Microscopy of the Umbilical Cord of Rock Cavies—*Kerodon rupestris* Wied, 1820 (Rodenta, Caviidae)

MARCIO NOGUEIRA RODRIGUES,[1]* GLEIDSON BENEVIDES DE OLIVEIRA,[2] VALÉRIA VERAS DE PAULA,[2] ALEXANDRE RODRIGUES SILVA,[2] ANTONIO CHAVES DE ASSIS NETO,[1] MARIA ANGELICA MIGLINO,[1] AND MOACIR FRANCO DE OLIVEIRA[2]

[1]*Department of Surgery, Faculty of Veterinary Medicine and Animal Science (FMVZ-USP), São Paulo University (USP), Cidade Universitária, Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo SP, 05508270, Brazil*
[2]*Department of Animal Science, Federal Rural of Semi Arid University, Mossoró, Rio Grande do Norte-Brazil. Av. Francisco Mota, 572, costa e silva, 59625900, RN Mossoró, Brazil*

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ABSTRACT The rock cavies (*Kerodon rupestris*) are rodents belonging to family Caviidae, with habitat restricted to northeastern Brazil. Current studies have shown that blood cord has stem and hematopoietic cells both with a high regenerative potential, microscopic studies about the description in rodents and other mammals are scarce. The aim of this study was to describe the structural components of the umbilical cord of rock cavies. Rock cavies embryos at different stages of pregnancy were used, three in the first third, five in middle third, and six in the final third of gestation. They were obtained at the Centre for Wild Animals Multiplication of Federal Rural University of Semi-Arid. In rock cavies was observed that the number of vessels involved in the transport of maternal substances was represented by two major arteries, veins and an allantois duct. The umbilical cord was delimited by amniotic epithelium, formed by a layer of squamous cells, connective tissue with characteristic of the mucosal tissue, corresponding to the Wharton jelly (fetal mesenchyme), where elastic fibers were observed and intercellular spaces were arranged in concentric blades. The morphology observed in rock cavies is similar to that described in the literature for other mammals, including man. The rock cavies are well adapted to captivity and are docile, which makes this animal an important model for study within the field of regenerative medicine. The knowledge of the umbilical cord morphology represents a base point for using this animal as a model for culture and cell therapy. *Microsc. Res. Tech. 76:419–422, 2013.*

INTRODUCTION

The rock cavies are mammals rodents belonging to family Caviidae with habitat restricted to northeastern Brazil. These are characterized by presenting the dorsal surface of the body with light gray color mixed with black and white and the posterior part of the limb with staining brown-ferriginosa (Moojen, 1962; Rood, 1979). Adults' measure 410 mm and can reach 1 kg of body weight. They are gregarious with twilight habits, spending the day sheltering in rocks holes, leaving in the evening and at dawn to feed of trees branches, shrubs and small branches of some climbing plants' species (Mendes, 1987).

The umbilical cord has origin from the embryonic stem, which connects the bladders of the yolk sac and amniotic. It is shifted ventrally and is called abdominal stalk, having an important role in the transport of maternal nutrients to the developing fetus and the elimination of excreta from the fetus to the mother (Keeth et al., 2000).

Current studies have shown that blood cord has stem cells, hematopoietic with regenerative potential of solid organ and stem cells. The mesenchymal cells derived from Wharton's jelly from the stromal cells have great potential for use in engineering tissues and may contribute in the regeneration of cartilage, fat, bone, muscle, and stroma (Jiang et al., 2007).

The umbilical cord is a structure discarded after the birth and the transplant of their cells may present less risks of causing immune reactions, resulting in minimum risk for the recipient of its cells (Bydlowsk et al., 2009). The use of stem cells from umbilical cord has created scientific expectations for its use in cell therapy and regeneration of organs affected by the so called incurable diseases (Zucconi et al., 2009).

The rock cavies represent an excellent biological model since they are wild rodents well adapted to captivity. Microscopic studies about the umbilical cord description of rodents and other mammals are not common. The aim of this study was to describe the structural components of the umbilical cord of rock cavies emphasizing its importance in the transport of substances between mother and fetus and as a potential source of stem cells.

