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ORIGINAL ARTICLE

Embryonic and Fetal Development in – Pigmy Rice Rat – *Oligoryzomys* sp. (Rodentia, Sigmodontinae) and its Significance for Being a new Experimental Model

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Summary

Oligoryzomys (Cricetidae, Sigmodontinae) is a common rodent genus from South America that includes a couple of very similar species. Related species have been used as experimental model for understanding several diseases for which these species are reservoirs. In order to provide a better understanding of the embryological aspects of this group, herein we showed data on the embryonic and fetal development in *Oligoryzomys* sp. Eight specimens of different stages of gestation were obtained from the Collection of the Zoology Museum of University of Sao Paulo, Brazil. Gestational ages were estimated by crown-rump-length according to Evans and Sack (1973). To address our analysis after examining the gross morphology, tissues from several organs were processed for light and scanning electron microscopy. Morphological data on the systems (nervous system, cardiorespiratory system, intestinal tract and urogenital system) were described in detail. Finally, the findings were compared with what is known about embryological aspects in other rodent species in order to establish similarities and differences during the organogenesis in different species.

Introduction

Muroid are the most abundant group of rodents in the world (Musser and Carleton, 2005). The families Muridae and Cricetidae together include more than 1500 species. Within Cricetidae, the subfamily Sigmodontinae has 377 recognised species in 74 genera (Musser and Carleton, 2005; D'Elía et al., 2008). Sigmodont rodents are mainly confined to the Neotropics, known as 'New world rats and mices' (Eisenberg and Redford, 2000). Sigmodontinae reached South America in the Great American Interchange in the Pliocene, or possibly even earlier (Almeida et al., 2006). Starting from this point of immigration, they underwent to a rapid radiation that makes them to be able to survive in a variety of habitats (Bonvicino et al., 2005; Almeida et al., 2006).

Most species belonging to Sigmodontinae have restricted home ranges being vulnerable to habitat loss and destruction, so these species can be considered important bioindicators (Cademartori et al., 2004). On the other hand, they are also considered as pests in many countries, due to the fact that they transmit many diseases not only to humans but also to other domestic animals. Antibody prevalence indicates that Sigmodontinae species are carriers of Hantaviruses in the mainland regions of Brazil, as well as in other countries of South and North America. The evolutionary pattern of Sigmodontinae indicates that they may be the most important carriers of zoonotic cutaneous leishmaniasis (Weksler, 2003; Jansa and Weksler, 2004). For this reason, it seems that there is a need of knowledge of the biology including reproduction and embryology of the species belonging to this group. Where hopefully that will be possible to establish breeding groups of these species in captivity in order to use them as experimental models.

Oligoryzomys is a genus that includes around 10 species, all exhibiting very similar characteristics. It is widespread in South America, especially in Brazil, associated with the Amazon rainforest, Atlantic rainforest, Cerrado, Caatinga and Pantanal (Bonvicino et al., 2008). With a

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standard length of 83–110 mm and an average weight of 14–35 g, it includes quite small animals. In addition, *Oligoryzomys* exhibits a short gestation period of 25–26 days with two or four offspring (Brasil, 2002).

In order to better understand the development of *Oligoryzomys* sp., herein we provide a comprehensive investigation about the embryo and fetal development in this specie comparing our results with the available data on other rodent species and their relatives like mice, mouse, rat and rabbit.

Materials and Methods

Eight *Oligoryzomys* specimens in different gestational ages were obtained from the collection of Zoology Museum of Sao Paulo University, Sao Paulo, Brazil (see Table 1).

The material was preserved in formaldehyde (4%), alcohol (70%) or glutaraldehyde (2.5%). Gestational ages were estimated by considering the crown-rump-length (CRL) and weight (by using a digital balance 0.001 g – MARTE model) of the embryos and fetuses according to Evans and Sack (1973). To describe the embryogenesis in O*ligoryzomys*, the investigated material was divided into three groups (Group I with three embryos with CRL between 0.4 and 0.5 cm; two fetuses belonging to Group II with 1.0–1.4 cm CRL and, finally, Group III with three fetuses with CRL between 1.8–2.2 cm).

First, the gross morphology using a magnifying glass (Zeiss Stemi SV6; Carl Zeiss, Gottingen, Germany) was examined and photographed (Sony MVC – CD500).

Then, the specimens MZUSP31178, MZUSP32735/1 and MZUSP32735/2 (Group I), MZUSP32729-3 (Group II) and MZUSP32729-2 (Group III) were processed for histology using standard histological methods, embedded in paraffin (Paraplast; Oxford Labware, St Louis, MO, USA) and longitudinally sectioned. Owing to the larger size, the specimen MZUSP32798 (Group III) had pieces of tissue belonging to various organs excised, which were processed together with the previous specimens. All samples were sectioned at 5 μm using an automatic microtome (RM2155; Leica, Berlin, Germany) and stained with haematoxylin and eosin (Lillie and Fulmer, 1976; Tolosa et al., 2003). Then, sections were examined in Olympus BX40 microscope (Zeiss KS400 image analysis system 3.4; Carl Zeiss Vision, Munich, Germany).

Table 1. Material collected and values for fetal length (cm), fetal weight (g) and estimated age (according to Evans and Sack, 1973) of embryos and fetus of *Oligoryzomys* sp. (Rodentia, Cricetidae, Sigmodontinae). The specimens were loan from the collection of the Museum of Zoology, University of Sao Paulo, SP, Brazil (MZUSP)

Groups	Collection number	Size (cm)	Weight (g)	Estimated age (days)
I	MZUSP31178	0.4	0.038	12
	MZUSP32735/1	0.5	0.036	12.5
	MZUSP32735/2	0.5	0.031	12.5
II	MZUSP31167	1.0	0.118	14.5
	MZUSP32729-3	1.4	1.200	16
III	MZUSP32729-2	1.8	0.665	17
	MZUSP32729-4	1.8	1.327	17
	MZUSP32798	2.2	2.327	18.5

Tissues for scanning electron microscopy (SEM) were obtained from two embryos, MZUSP31167 (Group II) and MZUSP32729-4 (Group III), and post-fixed in 2% osmium tetroxide for 10 min at 4°C. These tissues were dehydrated in crescent series of ethanol. After drying by critical point with liquid CO2, the material was sputtered by gold and viewed using SEM (LEO VP 435; Carl-Zeis, Oberkochen, Germany).

The nomenclature used is based on International Committee on Veterinary Gross Anatomical Nomenclature (1994), International Committee on Veterinary Embryological Nomenclatura (1994) and International Committee on Veterinary Histological Nomenclature (1994).

Results

Gross morphology

The CRL and the weight of the five embryos and the three fetuses varied from 0.4 to 2.2 cm and from 0.031 to 2.327 g, respectively (Table 1).

