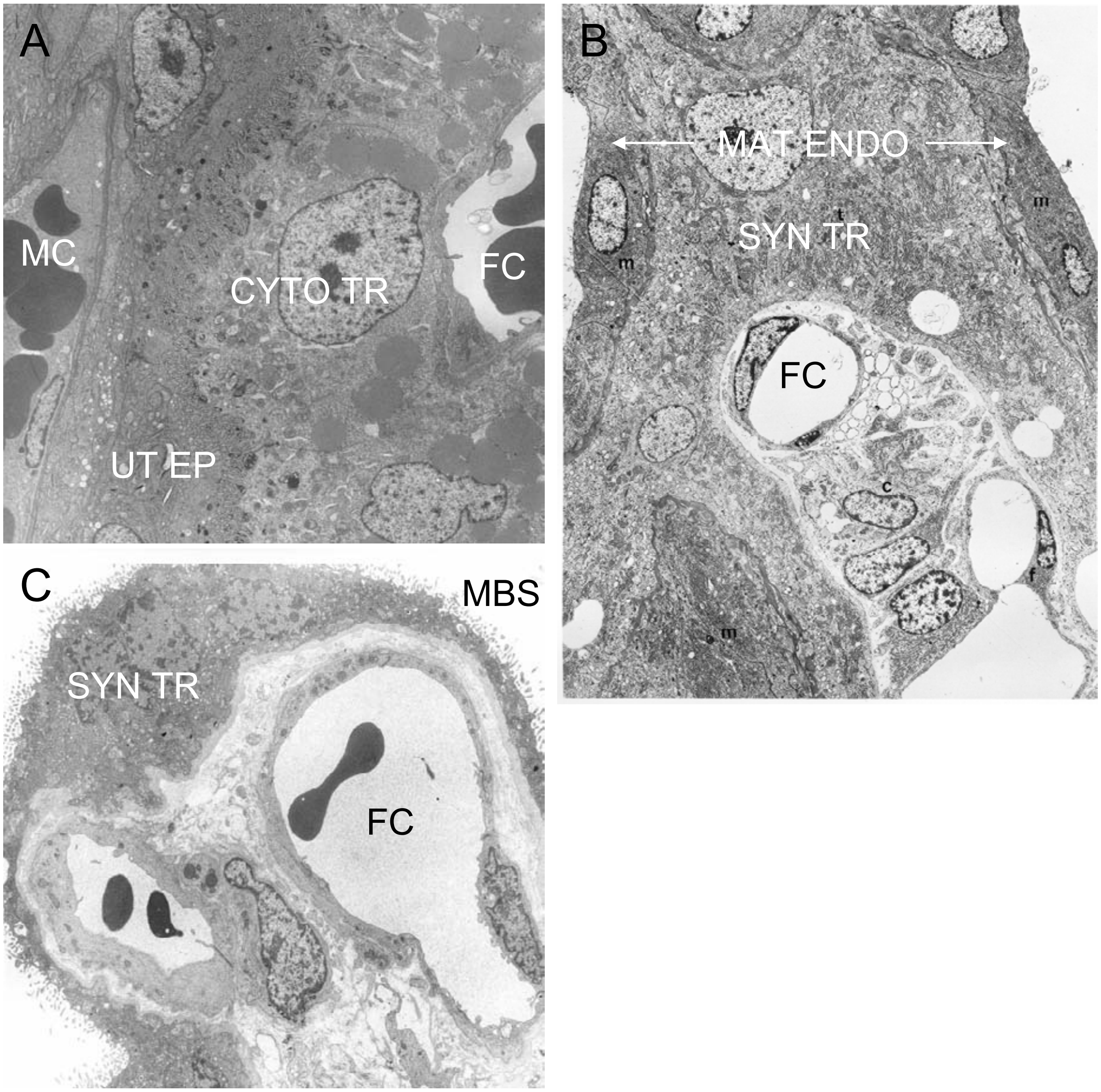

Figure 5
Placentation in primates (Euarchonta). (A) Epitheliochorial placenta of a bush baby, *Galago crassicaudata*. The interhemal membrane comprises maternal capillary (MC) endothelium, uterine epithelium (UT EP), cellular trophoblast (CYTO TR) and fetal capillary (FC) endothelium. (B) Endotheliochorial placenta of a tree shrew, *Tupaia glis*. The interhemal region contains maternal endothelium (MAT ENDO), syncytiotrophoblast (SYN TR) and the endothelium of the fetal capillary (FC). Reprinted from reference [52] with permission ©Springer-Verlag 1985. (C) The villous, hemomonochorial human placenta. The maternal blood space (MBS), or intervillous space, is lined by a thin layer of syncytiotrophoblast (SYN TR).

