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Gabriel Álvarez Otero

Universidad de Córdoba, galvarezmvz@gmail.com

Cesar Rojano Bolaño

Fundación Cunaguaro, c.rojanob@gmail.com

Juan Carrascal Velásquez

Universidad de Córdoba, juancarrascal@correo.unicordoba.edu.co

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Histological and Histochemical Description of the Pancreas in the Adult Lesser Capybara (*Hydrochoerus isthmus*)*

Gabriel Álvarez Otero¹ / Cesar Rojano Bolaño² / Juan Carrascal Velásquez³

Abstract

The aim of this study was to describe histologically and histochemically the pancreas of three adult *Hydrochoerus isthmus*, attacked by feral dog in Buenavista, Córdoba, Colombia. A complete necropsy was performed and pancreatic fragments were collected and stored in 10% buffered formalin and Bouin liquid. Then, they were dehydrated, diaphanized and embedded in paraffin, stained with Hematoxylin & Eosin, P.A.S., Gomori trichrome, Grimelius and Masson Fontana modified. The *H. isthmus* pancreas presented a duodenal mesenteric pattern. The exocrine portion was described as a composite tubuloacinar gland. The endocrine portion of the pancreas was constituted by pancreatic islets of Langerhans and a diffuse neuroendocrine system. The histological and histochemical techniques used allowed us to identify the exocrine and endocrine portion of the organ. It is suggested to complement this study with some special techniques for the identification of specific endocrine cells, such as Beta, Alpha, Delta, Epsilon, PP or Gamma.

Keywords: histology, digestive tract, exocrine, endocrine, rodent.

* Research article.

1 MSc, Universidad de Córdoba.
Facultad de medicina veterinaria
y zootecnia.

✉ galvarezmzv@gmail.com
ORCID: <https://orcid.org/0000-0002-2781-9935>

2 MSc, Fundación Cunaguaro
✉ c.rojanob@gmail.com
ORCID: <https://orcid.org/0000-0001-9481-0976>

3 PhD, Universidad de Córdoba.
Facultad de medicina veterinaria
y zootecnia
✉ juancarrascal@correo.unicordoba.edu.co
ORCID: <https://orcid.org/0000-0001-7178-3362>

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Descripción histológica e histoquímica del páncreas en cacós (*Hydrochoerus isthmus*) adultos

Resumen

El objetivo del presente estudio fue describir histológica e histoquímicamente el páncreas de tres individuos adultos de *Hydrochoerus isthmus* atacados por perros ferales en Buenavista, Córdoba, Colombia. Se realizó una necropsia completa y se recolectaron fragmentos pancreáticos, los cuales se almacenaron en formol tamponado al 10 % y líquido Bouin. Luego, fueron deshidratados, diafanizados y embebidos en parafina; teñidos con hematoxilina y eosina, P.A.S., tricrómico Gomori, Grimelius y Masson Fontana modificado. El páncreas de *H. isthmus* presentó un patrón mesentérico duodenal. La porción exocrina fue descrita como una glándula tubuloacinar compuesta. La porción endocrina del páncreas estaba constituida por islotes pancreáticos o Langerhans, y un sistema neuroendocrino difuso. En ese contexto, las técnicas histológicas e histoquímicas utilizadas permitieron identificar la porción exocrina y endocrina del órgano. Se sugiere complementar este estudio con algunas técnicas especiales para la identificación de células endocrinas específicas, como Beta, Alpha, Delta, Epsilon, PP o Gamma.

Palabras clave: histología, tracto digestivo, exocrino, endocrino, roedor.

INTRODUCTION

The lesser capybara (*Hydrochoerus isthmius*, Goldman, 1912) is a rodent distributed in Colombia, Venezuela and Panama. In Colombia, it is present in the Andes, the Caribbean and the Pacific regions (1). For many years it was classified as a subspecies of *Hydrochoerus hydrochaeris*. However, it is currently considered as a different species (2, 3).

