Electron microscopic study of the chorioallantoic placenta of the rock hyrax (Heterohyrax brucei)

D. Oduor-Okelo, V. O. Musewe*† and S. Gombe*

Departments of Veterinary Anatomy and *Animal Physiology, University of Nairobi,
P.O. Box 30197, Nairobi, Kenya

Summary. The interhaemal membrane consisted of only two cellular elements: a single layer of cellular trophoblast and the fetal capillary endothelium. The hyrax is therefore one of the few mammals known to possess the cellular haemomonochorial type of placenta. The trophospongium was also cellular while the basal trophoblastic cells were strongly phagocytic. The giant multinucleate cells at the feto-maternal junction were ultrastructurally different from the trophoblast cells and showed no signs of degeneration. Their appearance suggests that they are of maternal rather than fetal origin.

Introduction

Placentation in the rock hyrax (Procavia capensis: Order Hyracoidea) has been studied macroscopically and histologically and comparisons have been made between this placental type and that of the carnivores (Turner, 1875) and the rodents, insectivores and man (Assheton, 1906). In her detailed description of the histology of the hyrax placenta, Thursby-Pelham (1924) reported that the thin layer of cells lining the maternal blood lacunae was maternal endothelium. This interpretation implied that the hyrax placenta was of the "endothelio-syndesmal" or "endothelio-endothelial" type in Grosser's (1927) terminology. Thursby-Pelham's (1924) interpretation contradicted the earlier one by Assheton (1906) in which it was clearly implied that the placenta of the hyrax was haemochorial. This confusion has, however, since been clarified: Wislocki & Van der Westhuysen (1940) and Sturgess (1948) confirmed that the hyrax placenta is haemochorial and Dempsey (1969) demonstrated that the trophoblast of the interhaemal membrane was cellular rather than syncytial.

In this paper a more detailed ultrastructural description of the chorioallantoic placenta of the rock hyrax, Heterohyrax brucei, is reported.

Materials and Methods

All 41 pregnant hyraxes in this study were trapped in the wild between September 1979 and January 1980 at Lukenya, about 35 km south-east of Nairobi, Kenya. The animals were brought live to the laboratory where they were killed by an overdose of anaesthetic (chloroform or ether). The crown-rump length of the fetuses ranged from 4.3 cm (trapped in October 1979) to 22.5 cm (trapped in September 1979) and fetal weight ranged from 0.6 to 93.1 g. This range represented stages from limb-bud to near-term. The gestation period in the hyrax has been estimated at about 7½

† Present address: International Centre of Insect Physiology and Ecology (I.C.I.P.E.), P.O. Box 30772, Nairobi, Kenya.

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months (Sale, 1965). Small pieces of placental tissues were immediately immersed in formaldehyde–glutaraldehyde–trinitro cresol solution (Ito & Karnovsky, 1968), post-fixed in osmium tetroxide and embedded in Epon araldite. For orientation purposes thick sections were cut with glass knives, stained with toluidine blue and examined with the light microscope. Thin sections, cut with a diamond knife, were stained successively with uranyl acetate and lead citrate and examined under a Zeiss 9S electron microscope.

Observations

There were 25 singletons and 16 sets of twins in this sample of hyraxes. The ovary bearing the corpus luteum did not always correspond with the pregnant uterine horn and there were frequently 2 corpora lutea in one ovary when fetuses were present in each horn, indicating a transuterine migration of the fertilized egg.

The histology of the placenta of this species of rock hyrax (Heterohyrax) was similar in every detail to that of Procavia and was divisible into three zones (according to Wislocki & Van der Westhuysen, 1940): (1) Zone I where the trophoblast was richly vascularized by fetal mesenchyme. The trophoblastic tubules were arranged in tall columns with extensive anastomosis of the trophoblastic cells. These anastomoses resulted in the compartmentalization of the maternal blood spaces. (2) Zone II where the trophoblast formed vascular channels through which maternal blood percolated. This zone was not invaded by fetal mesenchyme. (3) Zone III which consisted of a layer of columnar basal trophoblast. This layer of trophoblast was in direct contact with the maternal decidua.

Ultrastructure

Electron microscopic examination of Zone I tissues revealed that the placenta of the rock hyrax at all stages studied was haemochorial. The interhaemal membrane consisted of a single layer of cellular trophoblast, a basal membrane and a fetal capillary endothelium (Pl. 1, Figs 1 & 2). The

PLATE I

Fig. 1. The zone of vascular exchange in hyrax placenta. A single layer of cellular trophoblast intervenes between maternal blood space (m) and the fetal capillaries (fc). Note the compartmentalization of the maternal blood space by trophoblastic extensions held together by desmosomes (see Fig. 4). Epon section at 60 nm stained with uranyl acetate and lead citrate. × 1700.

Fig. 2. The interhaemal membrane. The cellular trophoblastic cells have numerous large mitochondria, well developed rough endoplasmic reticulum (rER), Golgi complex (G) and bundles of tonofilaments, some of which converge upon the desmosomes (arrows). Towards the fetal side the intercellular spaces are dilated. Inclusion bodies are deposits of glycogen granules, lysosomes and membrane-bound secretory bodies (g). Epon sections at 60 nm stained with uranyl acetate and lead citrate. ×9500.