Three embryos from the Group I (CRL 0.4–0.5 cm) were 'C' shaped with a prominent cervical curvature (Fig. 1a). Embryo MZUSP31178 (CRL 0.4 cm) shows an early developmental stage of the cephalic region with the open anterior neuropore displaying the 4th ventricle. The nose was short, the optic vesicle was spherical in shape, and the eyes were not pigmented (Fig. 1a). The somites were 35–40 in number and clearly visible. The forelimb bud and the hindlimb were present, their extremities were in the initial stage of development, and in this way the digits were not present yet (Fig. 1b). Tail bud was observed. Proportionally, the heart and liver grow more than the embryo, because of the important role of these organs during early organogenesis. Therefore, both heart and liver form a prominent bulge in the thoracic and abdominal cavity, respectively. The beginning of separation of the digits in the forelimb was observed in embryos with CRL = 0.5 (Fig. 1c). The two more developed embryos from Group II (1.0–1.4 cm CRL) possessed a pigmented retina, elongated nose and an easily observable otic vesicle. The forelimb (with five digits) and the hindlimb (with four digits) were considerably enlarged (Fig. 2a). All digits were separated and the tail was elongated. The cephalic, scapular, abdominal and femoral

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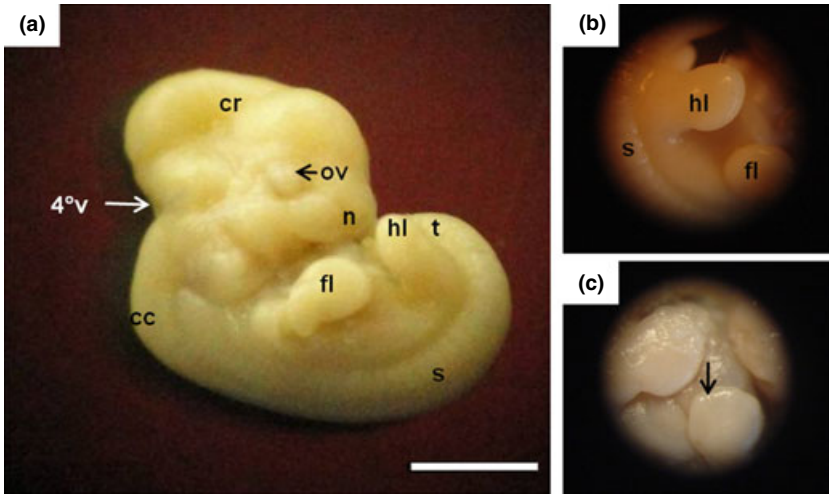

Fig. 1. Macroscopic view of embryo with 0.4 cm of crown-rump-length. In (a) observe: cephalic region (cr), forth ventricle (4v), nose (n), optic vesicle (ov), cervical curvature (cc), somites (s), forelimb (fl), hindlimb (hl), and tail (t). Scale Bar: 0.1 cm. Note in (b) the similar characteristics between hindlimb (hl) and forelimb (fl). In (c) Begin of the forelimb extremities differentiation in separated fingers.

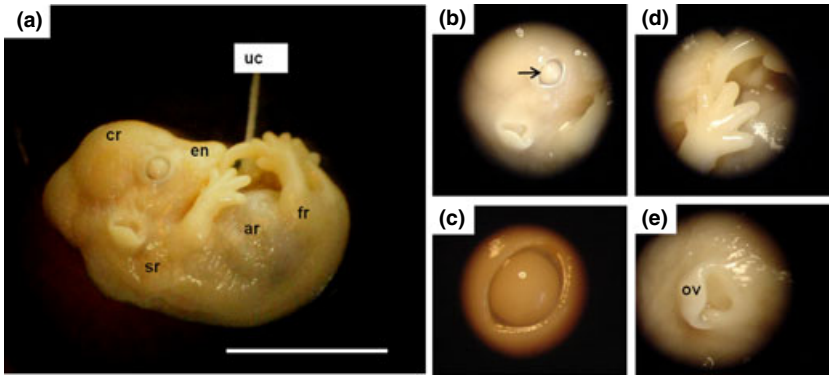

Fig. 2. Macroscopic view of embryo with 1.0 cm of crown-rump-length. In (a) note the regions: cephalic (cr), scapular (sr), abdominal (ar), femoral (fr), the elongated nose (en) and the umbilical cord (uc). Scale Bar: 0.5 cm. In (b–e) pigmented retina (arrow), eye, hindlimb separated in five fingers, and detail of the otic vesicle, respectively.

regions were easily recognised (Fig. 2a–e). The heart and liver were not prominent as in individuals from Group I.

In contrast, the individuals of the Group III (CRL 1.8–2.2 cm) were considered fetuses, because they were in advanced stage of growth (gestational age) and large body regions, the head, limb and tail were clearly visible (Fig. 3a). The nose was elongated showing the characteristic shape of the adult individual (Fig. 3b). Numerous sensory hairs were present in the skin. The limb completed its development together with its tegument and claw (Fig. 3c,e). The genital tubercle was observed in the inguinal region (Fig. 3d).

Microscopic analysis

Figure 4 shows overview of embryonic and fetal development in the three groups (Groups I, II and III), respectively. The main gross anatomical and microscopic characteristics during the development of *Oligoryzomys* sp. can be observed in the Table 2. In addition, details on the systems were given below.

Nervous system

Three brain vesicles were present in the embryos of the Groups I and II, as follows: prosencephalon, mesencephalon and rhombencephalon (Fig. 4a,b). The pituitary gland was connected to the hypothalamus by a stalk, the infundibulum, and it was located in a bone cavity of the sphenoid bone, the sella turcica (Fig. 5a). The choroid plexus was present in the embryos of 14.5 days of gestation (Fig. 5b). Morphologically, the choroid plexus was formed by vascularised folds from the pia mater and covered by an ependymal epithelium.

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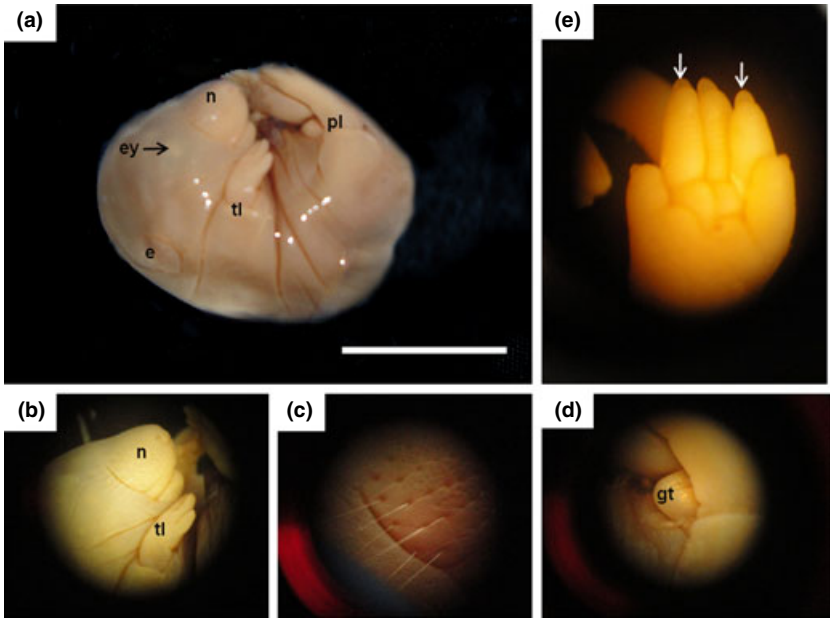

Fig. 3. Macroscopic view of fetus with 1.8 cm of crown-rump-length. In (a) observe: nose (n), eye vesicle (ey), ear (e), and thoracic (tl) and pelvic limbs (pl). Scale Barr: 1 cm. In (b–e): detail of nose (n) and thoracic limb (tl), sensorial hair, genital tubercle (gt), and claw (arrows), respectively.

