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Placentation in the Egyptian Slit-faced Bat *Nycteris thebaica* (Chiroptera: Nycteridae)

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ABSTRACT

Bats are a highly successful, widely distributed group, with considerable variation in placental structure. The Egyptian slit-faced bat *Nycteris thebaica* is a member of one of the few families with previously undescribed placentation. It was found that, although the interhemal type of the *Nycteris* placenta is endotheliochorial with a single layer of cytotrophoblast, the arborizing pattern of the maternal vessels and especially the extraordinary major placental artery differs from the placenta of the emballonurid bats to which this family is considered to be most closely related. The major placental artery providing maternal blood to the vessels of the placental disk has a highly glycosylated matrix surrounded by two-layered folds of trophoblast, forming an apparently rigid structure of unique morphology. The yolk sac is collapsed, with hypertrophied endodermal and mesothelial cells similar to many other bat species. The paraplacenta is extensive with abundant fetal vessels underlying cytotrophoblast and syncytial trophoblast layers, fronting on an endometrium that largely lacks uterine epithelial cells but has large decidual cells and is poorly vascularized. The placenta of *Nycteris* lacks a hemophagous region, unlike the emballonurid bats *Taphozous* and *Saccopteryx*. Although the latter two species have similar placentas, the placental structure of *Nycteris* does little to relate it to the other family within the Emballonuroidea. Shared and divergent reproductive characters are discussed in relationship to bat phylogenetic relationships.

1. Introduction

Bats underwent a rapid radiation in the early Eocene coincident with an increase in plant and insect diversity [1]. One consequence seems to have been a much greater variation in placentation than seen in other orders of placental mammal [2,3]. Some information on the placenta is available for 15 of the 19 extant families and several species have been studied in great detail [4,5]. Two of the remaining families are Craseonycteridae and Mystacinidae, each represented by a single, vulnerable species. Until now, Nycteridae has not been studied although there are 16 species and at least one of which is widely distributed. It has been suggested that the relative paucity of information on *Nycteris* reflects the geographical distribution of the genus [6].

Even in this era of molecular phylogenetics, relations between bat families continue to stir controversy [7–9]. Smith [10] suggested classification of microbats in four superfamilies. This concept has proven to be robust and is embraced by those working with morphological and molecular approaches [11–13]. Superfamily Emballonuroidea comprises just two families, Emballonuridae and Nycteridae, although it is part of a larger assemblage constituting Suborder Vespertilioniformes [14]. In a recent analysis of bat placentation that attempted to trace evolutionary trends, it was noted that Emballonuroidea had some unique features [15]. The data were, however, derived entirely from studies of emballonurid bats. *Taphozous* had been looked at in the greatest detail although for one defining character, the hemophagous region, information was available from six genera [16].

The placenta of *Nycteris* was examined to determine the major features of this placenta and to assess which characteristics it might share with emballonurids. The present study determined that *Nycteris* shares some characteristics with emballonurids but lacks a hemophagous region and in addition has at least one feature of placentation unique to those bats studied thus far.

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2. Materials and methods

Two pregnant Egyptian slit-faced bats *Nycteris thebaica* were collected in Bluff, Durban, South Africa. The uteri were removed, placed in Karnovsky glutaraldehyde/formaldehyde fixative, and sent to Davis, where they were dissected. Both specimens were from late gestation judging by the hairy nature of the fetuses, the complete development of the wings, and the large ears. One fetus had a crown-rump length of 22 mm, the other 24 mm. Representative slices of different areas of the placentas were selected. After rinsing in buffer overnight, specimens were dehydrated and embedded in Araldite resin, some with and others without postfixation in 1% osmium tetroxide. Sections of the postfixed blocks were cut at approximately 1 μm and stained with Azure B for examination by light microscopy. Thin sections of these blocks were stained with uranyl acetate and lead citrate for examination by electron microscopy. Sections from the non-osmicated blocks were used for the lectin studies, methods for which and the binding characteristics of the lectins used can be found as previously described [17].

3. Results

The placental disks are roughly mesometrially situated, and the fetus is attached to the disk by a very short umbilical cord containing a single artery and two veins. The cord insertion is eccentric (Fig. 1A). The large collapsed yolk sac, which extends in an arc overlying the margin of the placenta, is free in the exocelom except for its attachment at the cord. Large fetal vessels extend over the disk of the chorioallantoic placenta, and smaller fetal vessels extend to the paraplacental regions. The paraplacenta encompasses the area of the site not occupied by the placental disk. The placental disks are slightly oval, being 10 × 12 mm in one specimen and 11 × 12 mm in the other. The placental disks are not lobulated (Fig. 1B) and are composed almost entirely of a labyrinthine arrangement of maternal and fetal vessels in an endotheliochorial relationship (Fig. 1C,D).

