Immunohistochemical Identification of the Extravillous Trophoblast During the Placentation of the Degu (*Octodon degus*)

CLEOFINA BOSCO* AND CHOSICA BUFFET
Programa de Anatomía y Biología del Desarrollo, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile

ABSTRACT Recent data indicate that placentation in *Octodon degus* is similar to that in humans, making it a potential animal model for studies in human placental pathologies related to alterations in the migration of the extravillous trophoblast (EVT). Our objective was to immunohistochemically identify degu EVT during placentation by using cytoskeletal protein markers to establish the normal migratory pattern of the EVT. Fifteen *O.degus* were divided into three equal groups: day 27, 60, and 84 of gestation. The placentas were immunostained for cytokeratin (CK) and α smooth muscle actin (SMA). At day 27, the migrating EVT immunostained for SMA but not for CK. Once the EVT was incorporated in the maternal vessels (day 60) it was positive for CK but negative for SMA. The smooth muscle cells of the mesometrial arteries that remained after EVT invasion were positive for SMA. At day 84, the media muscular layer had partially regenerated but some EVT was still present. Furthermore, at day 27 cyclooxygenase-1 (COX-1) was detected in the endothelium of the maternal decidual vessels. Our results suggest that during the early stages of placentation, the cytoskeletal organization of the actin network of the migrating EVT corresponds to that of a cell with motile behavior. Once the EVT invaded the spiral arteries, the cytoskeleton reorganized, adopting the structure of an epithelial-like cell, expressing CK intermediate filaments. The media muscle layer regenerated near the end of gestation but some EVT remained. During EVT formation the endothelium of the maternal decidual vessels immunostained for COX-1. *J.*

The *Octodon degu*, commonly known as the degu, is a South American hystricognath rodent. It is similar to the rat in size and shape, the average litter is five, and the gestational period 90 days. The degu adapts easily and rapidly to laboratory conditions and has been used as an experimental animal in a variety of studies in reproduction, toxicology, diabetes, cataract development, and Alzheimer's (King, '92; Bosco, '97; Bosco et al., '97; Kertschanska et al., '97; Lee, 2004; Bosco, 2005; Inestrosa et al., 2005).

The degu shares characteristics common to all hystricognath rodents, including a long gestational period (Roberts, '71), interstitial implantation (Rojas et al., '82; Bosco et al., 2007), inversion of the yolk sac, and a discoid, pedunculated placenta with a subplacenta (Roberts, '71; Bosco, '97; Mess, 2003; Rodrigues et al., 2006).

As in other hystricognath rodent species, the degu presents two types of placentas: the chorioallantoic placenta and the inverted yolk sac placenta (Bosco, '97; Mess, 2003; Bonatelli et al., 2005; Bosco et al., 2007). The chorioallantoic placenta has a placental barrier that is hemomonochorial and labyrinthine in structure with continuous capillaries, which is also observed in the placenta of the closely related guinea pig (King

Grant sponsor: Departamento Técnico de Investigación y Desarrollo (DTI), Universidad de Chile; Grant number: EDID 99-009.
*Correspondence to: Dr Cleofina Bosco, Laboratorio de Placenta y Reproducción, ICBM, Programa de Anatomía y Biología del Desarrollo, Facultad de Medicina, Universidad de Chile, Independencia 1027, Casilla 70079, Santiago 7, Chile. E-mail: cbosco@med.uchile.cl

and Enders,'70; King, '92; Bosco, '97; Kertschanska et al., '97; Bosco et al., 2007; Mess, 2007a,b).

The placenta's principal function is to promote fetal growth and viability by providing an adequate nutrient supply from the mother to the fetus and allowing the removal of fetal toxic wastes. This exchange occurs across a large surface area composed of tissues that are located between fetal and maternal blood.

During the early stages of human pregnancy, the formation of the placental barrier and the conversion of the maternal spiral arteries into larger, more competent vessels (Bosco, 2005) are crucial for normal placentation. The remodeling of these vessels increases the volume of maternal blood available for exchange between both circulatory systems (Kaufmann et al., 2003; Bosco, 2005).