The habitats of these animals are diverse and they have as a common element a wetland and a grazing area that can be pastures, gallery forests, or shrubs (4). It is a rodent with a digestion similar to ruminants, and can compete with cattle for resources (4, 5). Currently, the species is listed as Data Deficient in view of the absence of information on population and on its extent of occurrence (1).

Its reproductive capacity, high growth rate, hardiness, social behavior and quality of its meat, makes the lesser capybara a promising resource for biomedical innovations and better productive management for protein in captivity (6). However, studies regarding its ecology, production, sustainability and morphophysiology are scarce in its distribution range (3, 4, 6).

One of the least explored knowledge areas for these mammals is the digestive morphophysiology, highlighting some descriptive studies done on different organs of *H. hydrochoerus* in Brazil (7, 8). The pancreas is a digestive organ located in the abdomen. In mammals, it contains exocrine glands that produce enzymes important to digestion, including trypsin, chymotrypsin, amylase and lipase. The endocrine component of the pancreas consists of islet cells (islets of Langerhans) that create and release important hormones like insulin and glucagon (9).

Based on this, and due to pancreas importance on the absorption, secretion and excretion (10), the aim of this study was to describe histologically and histochemically the pancreas of adult individuals of *H. isthmius*, including the identification of cellular components be-

longing to the exocrine and endocrine portion of this digestive organ.

MATERIALS AND METHODS

Type of study

The study was conducted as a cross-sectional descriptive study.

Study site

The study was conducted in the countryside of Buenavista, located south-east of the department of Córdoba between latitude 8°13'13" N and longitude 75°29'11" W, at 72 m.a.s.l.

Environmental conditions

The ecosystem corresponds to tropical dry forest. The climate in this region is of unimodal type with two hydrological periods. The annual temperature is 27.8 °C with average rainfall of 2,181 mm.

Study animals

Three adult individuals of the species *Hydrochoerus isthmius*. The corpses were opportunistically collected after finding these dead animals as a result of feral dog attacks between June and July of 2015.

Samples collection

The post-mortem examination of the individuals was carried out by making an incision in the midline (alba line) where the viscera were exposed, from the tongue to the anus. The stomach and small intestine (duodenum, jejunum and ileum) were removed from the rest of the organs, exposing the pancreas, and allowing its complete extraction, which allowed to appreciate its external morphological characteristics as well as its location and its ducts.

Sample analysis

Fragments of the pancreas were collected, until obtaining significant samples of each individual. Then, the samples were stored in vials with 10% buffered formalin and Bouin liquid for 24 hours, as tissue fixative, and after that they passed to the dehydration process in five ascending alcohol concentrations starting at 70%. Later, they were initially diaphanized in a 1:1 mixture of 100% alcohol + Xylol and then in two xylol solutions, embedded in paraffin and sectioned to a 4 µm thickness with a hand rotary microtome.

Hematoxylin & Eosin, PAS, Gomori, Grimelius and Masson Fontana modified staining techniques were used to remove the paraffin. Histological and histochemical characteristics were visualized and identified in the different portions of the pancreas (3, 4, 5). The samples were processed in the histopathology laboratory of the University of Cordoba, Colombia and in the laboratory of Veterinary Histology of the Department of Veterinary Medicine in the Federal University of Viçosa, Minas Gerais, Brazil.

Ethical aspects

Samples were collected taking into account the procedures of sampling, management and conservation, as well as the ethical, technical, scientific and administrative standards for animal research contained in Law 84 (National Congress of Colombia, 1989). This study has a scientific research license from the Autonomous Corporation of the Valleys of Sinu and the San Jorge river (CVS) through resolution No. 2-1033 of May 22, 2015.