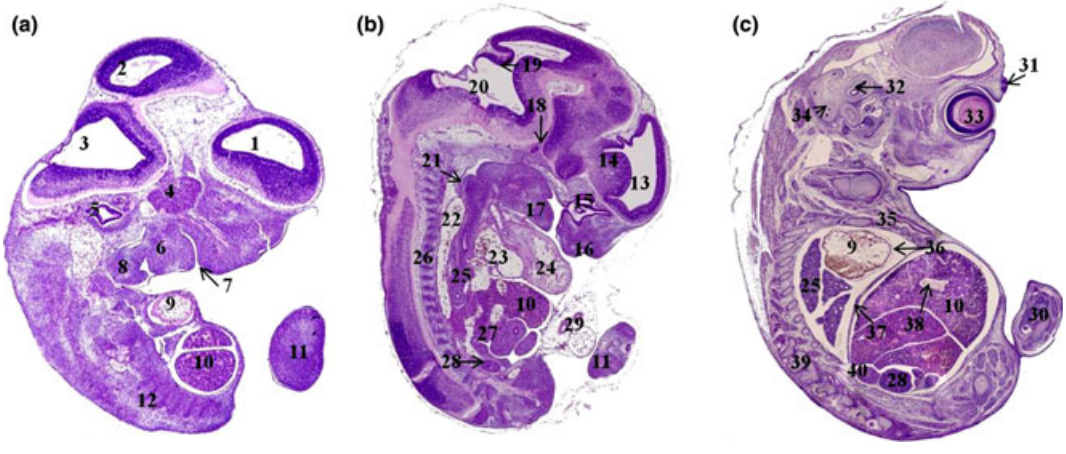

Fig. 4. Chronologic view of the embryo and fetal development from Groups I, II, and III, with 0.4, 1.0, and 1.8 cm of crown-rump-length (a–c), respectively. Observe: 1 – prosencephalon, 2 – mesencephalon, 3 – rhombencephalon, 4 – ganglium gasseri, 5 – ductus cochlearis, 6 – tongue, 7 – oral cavity, 8 – hindlimb, 9 – heart, 10 – liver, 11 – tail, 12 – somites, 13 – ventriculus lateralis, 14 – colliculus ganglionaris, 15 – septum nasi, 16 – nose, 17 – intrinsic muscle of the tongue, 18 – pituitary gland, 19 – cerebellum, 20 – forth ventricle, 21 – oesophagus, 22 – dorsal aorta, 23 – atrium, 24 – ventricle, 25 – lung, 26 – spinal cord, 27 – foregut, 28 – primitive gonad, 29 – umbilical cord, 30 – forelimb/cartilage of the fingers, 31 – upper lid, 32 – ductus cochlearis, 33 – eye, 34 – trigemial ganglia, 35 – sternum, 36 – pericardium, 37 – diaphragn, 38 – central vein, 39 – vertebrae, and 40 – kidney. Staining using haematoxylin and eosin. There are not relationship amount the size of the figures. They only represent a chronologic development of the mean structures in each Group.

The somites were already present in embryos belonging to Group I. As a temporary structures present in embryos, they derived from the paraxial mesoderm, followed by continuous process of differentiation and epithelialisation. The specimens of Group II developed a round shape (Fig. 5c). In the fetuses of Group III, the somites have been developed into vertebral bodies, constituted by cartilaginous tissue (Fig. 5d). The spinal cords (Fig. 5e) were tubular in structure, connected to the brain and extended until the last coccygeal vertebrae.

Arising from the optic vesicle (Group I, see Fig. 1a), the eyes have growth in embryos of Group II (Figs 2a–c

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Table 2. Development of the main characteristics of the body regions and organs observed in embryos and fetuses of *Oligoryzomys* sp. (Cricetidae Sigmodontinae) during the gestation

	Group I	Group II	Group III
Gross morphology	Initial development of the cephalic region Open anterior neuropore 4th ventricle Short nose Optic vesicle Somites Fore limb and hind limb buds Tail bud Pronounced heart and liver	Pigmented retina Elongated nose Otic vesicle Forelimb and hind limb enlarged Separated digits Elongated tail Scapular, abdominal and femoral region developed	Full development of the main body regions (head, thoracic and abdominal) Pronounced claw Sensory hair in the nasal region Developed genital tubercle in the inguinal region
Microscopic analysis	Brain vesicles (prosencephalon, mesencephalon and rhombencephalon) Pituitary gland Ganglium gasseri Elongated somites Optic vesicle Ductus cochlearis One atrial and one ventricular chambers Initial lungs like short tubes Thymus Tongue and submandibular gland Non-organised arrangement of the hepatoblasts Mesonephros	Choroid plexus Ventriculus lateralis Colliculus ganglionaris Cerebellum Somites with rounded shape Developed eyes and related structures (lens, vitreous humour, hyloid cavity, retina, cornea and conjunctival sac) Two atrial and two ventricular chambers Dorsal aorta and aortic valve Lung bifurcated into buds with pseudoglandular shape Oesophagus Foregut Small and large intestine Definitive kidney	Upper lid Lacrimal gland Trigeminal ganglia Increased number of bronchus and bronchioles Lung in canalisation phase divided in the main bronchi Developed trachea Vertebral bodies Definitive kidney Primitive gonad

and 4b) extending from each side of the prosencephalon and being in contact with the superficial ectoderm. The optic vesicle induces the lens formation. A characteristic epithelium was observed in the lens. The vitreous humour was observed occupying the hyloid cavity (between the retina and lens). A conjunctival sac was also present. The cornea was viewed as the outer membrane of the eyes. It was derived from condensations of mesenchyme and showed an avascular structure with a simple squamous epithelium, a thick basal membrane, stroma, posterior limiting lined by an epithelial layer, also called corneal endothelium (Fig. 5f–h). The lacrimal gland was identified with a glandular parenchyma organised in small lobules separated by septa of connective tissue (Fig. 5i).

Cardiorespiratory system

In embryos from Group I, the heart has one atrial and one ventricular chamber (Fig. 6a). The cardiac apex was directed to the ventral portion, the atrial chamber was in a craniodorsal position to the cranial region, and the ventricular chamber was directed to outside of the embryo, ventrocaudally to the atrium.

In the embryos and fetuses of Groups II and III, the division in two atrial and two ventricular chambers has been observed. The dorsal aorta and the aortic valve can also be identified (Fig. 6b,d). Three cardiac layers were present as follows: the pericardium, the myocardium and the endocardium, including the cardiomyoblasts and cardiomyocytes (Fig. 6c).