A lucid discussion of the evolution of placentation in primates is given by Luckett [55]. He noted that there is substantial homology in fetal membrane development between taxa with epitheliochorial placentation, but not between those with the endotheliochorial or hemochorial types. He argued that this was indicative of convergent evolution of hemochorial placentation, whereas the epitheliochorial type was part of the primitive eutherian condition.

On the grounds of parsimony, one might argue that the widespread occurrence of hemochorial placentation in Euarchonta reflects their common ancestry and follow Wislocki [10] in regarding the epitheliochorial placenta of strepsirhine primates as a derived state. Tree shrews have many conserved characters and it is possible that endotheliochorial placentation is one of these rather than being derived from a hemochorial type.

Placentation in Laurasiatheria

Many types of placentation are found in the Superorder Laurasiatheria. Not surprisingly for an order that has undergone such a high degree of adaptive radiation, the greatest diversity is found among the bats. The insectivores now included in Eulipotyphla also present a variety of placental types. Carnivores and pinnipeds have endotheliochorial placentation. The three remaining orders have epitheliochorial placentas. These are the whales and cloven-hoofed mammals (Cetartiodactyla); horses, tapirs and rhinoceroses (Perissodactyla); and pangolins (Pholidota).

Core insectivores

The order of insectivores has undergone steady attrition, the most recent exclusions being the tenrecs and golden moles. The relations between the core insectivores (shrews, moles, hedgehogs and *Solenodon*) continue to puzzle even the molecular phylogeneticists [57,58]. Most have discoid labyrinthine placentae. The interhemal area is hemochorial in hedgehogs and *Solenodon* [59]. The American mole *Scalopus* has a circumferential placenta that is considered to be epitheliochorial [60], whereas the European mole *Talpa* is definitely endotheliochorial [61]. However, Prasad et al. [60] had only a single midgestation specimen to examine by electron microscopy, and the looping arrangement of the maternal vessels is more similar to that seen in endotheliochorial placentas than that in typical epitheliochorial placentas. In the short-tailed shrew *Blarina* the interhemal area is endotheliochorial, but the trophoblast component is fenestrated [62]. In the musk shrew *Suncus* the placenta is endotheliochorial, but there is progressive thinning and fenestration of the trophoblast layer [63]. There are even areas where processes from the two endothelial layers contact one another through the pores in the trophoblast.

All insectivores have a partially or completely inverted yolk sac that is maintained to term. The allantoic sac is large in *Talpa*, medium sized in the hedgehog, small in *Scalopus* and small or rudimentary in shrews. This is a group of mammals that would repay close study using modern techniques.

Bats

As befits a widely diversified adaptive group of mammals [64], bats and especially microbats have evolved a multitude of endotheliochorial and hemochorial placental types (Table 1; reviewed by Badwaik and Rasweiler [65]). The most common type of definitive placenta is the hemodichorial placenta, where syncytial trophoblast that has intrasyncytial bays fronts the maternal blood space and is underlain by cytotrophoblast (Figure 6A). Endotheliochorial placentas are found both as a diffuse placenta in midpregnancy and as a cellular endotheliomonochorial discoid placenta in late pregnancy (Figure 6B). In some mollosid bats the central area of the placenta near term is cellular hemomonochorial (Figure 6C). Megabats (fruit eating bats) have hemochorial placentas with *Rousettus* having a syncytial hemomonochorial type and *Pteropus* having a hemodichorial one, at least in midpregnancy. Although 13 of the 18 families of bats have been studied [65], there is still a great deal to be learned both about the fine structure and about other advanced features of membrane development [14].