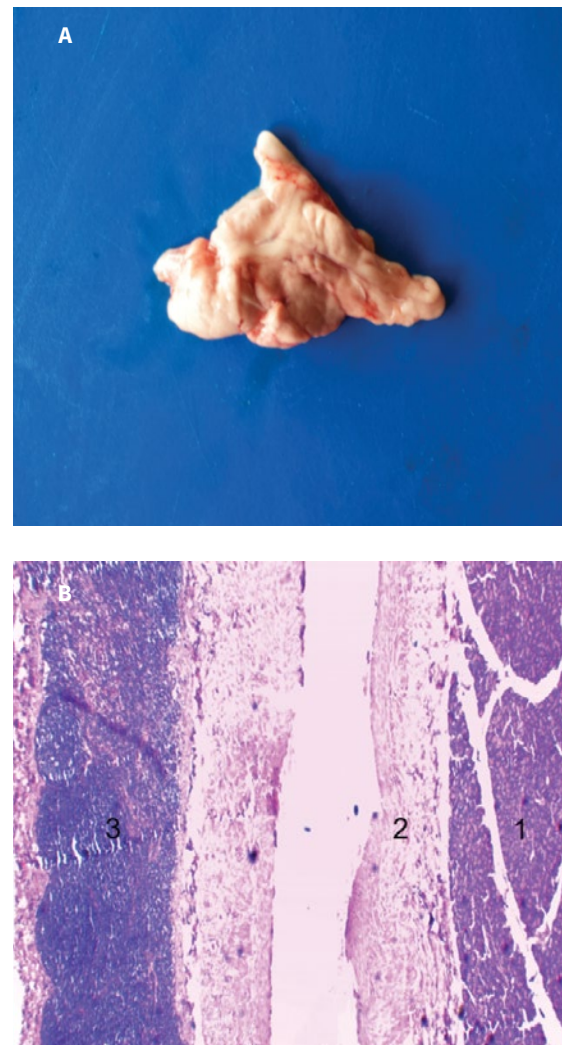
DATA ANALYSIS

The data obtained for this study was descriptive, since the identification and characterization of the different cellular components in the pancreas was performed, based on the cellular characteristics observable with the different staining methods proposed.

RESULTS

The pancreas (Figure 1A) of *H. isthmus* presented a duodenal mesenteric pattern. Separated lobes and segments are found; in some cases, largely, by elements of fat and mesenteric stroma. Likewise, lymphoid tissue was observed in the capsule of connective tissue that covers them (Figure 1B).

Figure 1. *Hydrochoerus isthmus* pancreas. A) Pancreas with mesenteric pattern; B) Panoramic view of the pancreatic parenchyma with presence of lymphoid tissue in the organ capsule (4X) H & E. 1. Exocrine portion. 2. Septum of loose connective tissue. 3. Lymphoid nodule.

