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International Journal of Animal Science

Research Article

Syncytiovascular Membranes in the Octodon Degus Placental Barrier: Morphological Evidence

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Article Information

Received date: Aug 29, 2017
Accepted date: Sep 14, 2017
Published date: Sep 19, 2017

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Keywords Octodon Degus; Placental barrier; Fine syncytium; Syncytial knots; Syncytiotrophoblast apoptosis

Abstract

Previous data indicates that placentation in the caviomorph rodent O. degus is similar to that in humans, regarding the migration of the Extra Sub Placental-Trophoblast (EST) to the uterine arteries to be remodeling. The aim of this paper was to determine the ultra structural morphological organization of the degu´s placental barrier, as part of a wider effort to understand their reproductive biology.

Four pregnant female Degus at 86 days of gestation, and their placentas were processed for histological analysis at electron microscopy levels. Our results demonstrate that at the pregnancy term, the placental barrier shows zone with presence of syncytial knots (defined as clusters of syncytiotrophoblast nuclei) in the fine syncytium or syncytiotrophoblast, zone with apoptotic knots evidenced by the accumulation of fragmented nuclei or apoptotic bodies with condensed chromatin, and the presence of zones with Syncytiovascular membranes (alpha zone). These Syncytiovascular membranes facilitate the exchange of metabolites between mother and fetus, and are exclusively observed in thin placental barrier zones where the syncytiotrophoblast nuclei are excluded. The presence of these Syncytiovascular membranes allowed us to conclude that they were formed as a consequence of the deportation of apoptotic bodies to the maternal blood, such as occurs in chinchilla, other caviomorph rodent. On the other hand, in human placental barrier several investigators have found that the syncytial apoptotic cascade is complete when apoptotic nuclei are deported to the maternal blood circulation and subsequently removed in the mother´s lung. Therefore, we concluded that the degu and human placentas share a number of structural and functional characteristics and this fact allows us to consider the degu as a potential animal model for studies related to human placental pathologies.

Introduction

Octodon Degus, commonly known as 'degu', is an endemic Chilean caviomorph rodent [1] whose habitat is principally located in the northern and central regions of the country. Adult individuals are similar to a rat in shape and size; the gestational period extends for 90 days and the average litter is composed of 5 offspring. Characteristically, as in all caviomorph rodents, the degu has a long gestational period of 90 days, presents interstitial implantation, inversion of the yolk sac, a discoid, pedunculated chorioallantoic placenta, along with a Sub placenta (SP) [2,3].

In rodent species, the transfer of substances and metabolites between the maternal and fetal bloodstreams occurs through two different types of placenta, the chorioallantoic and the inverted yolk sac placentas [4,5]. In particular, the degu's chorioallantoic placenta has been the subject of a number of studies that have demonstrated its hemomonochorial and labyrinthine morphological structure, as it is also the case in the guinea pig [5,6]. Specifically, the degu's chorioallantoic placenta is constituted by two lobes separated by the SP, from which a population of cells are the equivalent to the human Extra Villous Trophoblast (EVT). The latter cells migrate into the deciduas in order to remodel the mesometrial arteries of the endometrial, in a similar way to what occurs during human placentation. Based on these facts, some authors have defined the degu's SP as an organ with a similar role to the human anchoring chorionic villi [7,8].

Beneath the degus's SP, two types of labyrinthine trophoblast are observed: the labyrinthine interlobular trophoblast or Coarse Syncytium (CS) and the labyrinthine Fine Syncytium (FS). The CS is bathed only by maternal blood. The FS is the true placental barrier of the degu and, as such, establishes the first relationship between the embryonic and maternal blood flows [6].

In order to obtain a more detailed description of the organization of the degu's placental barrier, the aim of this study was to analyze the morphological organization of this barrier at ultra structural level, as part of a wider effort to understand their reproductive biology [9].

