Pancreas glucagon immunolocalization in the scorpion mud turtle, *Kinosternon scorpioides*

Running title: pancreas glucagon immunolocalization in turtle

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ABSTRACT

Jurará (*Kinosternon scorpioides*) is a semi-aquatic fresh water turtle. It is a species of the Brazilian fauna in imminent risk of extinction. Thus studies have been carried out to develop its rearing in captivity. In the present study pancreas fragments were used from six adult *K. Scorpioides* specimens (three males and three females) blocked in paraffin. Semi-seried3μm thick cuts were obtained in a rotating microtome and fixed on previously treated histological slides (sylanized). The pancreas cuts were submitted to the streptavidin-peroxidase technique
to detect glucagon antigens present in the pancreas parenchyma of jurará. Quantitative analysis of the slides was made by counting 20 distinct fields containing glucagon immunoreactive cells under an optical microscope with a 40X objective lens. Glucagon immunoreactive cells were identified in the pancreas parenchyma of the mud turtle, and they were more frequent in the pancreatic islets. They were also observed in the exocrine parenchyma and in the epithelial of the pancreatic duct cover. Histological analysis showed that the pancreatic islets did not present a capsule of conjunctive tissue. Quantitative analysis showed glucagon immunoreactive cells per field an average of cells 21.4 ± 11.4. Glucagon immunoreactive cells were observed throughout the organ.

**Keywords**: Testudinata. Parenchyma. Pancreaticislet. Chelonia.

**1 INTRODUCTION**

The reptile *Kinosternon scorpioides*, Linnaeus, 1766 of the class Reptilia, order Testudines, family Kinosternidae, is a semi-aquatic freshwater turtle, found from Costa Rica to Northern Argentina, East of the Andes (Acuña-Mesen, 1994). In Maranhão State/ Brazil, this turtle is also called jurará, and its capture is forbidden by the Brazilian Institute of the Environment and Renewable Natural Resources (Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis) IBAMA. This species, found on riverbanks and in fields in the Baixada Maranhense, is an excellent source of protein for the Riverside populations. Although turtles are still sold illegally, there are rearing establishments approved and inspected by IBAMA that are authorized to sell a determine percentages of their stock (BRASIL, 2007). Our group has been gradually developing important studies that consolidate information on...
the reproductive biology of the species *K. scorpioides* in the natural environment (VIANA et al., 2014; SOUSA et al., 2014). The present study, therefore, aims to increase the biological information of this species with the investigation of the digestive system, chiefly the pancreas.

There are few reports on the microscopic structure of the turtle digestive system such as Gapp et al. (1985), Madrid et al. (1989) and Beisser (1998), who studied the tongue and the esophagus. Some authors have described the endocrine cells of the pancreas (Garcia Ayala *et al.*, 1987; Rhoten, 1987), the intestines and the stomach of these animals (Muniz *et al*., 1991; Ivanova *et al*., 1997). The digestive tract and pancreas of *K. scorpioides* were studied morphologically and histochemically by Pereira (2000) but there are few data in the literature regarding the histological constitution of the digestive organs of this species by other authors. In order to fill the gap caused by the scarcity of biological and morphological data on *K. scorpioides*, this communication proposes to characterize histologically the pancreas of this species and verify glucagon immunolocalization in the endocrine cells in this organ so that these data can be used in the reptile clinic for specific diagnosis.

2 METHODOLOGY

Pancreas fragments were used from six adult *K. scorpioides* specimens (three males and three females) blocked in paraffin. The material used is from other research projects developed by Pereira (2000), licensed by the Brazilian Institute for the Environment and Renewable Resources Instituto Brasileiro do Meio Ambiente e Recursos Renováveis (IBAMA/MA), process Nº 001860/97 – 44.

The research was carried out in the Anatomopathology Laboratory/State University of Maranhão. All the procedures described were approved by the Committee of Ethics and Animal Well-being (UEMA), protocol 0044/2007.