Initial lung development took place in the embryos of Group I. The lungs resembled short tubes. This respiratory diverticulum appears ventrally to the caudal portion of the foregut. During further development, as viewed in the embryos of Group II, they have been bifurcated into buds with pseudoglandular format with few number of bronchus into the parenchyma (Fig. 6e). A thin membrane, that is, mediastinum, was formed by mesenchymal tissue and was separating the lung in development (Fig. 6f). In embryos and fetuses of the Groups II and III, respectively, an increased number of bronchus and bronchioles aroused, indicating that the lungs were in the canalisation phase (Fig. 6g). The bronchioles showed a simple columnar epithelium that was partly ciliated (Fig. 6h). In the fetuses of Group III, the lungs were divided into the main bronchi. In this group, the subsequent alveolar phase was

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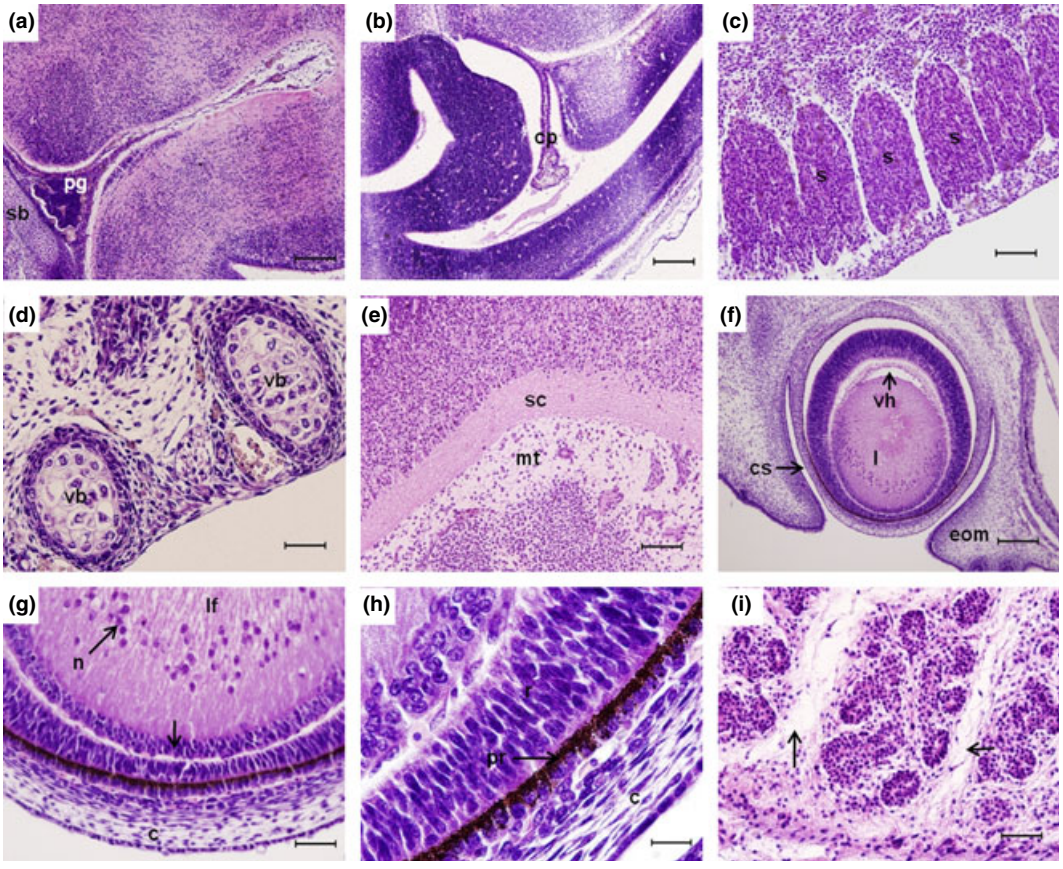

Fig. 5. Light microscopy of the nervous system in *Oligoryzomys* sp. (Rodentia, Cricetidae, Sigmodontinae). In (a) pituitary gland (pg) located in a bone cavity – sella turcica of the sphenoid bone (sb). In (b) choroid plexus (cp). Group II, Barr = 200 μm. In (c, d): development of the somites (s) in embryos from Group I and vertebrae bone (vb) in Group II. Bar = 50 and 100 μm, respectively. In (e) spinal cord (sc) surrounded by mesenchymal tissue (mt). Group I, Barr = 100 μm. In (f–h) development of the eye in embryos from Group II. Vitreous humor (vh), lens (l), conjunctival sac (cs), eye orbicularis muscle (eom), cornea (c), lens fiber (lf) and its nucleus (n), arrow (simple squamous epithelium), retina (r), and pigmented layer of the retina (pr). Barrs: 200, 50, and 20 μm, respectively. In (i): lacrimal gland, separated in lobules by connective tissue (arrows). Barr = 50 μm. Staining by haematoxylin and eosin.

observed. The lung parenchyma expanded and the smooth-walled saccules were observed. Later, the saccules transformed into alveolar sacs with alveoli, and the lung showed an adult appearance (Fig. 6g).

In embryos of Group II, a trachea with its tracheal rings of cartilaginous tissue with ciliated pseudo-stratified epithelium was presented. More pronounced development of the trachea occurred in the fetuses of the Group III (Fig. 6i).

Intestinal tract

In embryos from Group I, the thymus was identified in the cranial mediastinum in the neck region. The embryological origin of this important lymphatic organ results from the development of pharyngeal pouches derived from the foregut. It possessed numerous lobes, subdivided in two zones: outer cortical zone and inner medullar zone (Fig. 7a). In the parenchyma, epithelial reticular cells were present (Fig. 7b). In the fetuses from Group III, the thymus was in a maturation process. This process starts in the cortical zone, which became thin in shape.

The oral cavity in the early embryos of Group I was covered by a non-keratinised stratified squamous epithelium (Fig. 8a,b). In this region, the intrinsic muscles of the tongue and the submandibular gland arose (Fig. 8c). The foregut showed tubular sections with columnar absorptive epithelium covered by mesenchyme (Fig. 8d,e). Whereas in the early stages no differentiation between the small and large intestine was possible, this differentiation was visible in older embryos and fetuses (Groups II and III). In the small intestine, villi were present (Fig. 8f,g), and the large intestine was characterised by folds (Fig. 8h). In these stages, similar to the adult condition, the following layers inside the intestinal tract were observed: mucosa, lamina propria, muscularis and serosa (Fig. 8i).

