Growth and development of the placenta in the capybara (Hydrochaeris hydrochaeris)

Claudia Kanashiro¹, Tatiana C Santos², Maria Angelica Miglino¹, Andrea M Mess³ and Anthony M Carter*⁴

Address: ¹Department of Surgery, School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil, ²Department of Animal Science, State University of Maringá, Paraná, Brazil, ³Department of Research, Museum of Natural History, Leibniz-Community, Berlin, Germany and ⁴Department of Physiology and Pharmacology, University of Southern Denmark, Odense, Denmark

Email: Claudia Kanashiro - claudiakanashiro@uol.com.br; Tatiana C Santos - tcsantos@uem.br; Maria Angelica Miglino - miglino@usp.br; Andrea M Mess - andrea.mess@mfn-berlin.de; Anthony M Carter* - acarter@health.sdu.dk

* Corresponding author

Abstract

Background: The guinea pig is an attractive model for human pregnancy and placentation, mainly because of its haemomonochorial placental type, but is rather small in size. Therefore, to better understand the impact of body mass, we studied placental development in the capybara which has a body mass around 50 kg and a gestation period of around 150 days. We paid attention to the development of the lobulated arrangement of the placenta, the growth of the labyrinth in the course of gestation, the differentiation of the subplacenta, and the pattern of invasion by extraplacental trophoblast.

Methods: Material was collected from six animals at pregnancy stages ranging from the late limb bud stage to mid gestation. Methods included latex casts, standard histology, immunohistochemistry for cytokeratin, vimentin, alpha-smooth muscle actin, and proliferating cell nuclear antigen as well as transmission electron microscopy.

Results: At the limb bud stage, the placenta was a pad of trophoblast covered by a layer of mesoderm from which fetal vessels were beginning to penetrate at folds in the surface. By 70 days, the placenta comprised areas of labyrinth (lobes) separated by interlobular areas. Placental growth resulted predominantly from proliferation of cellular trophoblast situated in nests at the fetal side of the placenta and along internally directed projections on fetal mesenchyme. Additional proliferation was demonstrated for cellular trophoblast within the labyrinth.

Already at the limb bud stage, there was a prominent subplacenta comprising cellular and syncytiotrophoblast with mesenchyme and associated blood vessels. At 90 days, differentiation was complete and similar to that seen in other hystricognath rodents. Overlap of fetal vessels and maternal blood lacunae was confirmed by latex injection of the vessels. At all stages extraplacental trophoblast was associated with the maternal arterial supply and consisted of cellular trophoblast and syncytial streamers derived from the subplacenta.

Conclusion: All important characteristics of placental development and organization in the capybara resembled those found in smaller hystricognath rodents including the guinea pig. These features apparently do not dependent on body size. Clearly, placentation in hystricognaths adheres to an extraordinarily stable pattern suggesting they can be used interchangeably as models of human placenta.
Background

Rodents are useful models for human reproduction due to the ready availability of laboratory animals [1] and their closeness to the primate lineage [2-4]. Although four suborders are recognized, most species used in research are myomorph rodents [1]. A notable exception is the guinea-pig, which is a hystricognath rodent from the suborder Hystricomorpha [5]. The hystricognath rodents have adopted a reproductive strategy characterized by a relatively long gestation, small litter size and the delivery of well-developed (preocial) young [6]. This is in many respects similar to reproduction in higher primates [7]. For this reason among others [1,8], they offer more satisfactory models for human pregnancy than rodents that have short pregnancies and deliver large litters of poorly developed (altricial) young. As an example, events occurring during later stages of pregnancy in humans must be studied postnatally in rats and mice, introducing a wealth of confounding factors. There are several similarities in placentation between hystricognaths and higher primates including a single layer of syncytiotrophoblast in contact with the maternal blood space (i.e. haemomonochorial) as opposed to three trophoblast layers (i.e. haemotrichorial) in myomorph rodents. There are as well similar patterns of trophoblast invasion and placental growth [1,9-13].

Current concepts of palaeogeography favour an African origin for hystricognaths with dispersal to South America by a trans-Atlantic route in the Eocene or Oligocene [14]. The subsequent radiation resulted in the wide range of forms found in South America today [15,16]. The semi-aquatic capybara (Hydrochaeris hydrochaeris) is by far the largest extant species of rodent. Like other hystricognaths, it delivers precocial neonates after a relatively long gestation period [17,18].

Although the guinea pig is an attractive model for human pregnancy, the question arises whether it is possible to compare such a small animal with the condition in humans. To better understand this we have studied placental development in the capybara, which more closely approximates human dimensions with a maternal body mass around 50 kg, a delivery weight of around 1 kg and a gestation period of around 150 days [18]. The main aim of the study is to substantiate if the principle processes of placentation depend on body size or not. Special attention was paid to the following questions: How is the lobulated arrangement of the placenta developed in the capybara? Previous studies had shown only the architecture of the term placenta [19-21]. Does the labyrinth continue to grow in the course of gestation in the same way as in smaller hystricognaths? How do the ontogenetic differentiation of the subplacenta and the associated pattern of trophoblast invasion occur? These are both specialized features of hystricognath placentation. Finally, what is the significance of these findings on placental differentiation in the capybara for the choice of smaller species as models for human placentation?

