

Characterization of Embryonic and Fetal Development of *Necromys Lasiurus* (Rodentia, Cricetidae)

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Rec date: Mar 20, 2014, Acc date: Apr 23, 2014, Pub date: Apr 25, 2014

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Abstract

Background: *Necromys lasiurus* belongs to a group that includes New World rats and mice. The few studies concerning *N. lasiurus* consider this species to be a good experimental model because they adapt easily and because maintenance in captivity is low-cost. Moreover, Cricetidae are epidemiologically important as vectors of various diseases. The aim of the present study was to describe the macroscopic and microscopic features of embryonic and fetal development in this species.

Methods: Totally, 8 embryos and 12 fetuses were used and allocated in 6 groups. Gestational age was determined by the crown-rump length (CR) and weight. After the gross descriptions, samples were processed for light microscopy.

Results: The groups differed both macroscopically (due the characteristics related to the cervical curve, elongation of limbs and appearance of digits, closing of the auditory canal, blood vessels, elongation of the cranium, appearance of eyelids, skin thickness and coloration) and microscopically (the development of structures that constitute the facial region - mandibular, maxillary and nasal prominences). The structures preceding the appearance of the pituitary, lung, heart, brain cavity, fourth ventricle, liver development, the oral cavity, and layers that make up the cornea, retina, pigmented layer of the retina, and the lens were observed. Ossification of some regions was also observed.

Conclusion: The results showed that embryonic stage is present until day 15 of gestation. After this, during the fetal life the individuals showed a quickly development and maturation of the internal organs. These morphological results in regard to the development of *N. lasiurus*, can be used as a standard for interpretation of experiments that use this species as a model in science, including too compared with pathological results in disturbed gestations that can affect the normal development.

Keywords: Embryology; Sigmodontinae; Rodent; Animal models

Introduction

In science, rodents are one of the most important groups of experimental animals and have contributed significantly to the advancement of many areas of knowledge and health. The contributions of rodents range from the interpretation of the effects caused by drugs in the body and drug testing [1], to advances in molecular and genetic knowledge [2], to the development of knockout models for various genes and diseases [3]. Currently, knowledge of the embryotoxic effects of drugs and environmental factors such as pollution on embryonic and fetal development has gained special attention with the use of murine models, which allowed the elucidation of factors such as placental changes that lead to disturbances in the development of the uterus during pregnancy and consequently to changes in the normal pattern of fetal growth [4-6].

Although rodents are the most abundant group within the class Mammalia, with over 2000 species [7], few of these species have been studied with respect to embryonic and fetal development. This situation may pose a barrier to the advancement of knowledge,

especially for those species that could potentially be used as experimental models, including those belonging to the families Cricetidae and Murinae. These two families are the most abundant of the order Rodentia. Published data on embryology and organogenesis can be found for these groups [8-12], as well as for histricomorph rodents [13-17]; and related species such as the rabbit (*Oryctolagus cuniculus*) [18-21].

In the Americas, the rodent fauna are characterized by the abundance of rodents from the family Cricetidae-Sigmodontinae, which includes the so-called New World rats and mice [7,22-24]. Especially, *Necromys lasiurus* has a great importance for public health because it is a vector of several diseases (that are not transmitted by murine rodents), and may affect humans [7,25-27]. In addition, some of these diseases can also disturb the normal embryo development. Given the paucity of data on this species, especially with respect to embryonic and fetal development, the aim of the present study was to describe the gross and microscopic morphology of embryos and fetuses of this small rodent of 60 g body mass and gestational period of 23 days [10], from day 14 to 20.5 of gestation in order to standardize data on organogenesis in this species. Such data can be used

comparatively in experimental studies involving this and other related species, even to use the results obtained here in pathological analysis.

Materials and Methods

Animals

Totally, 8 *N. lasiurus* embryos and 12 fetuses were obtained from the Wild Animal Breeding Center (Centro de Multiplicacao de Animais Silvestres – CEMAS) of the Federal Rural University of the Semi-Arid, Mossoró–RN (IBAMA Number 14.492.004). The specimens were collected from July 2011 to July 2012 and were already fixed in 4% paraformaldehyde or 2.5% glutaraldehyde because they were derived from pregnant females used in other studies conducted in the Department of Domestic and Wild Animal Anatomy, School of Veterinary Medicine and Animal Science of the University of Sao Paulo [10,28-30]. The use of these experimental animals was duly approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Science, University of Sao Paulo (Protocol number 1766/2009).

Macroscopic analysis and biometrics

Measurements of the occipital-sacral distance of the head were taken with a stainless steel caliper, taking the nuchal crest at one end and the last sacral vertebra at the opposite end as references (Crown-Rump/CR), following the methodology proposed by Evans and Sack [31]. The CR distances as well as the external features of the embryos and fetuses were used to estimate the age of each individual, which could then be allocated in groups (I, II, III, IV, V, and VI). In addition, the weights (g) of embryos and fetuses were measured on a digital scale (0.001 g, model MARTE).

The external features of the embryos and fetuses were analyzed using a magnifying glass (Zeiss Stemi SV6, Germany). Photographic documentation was performed using a SAMSUNG ST64 14 MP camera.