Carnivores and pinnipeds

The terrestrial carnivores and their aquatic relatives the pinnipeds (sea lions, walruses and seals) usually have circumferential, labyrinthine and endotheliochorial placentas, although the hyena develops hemochorial placentation late in pregnancy [66,67]. Carnivore placentas have hemophagous regions of variable size and location [68]. Mossman [12] at one time considered the carnivore fetal membranes to be the most primitive type among extant eutherians, because both the yolk sac and allantoic sac are large and persist until term.

Cetartiodactyls, perissodactyls and pangolins

The remaining orders of Laurasiatheria are characterized by epitheliochorial placentation. The placenta may be diffuse with villi distributed over the surface of the chorioallantoc sac, as in whales [69], Suiformes (pigs, peccaries, and hippopotamus) [70], camels [71] and pangolins [13], or with microcotyledons as in the horse [16,72]. Most ruminants have cotyledonary placentas with the cotyledons varying in number from 5–8 in deer to 50–175 in bovids [8]. Chevrotains are considered to be the most primitive ruminants and, interestingly, they have diffuse placentas [73].

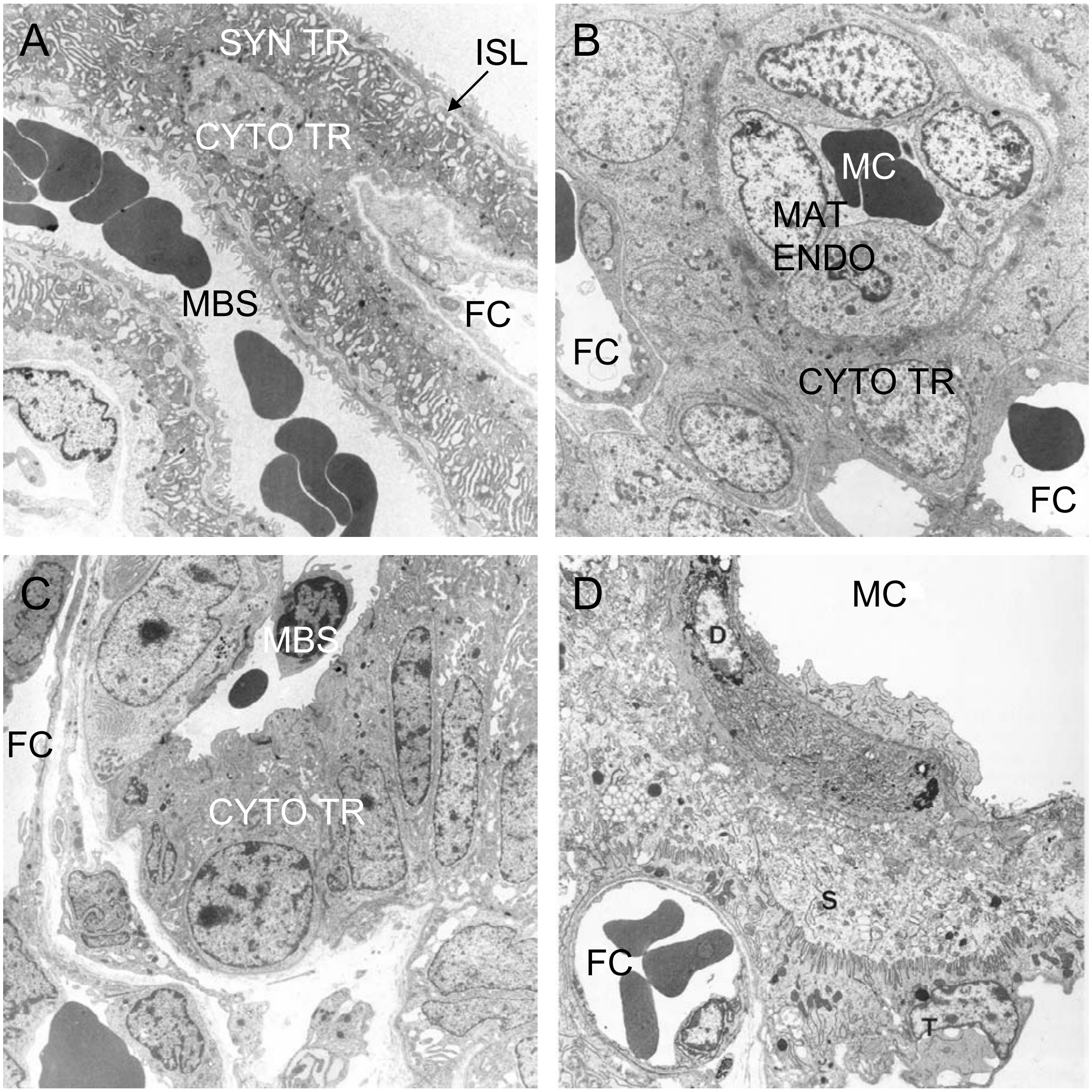

Figure 6
Placentation in bats and a ruminant. (A) Hemodichorial placenta of little brown bat, *Myotis lucifugus* (Vespertilionidae). There is an outer layer of syncytiotrophoblast (SYN TR) and an inner layer of cellular trophoblast (CYTO TR). Note the subsurface intrasyncytial spaces or bays (ISL) (B) Endotheliochorial placenta of funnel-eared bat, *Natalus sp*. (Natalidae) in late gestation. The interhemal region contains enlarged maternal endothelial cells (MAT ENDO) and cytotrophoblast (CYTO TR). (C) Hemomonochorial placenta of the Mexican free-tailed bat, *Tadarida brasiliensis* (Molossidae). The maternal blood spaces (MBS) are lined by cytotrophoblast (CYTO TR). (D) Epitheliochorial placenta of the sheep. The endometrial connective tissue has "decidual" cells whilst the fetal component comprises cellular trophoblast (T). Fusion of fetal and maternal cells gives rise to a syncytium (S). Therefore this type of placenta is sometimes referred to as synepitheliochorial. Reprinted from reference [8] with permission.