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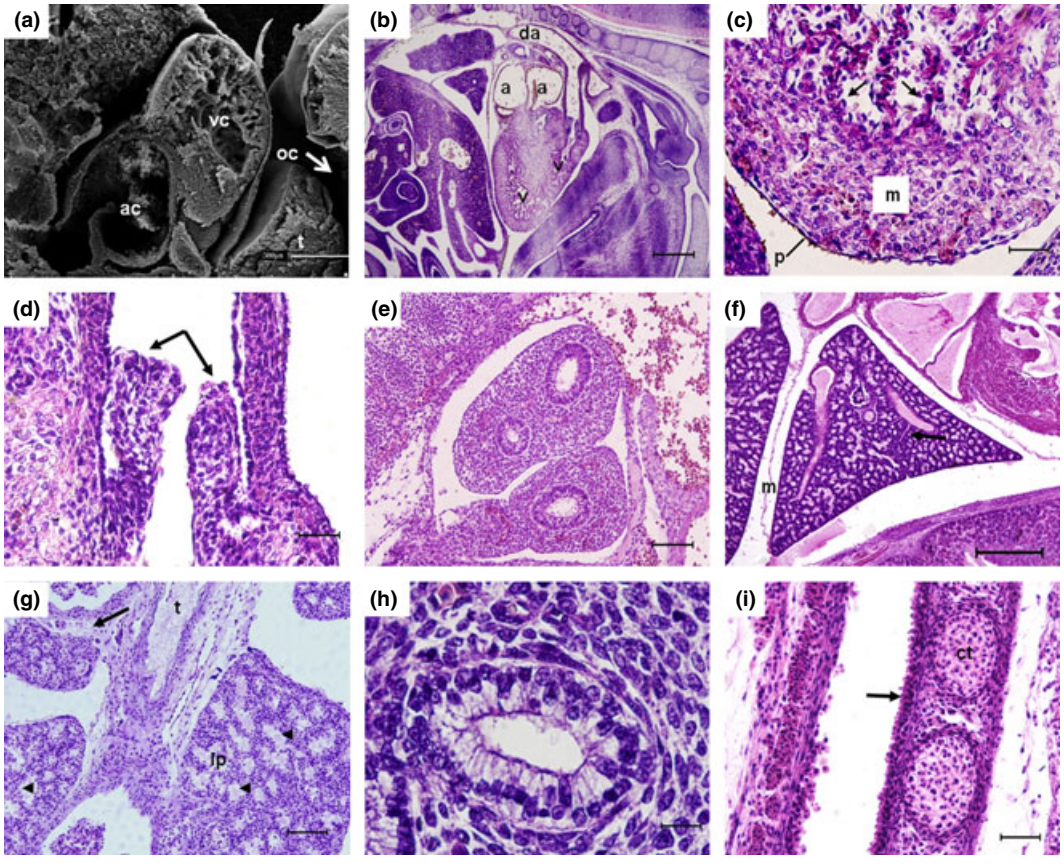

Fig. 6. Light and scanning electron microscopy of the cardiorespiratory system in *Oligoryzomys* sp. (Rodentia, Cricetidae, Sigmodontinae). In (a) septation of the heart in one atrial (ac) and one ventricular chambers (vc). Note the oral cavity (oc) and tongue (t). Group III, Barr = 300 μm. In (b) heart in later stage when observed both right and left atrium (a) and ventricle (v). Note the dorsal aorta (da). In (c) Detail of the cardiac layers: pericardium (p), myocardium (m), and endocardium (arrows). In (d) High magnification of the aortic valve (arrows). In (e) early development of the lung into buds. In (f) pseudoglandular phase of the lung. Observe the mediastinum (m) like a thin membrane separating the lung in development and note the bronchioles (arrow). Group I, Barrs: 100 and 500 μm, respectively. In (g) lung on canalization phase. Observe the aspect of the lung parenchyma (lp), primary bronchus (arrow), and trachea (t). In (h) note the simple columnar epithelium of the bronchiole. In (i) detail of the cartilaginous tracheal rings (ct) and the ciliated pseudo-stratified epithelium (arrow). Group II, Barrs = 200, 20, and 50 μm, respectively. Staining by haematoxylin and eosin.

The liver was the most prominent organ in the abdominal cavity of the embryos. Histologically, in the Group I, the hepatoblasts have a non-organised arrangement inside the hepatic parenchyma. These cells were surrounded by a number of erythroblasts (Fig. 9a). The liver of the specimens from Groups II and III revealed the glandular parenchyma organised and the development of the central vein (Fig. 9b,c). The glandular appearance of the hepatic parenchyma was elucidated by scanning electron microscopy (Fig. 9d).

Urogenital system

The development of the kidney is usually characterised by the following three phases: pronephros, mesonephros and metanephros. Owing to the advanced stage of development of the embryos from Group I, the identification of pronephros phase was not possible. The mesonephros occurred in embryos from Group I (Fig. 10a); they exhibited an elongated shape and were composed of numerous tubular formations with both proximal and distal convoluted tubules (Fig. 10c) that were surrounded by mesenchymal tissue. The mesonephros was already in a phase of degeneration, which was indicated by signs of apoptosis and replacement of mesonephric tissue. In the embryos and fetuses of Groups II and III, the definitive kidney or metanephros was present. It had a spherical shape and two distinct regions, the cortex and the medulla (Fig. 10b).

Medial to the kidney, the fetuses of Group I developed an associated organ, similar in structure to the kidney, the primitive gonad. It was in an undifferentiated stage

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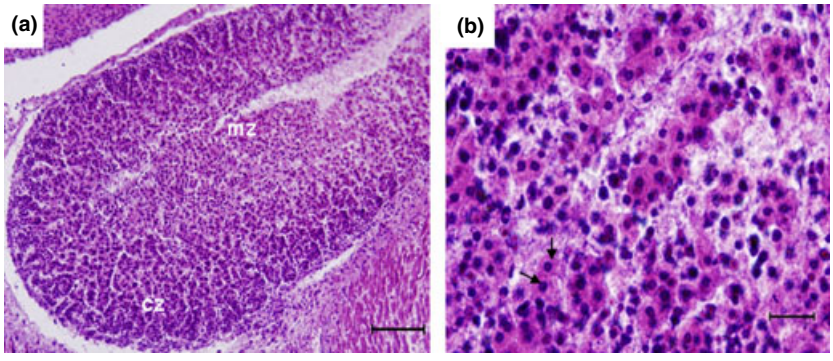

Fig. 7. Light microscopy of the thymus. In (a) cortical (cz) and medullary zones (mz). In (b) detail of the epithelial reticular cells into the parenchyma (arrows). Samples collected from Group II. Bars: 100 and 20 μm, respectively. Staining by haematoxylin and eosin.

which does not allow to differentiate the individuals as male or female (Fig. 10e). At this stage, the primitive gonad was composed of glandular tissue with numerous tubules inside. These tubular formations were identified as testicular cords (Fig. 10f).

Discussion

General features

The understanding of embryo development of many species lacks many details (Cademartori et al. (2004, 2005) and Bonvicino et al. (2008)). The sparse information regarding morphological changes that occur during the pregnancy can result in a wrong understanding of the reproductive biology of specific species.

Several continuous changes are correlated with the embryogenesis process (Knospe, 2002; Beaudoin et al., 2003; Hyttel et al., 2010). Despite similarities, described in the literature, there are some vital systems that are useful to classify the development phase of the embryo.

Felipe et al. (2006) described the external morphology of fetuses of *Myocastor coypus*. The authors reported that the Nomina embryological (1994) establishes 15 stages to the embryo development, while the system Cornegie appoints 23 stages, and the Theiler system (1972) and Dyban et al. (1975) provide 27 stages for rats, murine rodents and laboratory rabbits, respectively. We believe that these quantitative variations in the number of stages occur because of the use of different species that possesses different periods of gestation. As result of this statement, the quick appearance and disappearance of important morphological structures correlated with the different gestation periods of different species could interfere with the variation in the number of stages mentioned by these authors.

