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Placentation in the degu (*Octodon degus*): Analogies with extrasubplacental trophoblast and human extravillous trophoblast☆

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Abstract

This study examined the placentation in the degu, the origin of the extrasubplacental trophoblast (EST) (extravillous trophoblast in human), and the activity of Na^+/K^+ ATPase in the placental barrier during different gestational ages, as part of a wider effort to understand the reproductive biology of this species. Fifteen degus at the first stage of gestation, midgestation and at term of pregnancy were studied. At day 27 of gestation, the subplacenta is formed under the wall of the central excavation. Simultaneously, the outermost trophoblast of the ectoplacental cone differentiated into secondary trophoblast giant cells that lie on the outside of the placenta, forming an interface with the maternal cells in the decidua. These giant cells immunostained positive for cytokeratin (CK) and placental lactogen (hPL) until term. During this period, the EST merged from the subplacenta to the decidua and immunostained negative for CK, but at term, immunostained for CK and hPL in the maternal vessels. The vascular mesenchyme of the central excavation invaded the chorioallantoic placenta during this period, forming two fetal lobules of labyrinthine-fine syncytium, the zone of the placental barrier. The activity of Na^+/K^+ ATPase in the placental barrier was constant during the gestational period. The residual syncytium at the periphery of the placental disc and between the lobules was not invaded by fetal mesenchyme and formed the marginal and interlobular labyrinthine syncytium that immunostained first for CK, and later for hPL, as in the labyrinthine fine syncytium. The presence of intracytoplasmic electron-dense material in the interlobular labyrinthine syncytium suggested a secretory process in these cells that are bathed in maternal blood. Placentas obtained from vaginal births presented a large, single lobe, absence of the subplacenta, and a reduced interlobular labyrinthine syncytium. At day 27, the inverted visceral yolk sac is observed and its columnar epithelium immunostained for CK and hPL. This suggests that the yolk sac is an early secretory organ. The epithelium of the parietal yolk sac covers the placenta. The origin of the EST in the degu placenta and its migration to maternal vessels allows us to present this animal model for the study of pregnancy pathologies related to alterations in the migration of the extravillous trophoblast.

Keywords: *Octodon degus*; Hystricomorph rodent; Hemomonochorial placenta; Extravillous trophoblast; Syncytiotrophoblast; Cytokeratin; Placental lactogen; Subplacenta; Visceral yolk-sac placenta

1. Introduction

Octodon degus is a precocious, moderately sized, but slowly maturing hystricomorph rodent from central Chile (Lee, 2004). This animal has become increasingly popular as a research animal, especially as a model for diabetic cataracts due to its tendency to become hyperglycemic (Wright and Kern, 1992) and present islet amyloidosis (Nishi and Steiner, 1990).

In contrast, we have demonstrated that the degu is more sensible than the rat to the toxic effects of organophosphorous pesticides (Bosco et al., 1999). Degus exposed to low, chronic

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doses of malathion presented renal and morphological alterations (Bosco et al., 1997) which were not observed in rats exposed to malathion at doses that were three times higher (Desi et al., 1976). This response was related to the high activity of the hepatic oxidative enzymes in the microsomes (Letelier et al., 1985), the organelles in which maloxon, the toxic component of malathion, is generated.

The transfer of nutrients from the maternal organism to the fetus is mediated by the placenta, an organ which has undergone intense investigation for many years. In many species, including hystricomorph rodents, two types of placentas exist, the chorioallantoic placenta and the inverted yolk-sac placenta; the latter is always complementary to the definitive chorioallantoic placenta (Wimsatt, 1962).

In this aspect, the chorioallantoic placenta of the degu has been the subject of many studies which have concluded that its structure is hemomonochorial and labyrinthine as in the guinea pig (King and Enders, 1970).

The degu, as all hystricomorphs, has a long gestational period (90 days), presents interstitial implantation, inversion of the yolk sac, and a discoid, pedunculated placenta along with a subplacenta (Roberts, 1971; Mess, 2003). In addition, they give birth to offspring with open eyes, a fur coat, and complete dentition, which is the same as in the guinea pig (Rojas et al., 1982).