Processing the material for light microscopy

The fixed samples were dehydrated in a graded alcohol series (60% to 100%), cleared in xylene, and embedded in Merck Histosec paraffin [32].

Paraffin blocks were sectioned at 5 µm on an automated microtome (Leica RM 2165, Germany). Sections were stained with HE (hematoxylin/eosin). Photomicrographs were taken on a light microscope (Olympus BX40).

Results

Biometric and macroscopic descriptions of embryos and fetuses

The embryos and fetuses were allocated into 6 groups based on similarities with respect to the crown-rump measurements, weights, and external features, which were used to estimate the age of each individual (Table 1). To better clarify, the descriptions of macroscopic features for each group are listed.

Group I:

The embryos that comprised Group I exhibited CR length ranging from 9 to 11 mm, weighed 0.05 to 0.056 g and gestational age was 14 days. Regarding external features, individuals in Group I exhibited several structures in various developing body regions. In addition, the skin covering the body was thin and lean. The cephalic region was prominent; however, the external ear was short and slightly pronounced. At this stage, the optic vesicle exhibited retinal pigmentation. A pronounced cervical curvature was present at this stage of development (Figure 1A). The buds of the forelimbs and hindlimbs, still short, did not yet exhibit division of the digits at their distal ends (Figures 1A, 2A and 2B). Detailed analysis revealed that the jaw was forming, with emphasis on the nasal region, one of the first facial structures to form in the embryo along with the maxillary and mandibular prominences (Figure 1A). It was noted that the opening of the nostrils was located laterally in the nasal region. A short tail was visible (Figure 1A).

Groups	Animals	Crown-rump (mm)	Weight/g	Estimated age
1	1	9	0.05	14
	2	9	0.056	14
2	1	10	0.085	14.5
	2	10	0.111	14.5
	3	10	0.111	14.5
	4	10	0.12	14.5
	5	10	0.132	14.5
	6	11	0.146	15
3	1	16.5	0.513	16.5
	2	17.5	0.519	17
	3	18	0.59	17.5
	4	18	0.599	17.5
	5	18	0.638	17.5
4	1	20	1.09	18
	2	21	0.871	18
	3	22	0.929	18.5
5	1	25	1.09	19
	2	27	1.309	19.5
6	1	28	1.787	20
	2	30	2.154	20.5

Table 1: Biometry and estimated ages according to Evans and Sack (1973) for *Necromys lasiurus* (Rodentia, Cricetidae, Sigmodontinae) embryos and fetuses

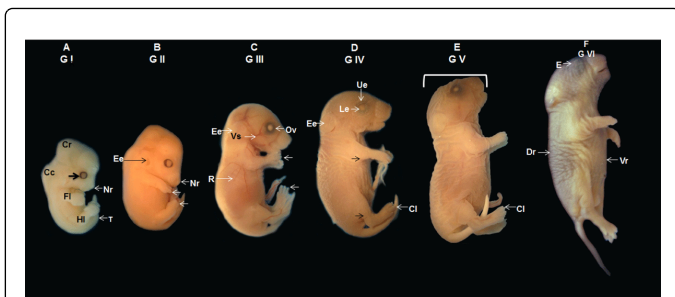


Figure 1: Macroscopic view of an embryo with a 9-m crown-rump distance. A Cephalic region (Cr), very prominent cervical curvature (Cc), pigmented optic vesicle (Arrow), and nasal region (Nr). The ends of the forelimb (Fl) and hindlimb (Hl) form the shape of a “paddle”. Note the beginning of digit differentiation and slightly pronounced tail (T). B An embryo with a CR distance of 10 mm. Note the forming external ear (Ee), ear canal covered by the pinna, longer Nasal region (Nr), and forelimbs and hindlimbs (Arrows) with differentiated extremities where deeper grooves are visible between the digits. C View of a fetus with a CR distance of 18 mm. The external ear (Ee) and pinna completely cover the ear canal. The vascularization (Vs) is visible over the fetus’ body due to little skin pigmentation. Also note the optic vesicle (Ov) and impression of the ribs (R). Fully separated digits with pronounced claws are visible at the ends of the forelimbs and hindlimbs (Arrows). D A fetus with a CR distance of 20 mm. Note the upper eyelid (Ue) and lower eyelid (Le) covering the eye, further projected external ear (Ee), longer forelimbs and hindlimbs (Arrows) with more pronounced claws (Cl) on the ends. E Fetus with a CR distance of 27 mm. Note the longer oral cavity and nasal region. F Full-term fetus with a CR distance of 30 mm. There is a marked change in skin pigmentation, with a darker dorsal region (Dr) and lighter ventral region (Vr). The open oral cavity and eyes (E) covered by the eyelids can be observed

distributed around the optic vesicle. There was greater vascularization of the skin surface over the entire body of the fetus; however, the skin was thin and remained transparent due to the lack of pigmentation, allowing the visualization of the ribs in the thoracic region (Figure 1C). The distal ends of the forelimbs and hindlimbs were well formed and had fully divided digits. The jaw and nose were formed, and vibrissae (sensory hairs) were visible in the nasal region (Figure 2D). The genital tubercle was forming, and the sex of individuals could not yet be determined.