Methods

Tissue collection and fixation

The observations are based on material collected from six animals at various stages of pregnancy (Table 1). Relevant placentation characteristics of the capybara and related hystricognath species investigated so far are summed up in Tables 2 and 3[6,9-13,19-50].

Capybara material was collected at hysterectomy from animals bred at the Centre for Experimental Breeding of Capybaras, Paulista State University, Araçatuba, São Paulo, as authorized by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA). Additional material (n = 3) was obtained at an IBAMA licensed slaughterhouse (Panamby-Porã, Miracatu, S.P.). The experimental protocol was approved by the Bioethics Committee of the School of Veterinary Medicine, University of Sao Paulo.

Bilateral hysterectomy was performed in 3 capybaras. The animals were premedicated with acepromazine (Univet, São Paulo, S.P., Brazil; 0.1–1.0 mg/kg I.M.). Anaesthesia was induced with xylazine (Dorcipec®, Vallée S.A., Montes Claros, M.G., Brazil; 0.5–1.0 mg/kg) and ketamine (Cristália, Itapira, S.P., Brazil; 5–10 mg/kg I.M.) and continued with halothane (Hoechst, Frankfurt, Germany; 1 per cent) or enflurane (Étrane®, Abbott, São Paulo, S.P., Brazil) in oxygen. Postoperative treatment included antibiotic coverage with benzyl penicillin and streptomycin (Pentabiotic®, Fort Dodge, Campinas, S.P., Brazil; 8000–24 000 IU/kg I.M.) and analgesia as required with flunixin meglumine (Banamine®, Schering-Plough, Rio de Janeiro, R.J., Brazil).

Table 1: Fetal and placental size at the four stages of gestation studied

| Crown-rump length of fetus (cm) | Placental size (cm)* | Estimated gestational age (days) [18] | Number of placentas studied |
|--------------------------------|---------------------|--------------------------------------|-----------------------------|
| 1.2 (n = 1)                    | 1.5 × 1.3           | Late limb bud stage                  | 1                           |
| 5.5–5.8 (n = 2)                | 3.5 × 2.5           | 70                                   | 2                           |
| 8.0–13.0 (n = 3)               | 6.7 × 4.0           | 90                                   | 4                           |

*Greatest diameter × greatest depth (including subplacenta).
In one placenta from mid gestation the maternal and fetal vessels were injected with coloured latex (uterine artery white, uterine vein blue, umbilical artery yellow and umbilical vein red) in order to show the vessel distribution.

Tissues collected for histology and immunohistochemistry were immersion fixed in 10 per cent formalin in 0.1 M phosphate buffer, pH 7.4, for 24–48 h. After fixation they were submitted to dehydration and embedded in paraplast. Tissues for transmission electron microscopy were fixed in 2.5% glutaraldehyde or 2% paraformaldehyde/2.5% glutaraldehyde for 24 h and embedded in araldite as described below or in Spurr’s resin.

**Histology and immunohistochemistry**

The blocks were sectioned at 5 µm using an automatic microtome (Leica RM2155, Germany). Sections were stained by standard procedures with haematoxylin and eosin, Masson’s trichrome and the periodic acid-Schiff reaction (PAS).

Following an approach established by Carter at al. [51], immunohistochemistry was performed for cytokeratin to

### Table 2: List of major placental characters and conditions in Hystricognathi

| Nr. | Character                        | Character condition(s)                                      |
|-----|----------------------------------|------------------------------------------------------------|
| 1   | Organization of main placenta    | Moderate lobulation (1). Complex lobulation (2).            |
| 2   | Capsule around placenta          | Absent (1). Present until mid gestation (2).                |
| 3   | Labyrinth I                      | Radial arrangement of the exchange areas around the maternal blood lacunas (1). |
| 4   | Labyrinth II                     | Countercurrent arrangement of the fetal and maternal blood flows (1). |
| 5   | Interhaemal barrier              | Haemochorial placenta with cellular and syncytial trophoblast in early ontogeny and mostly syncytial barrier later on (1). |
| 6   | Interlobium                      | Substantial areas of interlobus related with placenta (1).  |
| 7   | Placental growth                 | Essentially the result of specialised growing zones at the outer margin, associated with internally directed projections on fetal mesenchyme (1). |
| 8   | Additional proliferation          | Insignificant proliferation activity in trophoblast in the labyrinth (1). Remarkable proliferation in this trophoblast in early and mid gestation (2). |
| 9   | Presence of subplacenta          | Distinct and specialised area, consisting of layers of cellular and syncytial trophoblast on fetal mesenchyme, occurring from early ontogeny to near term or term (1). |
| 10  | Blood supply of subplacenta      | Associated with the maternal circulation in early ontogeny and supplemented by the fetal system later, without overlap between the two systems (1). Same composition, but some overlap between the systems during mid gestation (2). |
| 11  | Extraplacental trophoblast       | Both cellular trophoblast and syncytial streamers in the decidua between the placenta and the maternal arterial system, probably responsible for the replacement of the maternal arterial endothelium (1). |
| 12  | Visceral yolk sac                | Inverted yolk sac with conspicuous villous areas (1).       |
| 13  | Fibrovascular ring               | Specialised arterial and capillary system within the yolk sac near the attachment to the main placenta (1). |
| 14  | Parietal yolk sac                | Present, usually multi-layered from mid gestation on (1).   |