The extravillous trophoblast (EVT) consists of placental epithelial cytotrophoblastic cells that remodel the vessels during placentation. In humans, these cells originate from the anchoring chorionic villi of the placenta and subsequently migrate to the *decidual basalis* to invade the maternal spiral arteries. Recent data indicate that degu placentation is similar to that in humans (Bosco et al., 2007; Mess et al., 2007). In this hystricognath rodent, the EVT emerges from the subplacenta, a structure that is analogous to the anchoring chorionic villi in humans (Bosco et al., 2007; Mess et al., 2007).

Pre-eclampsia (PE), the leading cause of maternal death during human gestation, is a disease characterized by maternal hypertension, proteinuria, and edema (Goldman-Wohl and Yagel, 2002). PE affects 7–10% of pregnancies and was first described by Brosens et al. ('72). Although its etiology remains largely unknown, PE causes placental hypoxia, which leads to maternal endothelial injury (Kaufmann et al., 2003).

Studies have shown that PE is characterized by inadequate EVT invasion resulting in subsequent failure of spiral artery remodeling. From the time Brosens first described PE, EVT has become a major foci of placental research (Myatt, 2002; Kaufmann et al., 2003; Naicker et al., 2003; McMaster et al., 2004; Lyall, 2005; Soleymanlou et al., 2005; Harris et al., 2007). Nevertheless, in spite of these studies, the molecular mechanisms that regulate trophoblast invasion and uteroplacental artery remodelling remain controversial.

PE has been reproduced in the guinea pig, another hystricognath rodent (Golden et al., '80; Hees et al., '87; Verkeste et al., '98) whose EVT behavior, as in the degu, is similar to human placentation (Mess et al., 2007). Furthermore, Nanaev et al. ('95) have demonstrated in the guinea pig that the uteroplacental arteries begin to dilate when the interstitial trophoblast expresses endothelial nitric oxide synthase . Once dilated, the arteries are subsequently invaded by the EVT.

As human EVT is not available for in vivo studies owing to ethical reasons, adequate animal models must be obtained to study the cellular expression and activity of the EVT during placentation.

It is important to emphasize that the ability of eukaryotic cells to adopt a variety of shapes and carry out coordinated movements depends on the cytoskeleton, a complex network of protein filaments that extends through the cytoplasm. The cytoskeleton consists of three types of protein filaments: actin filaments, microtubules, and intermediate filaments (cytokeratin, CK) (Alberts et al., '94).

To further our understanding of the placentation process, we hypothesize that the EVT cytoskeleton undergoes a series of changes during placentation. To test this hypothesis, we immunohistochemically identified degu EVT during the early stages of placentation, using cytoskeletal markers present in moving cells, which will enable us to establish the normal migratory pattern of the EVT.

MATERIALS AND METHODS

We used a colony of 15 adult female *O. degus* weighing 190 ± 8 g, which were inbred in our Department of Anatomy and Developmental Biology. The animals received food and water ad libitum and the days of gestation were determined using timed matings. Implantation sites were easily identified as swellings in the uterine horn. Five animals were near one-third of gestation (day 27), five at mid-gestation (day 60), and five near term (day 84). The handling of the degus was carried out according to internationally accepted ethical rules after approval by the institutional animal care Committee of the Faculty of Medicine of the University of Chile.

The animals were anesthetized with ether (Merck, Darmstadt, Germany) and were subsequently sacrificed using an overdose of sodium pentobarbital (80 mg/kg i.p). The abdomen was opened and both uterine horns were exposed. For day 27 specimens, the uterine swelling was fixed intact, and for days 60 and 84, the placentas were

extracted and fixed intact in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 hr, embedded in paraffin wax, and made into 5 μm sections. Standard immunoperoxidase techniques were used to show CK and smooth muscle actin (SMA) in all three groups and cyclooxygenase-1 (COX-1) in the first group only (day 27), to observe their distribution in the tissue sections. Mouse anti-human CK monoclonal antibody, diluted 1:50 (v/v) (M3515 DAKO, USA); mouse anti-human α SMA monoclonal antibody (diluted 1:50 v/v M0851 DAKO, USA), and mouse anti-human COX-1 monoclonal antibody diluted 1:100 (v/v) (12E 12 Novocastra, UK) were applied individually to each section for 30 min at 37°C. Microwave heat-induced antigen retrieval in citrate buffer, pH 6.0, was required for optimal staining with α anti-human SMA antibody.