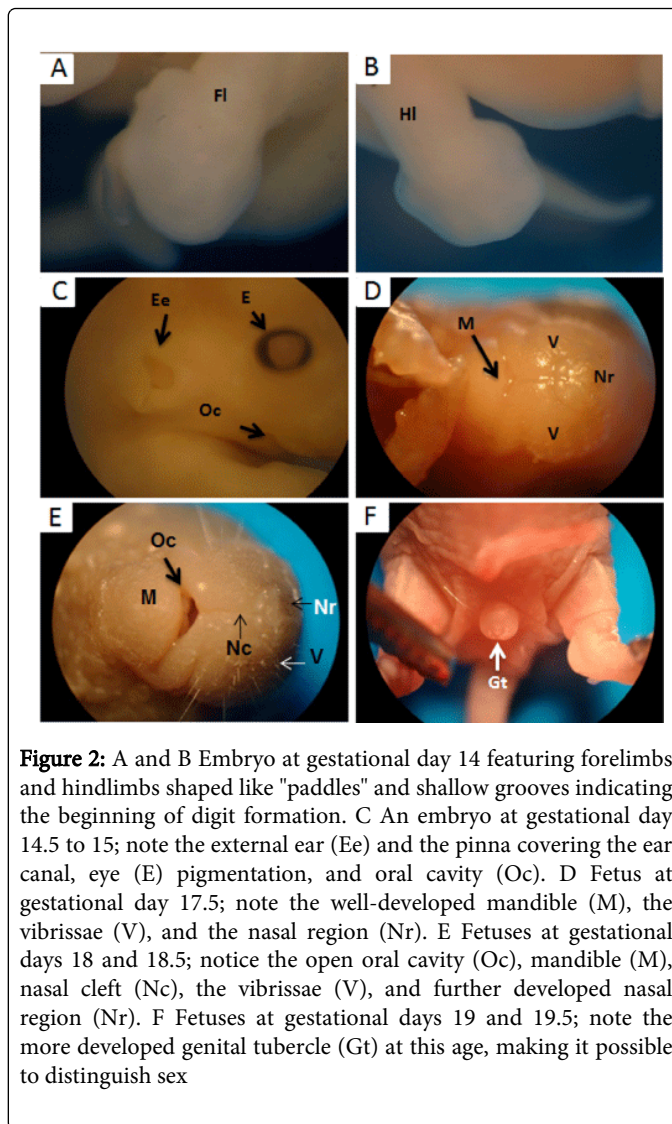


Figure 2: A and B Embryo at gestational day 14 featuring forelimbs and hindlimbs shaped like “paddles” and shallow grooves indicating the beginning of digit formation. C An embryo at gestational day 14.5 to 15; note the external ear (Ee) and the pinna covering the ear canal, eye (E) pigmentation, and oral cavity (Oc). D Fetus at gestational day 17.5; note the well-developed mandible (M), the vibrissae (V), and the nasal region (Nr). E Fetuses at gestational days 18 and 18.5; notice the open oral cavity (Oc), mandible (M), nasal cleft (Nc), the vibrissae (V), and further developed nasal region (Nr). F Fetuses at gestational days 19 and 19.5; note the more developed genital tubercle (Gt) at this age, making it possible to distinguish sex

Group II:

The embryos that comprised Group II exhibited CR length ranging from 10 to 11 mm, weighed 0.085 to 0.146 g and gestational age was 14.5 to 15 days. The individuals in Group II exhibited more developed body regions than Group I (Figure 1B). The external ear was more developed and projecting in the temporal region of the skull (Figures 1B and 2C). The skull was generally longer, especially in the nasal area, although the oral cavity had similar characteristics to Group I. The forelimbs and hindlimbs featured digits at the beginning of the division process. The nose jaw and were slightly more developed (Figure 1B).

Group III:

The individuals that possessed great level of body development and characteristics that allowed them to identify as rodent members were considered fetuses. The fetuses that comprised Group III exhibited CR length ranging from 16.5 to 18 mm, weighed 0.513 to 0.638 g and gestational ages ranged from 16.5 to 17.5 days. The individuals in this group exhibited more developed external features than the previously analyzed groups. There was a pronounced cervical curvature, characteristic of the embryonic period. The external ear was more developed (Figure 1C). Retinal pigmentation was evident and broadly

Group IV:

Fetuses that comprised Group IV exhibited CR length ranging from 20 to 22 mm, weighed 0.871 to 1.09 g and gestational ages ranged from 18 to 18.5 days. Compared with previous groups, individuals from Group IV exhibited more intense skin pigmentation that prevented visualization of internal organs. In general, the entire body was more developed than the earlier groups. The cranial region remained similar to the individuals in Group III. The eyes were covered by upper and lower eyelids, and the pigmentation remained somewhat transparent in this region (Figure 1D). The nose exhibited very apparent vibrissae, and the oral cavity exhibited an opening (Figure 2E). The ends of the

outer ear, more developed than in the previous group, were elongated (Figure 1D). The developed and well-pigmented limbs were elongated, and the highly characteristic regions of the joints were evident. The digits were fully divided, and claws were beginning to form. In a frontal view of the oral cavity, the opening could be observed along with vibrissae near the region (Figure 2E). An obvious nasolabial sulcus was present (Figure 2D).