Materials and Methods

A colony of 4 adult female O. Degus weighing in average 195 ± 5 g, inbred at the Animal Facility of the Department of Anatomy and Developmental Biology was used in this study. The animals received food and water ad libitum and the days of gestation were determined using timed

mating. The handling of the subjects was carried out according to internationally accepted ethical rules, after approval by the Animal Care Committee of the Faculty of Medicine of the University of Chile.

Four animals were culled near the gestation term (at day 86). They were sacrificed by an overdose of sodium pento-barbital (80 mg/kg i.p), and the abdomen was surgically opened and both uterine horns were exposed and opened. The placentas were then removed and cut into small blocks and fixed by immersion in 3.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, at 4°C for 3 h. For the Transmission Electron Microscopy (TEM) study, the samples were subsequently washed in the same buffer and post-fixed for 1 h in 2% osmium tetroxide prepared in the same buffer. The samples were then dehydrated in ascending grades of ethanol, cleared in propylene oxide and embedded in Araldite 502. For orientation purposes, semi-thin sections were collected and stained using 1% toluidine blue in 1% sodium tetraborate and examined under light microscopy. Ultra-thin sections were obtained using a Reichert OM-U2 ultra microtome, contrasted with uranyl acetate and lead citrate [8], and examined and photographed in a JEOL/JEM 100 SX electron microscope.

Results

At TEM level, the presence of a considerable number of clusters of syncytiotrophoblast nuclei, similar to the syncytial knots of human placenta [11] was observed in the FS or placental barrier (Figure 1A). These nuclei, which also showed pronounced indentations, have the appearance of nuclei in initial stages of the apoptosis process [10,11]. In other zone of the placental barrier, the nuclei appear finally packaged into apoptotic bodies, which were clearly observed (Figure 1B).

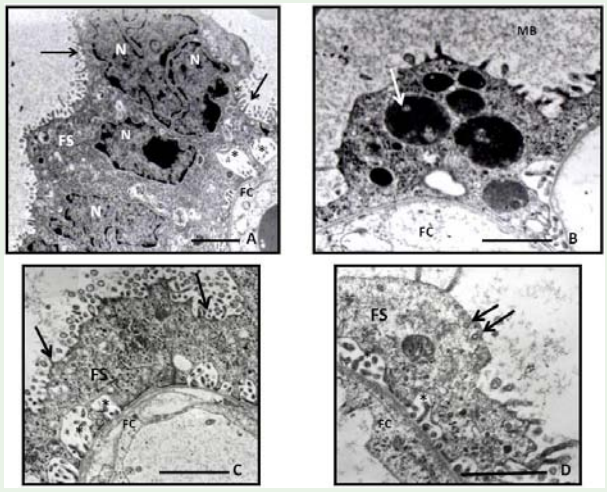

Figure 1: Transmission electron micrographs at day 86 (term) of the degu's gestation showing the placental barrier of the chorioallantoic placenta. **A)** Placental barrier formed by the syncytiotrophoblast or Fine Syncytium (FS), presenting microvilli in the apical zone (arrows) and basal infoldings (asterisks). A double basal laminae separate FS from a Fetal Capillary (FC). In the FS it is also possible to observe cluster of syncytial Nuclei (N), similar to the syncytial knots of human placenta. The nuclei showing very little heterochromatin with the exception of a Nucleolus. **B)** Syncytial knot evidencing apoptotic process in the fragmented nuclei at different stage of degeneration (arrow) displaying highly condensed chromatin. Note the Maternal Blood space (MB) and the endothelium of the fetal capillary (FC). **C)** Syncytial vascular membranes in the FS showing microvillus in the apical zone (arrows) and basal in folding (asterisks), the endothelium of the fetal capillary and the basal lamina. **D)** Syncytial vascular membranes in the FS showing small pinocytotic vesicles at the apical zone (arrows) of this syncytium, basal in folding (asterisk) and endothelium of the fetal capillary. Calibration bars: A, B, C and D: 2 µm.