Immunostaining was performed using a horseradish peroxidase-labeled streptavidin biotin kit (DAKO) following the manufacturer's directions and using diaminobenzidine as the chromogen. The sections were counterstained with Mayer's hematoxylin (DAKO) and mounted with Entellan (Merck). Immunohistochemical negative controls were done by replacing the primary antibodies with phosphate-buffered saline. All sections were examined by light microscopy (Zeiss Axioplan 2, Germany).

Routine histological analysis was performed on the 5 μm sections stained with hematoxylin–eosin.

RESULTS

The degu placentation was studied from day 27 of gestation, which corresponds to the period during which the subplacenta has formed (Bosco et al., 2007). We observed invasive spherical sprouts of trophoblast emerging from the lateral zone of the subplacenta (Fig. 1A) and migrating to the maternal vessels. These cells immunostained for α actin (Fig. 1A) but not for CK (Bosco et al., 2007). Figure 1B shows the negative control for α actin. The EVT in the mesometrial artery walls is evident during mid-gestation (60 days) and near term of gestation (84 days). The EVT immunostains negatively for α actin but positively for CK (Fig. 1C and E). In addition, the remaining degu smooth muscle cells in the inner layer of the muscular layer (60 days) that were not destroyed during the trophoblast invasion were positive for α actin, (Fig. 1D). By the end of gestation (day 84), the inner media smooth muscle cells regenerated almost completely, however, some EVT was still present and immunostained CK positive. (Fig. 1E). The endothelium of the blood vessels of the maternal *decidua* immunostained positively for COX-1 (Fig. 1F) during the early gestational period (27 days).

DISCUSSION

It is difficult to find animal models with migrating EVT invasion that is comparable to the human pattern (Enders and Carter, 2004; Carter, 2007). Nevertheless, it has been recently proposed that degu placentation would be an adequate animal model for the study of numerous human pathologies such as PE (Bosco et al., 2007; Mess et al., 2007).

PE is characterized by incomplete remodelling of the uterine spiral arteries by the EVT during the early gestational period (Kaufmann et al., 2003). Human EVT is not available for in vivo studies, thus, it is essential that an adequate animal model be obtained to study the mechanisms leading to abnormal placentation.

The results of our study suggest that during the early stages of gestation in the degu, the organization of the actin filament cytoskeletal network of the EVT is compatible with a cell with motile capacity (Fig. 1A). However, at mid-gestation (day 60), the EVT that forms the wall of the maternal vessels, reorganizes its cytoskeletal network, adopting the structure of epithelial-like cells (Fig. 1C). These cells are CK positive and will remain so until the end of gestation (Fig. 1E). These epithelial-like cells also begin to secrete hormones (Bosco et al., 2007).

The shape and function of animal cells is largely determined by the organization of their internal structural elements, including the filamentous structures of their cytoskeleton. Motile cells must assemble their cytoskeletal actin filaments in a spatially organized way, such that net filament growth and cell protrusion occur at the front of the cell. Actin filament dynamics, in turn, influence the overall shape and behavior (Alberts et al., '94; Lacayo et al., 2007). This correlates with the study of Mess et al. (2007), which has demonstrated that EVT migration routes and kinetics under in vivo conditions in the degu and guinea pig are 300–350 μm/day. CK is an intermediate cytoplasmic filament found in epithelial cells, whose function is to resist the mechanical stress; this could explain its presence in the walls of the mesometrial arteries (Fig. 1C). Degu EVT immunostained negative for CK at day 27 (Bosco et al., 2007), a result that was also described by Carter et al., '98 in the guinea pig.