### Table 3: Distribution of placental characteristics in hystricognath rodents

| Taxon                   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Principle source |
|-------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|------------------|
| Capybara (Hydrochaeris) | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | [19-21], own data |
| Guinea pig (Cavia)     | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | [6,9,12,21,27,32,33,38-41] |
| Prea (Galea)           | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | [30,31] |
| Rock cavy (Kerodon)    | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | [28], own data |
| Paca (Cuniculus)       | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | [20,21,42], own data |
| Agouti (Dasyprocta)    | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | [20,21,30,34,42], own data |
| Chinchilla (Chinchilla) | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | [26,43,44] |
| Degu (Octodon)         | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | [6,9,12,13,35-37,45,46] |
| Nutria (Myocastor)     | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | [25,47] |
| Canadian porcupine (Erethizon) | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | [24] |
| Dassie rat (Petromus)  | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | [6,9,29,35,45] |
| Canine rat (Thryonomys) | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | [6,48-50] |
| African porcupine (Hystrix) | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | [22] |
| Mole rat (Bathyergus)  | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | [22] |

The principle sources and the various character conditions are given. See Table 2 for a description of characters and character conditions. Unknown conditions are referred to by a question mark. The capybara, guinea pig, prea and rock cavy are members of the same family (Caviidae). Each of the other species represents a different family.
identify epithelial cells and trophoblasts; vimentin to identify mesenchymal cells and stromal decidua; and \(\alpha\)-smooth muscle actin to identify vessel walls. As a proliferation marker we used a mouse monoclonal antibody to human proliferating cell nuclear antigen (PCNA).

Sections were dewaxed then rehydrated in an ethanol series and in the course of this they were submitted to endogenous peroxidase blockage in 3% hydrogen peroxide (v/v) in ethanol for 20 minutes. They were then placed in 0.1 M citrate buffer, pH 6.0, and submitted to microwave irradiation at 700 MHz for fifteen minutes. The sections were equilibrated in 0.1 M phosphate-buffered saline (PBS), pH 7.4, and non-specific binding was blocked using Dako Protein Block (DakoCytomation, Carpinteria, California, USA) for 20 minutes.

Tissues were incubated with primary antibodies overnight at 4°C in a humid chamber. Cytokeratin was detected by a rabbit polyclonal antibody (1:500; PI071-UP, Biogenex, San Ramon, California, U.S.A.). Mouse monoclonal anti-human primary antibodies were used to detect vimentin (1:200; V9, sc-6260, Santa Cruz Biotechnology, Santa Cruz, California, USA), \(\alpha\)-smooth muscle actin (1:300; Clone 1A4, DakoCytomation, Carpinteria, California, USA), and PCNA (1:100; PC10, sc-56, Santa Cruz Biotechnology, Santa Cruz, California, USA). The slices were then rinsed in PBS and incubated with the biotinylated secondary antibody for 45 minutes, followed by streptavidin-HRP for 45 minutes (LSAB®+ System-HRP, DakoCytomation, Carpinteria, California, USA). After rinsing in PBS, the binding was visualized using aminobenzidine (DAB) as the chromagen. The sections were counterstained with haematoxylin and mounted in Faramount® (Fisher Scientific, Fair Lawn, New Jersey, USA). Negative controls were performed using PBS instead of primary antibody solution.

**Transmission electron microscopy**

Post fixation was in 2% phosphate-buffered osmium tetroxide, pH 7.4, for 2 h. Tissues were then washed in PBS (3 × 10 min) and immersed in a saturated uranyl acetate solution for 1 h. After washing in distilled water (3 × 10 min), they were dehydrated in alcohol and immersed in propylene oxide for 15 min. They were then immersed in a 2:1 mixture of propylene oxide and araldite (Polysciences Inc., Warrington, Pennsylvania, USA) for 1 h, in a 1:1 mixture for 30 min, in a 1:2 mixture for 2.5 h and in araldite for 3 h. Finally, they were embedded in araldite and baked in an oven at 70°C for 2–3 days to complete polymerization.

Semithin sections were cut at 1 μm on an automatic ultramicrotome (Ultracut R, Leica Microsystems, Nussloch, Germany) and stained with a 1% aqueous solution of toluidine blue to identify areas of interest. Ultrathin sections 70 nm thick were collected on copper mesh and contrasted with 2% uranyl acetate for 7–10 min and with 0.5% lead citrate for 7–10 min. Finally, the sections were studied in a transmission electron microscope (Morgagni 268D, FEI Company, Eindhoven, the Netherlands). Images were captured with a MegaView III camera linked to an image analysis system (Soft Imaging System, Münster, Germany).

**Results**

**General description**

Six placentas were processed for histology and three for transmission electron microscopy (Table 1). At the earliest stage available, a late limb bud stage, the embryos were deep in the decidua and covered by a thick capsule (Figure 1A–B, Table 2 and 3, character 2). The five embryos had an average crown-rump length of 11.5 mm. The forelimb and hind limb buds were present, with the forelimbs being larger and close to the somites. The cervical flexure was evident. The chorionicallantoic placenta was a pad of trophoblast (Figure 2A) covered by a layer of allantoic mesoderm from which fetal vessels were just beginning to penetrate the trophoblast at folds in the surface. There was a prominent subplacenta that occupied about half the total depth of the placenta (Figure 2A). It was situated deep in the decidua and associated with the maternal vasculature (Table 2 and 3, characters 9, 10). The yolk sac was inserted on the marginal surface of the placenta (Table 2 and 3, character 12) and is described below.