*Correspondence to: Marcio Nogueira Rodrigues, Av. Prof. Melo Morais, 1235, Bl. C Apto 206. Zip Code: 05508030, Butanta, São Paulo, SP, Brazil. E-mail: marcio_medvet@hotmail.com

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MATERIAL AND METHODS

Were used embryos of rock cavies at different stages of pregnancy, three in the first third, five in middle third, and three in the final third of gestation. They were obtained at the Centre for Wild Animals Multiplication of Universidade Federal Rural do Semi-Arid, registered with IBAMA and scientific breeding under number 1478912. To collect the samples, the pregnant females were subjected to a solid and water fasting for 4 and 2 h, respectively, receiving anesthetic as premedication intramuscularly association of ketamine (15 mg/kg) and midazolam (1 mg/kg). After 15 min, anesthesia was induced using inhalation mask craft with 100% oxygen associated with isoflurane. Then ovariohysterectomy procedure was performed to remove the gravidic uterus.

For microscopic analysis, the samples were collected from the umbilical cord with a maximum length of 1 cm and fixed in 4% paraformaldehyde buffered with 0.1 M sodium phosphate, pH 7.4 at 4°C. After fixation, the material was dehydrated in an ascending series of ethanol, diaphanized in xylene and embedded in histology paraffin (Paraplast). Cuts of 5 μm were obtained in microtome (Leica MR 2065). The slides were stained in Picrosirius-fuchsin paraldehyde+Gomori Trichrome and then photo documented. The histological procedures used followed that described by Tolosa et al. (2003).

RESULTS

In rock cavies, it was observed that the number of vessels involved in the transport of maternal substances was represented by two major arteries, veins and one allantois duct. In addition, two vessels were identified, represented by a vitelline artery and vein, which are distributed along the length of the coriovitelline membrane (Fig. 1A).

The umbilical cord was delimited by amniotic epithelium, formed by a layer of squamous cells, connective tissue with characteristic of the mucosal tissue corresponding to the Wharton jelly (fetal mesenchyme), where elastic fibers were observed and intercellular spaces were arranged in concentric blades (Fig. 1B).

The arteries showed two distinct layers forming their wall. The outermost was consisted by circular longitudinal smooth muscle fibers and the innermost was also formed by smooth muscle fibers but only with longitudinal disposal. More internally, near the lumen, internal elastic lamina was identified (Figs. 1C–1E).

The veins showed a homogeneous layer on his wall, where smooth muscle cells predominated, with circular disposal, possessing the internal limiting elastic lamina, showing up mostly disrupted (Figs. 2A and 2B). The allantoic duct consisted of a transitional epithelium with irregular light and rich in collagen fibers (Figs. 2C and 2D).

In all cases analyzed, the umbilical cord was inserted centrally to the placenta by a vascular hilum resulting inbranching structures represented by arteries, veins and allantoic duct.

DISCUSSION AND CONCLUSION

The umbilical cord is a unique mammalian fetal attachment (Blanchette, 2011) and was attached to the center of the placental disk (Favaron et al., 2011). It plays an important role in the transport of maternal nutrients to the developing fetus and the elimination of feces from the fetus to the mother (Keeth et al., 2000). Formed by two arteries and one vein, it has a main function of making the connection between the fetus and placenta, ensuring its viability especially in the later stages of pregnancy (Caughey et al., 2009). Lordy (1940) described that the umbilical cord is coated by amniotic epithelium and considers that the conjunctive amniotic layer is adhered closely to the fundamental substance of the cord known as fetal mesenchyme, which was composed of mesenchymal tissue