Group V:

Fetuses that comprised Group V exhibited CR length ranging from 25 to 27 mm, weighed from 1.09 to 1.309 g and gestational ages ranged from 19 to 19.5 days. Externally, there were not many significant changes relative to Group IV, except for an increase in the size of individuals in this group. In contrast, individuals into group V were longer than individuals from previous groups, with more developed cranial regions (Figure 1E). Especially, they presented peripheral blood vessels, a well-developed nose and jaw, and the opening of the oral cavity. The sensory hairs on the nasal region were thicker and pigmented. Divided digits featured claws similar to the previous group, but the claws were thicker due to differentiation and calcification (Figure 1E). The genital tubercle was prominent in the inguinal region. The fully formed tubercle allowed the sex of individuals to be determined (Figure 2F).

Group VI:

Fetuses that comprised Group VI exhibited CR ranging from 28 to 30 mm and weighed 1.787 to 2.154 g and gestational ages ranged from 20 to 20.5 days. Individuals in Group VI exhibited similar features to neonates, with elongated, well developed bodies and longer tails (Figure 1F). Due to the intensification of pigmentation, two colorations were evident on the body, one dark and dorsal and one light and ventral. The cranial region was well defined by the eyes, nose, jaw and oral cavity. The well-developed limbs were fully formed with digits (Figure 1F). The fully formed oral region had much more hair, especially sensory hairs near the sensory region.

Microscopic features

General features of embryos and fetuses and syntopy of the internal organs:

Embryos in Group I (gestational day 14) exhibited the following structures: brain cavity; a pituitary gland near to the brain; the formation of the mandible near the nasal area; the tongue between the nasal and mandibular prominences; atrium and ventricle forming the external protuberance in the ventral region; the pericardium covering the heart; the lung lobe near to the liver; and the interlobar area. At this stage, the forming somites, intestinal loops and hindlimb bud could also be observed (Figure 3).

Embryos between gestational days 14.5 and 15 (Group II), showed the brain, fourth ventricle and pharyngeal pouch. In the nasal region, the vomeronasal organ and oral cavity could be observed. The heart containing an atrium and ventricle, a formed diaphragm, and more developed lungs than in the previous group was observed forming the ventral external protrusion. The vertebrae, stomach forming near the liver, and the forelimb and hindlimb buds were noted (Figure 3).

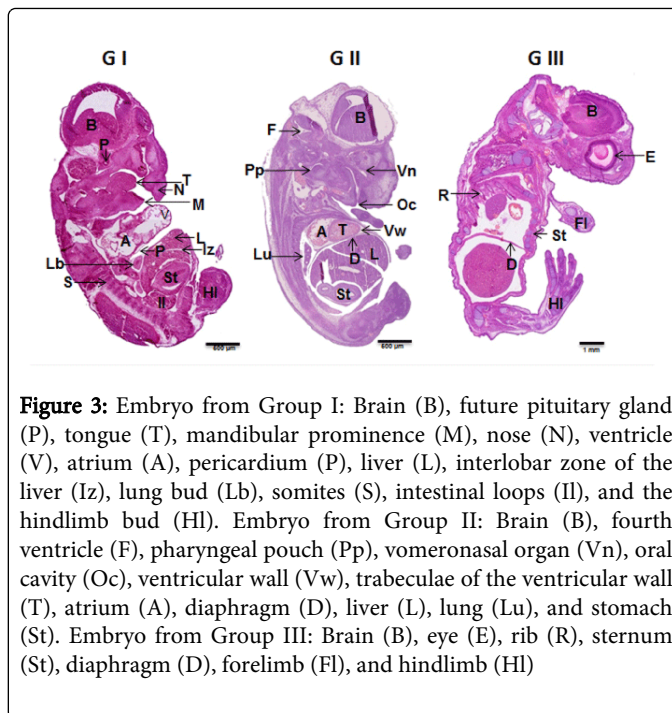


Figure 3: Embryo from Group I: Brain (B), future pituitary gland (P), tongue (T), mandibular prominence (M), nose (N), ventricle (V), atrium (A), pericardium (P), liver (L), interlobar zone of the liver (Iz), lung bud (Lb), somites (S), intestinal loops (II), and the hindlimb bud (HL). Embryo from Group II: Brain (B), fourth ventricle (F), pharyngeal pouch (Pp), vomeronasal organ (Vn), oral cavity (Oc), ventricular wall (Vw), trabeculae of the ventricular wall (T), atrium (A), diaphragm (D), liver (L), lung (Lu), and stomach (St). Embryo from Group III: Brain (B), eye (E), rib (R), sternum (St), diaphragm (D), forelimb (Fl), and hindlimb (HL)

Fetuses in Group III, at gestational day 18, exhibited a developed brain cavity, and the eyes and their constituent layers (retina, pigmented layer of the retina, and cornea) were visible nearby. The ribs, sternum, and lungs were visible in histological sections of the thoracic cavity. The diaphragm marked the area between the thoracic and abdominal cavities. Bone development was present in the forelimbs and hindlimbs (Figure 3).