At the next stage, estimated at 70 days gestation [18], the capsule was present as a thin membrane (Figure 3A–B). Differentiation of the placenta into areas of labyrinth was well advanced (Figure 3C) and these areas were separated by bands of trophoblast without fetal capillaries, the interlobular trophoblast (Table 2 and 3, character 1). The areas of labyrinth extended from near the surface of the placenta. There was a substantial amount of trophoblast beneath them that still was not differentiated into labyrinth or interlobum. The subplacenta was fully developed (Figure 3D) and extraplacental trophoblast could be followed from this organ into the decidua (Table 2 and 3, character 11). The yolk sac was largely villous except for the fibrovascular ring at the attachment to the placental disk (Figure 3C, Table 2 and 3, characters 12, 13).

By the following stage, estimated at 90 days gestation [18], the capsule no longer covered the conceptus and the visceral yolk sac was exposed to the uterine lumen. The placenta now had the lobulated appearance typical of the
Figure 1
Implantation site in the capybara at the limb bud stage of development. (A) Median sagittal section of capybara implantation site at the limb bud stage of development. (B) Schematic drawing of the same stage. The embryo and membranes are enclosed within the uterine wall. A thick capsular decidua faces the antimesometrial wall of the uterus. The main placenta and subplacenta are attached to the basal decidua on the mesometrial side. Membranes include the amnion and visceral yolk sac. The latter is supplied by vitelline vessels whilst umbilical vessels supply the placenta. The uterine cavity is also shown. Am = amnion, ant = antimesometrial wall of the uterus, bd = basal decidua, cd = capsular decidua, em = embryo, mes = mesometrial side, mp = main placenta, sub = subplacenta, uc = uterine cavity, vys = visceral yolk sac, vv = vitelline vessels. Scale bar = 1 cm.

Figure 2
Placentation in the capybara at the limb bud stage. (A) The chorioallantoic placenta is a pad of trophoblast covered by allantoic (fetal) mesoderm from which vessels are beginning to penetrate the trophoblast at several folds (arrows). The subplacenta is situated deep within the basal decidua. The visceral yolk sac is inserted on the marginal surface of the placenta and is largely unfolded. Haematoxylin and eosin. (B) The interhaemal membrane is haemomonochorial from an early stage. A superficial maternal vessel is seen from which blood enters maternal blood spaces separated from fetal capillaries (arrows) by syncytiotrophoblast. Haematoxylin and eosin. Bd = basal decidua, fm = allantoic or fetal mesoderm, mbc = maternal blood space, mv = maternal vessel, sub = subplacenta, syn = syncytiotrophoblast, vys = visceral yolk sac. Scale bars = 1.5 mm (A), 40 μm (B).
Reproductive Biology and Endocrinology 2009, 7:57

hystricognath placenta, with some of the lobes far from the surface and surrounded by interlobular trophoblast (Figure 4A–B, Table 2 and 3, characters 1 to 6). The subplacenta remained about the same size whereas the disk above it had continued to grow.

**Growth of the main placenta**

**Limb bud stage**

At this stage, most of the trophoblast formed a spongy pad containing maternal blood spaces (Figure 2A). The centre of the disk was covered on the fetal side by connective tissue (allantoic mesoderm) containing the fetal blood vessels. On the fetal side of the pad there were large nests of cellular trophoblast (Figure 5A–B). More centrally the pad consisted of both cellular and syncytial trophoblast. In addition spurs of connective tissue lined by cytotrophoblasts passed into the pad both in the region of the central excavation and more laterally. The incipient vascularization of the placenta by vessels emanating from these folds of connective tissue could be confirmed by immunostaining for vimentin and actin (Figure 5C–D). The nuclei of the cytotrophoblasts were shown to have high proliferation activity by immunostaining for PCNA (Figure 6A). In addition positive records for immunostaining for PCNA occurred within the spongy trophoblast pad (Figure 6B). At the ultrastructural level (Figure 6C–D), we saw nests of cellular trophoblast with several mitotic figures and large intercellular spaces (Table 2 and 3, character 7).

**Gestational age 70 days**

By this stage the main placenta was composed of areas of labyrinth, or lobes, with interlobular areas between them (Figures 3C, 7A–B). Large arterial blood channels lined with trophoblast were found at the centre of each lobe (Figure 7A). The labyrinth consisted of maternal blood spaces lined by syncytial trophoblast and fetal capillaries that were immunopositive for vimentin (Figure 7B).
maternal blood spaces could be seen to radiate from the larger blood channels at the centre of the lobe (Table 2 and 3, characters 3, 4). The interlobular areas between the placental lobes had a different organization. These areas were mostly composed of syncytiotrophoblast. The syncytial nuclei were dispersed with euchromatin and were spherical in shape. The cytoplasm was more eosinophilic than in the labyrinth. Within the syncytial mesh were maternal venous blood channels. However, nests of proliferating cytotrophoblast were still present at the fetal border of the placenta (Figure 7C) and along the strands of fetal mesoderm that carried fetal vessels towards the labyrinth (Figure 7D). Proliferating cellular trophoblast was even present within the labyrinth (not shown, Table 2 and 3, character 8). As at the stage described above, large intercellular spaces occurred between the cytotrophoblasts as well as between them and the layer of syncytiotrophoblast that lined the maternal blood channels (Figure 7E–F, Table 2 and 3, character 5).