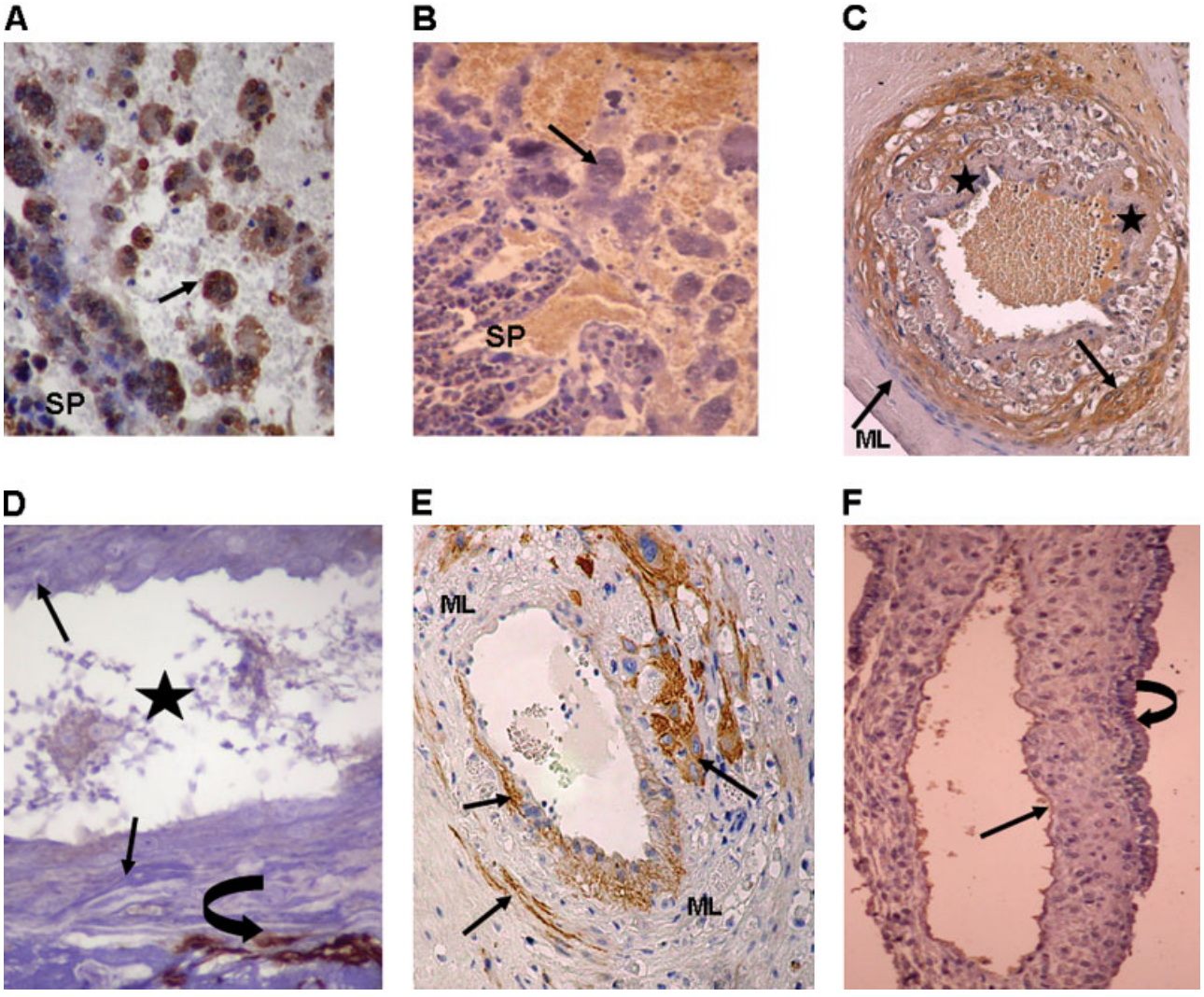

Fig. 1. (**A**) The spherical sprouts of EVT (arrow) that emerge from the degu subplacenta (SP) and migrate to the maternal endometrial degu vessels at day 27 of gestation, immunostain positive for α actin. 20 ×. (**B**) Negative control of EVT for α actin at day 27 of gestation. EVT (arrow). SP. 20 ×. (**C**) The EVT that infiltrates the wall of a maternal degu mesometrial artery immunostains positive for CK at day 60 of gestation (arrows). Note the destruction of the musculo-elastic structures of the vessel (stars) by the invading trophoblast. Some cells of the media smooth muscular layer (ML) remain under the EVT layer 20 ×. (**D**) Some smooth muscle cells of the inner media of a maternal degu mesometrial artery are not destroyed by trophoblast invasion at day 60 of gestation. These cells are positive for α actin (curved arrow) but not for EVT (arrows). The star shows the lumen of the artery. 40 ×. (**E**) The EVT in the mesometrial artery wall immunostains positive for CK at term of gestation (84 days), (arrows). Note the diminished EVT during this gestational period and the regenerated muscular layer of the vessel (ML). 20 ×. (**F**) Longitudinal section of dilated, medium-sized, maternal vessel in the decidua at day 27 of gestation. The endothelium immunostained positive for COX-1 (right arrow). Endometrial epithelium in the lumen of the uterus (curved arrow). Note the separation between the smooth muscle cells of the vessel wall. 20 ×.