Evans and Sack (1973) studied the prenatal development of several mammalian species. The authors described the morphological characteristics of mouse embryos from day 8.5 of gestation to the 22nd day (delivery day). These data were used in a comparative way to estimate the age of embryos and fetuses in our study using *Oligoryzomys* sp. as a model.

Because of the similar gestational period for the rat (22 days) described by Evans and Sack (1973) in comparison with 23–24 days for *Oligoryzomys*, we used this data as a reference to carry on our analysis. One example was observed in specimens with 12 days of gestation where the buds of forelimb and hindlimb, the optic vesicle and tail were visible similar to that described by Evans and Sack (1973) in rats with 11 days of gestation. These data gave support to compare both studies.

Nervous system

The nervous system has its origin in the neural plate (ectoderm) of the embryonic disc. The latter parts of the nueral tubes to remain open are the anterior and posterior neuropore, which close in the rat around days 10.5–11.0 of the development, in mice 9.0–9.5 days, in rabbit 9.5–10.5 days, hamster 8.25–8.5 days and in the guinea pig 15.25–16.5 days (Monie, 1976), respectively. In *Oligoryzomys*, the anterior neuropore closes around the day 14.5 of gestation, similar to what is known to the rat.

As shown in the results chapter, in embryos of *Oligoryzomys* sp. with 12 and 12.5 days of gestation, three brain vesicles were identified: the prosencephalon, mesencephalon and rhombencephalon. Their embryologic origin is related to the fusion process of the neural folds in the anterior region and closure of the anterior neuropore (Hyttel et al., 2010). The prosencephalon gives rise to the telencephalon and diencephalon. The mesencephalon and rhombencephalon divide into the metencephalon and myelencephalon (Hoar and Monie, 1981), respectively. Beaudoin et al. (2003) showed in their results a comparison of the embryonic developmental stages in rabbits and

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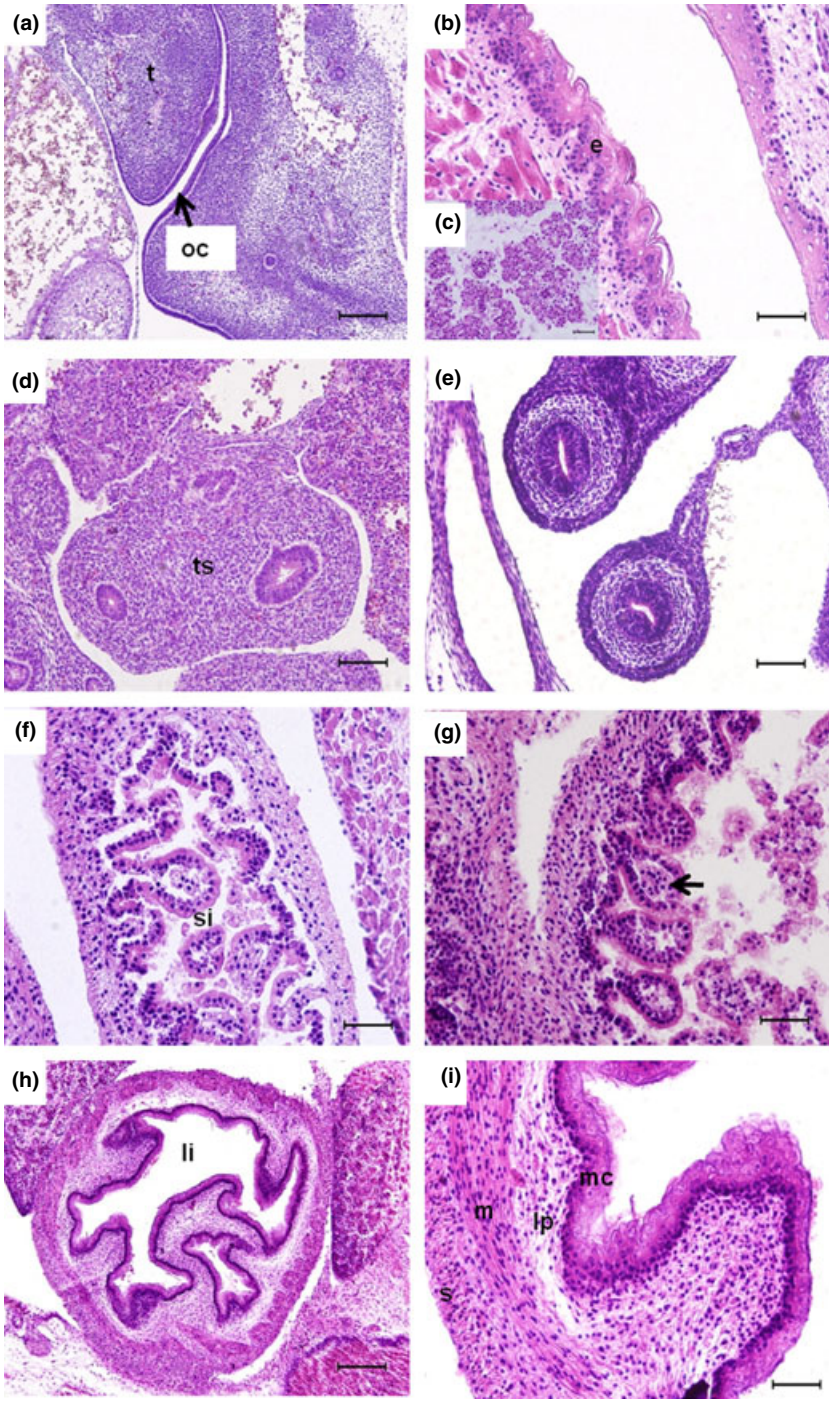

Fig. 8. Light microscopy of the intestinal tract. In (a, b) oral cavity (oc) covered by a non-keratinized squamous epithelium (e). Note the intrinsic muscle of the tongue (t). Group I, Barrs: 200 and 50 μm, respectively. In (c) submandibular gland. Group II, Barr: 50 μm. In (d, e) tubular sections (ts) of the foregut with columnar absorptive epithelium covered by mesenchymal tissue. Group I, Barrs: 100 μm. In (f, g) detail of the villi (arrow) from small intestine (si). In (h, i) large intestine (li) and high magnification of the layers of the intestinal tract: mucosa (mc), lamina propria (lp), muscularis (m), and serosa (s). Group III, Barrs = 50, 50, 200, and 50 μm, respectively. Staining by haematoxylin and eosin.

humans during early stages, but the authors did not show data about the neural growth, and only described the gross morphology of the embryos.