**Gestational age 90 days (mid term)**
The labyrinth was fully organized by mid gestation with fetal capillaries running parallel to maternal blood channels in a countercurrent arrangement (data not shown). The proliferation marker still revealed nests of proliferating cytotrophoblast near the surface of the placenta, associated with fetal mesoderm in deeper layers and within the labyrinth itself (Figure 8A–B).

**Subplacenta**
**Limb bud stage**
At this early stage the subplacenta was situated deep in the decidua (Figure 2A) and associated with maternal vasculature (Figure 9A). It consisted of several layers of cellular and syncytiotrophoblast with fetal mesenchyme and associated blood vessels separating the various lobes (Figure 9B). The cytotrophoblasts were highly proliferative (Figure 9C). At the ultrastructural level (Figure 9D–F), desmosomes were seen between adjacent trophoblast cells. The syncytiotrophoblast contained many vacuoles and its surface was covered with microvilli that projected into large extracellular spaces. Some of these spaces contained maternal blood cells.
Reproductive Biology and Endocrinology 2009, 7:57

From 70 days onwards the subplacenta was more fully differentiated (Figure 3D). Fetal vessels were found within the fetal mesenchyme, adjacent to the layer of cellular trophoblast. In addition, maternal blood lacunae were still present facing the syncytiotrophoblast (Figure 10A, Table 2 and 3, character 10). Much of the cytotrophoblast was in a single layer (Figure 10B). It was lined on one side by a band of fetal mesenchyme and on the other by a layer of syncytiotrophoblast. The cytoplasm of the syncytiotrophoblast contained spaces that gave it a fragmented appearance (Figure 10C). Fetal vessels within the mesenchyme were in close proximity to the cytotrophoblast (Figure 10D). There was a strong PAS positive reaction in the syncytiotrophoblast consistent with the presence of glycogen and/or glycoprotein (Figure 10E). The cytotrophoblast remained highly proliferative at mid gestation (Figure 10F).

Even at mid gestation both fetal arteries and maternal blood lacunae were present in the subplacenta as could be confirmed by injection of the vessels with latex (Figure 4B, Table 2 and 3, character 10).

**Junctional region and decidua**

**Limb bud stage**

At the ventral and lateral borders of the subplacenta, extraplacental trophoblast was evident, consisting of large cellular trophoblast and syncytial streamers. Near the subplacenta, extraplacental trophoblast had started to rebuild the walls of maternal arteries, although remnants of the vessel endothelium were still present (Figure 11A–B, Table 2 and 3, character 11).

**Gestational age 70 days and mid term**

At 70 days the subplacenta was associated with prominent areas of extraplacental trophoblast that could be followed deeply within the decidua. This included large and small trophoblast cells (extraplacental cytotrophoblast) and groups of trophoblast giant cells (Figure 11C) as well as strands of syncytiotrophoblast or syncytial streamers. These trophoblast cells were responsible for the destruction and replacement of the maternal vessel walls (Figure 11D). At 90 days this region was very similar except for thinning of the decidua consequent on fetal growth.

**Parietal and visceral yolk sac**

**Limb bud stage**

Rodents retain an inverted yolk sac placenta throughout gestation (Figures 3B, 12A). In the capybara at this stage the visceral yolk sac was fairly smooth (Figure 12A, E–F). It was attached to the placental disk and lateral to the attachment the endoderm continued as a thin epithelium referred to as the parietal yolk sac (Figure 12A). The cells rested on Reichert’s membrane, but it was poorly developed at this time. At the ultrastructural level the lateral cell membranes were attached by desmosomes. There were numerous microvilli on the apical surface of the cells (Figure 12C).

**Gestational age 70 days and mid term**

The attachment of the visceral yolk sac to the disk now had the distinctive form of a fibrovascular ring as known...
from other hystricomorph rodents (Figure 3C, Table 2 and 3, character 13). In the area near the disk the visceral yolk sac was villous in structure (Figure 12G). Most of the parietal yolk sac consisted of a single cell layer separated by Reichert’s membrane from the cytotrophoblast layer of the main placenta (Figure 12B). Near the base of the disk it took on a villous appearance (Figure 3C, Table 2 and 3, character 14). The ultrastructure of the cells had not changed greatly since the previous stage but Reichert’s membrane was noticeably thicker (Figure 12D). By 70 days the yolk sac had achieved the form previously described in detail for the term placenta [20].

Discussion

Among rodents, and especially within the subgroup Hystricognathi, there is an enormous variation in size. With a body weight of around 50 kg the capybara is by far the largest living rodent. Thus, the question arises of whether placental development in the capybara follows the same course as in its much smaller relatives. The capybara is the only rodent species that approximates human dimensions in body mass of mother and offspring. Our findings on placental development in the capybara are therefore a useful test of the presumed suitability of hystricognath rodents, particularly the guinea pig, as animal models for human placentation.