The epitheliochorial type of arrangement has as its advantage the safety factor of the isolation of fetal and maternal components. The presence of two complete epithelia should diminish immunological problems as well as the deportation of fetal cells to the maternal organism. The disadvantage is the greater difficulty in passage of materials between organisms, but this is overcome by a variety of mechanisms. Most importantly, indenting of trophoblast and uterine epithelium by capillaries decreases the interhemal distance. There are often, but not always, small absorptive and phagocytic areas, for example the areolae of the pig placenta. In equids, girdle cells of fetal origin invade the uterine epithelium to form endometrial cup cells that secrete equine chorionic gonadotropin [74-76]. However, not all perissodactyls have endometrial cups [8]. In ruminants such as sheep and cow, binucleate trophoblast cells fuse with maternal epithelial cells and this facilitates passage of prolactin to the maternal organism [77]; this type of placenta is sometimes referred to as synepitheliochorial.

The diffuse type of epitheliochorial placenta usually is associated with an extensive allantoic sac. The cotyledonary type tends to be associated with a long, tubular allantoic cavity, although it is still large. There is a temporary yolk sac placenta early in development, where this has been examined, and a free yolk sac later in gestation. Mossman [13] noted the resemblance in definitive yolk sac placentation between whales, artiodactyls, perissodactyls and pangolins, but grouped them together with some non-laurasiatherians.

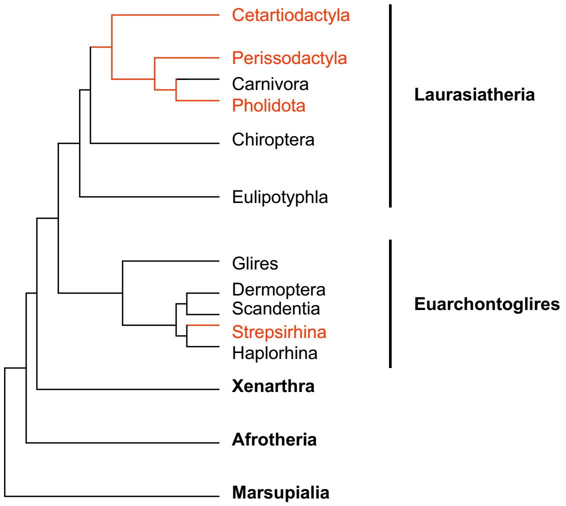

Figure 7
Cladogram based on nucleotide sequence analysis. The four superorders of eutherian mammals and the marsupial outgroup are shown in bold face. Epitheliochorial placentation, indicated in red, occurs in three orders of Laurasiatheria and one suborder of Euarchontoglires. These are the orders Cetartiodactyla (whales, pigs and hippopotami, camels, ruminants); Perissodactyla (horses, tapirs and rhinoceroses); Pholidota (pangolins or scaly anteaters); and the primate suborder Strepsirhina (lemurs and lorises).