Fig. 3. The interhaemal membrane of the rock hyrax placenta. The cellular trophoblast contains rough (rER) and smooth (sER) endoplasmic reticulum. The smooth membranes are associated with glycogen particles. Note also the numerous coated vesicles, lysosomes (L) and crystalloid structures (C). Epon section at 60 nm stained with uranyl acetate and lead citrate. × 10 000.

Fig. 4. High-power magnification of an area similar to that in the square in Fig. 1. The maternal blood space (m) is compartmentalized by these two cellular trophoblastic cells which are held together by desmosomes. Note the concentration of large mitochondria in this area. Note also the pinocytotic vesicles (arrows). Epon section at 60 nm stained with uranyl acetate and lead citrate. × 9000.
cellular trophoblast had numerous slender microvilli abutting into the maternal blood spaces. The cells were held together by tight junctions and desmosomes, the latter being associated with cytoplasmic tonofilaments which converge upon the attachment plaques (Pl. 1, Figs 2 & 3). Between the junctional specializations, and especially towards the basal region, the intercellular spaces were dilated.

The trophoblastic nuclei tended to be oval or round in shape with a more or less even distribution of heterochromatin material. Some nuclei were greatly indented. They had distinct nucleoli. The cytoplasm contained short stacks of rough endoplasmic reticulum (Pl. 1, Figs 1, 2 & 3) as well as profiles of smooth endoplasmic reticulum that was associated with glycogen granules (Pl. 1, Fig. 3). Also present in the cytoplasm were large mitochondria, Golgi complex, numerous microfilaments and free ribosomes. Lysosomes were present in various shapes: there were primary and secondary lysosomes, some of which contained crystalloid contents (Pl. 1, Figs 2 & 3). Inclusion bodies present were lipid droplets and glycogen particles scattered throughout the cytoplasm but some of the glycogen particles were closely associated with profiles of smooth endoplasmic reticulum. Numerous coated vesicles were seen on both the luminal and basal areas of the cells (Pl. 1, Fig. 4). When the trophoblast cells lining the maternal channels anastomosed to interrupt the flow of blood in the channels, the cells were held together by desmosomes. At such sites there was an unusual concentration of large mitochondria (Pl. 1, Figs 1 & 4).

The Zone II trophoblast, i.e. the spongy trophoblast, was also cellular. The nuclei were indented and the chromatin material was concentrated in lumps along the periphery. The cytoplasm had a well developed rough endoplasmic reticulum, numerous mitochondria, Golgi complexes, secretory granules and many lysosomes (Pl. 2, Fig. 5). Intracellular canaliculi with microvilli were also present but these could have been artefacts. The microvilli from the cell surface were slender. Adjacent cells were held together by tight junctions, desmosomes and an intricate system of interdigitations of their lateral surface membranes.

The basal trophoblastic cells of Zone III were a mixture of columnar and cuboidal cells. The columnar cells had longitudinally orientated nuclei with distinct nucleoli. The basal trophoblastic cells were phagocytic (Pl. 2, Fig. 6) and contained remnants of disintegrating phagocytosed cells. These cells were also rich in lysosomes and residual bodies and contained rough endoplasmic

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**PLATE 2**

**Fig. 5.** Cellular trophoblast of the trophospongial zone. The cells are held together by tight junctions, desmosomes and an intricate system of interdigitations (arrow). Note the numerous dense bodies and crystalloid structures in the cytoplasm. Epon section at 60 nm stained with uranyl acetate and lead citrate. × 2900.

**Fig. 6.** Cellular trophoblast of the basal zone. The intercellular spaces (*) are dilated and filled with a dense secretory material. Lipid droplets can be seen in the cytoplasm. Lysosomes and rough endoplasmic reticulum are also present. Note the remnants of a phagocytosed cell (arrow) lying in the trophoblastic cytoplasm. N = nucleus. Epon section at 60 nm stained with uranyl acetate and lead citrate. × 2400.

**Fig. 7.** Light microscopic picture of the placenta of the rock hyrax to show the trophospongium (T), basal zone (B) and the giant multinucleate cells of the decidua (arrows). Paraffin wax section stained with H & E. × 180.

**Fig. 8.** A giant multinucleate decidual cell. The cell does not show any signs of degeneration. There are numerous mitochondria and the nuclei are more centrally located in the cytoplasm. Note also the numerous microfilaments (arrows). Epon section at 60 nm stained with uranyl acetate and lead citrate. × 2300.

**Fig. 9.** High-power electron micrograph of the area in the square in Fig. 8. Note the mitochondria, short stacks of rough endoplasmic reticulum (arrows) and the microfilaments. Epon section at 60 nm stained with uranyl acetate and lead citrate. × 8500.

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reticulum and numerous lipid droplets. The cells were held together by numerous desmosomes and in some sections the intercellular spaces were extensively dilated and contained a finely granular precipitate of moderate electron density.