Firstly, how is a fetus of this size supported and how does the associated placental architecture develop? Even in rodents that deliver smaller and less well developed young, placental gas exchange is optimized by countercurrent arrangement of the maternal and fetal blood vessels. As shown by Mossman [52], no further improvement in efficiency can be gained by increasing the length of the capillaries. The solution adopted by the hystricognath rodents was to fold the labyrinth, thus increasing the exchange area while keeping the placenta quite compact. The lobulated appearance of the placenta in cross section is a result of this and has been considered a defining feature of the hystricognath placenta [9,10,21-23]. In the capybara, first steps towards this arrangement are apparent at an ontogenetic stage of around 70 days. Full establishment of the highly complex, lobulated appearance is achieved by 90 days, and is then equivalent to the condition of the term placenta [19-21]. As in the mature placenta, by mid gestation the labyrinth shows a fully organized counter current arrangement of maternal blood channels, lined by trophoblast, and fetal capillaries. This is associated with the presence of large arterial blood channels at the centre of each lobe and interlobular areas to collect the maternal blood from the lobes. Thus, establishment of the placental architecture of the capybara is similar to that described for other hystricognath species [[6,9,10,19-51]; see Tables 2 and 3]. In the capybara material investigated, ranging from a limb bud stage to a mid gestation stage of 90 days, placental growth is predominantly the result of proliferation of cellular trophoblast situated in nests at the fetal side of the placenta and along internally directed projections on fetal mesenchyme. Additional proliferation has been demonstrated for cellular trophoblast inside the labyrinth. This pattern is present in later stages, too, and continues to near term (data not
shown). Thus, even though placental dimensions are much larger in the capybara, the essential growth processes are equal to those in other hystricognaths, especially those with a highly lobulated placental architecture [[11,13], Tables 2 and 3].

Secondly, a unique feature of the placenta of hystricognath rodents is the subplacenta. Its purpose is not fully understood but one function is to act as a source for the trophoblast that invades and transforms the maternal arteries [10,12]. This process is important in ensuring an adequate blood supply to the placenta and is analogous to the transformation of the spiral arteries in the human placenta [12]. As in the early development of other hystricognaths [[6,9,10,12,19-29,31-44,46-50], Tables 2 and 3], a subplacenta associated with the maternal vasculature and characterised by layers of syncytial and highly proliferative cellular trophoblast, can be confirmed at an early stage in the capybara. At mid gestation differentiation is typical of that in other hystricognaths, but there is an overlap of fetal arteries and maternal blood lacunae that could be confirmed by injection of the vessels with latex. In contrast, the subplacenta is supplied only by fetal vessels at
term. The overlap between the two systems in mid gestation is a rare feature among hystricognaths (Tables 2 and 3), known only for the degu [35] and the prea [31], whereas the ancient condition of hystricognaths does not include an overlap or fetomaternal exchange inside the subplacenta [35]. All stages of placentation in the capybara that we investigated had extraplacental trophoblast, i.e. large cellular trophoblast cells and syncytial streamers derived from the subplacenta, that was responsible for the destruction and replacement of the maternal vessel walls deep within the decidua. This likewise represents a typical hystricognath feature [10,12,19,31,35-37]. Thus apart from the difference in placental diameter, both the differentiation of the subplacenta and its role for trophoblast invasion is similar within hystricognaths.

Based on this and previous studies we are able to list 14 characteristics of placentation that hold across the 11 families so far studied, including African representatives such as the cane rat, African porcupine and mole rat (Table 2). As shown in Table 3, just four of these show sufficient variation between species to justify definition of two character states. This indicates an extraordinarily stable pattern that clearly was evolved before dispersal of the group to South America [14].

Figure 11
Junctional region and decidua at limb bud stage (A-B) and 70 days of gestation (C-D). (A) At the limb bud stage maternal vessels in the decidua retain most of their endothelium. There is, however, intrusion of a trophoblast giant cell into the decidua subjacent to the vessel wall. TEM (B) Immunostaining for actin confirms that vessel architecture is largely intact. (C) Immunostaining for cytokeratin at 70 days reveals the presence of trophoblast cells in the vessel walls and surrounding decidua. These include giant cells, large cells (arrows) and smaller cells. (D) Immunostaining for vimentin shows a discontinuous vessel endothelium. Decidual cells also are vimentin positive. End = endothelium, tgc = trophoblast giant cell. Scale bars 10 μm (A), 40 μm (B-D).

Figure 12
Parietal and visceral yolk sac at limb bud stage (A-D) and 70 days of gestation (E-G). (A) Overview to show attachment of the visceral yolk sac to the placental disk. Lateral to this the parietal yolk sac extends over the surface as a thin epithelial layer. The central part of the disk is covered with allantoic mesoderm. (B) Endoderm of the parietal yolk sac at the limb bud stage Note the apical microvilli and the desmosomes (arrowheads). TEM. (C) Visceral yolk sac; PAS. Beneath the endoderm is a layer of mesoderm carrying the vitelline vessels. (D) Visceral yolk sac; haematoxylin and eosin. (E) The yolk sac epithelium rests on Reichert's membrane, which separates it from the surface layer of cytotrophoblast; immunostained for cytokeratin. (F) Endoderm of the parietal yolk sac at 70 days. The cells rest on Reichert's membrane. (G) Visceral yolk sac at 70 days; immunostained for vimentin. Note the villous appearance of the yolk sac at this stage. Cyt = cytotrophoblasts, endo = endoderm, fm = allantoic or fetal mesoderm, pys = parietal yolk sac, Rm = Reichert's membrane, vys = visceral yolk sac, vv = vitelline vessels. Scale bars 500 μm (A), 5 μm (B), 40 μm (C-E), 10 μm (F) 100 μm (G).
Conclusion
In summary, our findings on the capybara, a rodent with around 50 kg body mass, show that all important characteristics of placental development and organization in guinea pig related rodents are similar and do not vary with body mass. Therefore it is not necessary to solve the challenges of animal husbandry that presently preclude use of the capybara as a laboratory animal. Rather, our data indicate that its smaller relatives, especially the guinea pig, are adequate models for human placentation and pregnancy.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TCS and MAM devised the study, participated in its design and coordination and helped to write the manuscript. CK and TCS performed the major part of the histological analysis. AMC and AMM participated in the study design and analysis and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico Tecnológico (CNPq).