However, our results differ from those of Rodrigues et al. (2006), whose study in the hystricomorph rodent red-rumped agouti showed that migrating EVT immunostained CK positive at mid-gestation. We suggest that the red-rumped agouti CK positive EVT at mid-gestation, represents EVT that did not reach the spiral arteries in time for arterial remodeling.

Figure 1C shows the structural changes undergone in the uteroplacental arteries during mid-gestation once the EVT has invaded vessels, inducing arterial dilatation. We observed trophoblastic replacement of the endothelium, destruction of the internal elastic limiting membrane, and partial destruction of the muscular media layer. Some of the inner smooth muscle cells in the

media layer were not destroyed during EVT invasion (Fig. 1D) and may contribute to the regeneration of this layer near term (Fig. 1E) as is seen in the guinea pig (Nanaev et al., 2000). A decrease in the EVT is observed in the vessels toward the end of gestation (Fig. 1E).

It has been demonstrated that during early pregnancy, placentation occurs in a relatively hypoxic environment (James et al., 2006). This low oxygen environment is essential for normal embryonic and placental development as the early conceptus has little protection against oxygen-generated free radicals (Caniggia et al., 2000). Genbacev et al. ('96, '97) have provided in vitro evidence to support the role of low oxygen tension in maintaining the cytotrophoblast in a proliferative, noninvasive, and immature phenotype that only differentiates to syncytium in the placental barrier. Furthermore, Caniggia et al. (2000) have demonstrated in in vitro human villous placental explants of 5–8 weeks of gestation that the expression of hypoxia-inducible factor-1 (HIF-1), a master regulator of oxygen homeostasis, and TGFβ_3, an inhibitor of EVT differentiation, are elevated in early human pregnancy. The expression of both the factors begins to fall around week 9 of gestation, a time period during which placental pO_2 levels increase, allowing the cytotrophoblast to differentiate from placental anchoring chorionic villi into invasive EVT that will deeply penetrate the maternal uterus. An earlier report (Bosco et al., 2007) demonstrated that at day 17 of degu gestation, a trilaminar embryo and a developing placenta without a subplacenta could be observed. At day 27 of gestation, the EVT differentiates from the subplacenta and begins to migrate. This process probably correlates with an increased in placental pO_2 levels, as is seen in human (Caniggia et al., 2000), but further studies are required to confirm this. Our results suggest that cell migration is facilitated by the cytoskeletal characteristics that are observed in the migrating cells (Fig. 1A). The study of Caniggia et al. (2000) is consistent with our observations, given that the immunohistochemical expression of COX-1 in the maternal decidual endothelium (Fig. 1F) could trigger the synthesis of factors associated with an increase in environmental pO_2 (Smith et al., '96; Janowiak et al., '98) as a result of the dilated lumen observed in the vessel. This requires future investigation, but it could be suggested that the trophoblast invasion is preceded by maternal endothelial activity.

In conclusion, our findings suggest that cytoskeletal dynamics, such as remodelling and reorganization, are characteristic of the EVT in the early placentation of the degu. This is the first time that the migrating EVT has been identified using its actin cytoskeleton. Once the EVT remodels the maternal vessels, its cytoskeleton changes adopting an epithelial-like cell configuration, enabling it to resist mechanical stress. Near term, the inner media smooth muscle cells regenerate the smooth muscle layer, but some EVT still remains. The expression of COX-1 in the vessels of the *decidua* should be further studied to determine if its presence is associated with the observed vasodilation, which serves to increase the placental pO_2, thus enhancing the development of the embryo and the differentiation of trophoblast into invasive EVT. In conclusion, further studies will be required to reproduce PE in this animal model and detect possible alterations in the EVT migration associated with this pathology.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Ricardo Contreras and the photographic work of Orlando Valladares.