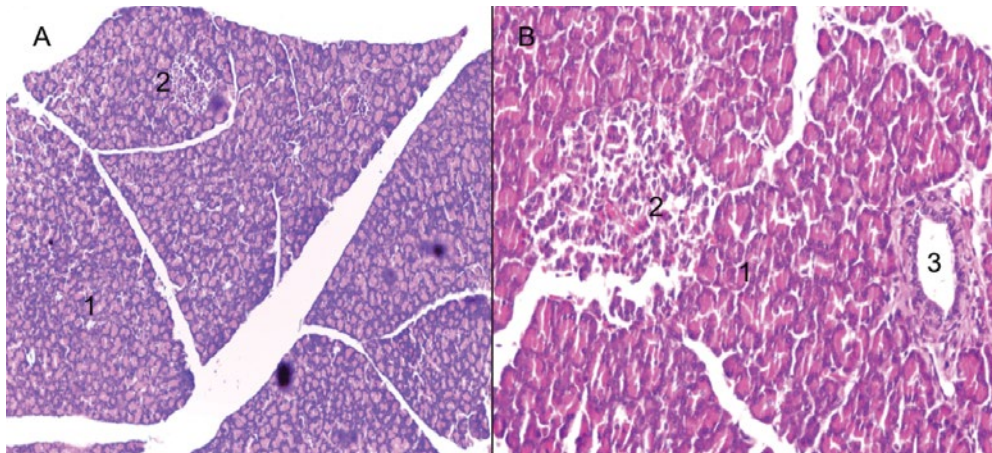


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On the other hand, an exocrine and endocrine portion was identified for the pancreas (Figures 2A and 2B). The exocrine portion was described as a composite tubulo-acinar gland, which presented clusters of interdigitating branches, that were grouped into lobules constituting the parenchyma, which was covered by a thin layer of loose or areolar connective tissue (Figures 3A and 3B).

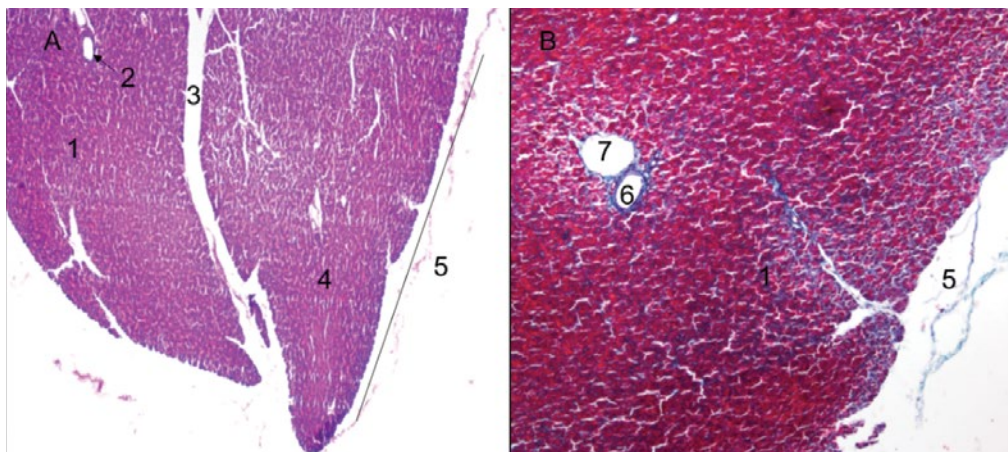
The parenchyma presented numerous serous acini, few mucous adenomers (mainly related to interlobular ducts and pancreatic islets), intralobular and interlobular ducts (Figures 4A and 4B). Likewise, pancreatic Langerhans islets and the diffuse neuroendocrine system were observed: this one constitutes the endocrine portion of the pancreas (Figure 5A).

Figure 2. *H. isthmus* pancreas. A) Panoramic view of the pancreatic parenchyma (10X); B) Pancreatic islet (40X), H & E. 1. Exocrine portion. 2. Pancreatic islets. 3. Interlobular excretory duct.



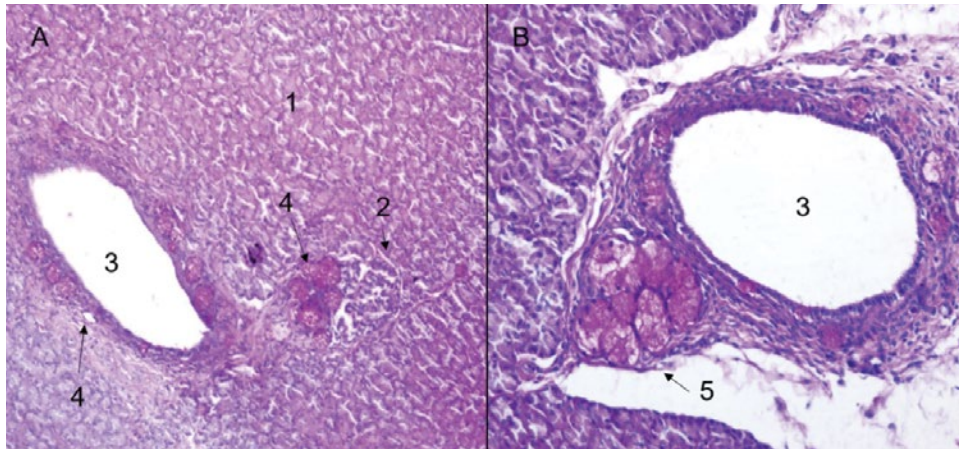
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Figure 3. Lesser capybara pancreas. A) Pancreatic lobes (4X), H-E. B) Details of the thin capsule of loose connective tissue covering the pancreas (40X), Gomori trichrome. 1. Exocrine portion. 2. Interlobular excretory duct. 3. Septum of loose connective tissue. 4. Pancreatic lobe. 5. Pancreatic capsule. 6. Artery. 7. Vein.