Morphology of the internal organs and their cellular constituents:

In fetuses at gestational days 19 and 19.5, the stomach contained zymogenic cells and parietal cells. The gastric pits of the forming stomach could be observed at higher magnification (Figure 4A). Fetal intestinal loops (Figure 4B) were evident, and villi in the lumen were visible in the same section. The intestine of the fetuses at gestational days 20 and 20.5 consisted of submucosal layers, muscularis mucosa, intestinal glands, and goblet cells. The villi featured goblet cells, intestinal glands, muscularis mucosa, and submucosa (Figures 4C and 4D).

The bladder at gestational days 20 and 20.5 exhibited lamina propria, an extensive muscle layer, and serosa (Figure 4E). At higher magnification, the muscle layer lacked distinction between the inner longitudinal muscle layer and the outer circular muscle layer (Figure 4F). The kidney of the fetuses at gestational days 20 and 20.5 consisted of a cortical region; medullary area, renal corpuscle, and renal pelvis (Figure 4G). The glomerulus was coated by Bowman's capsule. The distal convoluted tubule could be distinguished along with longitudinal tubules connected to the renal pelvis (Figure 4H). At higher magnifications, the glomerulus and Bowman's capsule could be observed forming the renal corpuscle (Figure 4I).

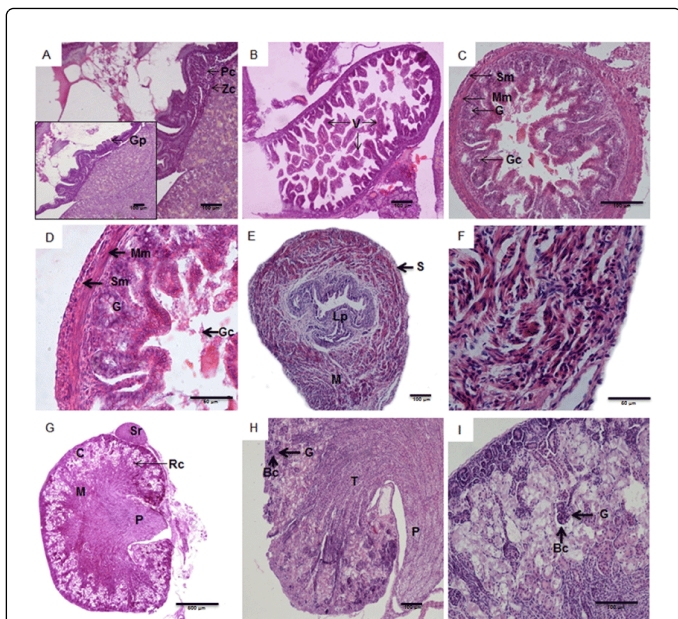


Figure 4: A Fetus at gestational days 18 and 18.5; notice parietal cells (Pc); zymogenic cells (Zc), and gastric pit (Gp). B In the same animal, note the villi (V) that were cut in the same section as intestinal loops. C Full-term fetus gestational days 20 and 20.5; note the layers of the intestine, which consists of submucosa (Sm), muscularis mucosae (mm), gastric gland (G), and goblet cell (Gc). D, Detail of the intestine, featuring the submucosa (Sm), muscularis mucosae (Mm), gastric gland (G), and goblet cells (Gc). E Full-term fetus at gestational days 20 and 20.5; note the lamina propria (Lp) in the bladder, the muscle layer (M), and the serous layer (S). F Thick muscular layer of the bladder. G Full-term fetus at gestational days 20 and 20.5; note the kidney with the following structures: suprarenal (Sr), cortical area (C), medullary region (M), renal corpuscle (Rc), and pelvis (P). H At higher magnification, Bowman's capsule (Bc) is observed surrounding the glomerulus (G), the longitudinal tubules (T), and pelvis (P). Detail of Bowman's capsule (Bc), which surrounds the glomerulus (G) and makes up the renal corpuscle

The embryonic heart at gestational days 14.5 and 15 consisted of the atrium, ventricle, and pericardium. The diaphragm and liver were evident at this age (Figure 5A). Figure 5B shows the heart of an animal at term from group IV (gestational days 20 and 20.5), including the structures that comprise the heart, where the atria, ventricles, endocardium, myocardium, and epicardium are indicated; these are the normal components of a rodent heart. The endocardium, myocardium, and epicardium are visible in detail where the arrangement of the muscle fibers in the myocardium was noted (Figure 5C).

The fetal thymus at gestational days 20 and 20.5 showed cortical and medullary regions. Internally, the organ was divided in lobes and the outer part was covered by capsule composed by connective tissue (Figure 5D).