LITERATURE CITED

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. 1994. The cytoskeleton. In: Robertson M, editor. Molecular biology of the cell, 3rd edition. New York: Garland Publishing. p 797–814.

Bonatelli M, Carter AM, Fernandez Machado MR, De Oliveira MF, Cardoso de Lima M, Miglino MA. 2005. Placentation in the paca (*Agouti paca* L). Reprod Biol Endocrinol 3:9–21.

Bosco C. 1997. Ultrastructure of the degu term placental barrier (*Octodon degus*): a labyrinthine hemomonochorial placental model. Med Sci Res 23:15–18.

Bosco C. 2005. Alcohol and xenobiotics in placenta damage. In: Preedy VR, Watson RR, editors. Comprehensive handbook of alcohol related pathology, Vol. 2. London: Elsevier Science Press. p 921–935.

Bosco C, Rodrigo R, Díaz S, Borax J. 1997. Renal effects of chronic exposure to malathion in *Octodon degus*. Comp Biochem Physiol 118C:247–253.

Bosco C, Buffet C, Bello MA, Rodrigo R, Gutierrez M, García G. 2007. Placentation in the degu (O*ctodon degus*) and analogies with extrasubplacental trophoblast and human extravillous trophoblast. Comp Biochem Physiol A Mol Integr Physiol 146:475–485.

Brosens IA, Robertson WB, Dixon HG. 1972. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu 1:177–191.

Caniggia I, Mostachfi H, Winter J, Gassmann M, Lye SJ, Kuliszewski M, Post M. 2000. Hypoxia-inducible-factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFβ_3. J Clin Invest 105:577–587.

Carter AM. 2007. Animal models of human placentation. Placenta 28:S41–S47.

Carter AM, Tanswell B, Thompson K, Han VK. 1998. Immunohistochemical identification of epithelial and mesenchymal cell types in the chorioallantoic and yolk sac placentae of the guinea-pig. Placenta 19:489–500.

Enders AC, Carter AM. 2004. What can comparative studies of placental structure tell us? A review. Placenta 25:S3–S9.

Genbacev O, Joslin R, Damsky CH, Polliotti BM, Fisher S. 1996. Hypoxia alter early gestation human cytotrophoblast differentiation/invasion in vitro and models the placenta defects that occur in preeclampsia. J Clin Invest 9:540–550.

Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. 1997. Regulation of human placental development by oxygen tension. Science 277:1669–1672.

Golden JG, Hughes HC, Lang CM. 1980. Experimental toxemia in the pregnant guinea pig (*Cavia porcellus*). Lab Anim Sci 30:174–179.

Goldman-Wohl D, Yagel S. 2002. Regulation of trophoblast invasion: from normal implantation to pre-eclampsia. Moll Cell Endocrinol 22:233–238.

Harris LK, Keogh R J, Wareing M, Baker PN, Cartwright JE, Whitley GS, Aplin JD. 2007. BeWo cells stimulate smooth muscle cell apoptosis and elastin breakdown in a model of spiral artery transformation. Hum Reprod 22:2834–2841.

Hees H, Moll W, Wrobel KH, Hees I. 1987. Pregnancy-induced structural changes and trophoblastic invasion in the segmental mesometrial arteries of the guinea pig (*Cavia porcellus L.*). Placenta 8:609–626.

Inestrosa NC, Reyes AE, Chacón MA, Cerpa W, Villalón A, Montiel J, Merabachvili G, Aldunate R, Bozinovic F, Aboitiz E. 2005. Human like rodent amyloid-beta-peptide determines Alzheimer pathology in aged wild-type *Octodon degus*. Neurobiol Aging 26:1023–1028.

James JL, Stone PR, Chamley LW. 2006. The regulation of trophoblast differentiation by oxygen in the first trimester of pregnancy. Hum Reprod Update 12:137–144.

Janowiak MA, Magness RR, Habermehl DA, Bird IM. 1998. Pregnancy increased ovine uterine artery endothelial cyclooxygenase-1 expression. Endocrinology 139:765–771.