According to Theiler (1972) and Junqueira and Carneiro (2008), the pituitary gland showed a quick development that showed two different embryological origins:

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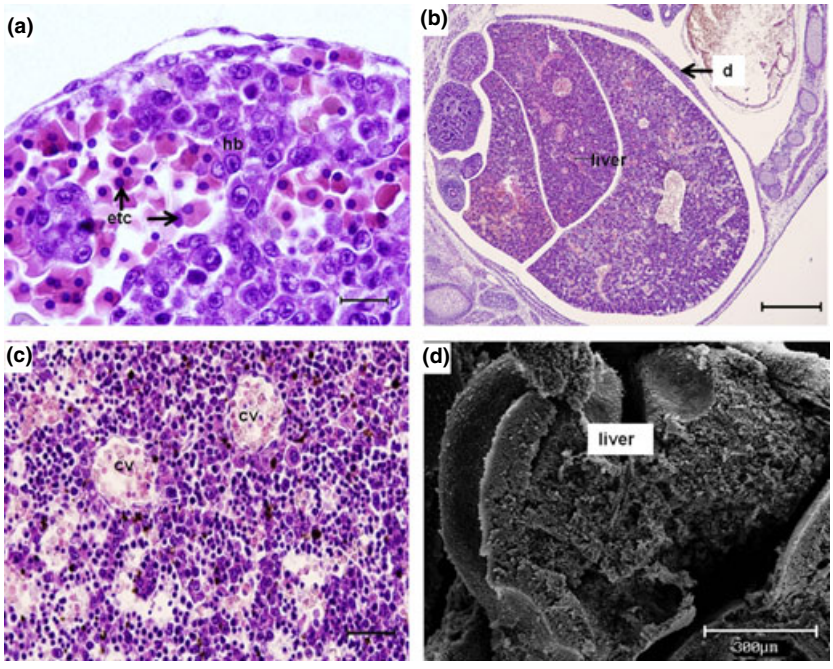

Fig. 9. Light and scanning electron microscopy of the liver. In (a) non-organized arrangement of the hepatoblasts (hb), which were surrounded by erythroblasts (etc). Group I, Bar: 20 μm. In (b–d) overview of the glandular parenchyma limited in the abdominal cavity by the diaphragm (d). Observe the organized parenchyma and the central vein (cv). Group II, Bars: 200, 50, and 300 μm. Staining by haematoxylin and eosin.

nervous and ectodermal. In our histological slides, we found the pituitary gland completely formed in embryos of *Oligoryzomys* with estimated age from 12.5 days. These results related to the quick development of this endocrine gland are in accordance with the descriptions of the authors. The choroid plexus was also observed in embryos with 14.5 days of gestation, as mentioned by Kaufmann (2008) for rats with 15.5 days of gestation.

The somites arise by the paraxial mesoderm that divides in couples of cuboids structures. In *Oligoryzomys*, the somites are located on each side of the neural tube in a cephalocaudal direction. We compare the number of somites (35–40 somites) observed in embryos of *Oligoryzomys* with 12 days of gestation with the available data for mouse according to Theiler (1972), and we observed that these characteristics (age, size and number of somites) are very similar between both species. We observed around the day 14.5 three distinct regions in the somites, myotome (muscle tissue), sclerotome (cartilage and bone) and dermatome (subcutaneous tissue). These characteristics were similar to the descriptions of Kaufmann (2008) for mouse embryos. Initially, we note that the somites were elongated in shape. During its development, in embryos and fetuses with more advanced stages of pregnancy (Groups II and III), these structures became rounded in shape.

Hoar and Monie (1981) studied the mice development, and these authors demonstrated that the embryonic optic vesicle arises from the diencephalon, and the cornea, sclera and choroid arise from condensed mesenchymal tissue. In *Oligoryzomys* with estimated age of 14.5 days were identified some components of the eyes: the lens, cornea, retina (and their respectively layers). Near to the eyes was possible to identify the lacrimal gland in embryos and fetuses with estimated age of 14.5 days of gestation. The literature showed controversial data about its origin (Cuadra-Blanco et al., 2003). Recently, several authors studying the embryology of the lacrimal gland (Lovicu et al., 1999; Wahl and Noden, 2000) confirmed the origin of the lacrimal gland from the surface of the ectoderm and not from the cells deriving from the neural crest as first described by Tripathi and Tripathi (1990).

According to Theiler (1972), embryos of mouse with 14 days of gestation show numerous hair follicles on the skin, except in the head region. In our study, we observed the sensorial hair follicles and sebaceous glands in fetuses of 17 days of gestation, which were in the nasal region. Before this age, hair follicles were not observed, differently of the descriptions for mouse embryos (Theiler, 1972).

Cardiorespiratory system

The survival of the neonate depends on hard maturity of the respiratory apparatus. The structural development of

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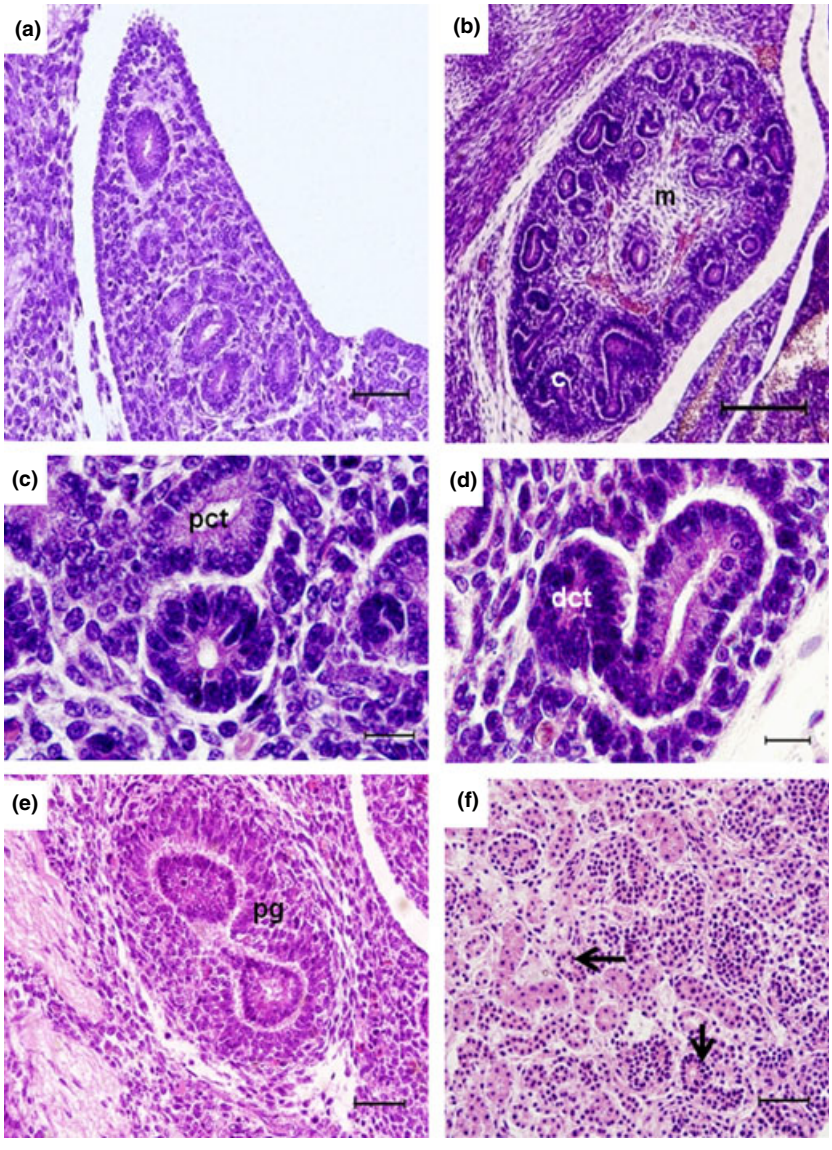