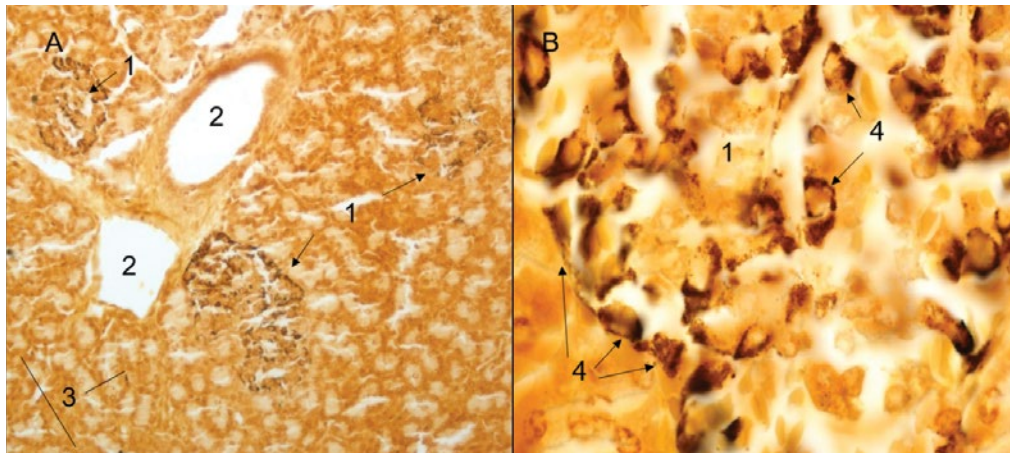
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Figure 4. *H. isthmus* pancreas. A) Panoramic of an excretory duct, mucous glands and periductal pancreatic islet (10X). B) Detail of interlobular excretory duct with presence of periductal PAS positive mucous glands (40X), PAS. 1. Exocrine portion. 2. Pancreatic islets. 3. Interlobular excretory duct. 4. PAS positive mucous glands. 5. Periductal PAS - Positive mucous glands.



Source: own work

Figure 5. A) Panoramic view of pancreatic parenchyma with presence of periductal pancreatic islets composed of argyrophyll cells and presence of isolated endocrine cells (40X). B) Detail of a pancreatic islet with presence of argyrophyll cells (100X), Grimelius. 1. Pancreatic islet. 2. Interlobular excretory duct. 3. Isolated endocrine cells. 4. Argyrophyll cells.



Source: own work

The pancreas presented connective formations of sustentation, like the capsule that surrounds it. We found a conformation of dispersed, disorganized fibers between the fibroblasts. From there depart the connective septa that are directed towards the interior of the organ, which contain varying amounts of an amorphous substance, blood vessels and lymphatics, as well as adipose tissue, nerves and interlobular pancreatic ducts (Figures 3A and B, and 6A).

From the interlobular septa, delicate connective projections arise, which contain blood vessels into the lobes, wrapping the secretory acini and reaching the pancreatic islets (Figure 7A). Acinar cells are indistinct; and, in the middle of the pancreatic acini, intralobular or intercalary ducts were observed (Figures 6B, 7A). The centroacinar cells were observed: flattened, oval and cubic, depending on the contents of the conduit.