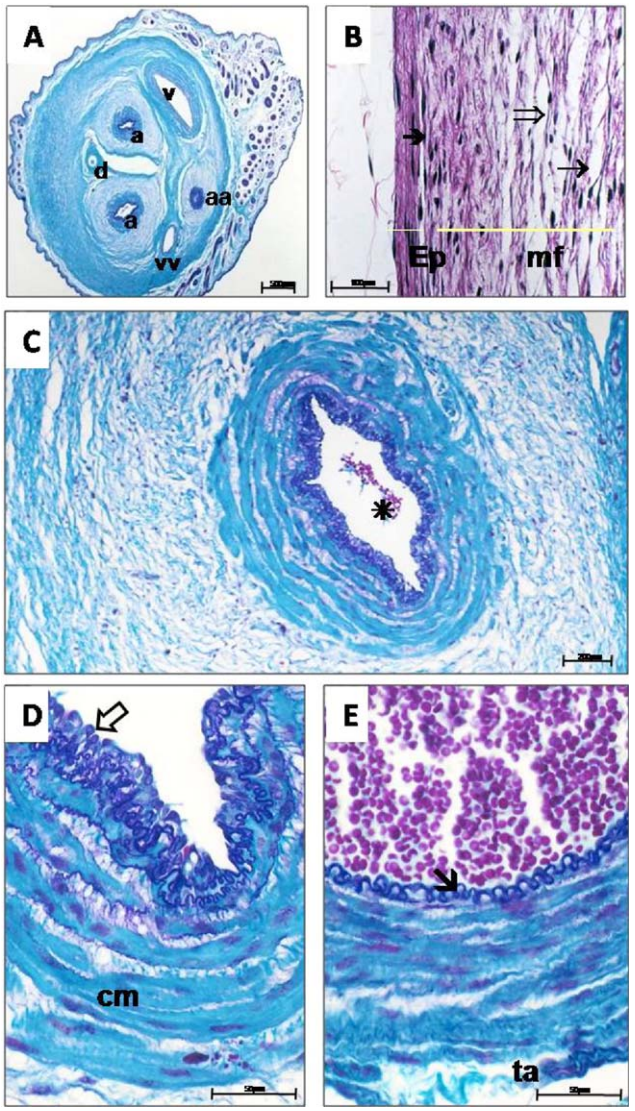

Fig. 1. Photomicrograph of the umbilical cord of rock cavies fetuses. In (**A**), note the umbilical arteries (a), the umbilical vein (v), the allantoic duct (d) and the vitelline artery (aa) and vein (vv). In (**B**), amniotic epithelium (Ep) and fetal mesenchyma (mf). Note the squamous epithelial cells (➔), mesenchymal cells (⟶) and elastic fibers (⟹). In (**C**), one of fetal arteries with irregular light (*). In (**D**), the same detail which identifies the vessel endothelium (⇖) and middle layer (cm). In (**E**), vitelline artery, which identifies the internal elastic (⇘) and the tunica adventitia (ta). A, C, D, and E: Fuchsin-Paraldeido + Gomori's trichrome. B Picrossirius. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