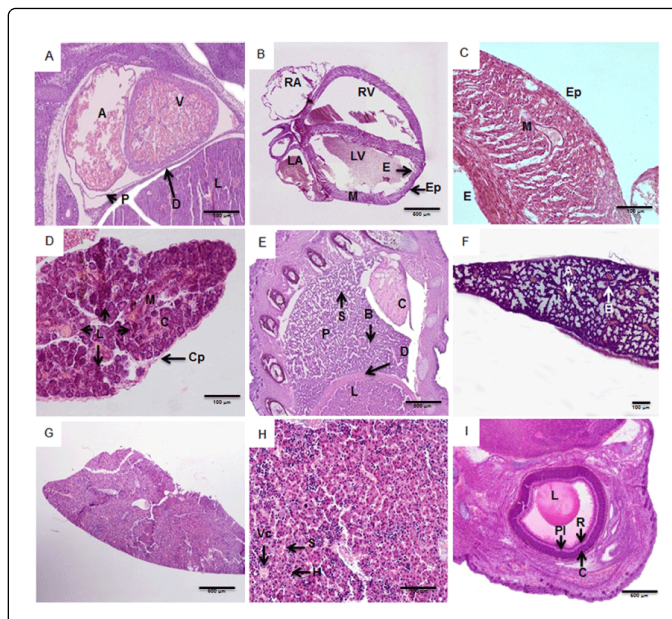


Figure 5: A Embryo at gestational days 14.5 and 15. It is possible to see the forming heart, ventricle (V), atrium (A), the pericardium (P), and the diaphragm (D) outlining the cavities. Note also the liver (L). B The heart of a full-term fetus at gestational days 20 and 20.5, note structures such as the right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), endocardium (E), myocardium (M), and epicardium (Ep). C Detail of the heart from the same animal; note the endocardium (E), myocardium (M), and epicardium (Ep) and the arrangement of the muscle fibers. D Fetuses at gestational days 20 and 20.5; note the thymus dividing in the cortical region (C) and medullary region (M); lobes (L) can be observed along with the capsule (Cp) covering the thymus. E Alveolar sacs (S) and bronchioles (B), components of the lung (Lu). F, alveolus (A) and bronchioles (B). G Note the fetal liver at gestational days 20 and 20.5. H, Note the centrilobular vein (Vc), sinusoids (S), and hepatocytes (H). I Note the layers constituting the fetal eye at gestational days 16.5 to 17.5: lens (L), retina (R), cornea (C), and retinal pigmented layer (PI)

The fetal lung at gestational days 19 and 19.5 was composed by few alveoli and many alveolar sacs and bronchioles. Later, the lungs of fetus from Group VI (gestational days 20 and 20.5) showed bronchioles. Few alveolar sacs were present (Figures 5E and 5F).

The liver of fetuses at gestational days 20 and 20.5 consisted of hepatocytes, sinusoids, and a probable centrilobular vein. Lobation of the liver was not visible even in full-term fetuses (Figure 5H).

At gestational days 16.5 and 17.5, the presence of the cornea, retinal pigmented layer, retina, and lens were evident (Figure 5I).

A comparison of the development of the external features and internal organs could be observed on Table 2.

Group I	Group II	Group III	Group IV	Group V	Group VI
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Gross Morphology	<ul style="list-style-type: none"> - Pronounced cervical curvature; - Retinal pigmented; - Open pavilion; - Short limbs; - Digits with "paddle" shape. 	<ul style="list-style-type: none"> - Pronounced nasal region; - Elongated limbs; - Deep grooves between the digits. 	<ul style="list-style-type: none"> - Acoustic meatus covers the pavilion of the external ear; - Nasal region with sensory hairs; - Open the oral cavity; - Thin skin shows with peripheral vessels; - Separated digits; - Early formation of claws. 	<ul style="list-style-type: none"> - Thickening of the skin; - Eyelid divided into upper and lower; - Well-defined digits; - Apparent claws. 	<ul style="list-style-type: none"> - Not Presents cervical curvature. 	<ul style="list-style-type: none"> - Skin pigmentation; - Well-developed fetus with neonate features.
Histology	<ul style="list-style-type: none"> - Brain; - Pituitary gland; - Tongue; - Nasal prominence and mandibular; - Heart (atrium, ventricle and pericardium); - Somites; - Lung bud; - Liver – interlobar zone; - Stomach; - Intestinal loops; - Pelvic limb bud; 	<ul style="list-style-type: none"> - Fourth ventricle; - Pharyngeal pouch; - Vomeronasal organ; - Oral cavity; - Ventricular wall; - Lung; - Diaphragm; - Stomach. 	<ul style="list-style-type: none"> -Eye (retina, pigmented layer of the retina and cornea); - Forelimb; - Ribs; - Sternum; - Hindlimb. 	<ul style="list-style-type: none"> - Immature Liver; - Muscle layer stomach; - Lung (Canalicular phase). 	<ul style="list-style-type: none"> - Stomach (zymogen and parietal cells); - Intestinal loops; - Lung (alveolar sacs, alveolus and bronchioles). 	<ul style="list-style-type: none"> - Intestine (Submucosa, Muscular mucosa, caliciform cells and intestinal glands); - Bladder (muscular and serous layer, no distinction between internal longitudinal muscle and external circular muscle_; - Kidney [cortical and medullary region, renal corpuscle (glomerulus and Bowman's capsule), renal pelvis]; - Heart –(Atriums, ventricles, endocardium, myocardium and epicardium); - Lobed thymus with cortical and medullary region enclosed by the capsule; - Liver (hepatocytes, sinusoidal and lobular central vein); - Lung (alveolus, bronchioles and alveolar sacs)

Table 2: Comparison of the gross and microscopic features of embryos and fetus of *Necromys lasiurus*

Discussion

Macroscopic features

Data on embryology and organogenesis in rodents are available for the mouse (*Mus musculus*) [8,9,11]; rat (*Rattus norvegicus*) [12]; *Oligoryzomys* sp. [10]; guinea pig (*Cavia porcellus*) [15,16]; some hystricomorphic species, such as the paca (*Cuniculus paca*), agouti (*Dasyprocta leporine*), cavy (*Galea spixii*) and capybara (*Hydrochaeris hydrochaeris*) [13,17]; coypu (*Myocastor coypus*) [14]; and related species such as the rabbit (*Oryctolagus cuniculus*) [18-21]. In contrast, embryology references for *N. lasiurus* are not yet available in the literature.