The endocrine portion of the pancreas was constituted by pancreatic islets of Langerhans (Figures 5B, 7B), which were presented in a variable number and generally in a rounded form. These tend to be unevenly distributed throughout the pancreas, being present in interlobular, periductal and occasionally intralobular sites. However, it was possible to observe a lack of islets sections (Figure 3A). In the middle of the constituent endocrine cells, a dense network of blood capillaries was observed (Figure 7B). It was also possible to observe beta cells, using the Gomori trichromic immunohistochemical staining (Figure 7B).

When staining argyrophyl (Grimelius) and argentaffin cells (Masson Fontana modified), a reaction was observed for argyrophyl cells; on the contrary, we found no reaction for argentaffin cells.

DISCUSSION

The lesser capybara presents a pancreas with macroscopic characteristics compatible with a mesenteric pattern similar to that of the rabbit, mouse and rat (11). In this model, the lobes and segments are separated, some-

times broadly, by elements of fat and mesenteric stroma; and lymphoid aggregates have been reported in the pancreas of *H. hydrochaeris* (8, 12). These aggregates have also been observed during this study.

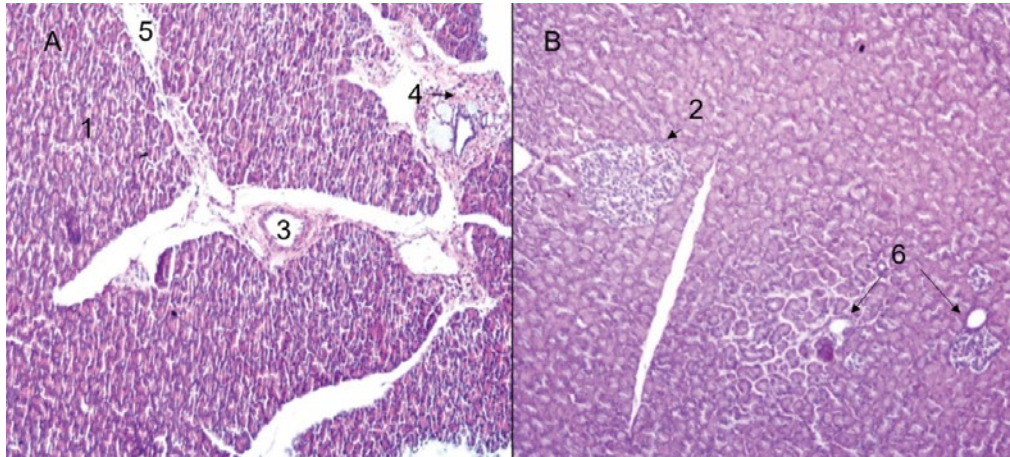
The structural features of the exocrine portion of the pancreas of *H. isthmus* are similar to those of other domestic mammals (13, 14). Mucous glands were associated with interlobular ducts and in some cases with pancreatic islets. Such glands are not commonly reported in the pancreas; however, some studies report the presence of different cell types producing mucin in the pancreas (15–17), whose main functions are lubrication, hydration and protection of the epithelium; in the same way, mucin has been shown to play an important role in innate immunity to microbial infections, and has highly immunogenic characteristics, such as blood group antigens (17).

The pancreatic parenchyma is covered by a thin layer of loose or areolar connective tissue, similar to the other domestic mammals (13, 18). Such feature contrasts with the report in *H. hydrochaeris*, described as a layer of dense connective tissue covering the pancreas (12).

The acinar cells are indistinct; in the middle of the pancreatic acini, intralobular or intercalary ducts were observed. These cells have a basal or centrobasal nucleus and, in the case of *H. isthmus*, as in other rodents, they can have two nuclei (18, 19). Likewise, centroacinar cells were observed with flattened, oval and cubic forms, depending on the content of the canal: this is compatible with the reported form for the other rodents (20, 21).

