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# Placentation in the colugos *Cynocephalus volans* and *Galeopterus variegatus* (Dermoptera) and the transition from labyrinthine to villous placentation in primates

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ABSTRACT

*Introduction:* Phylogenetics and genomics place colugos as the sister group to primates. Therefore their placentation is of interest in an evolutionary perspective. Previous accounts are fragmentary, not readily accessible and sometimes contradictory.
*Methods:* We have examined archival material covering the early development of fetal membranes and placenta, the fate of the yolk sac and definitive placentation.
*Results:* Initially the trophoblast extended over a rather broad but shallow area, enclosing maternal blood spaces. After expansion of the exocoelom it became covered by somatic mesoderm. The mature chorioallantoic placenta was haemochorial and characterized by a labyrinth with markedly dilated maternal blood spaces. Blood vessels appeared in the splanchnopleure early in development and later extended to the yolk sac, but we found no evidence of a choriovitelline placenta at any stage of gestation. There was, however, an extensive paraplacenta.
*Conclusions:* A choriovitelline placenta is not formed early in gestation nor is it present at term. Early in development invasive trophoblast spreads laterally to form a trophoblastic plate. We found evidence to support the idea that the colugo placenta is intermediate between the labyrinthine placenta of rodents and the trabecular type of Neotropical primates.



## 1. Introduction

Based on molecular and morphological phylogenies, the mammals closest to primates (Order Primates) are tree shrews (Scandentia) and colugos (Dermoptera) [1,2]. The endotheliochorial placenta of tree shrews has been well studied [3,4], but for colugos we must rely on papers by Hubrecht that are restricted to gross anatomy [5,6] and early development up to emergence of the allantoic bud at the 18-somite stage [7]. Later stages of placentation have been dealt with only in outline [8,9]. The situation is unsatisfactory because of perplexing and inaccurate statements in standard works of reference. Thus Mossman [10] presented two interpretations of the fetal membranes, one with a persistent, partially inverted yolk sac placenta, the other with the yolk sac floating free in the exocoelom.

The chorioallantic placenta of colugos could represent a stage in the evolution towards villous placentation, as first suggested by Wislocki [11]. Rodents and lagomorphs have labyrinthine placentas, one advantage being that fetal capillaries and maternal blood channels can be packed together with countercurrent flow to support highly efficient exchange of blood gases. This arrangement has been abandoned by catarrhine primates (Old World monkeys, apes and human) in favour of villous placentation, which allows a much greater volume flow and thus increased oxygen delivery to the placenta. Wislocki [11] suggested two intermediate steps. The first retained a labyrinthine structure, but with greatly widened maternal blood channels as seen in colugos. The second led to the placenta of platyrrhine (Neotropical) primates such as the spider monkey (*Ateles geoffroyi*), where the villi are connected by strands of syncytiotrophoblast in a trabecular arrangement.

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Here we attempt to give an integrated account of early development and placentation in colugos based on the available archival material. In particular we try to clarify some early events in the establishment of the chorioallantoic placenta and provide evidence of the development and fate of the yolk sac. Our findings are discussed in the context of current evolutionary perspectives.

## 2. Materials and methods

To address the question of yolk sac placentation, we examined the relevant slides in the A. A. W. Hubrecht Collection from the Embryological Collection of Museum für Naturkunde, Berlin (Table 1). Since the origin of that material is known [6,7] and confirmed by the accompanying laboratory notebooks, they are of the Sunda Colugo, *Galeopterus variegatus*, which is distributed in Indochina, Java, Borneo and accompanying islands. The heading "*Galeopithecus volans*" in the plates prepared by De Lange for Hubrecht's posthumous paper [7] and the original drawings at Universiteits Museum Utrecht (www.universiteitsmuseum.nl) is incorrect (there was confusion as to terminology in the period between collection of the material and its description [12]).