Fig. 10. Light microscopy of the urogenital system. In (a) mesonephros with several tubular formations. Group I, Bars: 50 μm. In (b–d) metanephros divided in cortical (c) and medullary (m) regions. Observe the proximal (pct) and distal convoluted tubules (dct). Group II, Bars: 200, 20, and 20 μm. In (e) primitive gonad (pg). Group I, Bar: 50 μm. In (f) testicular cords (arrows). Group III, Bar: 50 μm. Staining by haematoxylin and eosin.

the mammalian lung is similar in all species of mammals studied (Tschanz, 2007 and Mess and Ferner, 2010). Its development follows several phases, which mainly are glandular, canalisation and vascularisation phases. During the analysis of the development of the lung in *Oligoryzomys* were also observed the saccular and alveolar phases.

In the hamster, the first respiratory rudiment appears on day 9 of gestation and in the guinea pig on day 17.5 (Monie, 1976). In our analysis, we observed that embryos from Group I (12 days of gestation) showed the lung bud with pseudoglandular appearance and showed inside the parenchyma little number of bronchi. In Group II (14.5 days of gestation), there was a considerable increase in bronchi, characterising the canalicular phase. In the fetuses from Group III, the lung was completely formed. These characteristics were in accordance with descriptions of Theiler (1972) using rats as experimental model and Monie (1976) and Hoar and Monie (1981) using hamsters.

An interesting point is that the stage of lung development when mammals are born is quite variable, but not the chronology of final lung maturation. According to Mess and Ferner (2010), it always follows three consecutive steps: increase in lung volume by expansion of the

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airspaces, increase in tissue mass because of alveolisation and microvascular maturation.

The heart and its respective structures are formed from the splanchnic mesoderm. This is the first organ to start its functions in the embryo. According to Theiler (1972) with 9 days of gestation, the heart of mice is able to maintain some blood flow. However, the atrium and ventricle are not formed yet. The developing heart becomes divided by continuous process, in which the different septa develop (Hyttel et al., 2010). When we analysed embryos of *Oligoryzomys* with 12 days of gestation were observed two chambers, an atrium and a ventricle. In embryos and fetuses from Groups II and III, a division into two atrial and two ventricular chambers was found, thus being comparable to descriptions for embryos of mice (Theiler, 1972).

According to Monie (1976) in embryos of hamsters and guinea pig, the complete cardiac septation occurs around the 11th day of gestation, similar to *Oligoryzomys* in which septation occurs around 13th day of gestation. In our studies, we observed that the ventricular cavity has many cardiomyoblasts that arranged in cords. At this early stage and surrounding the coelomic cavity was identified a thin layer of mesenchymal tissue, which will differentiate in the pericardium. The intra-embryonic coelom in this early stage will be divided into three different cavities: peritoneal, pleural and pericardial (Hyttel et al., 2010). According to Sadler (2005), the dorsal aorta has a bilateral embryonic origin. In some histological sections, the dorsal aorta was observed, as mentioned by the author but in an advanced stage of development, which is formed by a single structure after the fusion of both dorsal and ventral portions, resulting in a single aorta.

Intestinal tract

According to Sadler (2005), the liver arises from the endodermal layer. In embryos from Group I, the liver was the biggest organ inside the embryo, as observed by Kaufmann (2008) for embryos of mice with same age. Histologically, an organisation of liver parenchyma was observed during the embryo development. Inside the glandular parenchyma were identified hepatoblasts, typical cells of the liver as cited by Junqueira and Carneiro (2008). In the Groups II and III was observed a typical organisation of the hepatoblasts in the glandular parenchyma. The cellular arrangement in cords and the central position of the veins and ducts were also seen in embryos of *Oligoryzomys* of the Groups II and III.

In our histology analysis, embryos from Group I just displayed a primitive intestinal loop. In fetuses from Group III were observed both small and large intestines. In the small intestine was observed the development of the intestinal villi. In contrast, the large intestine showed coarser tissue formations, that is, folds. In both regions were identified the typical layers of these organs: mucosa, lamina propria, muscularis and serosa. The embryological development of the intestines in rodents has not been studied yet; however, it represents an important point of studies due to the fact that several organs arise from the intestine tract.

Another important organ identified in our study that was present mainly in the embryonic and fetal life was the thymus. This organ showed a double embryological origin. The lymphocytes arise from mesenchymal cells and invade an epithelial sketch formed from the endoderm of both third and fourth pharyngeal pouches (Theiler, 1972; Hyttel et al., 2010). In embryos from Group I, the thymus was observed with several lobes with cortical and peripheral layers. In addition, reticular epithelial cells were found inside the parenchyma. Similar data were described by Theiler (1972) who also mentioned that the thymus and the thyroid are easily recognizable in the mouse embryo with 14 days of gestation.

Urogenital system

The pronephros phase was not observed in embryos from Group I. Probably, this phase is usually visualised in young embryos (less than CRL 0.4 cm). The mesonephros phase was characterised by an elongated organ showing tubular formations and can be differentiated in proximal and distal convoluted tubules. In embryos from Groups II and III, we identified a round shaped metanephros. In this phase, the parenchyma was divided in cortex and medulla.

Each stage of the kidney development (pronephros, mesonephros and metanephros) showed a specific location in the embryo and a specific characteristic of the tissue arrangement (Junqueira and Carneiro, 2008; Hyttel et al., 2010).

The primitive gonad or also namely gonadal ridge was observed in embryos from Group I. During its initial development, the primitive gonad consisted of a mesenchymal and little specialised tissue. Later, we observed that the mesenchymal tissue was differentiated in gonadal cords. According to Hyttel et al. (2010), although the sex of mammalian embryos is determined genetically at the time of fertilisation, when a Y or X chromosome-bearing spermatozoon fuses with the oocyte, the differentiation of the testis occurs under the influence of a specific gene, namely Sry (a member of the Sox family of transcription factors). Without the expression of the products of this gene, the indifferent gonad develops later into an ovary. Kaufmann (2008) using mice at 13.5 days showed that sexual differentiation in males was identified by testis cords and in females by cortical cords.

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Final considerations

Researches that involve disorders during human development are often forced to work with experimental model. Furthermore, many techniques using embryology, including the production of chimaeric animals and stem cells, were pioneered using small rodents as animal model; therefore, the seek for new species becomes necessary because science, biotechnology and health science need new solutions to understand the morphogenesis. Comparative analysis of the embryology in *Oligoryzomys* with equivalent stages of development in other rodents will be helpful to establish similarities and differences that would be able to give support for a better understanding of the morphogenesis and organogenesis in these species.

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