This apoptotic bodies are similar to that observed by Smith et al. [12] in the ST of the human placenta. Furthermore, these nuclear apoptotic bodies are defined in the human placenta as syncytial knots and represents the final steps of the apoptosis of the syncytiotrophoblast [13,14] previous to its deportation to the maternal blood, an event that has also been described in the chinchilla [15,16]. The deportation of these apoptotic bodies allows the thinning of the placental barrier and thus favors the metabolic exchange [17] (Figures 1C and 1D).

Discussion

This work, by the first time, demonstrate the formation of Syncytiovascular membrane, in the degu´s term placental barrier, as the initial presence of syncytial knots followed by the formation of apoptotic bodies (Figure 1).

In the human placenta, the apoptotic syncytium is carried out by activation of the effectors Caspase 3 [18,19]. The activation of this cytosolic enzyme is related to the degradation of DNA and structural proteins. In this context, the pro-apoptotic caspase 3 produce the cleavage of cytokeratin 18, a thin filament of syncytium cytoskeleton [20] whose function is in part to support the nuclear position. Thus, in human the cleavage of Cytokeratin 18 (CK 18) allows the accumulation of nuclei to form the syncytial knots (Figure 1A).

In the apoptosis cascade the nuclei became fragmented, condensed and packaged within cell membranes into dense apoptotic bodies, as those observed in Figure 1B [21]. In human placenta, as the apoptosis continue the apoptotic bodies are finally deported to the maternal blood to be eliminated by the macrophage cells in the mother´s lungs [22]. In addition, several investigators have found that human placental syncytial knots undergo apoptosis before entering the maternal circulation [23].

Additionally, Bosco et al., [8] demonstrated by immunohistochemistry the presence of CK in the syncytial cytoskeleton of the placental barrier of the degu´s placenta. In other hand, this presence of syncytial knots to be deported are in agreement with the studies carried out by Billington and Weir [15], who observed the presence of syncytial knots in the lung of pregnant chinchillas. The epithelial syncytial apoptotic pathway in human [12,23], correspond to a normal process that increases towards the end of the gestation, being exacerbated in some cases of human pregnancy complications such as preeclampsia [12,23-26].

In mature tissue undergoing proliferation, cells must die or be lost in some proportions in order to maintain tissue homeostasis. Consequently, proliferation and programmed cell death or apoptosis are tightly regulated processes [21]. Apoptosis is also a normal constituent of trophoblast turnover and the release of apoptotic material does not lead to an inflammatory response in the mother [19]. It is important to emphasize that in human, the syncytial apoptotic knots released to the blood chamber travel through the endometrial veins until they reach the maternal lungs, where they are eliminated by the pulmonary macrophages, a process observed by the German anatomist and pathologist Georg Schmorl [27] as

early as the year 1893 in a woman deceased by PE. This fact has been reinforced by Ilha et al. [16] and recently by Yumusak et al. [28] who found a number of female chinchillas raised at a breeding farm that died as result of respiratory distress due to trophoblastic embolism causing fatal pneumonia, as a result of the abnormal proliferation of the trophoblastic epithelium. Embolism occurs secondarily in women, as a result of the abnormal trophoblastic proliferation of the placenta induced by stress and hypoxia, as well as a result of multiple abnormal pregnancies [29]. These facts allow us to state that syncytial knots occur in women [11,12,23], chinchillas [15,16], rats [30] and in degus (actual study) and its deportation to maternal blood favors the constitution of syncytial vascular membranes.

Conclusion

On the basis of the present study, added to other evidences reported in previous investigations [5,10] we concluded that the degu's placenta appears to be a very appropriate model for the study of placental growth processes and pregnancy pathologies present in human pregnancies.

Acknowledgmets

This research was partly supported by the Departament Técnico de Investigación y Desarrollo (DTI), Universidad de Chile, grants (EDID) 99-009, and was approved by the Bioethics Committee of the Faculty of Medicine. We gratefully acknowledge the technical ultra structural assistance of CESAT. Finally, the authors wish to thank Miss Paulina González Díaz for her kind assistance with language editing.

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