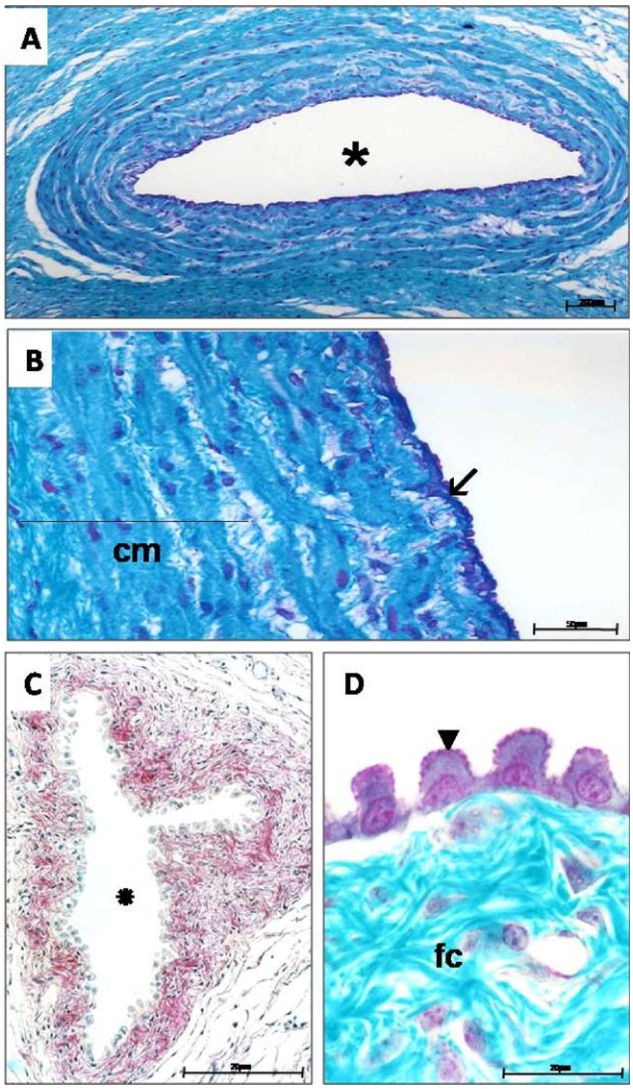

Fig. 2. Photomicrograph of the umbilical vein of rock cavies fetuses. In (**A**), note the vein, whose light showed the aspect more regular than in arteries (*). In (**B**), the muscle layer (cm) and the vessel endothelium (↙). In (**C**), observe the irregular light duct (✱). In (**D**), a detail showing the duct with transition epithelium (▼) and collagen fibers (fc). A, B, and D Fuchsin-Paraldeido+Gomori's trichrome, in C Picrossirius. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

with stellate cells and amorphous ground substance containing much glycogen. This gelatinous composition or jelly Wharton has been showing much interest in recent research regarding their potential for differentiation and tissue repair. However, numerous efforts have been inferred to the establishment of animal models that may be used in the preclinical studies (Sousa et al., 2011).

In rock cavies the results of the number of umbilical vessels are similar to those found by Hillemann and Gaynor (1961), Silva (2001), and Tibbits and Hillemann (1959), as the presence of the vitelline arteries and veins in the umbilical cord. The umbilical cord of rock cavies showed arterial and venous pattern similar to that described in the literature for pacas (Tibbits and Hillemann, 1959), nutria (Hillemann and Gaynor, 1961), chinchilla (Silva, 2001), human (Alp Can, 2007), and cavies (Dantas et al., 2009) when these authors report the presence of two umbilicals arteries and one vein. However, in rock cavies the results differ from those obtained by the authors regarding the type of tissue lining the lumen of allantoic duct. In rock cavies the duct epithelium is typically transitional, unlike the results found by Tibbits and Hillemann (1959) that describe the allantoic duct epithelium in chinchillas as being simple cubic type. Silva (2001) reported a bi-stratified cubic epithelium in pacas and Neves (2000) characterized the duct epithelium as being lined by endothelial cells, similarly to different rock cavies in our study.

Tibbitts and Hillemann (1959) studied the development and placental histology in chinchillas (*Chinchilla lanigera*) through injection techniques using latex, vinyl resin, India ink, lightening glycerin, and potassium hydroxide and histological techniques for light microscopy. When describing the umbilical cord in fetuses, they reported that the length thereof was equal to 20 mm on average and had five major vessels, two arteries and one umbilical vein, one artery and one vein vitelline, and one allantoid vesicle composed by simple cubic epithelium. Furthermore, smaller blood vessels were observed and blood cells were arranged in the gelatinous substance of the umbilical cord. Considering the fact that the fetal membranes have phylogenetic significance, Hillemann and Gaynor (1961) studied the nutria placentation (Myocastor coypus), comparing with other histricomorfs. Based on his observations in the rock cavies umbilical cord, it was covered by a simple squamous epithelium continuous represented by the amnion, inserted ventrally on the fetal surface of the placenta and harboring two arteries and one umbilical vein, and an artery and a vitelline vein. The amniotic ectoderm of the rock cavies was formed by squamous simple cubic epithelium, with ovoid nuclei occupying the central position of the cell, showing no evidence that it was vascularized near the disc and not by vitelline vessels. Similar description was reported by Lordy (1940) in rodents, Silva (2001) in pacas, and Dantas et al. (2009) in cavies.

The umbilical cord has been highlighted as a source of study in several areas. Whereas, it is usually discarded after birth, this caught the attention of researchers. The blood of the umbilical cord has hematopoietic stem cells with regenerative potential and mesenchymal stem cells (Jiang et al., 2007). As reported, the transplantation of these cells derived from umbilical cord presents less risk to cause immune reactions to donor and have quick availability to the receiver (Bydlowsk et al., 2009).

Based on this information and morphological similarity of the umbilical cord in the rock cavies with most mammals, including humans, along with the fact that this animal is well adapted to captivity this becomes an important model in the study in the area of regenerative medicine. The knowledge of the umbilical cord morphology accounts a base point for the use of this animal as a model for culture and cell therapy.

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