The sectioned material in the Hubrecht Collection covered the earliest stages in formation of the placenta up to the 18-somite stage and emergence of the allantoic bud. There were no sections to document the subsequent fusion of chorion and allantois, although one specimen from advanced pregnancy had been serially sectioned. To describe the mature placenta, we examined specimens from the J. P. Hill Collection also at Museum für Naturkunde, Berlin. With these as a guide, we were able to incorporate a larger number of slides made by Hill, but retained in the collection of his student E. C. Amoroso and now curated at The Royal Veterinary College London. Some of these were clearly labelled *Cynocephalus volans*, the Philippine colugo, but others were indeterminate as to species. Lastly, we examined sections through the mature placenta from the Harland W. Mossman Collection at the University of Wisconsin Zoological Museum (UWZ M20472). These are marked "*Cynocephalus*," which is ambiguous, but are catalogued as *Cynocephalus volans* from the Philippines.

Recent studies indicate hidden biodiversity among colugos with up to six Sundaic species (four subspecies were recognized on morphological grounds [13]) and two species of Philippine colugo [14]. Most of Hubrecht's description was based on specimens collected on Bangka, in southern Sumatra and East Java. The provenance of the specimens in the Hill and Mossman Collections is, however, not sufficiently detailed to relate to these proposed lineages.

## 3. Results

### 3.1. Yolk sac

In all sections where bilaminar omphalopleure was present, it was separated from the endometrium by the uterine cavity (Fig. 1A–C). Following expansion of the exocoelom, blood vessels began to form within the extraembryonic mesoderm of the yolk sac splanchnopleure. Otherwise the yolk sac remained bilaminar and there was no evidence to suggest close contact between the bilaminar omphalopleure and the uterine wall (Fig. 1C–D). At most later stages, the yolk sac had been removed together with the embryo or fetus during preparation of the histological material. Perhaps therefore we never saw a trilaminar omphalopleure (see Discussion).

### 3.2. Chorioallantoic placenta

#### 3.2.1. Early development

The first phase in formation of an ectoplacenta is shown in Fig. 2 A. There was a discontinuity in the uterine epithelium that suggested erosion by the trophoblast. Interestingly, there was some multinucleate trophoblast here, suggestive of syncytiotrophoblast. The epithelium of an adjacent gland was intact indicating that invasion may not occur by this route. Subsequently, the trophoblast expanded laterally to form a trophoblastic plate enclosing maternal blood spaces. However, the trophoblast appeared not to penetrate very deep at this stage (Fig. 2B–D). At its surface the trophoblast formed an epithelial layer to which the somatic mesoderm was closely applied (Fig. 2 C). Later the mesoderm expanded to occupy folds in the surface of the trophoblastic plate (Fig. 2 D). We concur with Hubrecht [7] that there are signs of blood vessel formation inside the somatic mesoderm.

**Table 1**
Specimens of colugo fetal membranes and placenta.

| Collection | Current Location | Voucher or Collection Nos. | Species | Provenance | Remarks |
|---|---|---|---|---|---|
| A. A. W. Hubrecht | Museum für Naturkunde, Berlin Embryological Collection (ZMB EMB) | 27241–27295 | *G. variegatus* | Indonesia: Bangka, Sumatra, Java, Belitung and Borneo. | |
| J. P. Hill | Museum für Naturkunde, Berlin Embryological Collection (ZMB EMB) | INS 116-119 | Slides labelled *Cynocephalus* or *C. volans* | INS 116 from W. L. H. Duckworth; INS 117–119 source unknown | |
| Ditto | Ditto | INS 125-127 | *G. variegatus* | INS 125 from J. L. Shellshear (Borneo); INS 126 from W. E. Le Gros Clark (Sarawak); INS 127 from C. Hose (Sarawak) | Wet collection; not available for dissection |
| E. C. Amoroso | Royal Veterinary College, London | Not catalogued | *C. volans*; sections are from same source as INS 117–119 in the Hill Collection | Unknown | Received by Amoroso from Hill; labels read, "1925, Institute of Anatomy, U.C.L., Galiopithecus" |
| H. W. Mossman | University of Wisconsin Zoological Museum, Madison Wisconsin (UWZ) | UWZ M20472 | *C. volans* | Philippines | |
| K. L. Duke | Brigham Young University, Provo, Utah | Not curated | *C. volans* | Philippines | Not available to us |