Evans and Sack [31] were the first to describe a methodology to estimate the gestational age of embryos and fetuses, the crown-rump (CR) distance. The CR distance of *N. lasiurus* was compared with the rat because they have similar gestational periods. There are notable differences between the two species; for example, a rat embryo of similar age to GI exhibits sensory hairs above the eyes and on the upper lip, whereas in the *N. lasiurus* embryo, the jaw is still forming, the eyes are still covered by a layer of skin with no distinguishable

upper or lower eyelid, and the pinna is still being formed. At this age in the guinea-pig, somites are forming and that the neural folds are beginning to fuse [31]. Compared with *N. lasiurus*, the guinea pig is more developed due to relatively faster gestation. The *Oligoryzomys* sp. at gestational day 14.5 showed a developed cephalic region, pigmented retina, and other well-formed organs [28]. In contrast, *N. lasiurus* embryos are still developing, the short tail is still forming, the cephalic region is developing but very prominent, the abdominal region is marked by a prominent liver, and the thoracic region has a prominent heart. In *N. lasiurus*, the limbs were still buds shaped like "paddles" at the distal end. Agouti embryos with CR distances from 0.9 cm to 1.1 exhibited similarities in the morphology of the limbs, which were "paddle" shaped; the "C" curvature and thoracic and abdominal prominences were due to the heart and liver, respectively [17].

Embryos at gestational days 14.5 and 15 days (GII) exhibited more developed forelimbs and hindlimbs with dividing distal ends. At this stage in mice, there are sulci between the posterior digits, and forelimbs already have fully formed digits [31]. Embryos of *Oligoryzomys* sp. in the same group between gestational days 14.5 and 16 already exhibit fully formed hindlimb and forelimb digits [28]. The facial cleft is closed in mice at 15 days [31]; however, the cleft is still

forming in *N. lasiurus* at the same time, and the nasal prominence is present along with the forming jaw. The nostrils are still forming on the side of the nasal region, which resembles the mouse with closed nostrils. The cephalic region in *Oligoryzomys* sp. is easily recognizable [28], similar to *N. lasiurus* with an elongated head.

In fetuses at gestational day 16.5 to 17.5 (GIII), the pinna fully covered the ear canal, similar to the mouse, in whom the pinna covers the ear canal at gestational day 17. This occurs at gestational day 18.5 in the rat according to Evans and Sack (1973) [31]. Vibrissae were observed in the nasal region, similar to mice with sensory hairs on the upper lip. In *Oligoryzomys* sp., claws and integuments are already formed in fetuses at gestational days 17 and 18.5. In *N. lasiurus*, the claws are just starting to form at gestational days 16 to 17.5 [28].

In fetuses at gestational days 18 to 18.5, skin pigmentation and thickening are evident, and the eyes are covered by lids; however, the pigmentation is transparent, allowing visualization of the retinal pigmentation. In rats at gestational day 18.5, lids cover the eyes but are not fused. In the guinea-pig, eyelids appear at gestational day 27 [31]. At this stage, *N. lasiurus* already exhibits a developed ear with elongated tips, unlike the rat with pinna completely covering the canal. In *N. lasiurus*, the forelimbs and hindlimbs are elongated and developed with fully formed digits and evident keratinization of the claws. The entire cranial and nasal regions are well-formed with an open oral cavity and a naso-labial cleft in *Oligoryzomys* sp. at gestational days 17 and 18.5; the following body regions are fully formed: head, limbs, claws, and integument [28]. The forelimbs and hindlimbs are fully formed at gestational day 18 in mice [11].

Fetuses at gestational days 19 and 19.5 (Group V) exhibited longer bodies than the previous group. The cranial region was well formed with a developed jaw and fully formed nasal region containing thicker whiskers. The inguinal region had a developed genital tubercle allowing the distinction of sex. In rats on gestational day 19, at the end of pregnancy, the eyelids were fused, and the auditory canal had an epithelial plug [31].

Neonates at gestational days 20 to 20.5 exhibited fully formed body regions. The neonates were similar to rats at the end of gestation (22 days) [31].

Microscopic features

The nervous system originates from the neural plate in the ectoderm of the embryonic disc. During development, this plate grows, and its edges become folds forming the neural tube. The medial portion of this tube closes, and the two ends, called the rostral and caudal neuropores, are closed last. Monie [33] described this closing in some animals such as the rat (10.5 to 11 days), mouse (9 to 9.5 days), hamster (8.25 to 8.5 days), guinea pig (15.25 - 16.5 days), rabbit (9.5-10.5 days), and even men (25-27 days). In *N. lasiurus*, the neuropores are closed by gestational day 14.