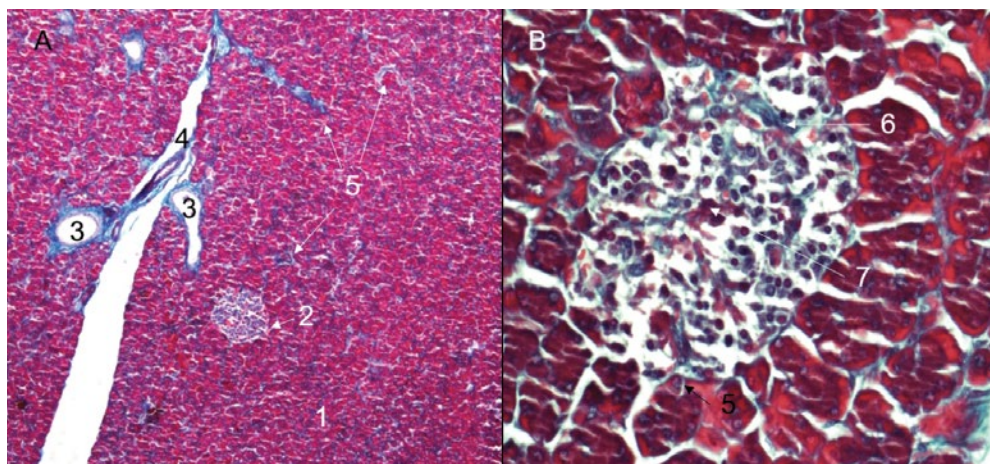
The endocrine portion is composed of pancreatic islets that are presented in varying numbers and generally in a rounded form, which is consistent with *H. hydrochaeris* (12). The pancreatic islets tend to be distributed unevenly throughout the pancreas, which makes possible to observe sections with lack of islets; however, it is not possible to state that there are pancreatic lobes with total islet absent, due to the conformation and three-dimensional location of the same inside the lobe. These

Figure 6. A) Interlobular septum of loose connective tissue (10X), H-E. B) Pancreatic parenchyma with interlobular pancreatic islet presence and intralobular or intercalary excretory ducts (10X), PAS. 1. Exocrine portion. 2. Pancreatic islet. 3. Interlobular excretory duct. 4. Septum of loose connective tissue. 5. Vein. 6. Interlobular excretory duct.



Source: own work

Figure 7. A) Pancreatic parenchyma with presence of interlobular septum of loose connective tissue and interlobular pancreatic islet (10X), Gomori trichrome. B) Detail of the pancreatic islet showing that the cytoplasm of the beta cells adopts a blue-violet (40X) coloration, Gomori trichrome. 1. Exocrine portion. 2. Pancreatic islet. 3. Interlobular excretory duct. 4. Artery. 5. Connective projections. 6. Beta cell. 7. Capillary network.



Source: own work

are present in interlobular, periductal and occasionally intralobular sites, in agreement with reports for rodents (18, 20).

The pancreas has isolated endocrine cells, which are part of the diffuse neuroendocrine system (12, 18, 20). These cells are argyrophyls or argentaphins, with the exception of immunoreactive insulin producing cells, cholecystokinin and somatostatin, which do not stain with silver (22). When staining the argyrophyl (Grime-lius) and argentafines cells (modified Masson Fontana), a reaction was observed for argyrophyl cells and, on the other hand, there was no reaction for argentaffin cells (22, 23).

CONCLUSIONS

The histological and histochemical techniques used allowed us to identify the exocrine and endocrine portion of the pancreas in this rodent, which has similar characteristics to other mammals. It is suggested to complement this study with some special techniques for the identification of specific endocrine cells, such as Beta, Alpha, Delta, Epsilon, PP or Gamma.

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