How did the placenta evolve?

Although placental structure is directly involved in the success of fetal growth, it is only one aspect of the complex interrelationship between reproductive success of individual matings and the overall reproductive success of a given female [78]. Consequently different aspects of the total reproductive process may be more or less important depending on the relationship of a given species to its environment. Nevertheless placental structure has been considered useful as one of the characters to be used in tracing relationships as it is considered conservative [14]. Although physiological data would be useful, unfortunately such data as the increase in fetal weight per weight of placenta per day is available for only a few domestic and laboratory animals, limiting our ability to use such data on a comparative basis [79].

The evolutionary pressure favoring some type of hemochorial placenta has evidently been great. Hemochorial placentas are found in insectivores, primates, tenrecs, rodents, bats, hyraxes, elephant shrews, anteaters, armadillos, flying lemurs and even hyenas. The large variation in the definitive form of the placenta, the divergent way in which the hemochorial condition is achieved, and the variety of unrelated orders in which it is found might seem to suggest convergent evolution [15].

An endotheliochorial condition is succeeded by hemochorial placentation in the hyena. This could very well have been the path of evolution in Afrotheria and perhaps in Xenarthra and some bats. The question then arises of whether either placental type arose from an epitheliochorial condition. It vexed the pioneers in this field, who were puzzled to find that the tenrecs, which were mammals with many conserved characters, nonetheless had hemochorial placentas (see [33]). Later, Wislocki [10] advanced the view that hemochorial placentation was the more primitive type and that epitheliochorial placentation was the result of a secondary simplification. A similar opinion was held by Portmann [11]. Certainly, it is more parsimonious to assume that epitheliochorial placentation is a derived state arising perhaps twice, in Laurasiatheria and in the strepsirhine primates (Figure 7). Of interest, invasive trophoblast occurs in marsupials, although there is too little information to tell whether invasiveness is part of the stem species pattern or arose independently in metatherian and eutherian mammals [2].

Additional clues to placental evolution are provided by the early development of the fetal membranes. Luckett [14,55] argued that ancestral mammals would have had an amnion formed by folding rather than cavitation and possessed a large allantoic vesicle. A large vascular yolk sac would have been present early in development, forming a choriovitelline placenta, but the yolk sac would have been reduced in later stages [14]. These features are found with endotheliochorial and hemochorial placentas in Afrotheria as well as with epitheliochorial placentas in some Laurasiatheria.

Portmann [11] argued that the allantoic sac had evolved along two paths. Mammals with epitheliochorial placentation had retained a large allantoic sac as a receptacle for the dilute urine produced by a large and persistent mesonephros. The carnivores had also followed this route. In contrast the allantoic cavity had become reduced or was absent in mammals that had a poorly developed mesonephros, such as rodents, higher primates, bats and xenarthrans. Most of these had a hemochorial placenta that was thought to have assumed the function of the mesonephros as first proposed by Bremer [80]. This hypothesis needs to be tested and refined in the light of current concepts of mammalian phylogenetics and kidney development [81].

Conclusions

The position argued here resembles that originally adopted by Mossman [12]. He considered that the endotheliochorial and hemochorial conditions were closely related and that the endotheliochorial type was the more primitive. We have shown that the likely path of evolution was from endotheliochorial to hemochorial in the Afrotheria. It certainly occurred with the carnivores and more than likely in the bats and Xenarthra. There may be an epitheliochorial stage during placental ontogeny, but Mossman [12] argued that a definitive epitheliochorial placenta was a secondary specialization; he regarded the cotyledonary placentas of ruminants as among the most highly specialized. We are inclined to agree. Consideration of placental type in relation to the findings of molecular phylogenetics suggests that epitheliochorial placentation evolved just twice, once in the Laurasiatheria and once in Euarchontoglires.

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