3.1. Placental labyrinth

The most conspicuous feature of the labyrinth is the presence of numerous maternal capillaries composed of darkly stained hypertrophied endothelial cells (Fig. 1E). The smaller branches of these capillaries tend to be relatively uniform in diameter. The cuboidal arrangement of the endothelial cells produces an irregular luminal

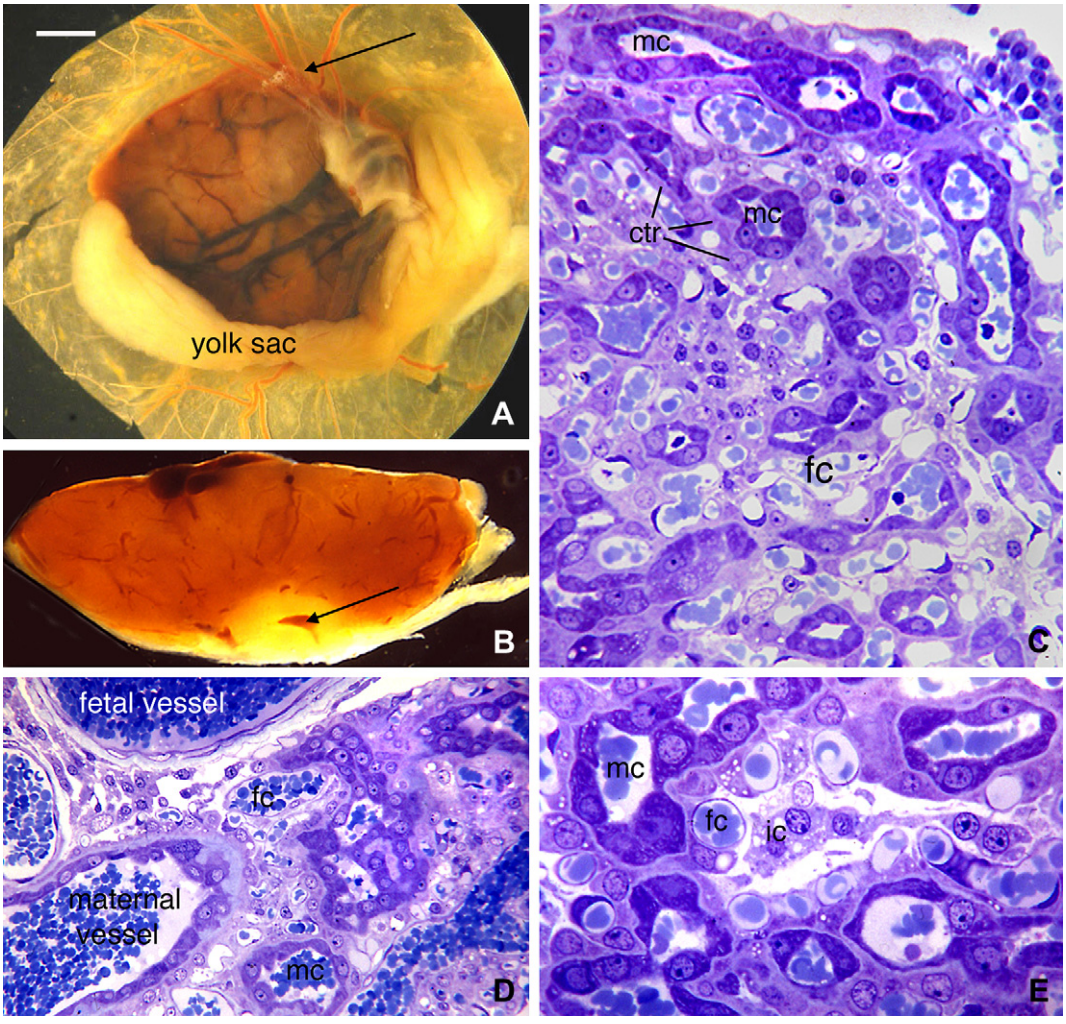

Fig. 1. (A) View of the placenta from the exocelom after removal of the embryo. Note the U-shaped yolk sac lying on the placental disk, the cut umbilical cord, the large dark allantoic vessels stretching across the placental disk, and the spray of fine vessels going to the paraplacenta (arrow). (B) Slice through the placental disk, showing large fetal vessels at the exocelomic surface. Note the main maternal artery in the lower center (arrow), and a major maternal vein to the left of the artery. (C) Section through the placental disk showing the numerous maternal capillaries (mc) with darkly stained hypertrophied endothelial cells, surrounded by cytotrophoblast cells (ctr). Fetal capillaries (fc). (D) Placental labyrinth showing intermediate-sized maternal and fetal vessels as well as maternal capillaries. Note thick endothelium and interstitial lamina of the maternal vessel in the lower left, and the thin endothelium in the fetal vessel in the upper left. (E) Higher magnification of the labyrinth showing that the darkly stained maternal capillaries are invested by a layer of cytotrophoblast cells of variable thickness. Note the cluster of fetal interstitial cells (ic) and the very thin wall of the fetal capillaries (fc). Scale bar: 2.5 mm (A), 1.45 mm (B), 27 μm (C), 40 μm (D), and 15 μm (E).