The pituitary originates from two separate parts: Rathke's pouch, which forms the anterior pituitary, and an inferior ventral growth of the diencephalon, the infundibulum, which forms the neurohypophysis [9,34]. In *N. lasiurus* at gestational day 14, it was possible to see the lumen of Rathke's pouch and the future pituitary. The lumen of Rathke's pouch can be observed in rats of the same gestational age. A fully formed pituitary is visible in mice at gestational day 15.5 [9].

The first recognizable structures in the facial region are the frontonasal, maxillary, and mandibular prominences [34]. In *N. lasiurus* at gestational day 14 (Group I), the nasal prominence was more developed than the mandibular prominence. In *Oligoryzomys* sp. of the same age, the nasal prominence formed together with the mandibular prominence, and the tongue was evident between them.

The following components of the eye were visible in fetuses at gestational days 16.5 to 17.5: cornea, retina, the pigmented layer of the retina, and the lens. Similar findings were described in *Oligoryzomys* sp. [28] and in transgenic and non-transgenic mouse fetuses at gestational day 15.5 [35].

The circulatory system is essential for embryonic and fetal growth. The embryo requires a functional circulatory system when it reaches a greater size and complexity. Before the circulatory system is needed, the embryo is nourished by diffusion from the fluid that is secreted by the uterine glands in the uterine cavity [34,36]. Monie [33] described the origin of the heart, where two heart tubes are formed at the end of the embryonic disk head and merge to form a single tube, the heart. In *N. lasiurus*, the atrium and ventricle were visible in the developing heart at 14 gestational days. The heart of *N. lasiurus* fetuses at gestational day 20.5 is similar to the guinea pig [15]. The authors noted the layers of the heart wall, endocardium, myocardium, and epicardium and the structure of the muscle fibers, making it possible to examine similarities between the two species [1].

The origin of the liver is intimately linked with the intestine and heart. At gestational day 10, the anterior and posterior intestine become visible, and liver development begins with binding of the foregut endoderm to the developing heart. Bone morphogenetic proteins (BMPs) and fibroblast growth factor (FGF), signals arising from the transverse septum and heart development, respectively, and transcription factors, such as GATA, FOXA1 and FOXA2, activate specific liver genes that regulate hepatogenesis [37-40]. On gestational day 12, the liver develops [41]. In fetuses at gestational day 20 and 20.5 (full term), the following structures are visible in the liver that resembles those in adults: hepatocytes, sinusoids, and centrilobular vein. Similar features were observed for liver development in rats and mice [11,42].

The development of the respiratory system begins in the embryonic period when the primitive trachea forms lung buds as caudal-lateral branches of the main bronchi. These main bronchi become lobar and determine how the lung divides into lobes [34]. Lung lobes were visible in *N. lasiurus* at gestational day 14. Monie [33] reported that the rudimentary respiratory system originates from the laryngotracheal groove on the ventral side of the foregut; it appears on gestational day 9 in the hamster and day 17.5 in the guinea pig. Sacs, bronchioles, and the diaphragm outlining the cavities were evident in the lungs of *N. lasiurus* fetuses at gestational days 19 and 19.5 (Group V). The lungs of fetuses at gestational days 20 and 20.5 were better developed, with sacs and bronchioles and some alveoli, which are characteristic of adulthood.

In the rat, thymus development begins at gestational day 10.5 with the segmentation of the posterior pharynx, which is the first step in thymus formation [43]. The *Tbx1* gene is critically important for the organogenesis of the thymus and has been the target of studies to understand its functions. In the thymus of full-term *N. lasiurus* (gestational day 20.5), cortical and medullary regions were visible, even the lobes and capsule of connective tissue that covers the organ externally.

Fetuses of *N. lasiurus* at gestational days 19 and 19.5 exhibit stomach zymogenic cells, as reported in adult animals [44]. The zymogenic cells are cells that make up the lower region of gastric glands, in addition to the parietal cells that are rare at the base, and form the upper part of the gastric gland [45]. Such cells were observed in *N. lasiurus* at gestational days 19 and 19.5.

The intestine of full-term fetuses (gestational day 20.5) exhibited the following structures: submucosa, muscularis mucosa, glands, and goblet cells. Similar findings were reported in other species [44,45].

The bladder originates from the proximal portion of the urachus and the pelvic region of the urogenital sinus [34]. In *N. lasiurus* at gestational days 20 and 20.5, the following layers of the bladder were observed: a serous layer, muscle, and lamina propria. The muscle layer was thick, and the bladder observed to be empty.

The kidney development along three distinct stages: pronephros, mesonephros, and metanephros. Maturation of the metanephros forms the kidneys [34]. The kidneys of *N. lasiurus* at gestational days 20 and 20.5 are similar to those of adult animals such as the guinea pig and mouse; the kidneys contain many glomeruli and have a similar multipyramidal shape [46]. In addition, many longitudinal tubules were observed in the pelvis.

Conclusions

Rodents are a very important group of experimental models for science. The present study described in detail for the first time the morphological features related to organogenesis and development of *N. lasiurus*. Although the order Rodentia is very rich in number of species, few studies are available on the embryology of the various families inside this order. In conclusion, the species *N. lasiurus* has features similar to murine rodents and other rodents that are conventionally used as experimental models. Thus, the results showed in this study will help to establish a normal pattern of morphological parameters for embryology that can be used to compare and understand experimental results, especially those that may affect embryonic development which may even result in malformations.

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