**Giant decidual cells**

Giant cells were found in the junctional zone between the basal trophoblastic zone and the maternal decidua. These cells did not form a continuous layer but rather appeared at intervals all around the placental belt. In histological preparations these cells had acidophilic cytoplasm with numerous nuclei, sometimes as many as 25–30 in one cell (Pl. 2, Fig. 7). These nuclei were arranged randomly in the cytoplasm or along the periphery of the cell. These multiple nuclei were oddly shaped and had deeply indented membranes. There were numerous mitochondria in the giant cells and many microfilaments were distributed randomly within the cytoplasm. Other cell organelles included a few strands of rough endoplasmic reticulum polyribosomes, coated vesicles, multivesicular bodies and lysosomes (Pl. 2, Figs 8 & 9).

**Discussion**

The ultrastructure of the hyrax placenta has been described only briefly by Dempsey (1969). He observed that the interhaemal membrane consisted of a layer of cellular rather than syncytial trophoblast. This observation is confirmed in the present study. The interhaemal membrane in *Heterohyrax* consists of a single layer of cellular trophoblast, a fetal capillary endothelium and the basal laminae of both cell types. The placenta of the hyrax can therefore be regarded as haemomonochorial according to Enders' (1965) classification. In the majority of the haemomonochorial placentas that have been studied the single layer of trophoblast is considered to be syncytial (see Enders, 1965). However, there are some haemomonochorial placentas in which the trophoblastic layer is cellular: e.g. the placentas of the bat, *Tadarida brasiliensis cynocephala* (Stephens, 1969) and the jumping mice, *Zapus hudsonius* and *Zapus princeps* (King & Mossman, 1974). In *Zapus*, it was reported that there is early arrest of development and eventual disappearance of cytotrophoblast and syncytiotrophoblast followed by the migration of trophoblastic giant cells into the allantoic mesenchyme to form the maternal channels of the labyrinth. In the hyrax there is no evidence of the migration of cellular trophoblast into the allantoic mesenchyme.

The cytoplasmic composition of the cellular trophoblast indicates that these cells are actively engaged in absorption and secretion. The production of placental gonadotrophic hormones is fairly well established in man and a number of other mammals (see Friesen, 1973). These hormones are glycoproteins and their synthesis requires the presence of rough endoplasmic reticulum and Golgi complexes (Rhodin, 1974). Chorionic gonadotrophin has been isolated (although in an impure form) from hyrax placental tissues (C. Bambra & S. Gombe, unpublished) and this correlates well with the ultrastructural findings in this study, especially with the presence of well developed rough endoplasmic reticulum. In addition the mammalian placenta is known to produce some steroid hormones in different stages of pregnancy (Ryan, 1973; Amoroso, 1981). Gombe, Heap & Sale (1976) found that the hyrax corpus luteum yielded large amounts of progesterone, but neither the placenta nor the rest of the ovarian tissue contained any significant quantities of this hormone. Gombe, Odor-Okelo & Amoroso (1977) subsequently showed that bilateral ovariectomy of pregnant hyraxes resulted in abortion within 48–72 h. In view of the ultrastructural observations in this study, especially the presence of smooth endoplasmic reticulum, it seems reasonable to suggest that the hyrax trophoblast may be involved in the synthesis of steroids other than progesterone.

The significance of the association between smooth endoplasmic reticulum and glycogen particles in the trophoblastic cells is not clear. The association of glycogen particles with smooth endoplasmic reticulum is a common feature in liver cells (Fawcett, 1981) and has also been
described in the extraocular muscle of the rabbit (Davidowitz, Philips, Pachter & Breinin, 1975), in the sensory nerve fibres of the cat muscle spindle (Corvaja, Magherini & Pompeiano, 1971) and in normal and abnormal muscle (Miledi & Slater, 1969). The smooth endoplasmic reticulum in these situations may be involved in glycogen metabolism but the functional significance of such a close association is still obscure.

The phagocytic activity of the basal trophoblast, which was suggested by Wislocki & Van der Westhuysen (1940) is confirmed by the electron microscopic findings in this study. These cells are rich in lysosomes and residual bodies. Whether the phagocytosed cells seen in Pl. 2, Fig. 6 are of maternal or fetal origin, is difficult to tell. The finely granular precipitate (Pl. 2, Fig. 6) in the dilated portions of the intercellular spaces may be a secretory product of the basal trophoblastic cells but its nature remains unknown.

The ultrastructure of the giant multinucleate cells at the fetal–maternal border shows that they are different from the trophoblastic cells. They show no sign of degeneration and have abundant cytoplasm containing many mitochondria, short strands of rough endoplasmic reticulum, multivesicular bodies, coated vesicles, polyribosomes and randomly distributed bundles of microfilaments. From their cytological appearance these cells can be assumed to be very active. Previous investigators (Thursby-Pelham, 1924; Wislocki & Van der Westhuysen, 1940) have shown that these giant multinucleate cells are derived from uninucleate decidual cells which form the bulk of the extensive compact zone of the maternal placenta. The present study (Pl. 2, Figs 7 & 8) supports this view. The physiological significance of these cells at the feto–maternal junction is still unknown, especially since they do not form a continuous layer at this junction.

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