For the histological processing of the material, semi-seried4μm thick cuts were obtained in a Micron HM – 360 – Zeissrotating microtome, distended in an OMA MJ72histological water bath, and fixed on previously treated (silanized) histological slides. The slides were analyzed using an Axiostar plus – Zeiss light microscope, at magnifications of 20, 40X. The slides were analyzed qualitatively by counting 20 different fields containing glucagon immunoreactive cells under electronic microscope, with 40X lens.

The pancreas cuts were submitted to the streptavidin-peroxidase technique to detect glucagon antibodies present in the pancreas parenchyma of the mud turtle. Next the tissue cuts were deparafinated in xylol for 20 minutes, hydrated in decreasing alcohol solutions (absolute
alcohol, 90%, 80% and 70%, respectively) and bathed in PBS (“Phosphate Buffer Saline”- pH 7.2, 0.01M). Later a block was made of the endogenous peroxidase by adding hydrogen peroxide (30 volumes diluted to 4% in PBS) to the PBS bath for 30 minutes at ambient temperature. The slides were then covered with block solution of non-specific linking sites (skim milk powder diluted in PBS -12g milk in 200 mL PBS) and incubated in a wet chamber for 30 minutes at ambient temperature. Shortly afterwards, the primary antibody5 (antibody of rabbit anti glucagon, diluted 1:50 in BSA – bovine albumin serum) was added in sufficient quantity to cover the fragments, and the slides were incubated for 18 to 24 hours in a wet chamber at 4ºC. Next the secondary biotinylated antibody (goat anti-rabbit antibody diluted at 1:100 - Ultra Streptavidin Detection System – NCL – GLUCP) was added and the slides were again incubated in a wet chamber for 30 minutes at ambient temperature. Then the streptavidin peroxidase complex was added followed by 30 minutes incubation in a wet chamber at ambient temperature. The reaction was developed using Diaminobenzidine (DAB) solution at 0.024% PBS Laboratory novocastra™ with the addition of hydrogen peroxide 40 volumes solution at 0.16% in PBS, for five minutes at ambient temperature. Afterwards the slides were washed in running water and counter stained with Harris Hematoxylin. The slides were then dehydrated in increasing alcohol (70°, 80°, 90° and absolute alcohol), diazophonized in xylol, and mounted in synthetic balsam. A negative and positive control was used for each batch of 20 slides; PBS was used as negative control, substituting the primary antibody. For positive control, a fragment of the dog pancreas a dog was used. The slides were observed under an Olympus BX61VS microscope.

3 RESULTS and DISCUSSION

Immunolabeling cells for glucagon were observed in whole organ as as blackened dots in in the pancreas of K. scorpioides. They were more frequent in the pancreatic islets. They were also observed in the parenchyma exocrine and in the epithelium of the pancreatic duct covering. The histological analysis showed that the pancreatic islets did not present a conjunctive tissue capsule. No difference in immunolocalization was seen among males and females.

According to Rhoten (1987), the reptilian endocrine pancreas can be even more complex, regarding the peptide localizations than the pancreatic islets of mammals. According to Miller and Lagios (1970), the endocrine pancreas tissue in reptiles differs from that of most mammals, because there is no demarcation of the exocrine pancreatic tissue. The pancreatic
islets are associated directly to the acinus and interlobular ducts, because there is no conjunctive capsule (Miller; Lagios, 1970).

Loosely arranged cells were observed, formed by glucagon immunoreactive cells. These results are similar to those found by Vydia and Prakash (2008), in research on the turtle *Melanochelystrijuga*. However, these authors reported that the glucagon immunoreactive cells were present only in the periphery of the pancreas of the turtle *Melanochelystrijuga*, while in *K. scorpioidea* they were distributed throughout the organ. Regarding quantitative analysis of glucagon immunoreactive cells, the mean was $21.4 \pm 11.4$ cells.

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