References
1. Carter AM: Animal models of human placentation. Placenta 2007, 28(Suppl 1):S129-S132.

2. Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ryder OA, O’Brien SJ: Molecular phylogenetics and the origin of placental mammals. Nature 2001, 409:614-618.

3. Murphy WJ, Eizirik E, O’Brien SJ, Madsen O, Scally M, Douady CJ, Teeling E, Ryder OA, Stanhope MJ, de Jong WVW, Springer MS: Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 2001, 294:2348-2351.

4. Springer MS, Murphy WJ, Eizirik E, O’Brien SJ: Molecular evidence for major placental clades. In The Rise of Placental Mammals: Origins and Relationships of the Major Extant Clades Edited by: Rose KD, Archibald JD. Baltimore: Johns Hopkins University Press; 2005:37-49.

5. Wilson DE, Reeder DM, editors: Mammal Species of the World: A Taxonomic and Geographic Reference. Baltimore: Johns Hopkins University Press; 2005.

6. Mess A, Mohr B, Martin T: Transformations in the stem species pattern of hystroicognath Rodentia and the climatic change in the Eocene to Late Oligocene time interval. Mitt Mus Naturk, Zoologische Reihe 2001, 77:193-206.

7. Martin RD: Evolution of placentation in primates: Implications of mammalian phylogeny. Evol Biol 2008, 35(2):125-145.

8. Carter AM: Animal models of human placentation – a review. Placenta 2007, 28(Suppl A):S41-47.

9. Kaufmann P, Guineia Pig Cavia porcellus – Comparative Placentation 2004 [http://medicine.ucsd.edu/ca/pca].

10. Mess A: The guinea pig placenta: model of placental growth dynamics. Placenta 2007, 28:812-815.

11. Mess A, Žák N, Kadýrov M, Korp H, Kaufmann P: Caviomorph placentation as a model for trophoblast invasion. Placenta 2007, 28:1234-1238.

12. Mess A: Development of the chorioallantoic placenta in Octodon degus – a model for growth processes in caviomorph rodents? J Exp Zool B Mol Dev Evol. 2007, 308(4):371-383.

13. Gheerbrant E, Rage J-C: How distinct from Gondwana and Laurasia? Palaeogeography, Palaeoclimatology, Palaeoecology 2006, 241:224-246.

14. Rowe DL, Honeycutt RL: Phylogenetic relationships, ecological correlates, and molecular evolution within the cavioidae (Mammalia, Rodentia). Mol Biol Evol 2002, 19:263-277.

15. Opazo JC: A molecular timescale for caviomorph rodents (Mammalia, Hystroicognathi). Mol Phylogenet Evol. 2005, 37(3):932-937.

16. Moojen J: Os Rodentes do Brasil Rio de Janeiro: Ministério de Educação e Saúde. Instituto Nacional do Livro; Biblioteca Científica Brasileira. Série A – II: 1952.

17. Barbella SL: Considerazioni generali sulla gestazione del chiquire (Hydrochoerus hydrochaeris). Acta Científica Venezolana 1987, 38:84-89.

18. Kaufmann P: Cavybara Hydrochoerus hydrochaeris. Comparative Placentation 2004 [http://medicine.ucsd.edu/ca/pca].

19. Miglino MA, Carter AM, dos Santos Ferraz RH, Fernandes Machado MR: Placentation in the capybara (Hydrochaerus hydrochaeris), agouti (Dasyprocta aguti) and paca (Agouti pacu). Placenta 2002, 23:416-428.

20. Miguelino MA, Carter AM, Ambrosio CE, Bonatelli M, De Oliveira MF, Dos Santos Ferraz RH, Rodrigues RF, Santos TC: Vascular organization of the hystriomorph placenta: a comparative study in the agouti, capybara, guinea pig, paca and rock cavy. Placenta 2004, 25:438-448.

21. Luckett WP, Mossman HW: Development and phylogenetic significance of the fetal membranes and placenta of the African hystroicognathous rodents Batyurus and Hystrix. Am J Anat 1981, 162:265-285.

22. Luckett WP: Superordinal and intraordinal affinities of rodents: Developmental evidence from the dentition and placentation. In Evolutionary Relationships among Rodents Volume 92. Edited by: Luckett WP, Hartenberger J-L. New York: Plenum Press; NATO ASI-Series: 1985:227-276.

23. Perrott CA: Fetal membranes of the Canadian porcupine, Erethizon dorsatum. Am J Anat 1959, 120:35-59.

24. Hillelmann HH, Gaynor AL: The definitive architecture of the placentae of nutria, Myocastor cynocephalus (Molina). Am J Anat 1961, 109:299-317.