Kaufmann P, Black S, Huppertz B. 2003. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod 69:1–7.

Kertschanska S, Schröder H, Kaufmann P. 1997. The ultrastructure of the trophoblastic layer of the degu (*Octodon degus*) placenta: a re-evaluation of the ''Channel Problem''. Placenta 18:219–225.

King B. 1992. Ultrastructural evidence for transtrophoblastic channels in the hemomonochorial placenta of the degu (*Octodon degus*). Placenta 13:35–41.

King BF, Enders A. 1970. Protein absorption and transport by the guinea pig visceral yolk sac placenta. Am J Anat 129:261–288.

Lacayo C, Pincus Z, VanDuijn M, Wilson C, Fletcher D, Gertler F, Mogilner A, Therlot J. 2007. Emergence of large-scale cell morphology and movement from local actin filament growth dynamics. PLoS Biol 5: 2035–2052.

Lee TM. 2004. *Octodon degus*: a diurnal, social, and long-lived rodent. Inst Lab Anim Res J 45:14–24.

Lyall F. 2005. Priming and remodelling of human placental bed spiral arteries during pregnancy—a review. Placenta 26:S31–S36.

McMaster MT, Zhou Y, Fisher S. 2004. Abnormal placentation and the syndrome of preeclampsia. Semin Nephrol 24:540–547.

Mess A. 2003. Evolutionary transformations of chorioallantoic placental characters rodentia with special reference to hystricognath species. J Exp Zoolog A Comp Exp Biol 299:78–98.

Mess A. 2007a. The subplacenta in *Octodon degus* and *Petromus typicus*—two hystricognath rodents without significant placental lobulation. J Exp Zoolog B Mol Dev Evol 308:172–188.

Mess A. 2007b. Development of the chorioallantoic placenta in *Octodon degus*—a model for growth processes in caviomorph rodents? J Exp Zoolog B Mol Dev Evol 308:371–383.

Mess A, Zaki N, Kadyrov M, Korr H, Kaufmann P. 2007. Caviomorph placentation as a model for trophoblast invasion. Placenta 28:1234–1238.

Myatt L. 2002. Role of placenta in preeclampsia. Endocrine 19:103–111.

Naicker T, Khedun SM, Moodley J, Pijnenborg R. 2003. Quantitative analysis of trophoblast invasion in preeclampsia. Acta Obstet Gynecol Scand 82:722–729.

Nanaev AK, Chwalisz K, Frank HG, Kohnen G, Hegele-Hartung C, Kaufmann P. 1995. Physiological dilation of uteroplacental arteries in the guinea pig depends on nitric oxide synthase activity of extravillous trophoblast. Cell Tissue Res 282:407–421.

Nanaev AK, Kosanke G, Reister F, Kemp B, Frank HG, Kaufmann P. 2000. Pregnancy-induced de-differentiation of media smooth muscle cells in uteroplacental arteries of the guinea pig is reversible after delivery. Placenta 21:306–312.

Roberts CM. 1971. The early development of some hystricomorph rodents with particular reference to *Chinchilla laniger*. J Reprod Fert 27:488–489.

Rodrigues RF, Carter AM, Ambrosio CE, Carlesso dos Santos T, Miglino MA. 2006. The subplacenta of the red-rumped agouti (*Dasyprocta leporina L*). Reprod Biol Endocrinol. 4:31–38.

Rojas M, Montenegro MA, Morales B. 1982. Embryonic development of the degu, *Octodon degus*. J Reprod Fert 66:31–38.

Smith WL, Garavito RM, Dewitt DL. 1996. Prostaglandin endoperoxide H synthase (Cyclooxygenase-1 and -2). J Biol Chem 271:33157–33160.

Soleymanlou N, Jurisica I, Nevo O, Ietta F, Zhang X, Zamudio S, Post M, Caniggia I. 2005. Molecular evidence of placental hypoxia in preeclampsia. J Clin Metab 90:4299–4308.

Verkeste CM, Slangen BF, Daemen M, van Straaten H, Kohnen G, Kaufmann P, Peeters LL. 1998. The extent of trophoblast invasion in the preplacental vasculature of the guinea-pig. Placenta 19:49–54.