Intrauterine growth restriction (IURG) is the second most important cause of perinatal and infant mortality and morbidity. The pathophysiological mechanisms underlying IURG are not well established. Impaired nutrient and oxygen supply to the developing fetus may be due to compromised uteroplacental circulation and/or placental transport. Many transport processes across the microvillous membrane syncytiotrophoblast (MVM) are Na^+-coupled and consequently depend on a low intrasyncytial Na^+ concentration. In this respect, Johansson et al. (2003) demonstrated that Na^+/K^+ ATPase activity was reduced in the MVM of placentas from IURG pregnancies and Bosco (2005) showed that pregnant degus exposed to malathion increased the activity of placental Na^+/K^+ ATPase, but not the fetal weight.

With regards to human pathologies during pregnancy, preeclampsia has one of the highest incidences, and has been reproduced in the guinea pig (Golden et al., 1980). This pathology has been related with alterations in the invasion of the extravillous trophoblast in the uterine arteries during the first half of gestation, a phenomenon that has been described by Brosens et al. (1972). Since then, endovascular trophoblast invasion has been one of the major foci of placental research (Kaufmann et al., 2000; McMaster et al., 2004). The molecular mechanisms that regulate trophoblast invasion and uteroplacental artery remodelling are still controversial. In addition, guinea pig uteroplacental arteries begin to dilate when interstitial trophoblast expressing endothelial nitric oxide synthase (eNOS) approaches, and the already dilated arteries are subsequently invaded by trophoblast (Nanaev et al., 1995).

Since the implantation in the degu is interstitial, as in humans and guinea pigs (Nanaev et al., 1995), placentation in the degu is a potential model for studies of trophoblast invasion. Moreover, it is an animal with a greater number of offspring per litter, thus increasing the chances of obtaining a greater number of placentas per animal.

The aim of this study was to describe the placentation of this rodent as part of a wider effort to understand their reproductive biology. This will allow resolve difficulties arising from carrying out controversial studies in human tissue and animals that result in methodological restrictions that presently limit investigations (Enders and Carter, 2004). Furthermore, the accurate characterization of these structures is key in presenting the findings of functional studies in a comparative context (Carter et al., 1998).

2. Materials and methods

We used a colony of 15 adult female *O. degus* weighing 195 ± 12 g which were inbred in our Department of Experimental Anatomy and Biology. Placentae were obtained from 15 pregnant degus. The animals received food and water ad libitum and the days of gestation were determined using fetal size and weight or timed matings. Implantation sites were easily identifiable as swellings in the uterine horn. Three animals were in the early gestational period (days 12, 14 and 17), two near 1/3 of gestation (day 27), three at 1/2 of gestation (day 45), three at 2/3 of gestation (day 60), three near term (day 87) and one at vaginalis partum. The handling of the degus was carried out according to internationally accepted ethical rules.

The animals were initially anesthetized with ether (Merck, Darmstadt, Germany) and subsequently sacrificed using an overdose of sodium pentobarbital (80 mg/kg i.p). The abdomen was opened and both uterine horns were exposed. The uterine swelling in the early stages was fixed intact. In the later stage (60–87 days) they were opened and the placentae removed. It was difficult to obtain the term vaginal placentas without anaesthesia due to the fact that the mother rapidly ingests the placenta; of the 8 offspring that were born, we were only able to obtain 3 placentas. For histological studies, the placentae were cut in half and the tissue was fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 h, embedded in paraffin wax and cut into 5 μm sections. Standard immunoperoxidase techniques were used to show: Cytokeratin (CK) and placental Lactogen (hPL) in order to observe their distribution in the tissue sections during the different days of gestation. Mouse anti-human CK monoclonal antibody, diluted 1:50 (v/v) (M3515 DAKO) or rabbit anti-human hPL polyclonal antibody, ready-to-use (N1548 DAKO) was applied individually to each section for 30 min at 37 °C. Immunostaining was performed using a horseradish peroxidase-labelled streptavidin biotin kit (DAKO) following the manufacturer's directions using diaminobenzidine as the chromogen. Sections were counterstained with Mayer's haematoxylin (DAKO) and mounted with Entellan (Merck). Immunohistochemical controls were done by replacing the primary antibodies with phosphate buffered saline. All controls were negative. All sections were examined by light microscopy (Zeiss Axioplan 2).

Routine histological analysis was performed in the 5 μm sections stained with haematoxylin-eosin.