25. King BF, Tiffet FD: The fine structure of the chinchilla placenta. J Exp Zool 1961, 145:93-110.

26. Kaufmann P, Davidoff M: The guinea pig placenta. Adv Anat Embryol Cell Biol 1977, 53:1-91.

27. Oliveira MF, Carter AM, Bonatelli M, Ambrosio CE, Miglino MA: Placentation in the rock cavy, Kerodon rupestris (Wied). Placenta 2002, 23:87-97.

28. Mess A: Chorioallantoic and yolk sac placation in the das ges rat Petromus typicus and its bearing to the evolution of hystroicognath Rodentia. Placenta 2007, 28(11-12):1229-1233.

29. Miglino MA, Francioli ALR, de Oliveira MF, Ambrosio CE, Bonatelli M, Fernandes Machado MR, Mess A: Development of the inverted yolk sac in three species of caviids (Rodentia, Caviomorpha, Caviidae). Placenta 2008, 29:748-752.

30. Oliveira MF, Mess A, Ambrosio CE, Dantas CAG, Favaron PO, Miglino MA: Chorioallantoic placation in Galea spixii (Rodentia, Caviomorpha, Caviidae). Reprod Biol Endocrinol 2008, 6:39.

31. Uhlenhord B, Kaufmann P: Die Entwicklung des Plazentastels beim Meerschweinchen. Zbl Vet Med C Anat Histol Embryol 1979, 23:23-247.

32. Wolff J, Kaufmann P: Die Ultrastruktur der Meerschweinchen-Subplazenta. Zbl Vet Med C Anat Histol Embryol 1980, 29:9-24.

33. Rodrigues RF, Carter AM, Ambrosio CE, Dos Santos TC, Miglino MA: The subplacenta of the red-rumped agouti (Dasyprocta leporina L). Reprod Biol Endocrinol 2006, 4:31.

34. Mess A: The subplacenta in Octodon degus and Petromus typicus – two hystroicognath rodents without significant placental lobulation. J Exp Zool (Mol Dev Evol) B 2007, 308:172-188.

35. Bosco C, Buffet C, Belo MA, Rodrigo R, Gutierrez M, Garcoa G: Placentation in the degu (Octodon degus): Analogies with extrauterine trophoblast and human pregnancies. Comp Biochem Physiol A Mol Int Physiol 2007, 146:475-485.

36. Bosco C, Buffet C: Immunohistochemical identification of the extravillous trophoblast during the placentation of the degu

37. Bosco C, Buffet C: Immunohistochemical identification of the extravillous trophoblast during the placentation of the degu
38. Davies J, Dempsey EW, Amoroso EC: The subplacenta of the guinea pig: An electron microscopic study. J Anat (London) 1961, 95:311-324.
39. Davies J, Dempsey EW, Amoroso EC: The subplacenta of the guinea pig. Development, histology and histochemistry. J Anat (London) 1961. 95(1):457-473.
40. Kaufmann P: Die Meerschweinchenplacenta und ihre Entwicklung. Z Anat Entw-gesch 1969, 129:83-101.
41. Kaufmann P: Electron microscopy of the guinea-pig placental membranes. Placenta 1981:3-10.
42. Bonazelli M, Carter AM, Fernandes Machado MR, De Oliveira MF, De Lima MC, Miglino MA: Placenta in the paca (Agouti paca L). Reprod Biol Endocrinol 2005, 3:9.
43. Tibbits FD, Hillemann HH: The development and histology of the chinchilla placenta. J Morph 1959, 105:317-366.
44. Benirschke K, Kaufmann P: Chinchilla Chinchilla laniger. Comparative placentaition 2004 [http://medicine.ucsd.edu/cpa].
45. Mess A: The fibrovascular ring: A synapomorphy of hystricognath Rodentia newly described in Petromus typicus and Octodon degus. Belgian Journal of Zoology 2005:31-38.
46. Mess A, Kaufmann P: Degu Octodon degus. Comparative placentaition 2003 [http://medicine.ucsd.edu/cpa].
47. Benirschke K: Nutria or Coypu Myocastor coypus. Comparative Placentaition 2004 [http://medicine.ucsd.edu/cpa].
48. Oduor-Okelo D: An electron microscopic study of the chorionic-alantoic placenta and the subplacenta of the cane rat (Thryonomys swinderianus Temminck). Placenta 1984, 5:433-442.
49. Oduor-Okelo D, Gombe SG: Placentaition in the cane rat (Thryonomys swinderianus). Afr J Ecol 1982, 20(1):49-66.
50. Oduor-Okelo D, Gombe SG: Development of the foetal membranes in the cane rat (Thryonomys swinderianus): a re-interpretation. Afr J Ecol 1991, 29(2):157-167.
51. Carter AM, Tanswell B, Thompson K, Han VKM: Immunohistochemical identification of epithelial and mesenchymal cell types in the chorioallantoic and yolk sac placenta of the guinea-pig. Placenta 1998, 19:489-500.
52. Mossman HW: The principal exchange vessels of the chorioallantoic placenta of mammals. In Organogenesis Edited by: DeHaan RL, Ursprung H. New York: Holt, Rinehart and Winston; 1965:771-786.

Octodon degus. J Exp Zoolog B Mol Dev Evol. 2008, 310(6):534-539.