Brazilian Journal of Development

Pancreas glucagon immunolocalization in the scorpion mud turtle, *kinosternon scorpioides*
Running title: pancreas glucagon immunolocalization in turtle

DOI:10.34117/bjdv6n3-166

Recebimento dos originais: 30/01/2020
Aceitação para publicação: 12/03/2020

José Gomes Pereira
Doutor em Medicina Veterinária pela Univer Estadual Paulista "Julio de Mesquita"
Instituição: Universidade Estadual do Maranhão, Centro de Ciências Agrárias
Endereço: Cidade Universitária Paulo VI – Caixa Postal 09, São Luís, Maranhão, Brasil
E-mail: josegomesnetoster@gmail.com

Mayra da Silva Oliveira
Mestre em Ciência Animal pela Universidade Federal de Minas Gerais
Instituição: Universidade Estadual do Maranhão, Centro de Ciências Agrárias
Endereço: Cidade Universitária Paulo VI – Caixa Postal 09, São Luís, Maranhão, Brasil

Diego Carvalho Viana
Doutor em Anatomia dos Animais Domésticos e Silvestres pela Universidade de São Paulo
Instituição: Universidade Estadual da Região Tocantina do Maranhão (UEMASUL), Centro de Ciências Agrárias, Curso de Medicina Veterinária; Programa de Pós-graduação em Ciência Animal, Universidade Estadual do Maranhão (UEMA)
E-mail: diego_carvalho_@hotmail.com

Ana Lúcia Abreu-Silva
Doutora em Ciência pela Fundação Oswaldo Cruz
Instituição: Programa de Pós-graduação em Ciência Animal, Universidade Estadual do Maranhão (UEMA)
Endereço: Cidade Universitária Paulo VI – Caixa Postal 09, São Luís, Maranhão, Brasil
E-mail: abreusilva.ana@gmail.com

Ferdinan Almeida Melo
Doutor em Patologia pela Universidade Federal de Minas Gerais
Instituição: Programa de Pós-graduação em Ciência Animal, Universidade Estadual do Maranhão (UEMA)
Endereço: Cidade Universitária Paulo VI – Caixa Postal 09, São Luís, Maranhão, Brasil
E-mail: ferdinanmelo@yahoo.com.br

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

Brazilian Journal of Development

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 parenchymaof 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 is lets 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 immmunoreactive cells were observed throughout the organ.

Keywords: Testudinata. Parenchyma. Pancreaticislet. Chelonia.

RESUMO

Kinosternon scorpioides é um cágado semi-aquático de água doce, que no Brasil está em risco iminente de extinção. Dessa forma é necessário realizar estudos para desenvolver sua criação em cativeiro. Nesta investigação, foram utilizados fragmentos de pâncreas de seis espécimes adultos de *K. scorpioides* (três machos e três fêmeas) que foram previamente emblocados em parafina. Cortes de 3µm de espessura foram colocados em lâminas histológicas previamente tratadas (silanizadas). Os cortes foram submetidos à técnica estreptavidina-peroxidase para detectar antígenos de glucagon presentes no parênquima do pâncreas da tartaruga. A análise quantitativa das lâminas foi realizada contando 20 campos distintos contendo células imunorreativas ao glucagon sob um microscópio óptico com objetiva de 40X. As células imunorreativas do glucagon foram identificadas no parênquima do pâncreas da tartaruga da lama e foram mais frequentes nas ilhotas pancreáticas. Elas também foram observadas no parênquima exócrino e no epitelial da cobertura do ducto pancreático. A análise histológica mostrou que as ilhotas pancreáticas não apresentavam cápsula de tecido conjuntivo. A análise quantitativa mostrou células imunorreativas de glucagon por campo uma média de células 21,4 ± 11,4. Células imunorreativas ao glucagon foram observadas em todo o órgão.

Palavras-chave: Testudinata. Parênquima. Ilhota pancreática. 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 do 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 40Xlens.

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 primaryantibody5 (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), diaphonized 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 anOlympus 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. scorpioides* they were distributed throughout the organ. Regarding quantitative analysis of glucagon immunoreactive cells, the mean was 21.4 ± 11.4 cells.

ACKNOWLEDGEMENTS

The authors thank the national Council for Scientific and Technological Development **(**Conselho Nacional de Desenvolvimento Científico e Tecnológico) (CNPq) and the Coordination for Training of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the graduate grant and financial help for the materials necessary to develop the project.

REFERENCES

Acuña-Mesen, R.A. (1994). Morphometric variation and ecologic characteristic of the habitat of the *Kinosternon scorpioides* turtle in Costa Rica. Revista Brasileira de Biologia, 3, 537-547.

Brasil. Instrução Normativa IBAMA *169, de 20 de fevereiro de 2008*. Aprova a Estrutura Regimental do IBAMA, publicada no Diário Oficial da União de 27 de abril de2007

Beisser, C.J., Weisgram, J., Hilgres, H., & Splechtna, H. (1998). Fine structure of the dorsal lingual epithelium of *Trachemys scripta Elegans* (Chelonia: Emydidae). The Anatomical Record, 2, 127-135. doi: 10.1002/(SICI)1097-0185(199802)250:2<127::AID-AR1>3.0.CO;2-0.

Garcia, A.A., Lozano, M.T., & Agulleiro, B. (1987). Endocrine pancreas of *Testudo graeca L.* (Chelonia) in summer and winter: an immunocytochemical and ultrastructural study. General and Comparative Endocrinology, 2, 235-248. doi: 10.1016/0016-6480(87)90035-9.

Gapp, D.A., Kenny, M.P., & Polak, J.M.(1985). The gastro-entero-pancreatic system of the turtle, *Chrysemys picta*. Peptídes, 3, 347-352. doi: 10.1016/0196-9781(85)90396-1.

Miller, M.R., & Lagios, M.D. (1970). The pancreas. (New York: Academic Press)

Muniz, W.W., Machado, V.L., & Marques, M. (1991). Immunohistochemical localization of insulin-like material in antral gastric mucosa and intestinal epithelial cells of the turtles *Chrysemys dorbigni* and *Phrynops hilarii*. Brazilian Journal of Medical and Biological Research, 3, 327-331.

Ivanova, V.F., Rossol'Ko, G.N., & Puzyrev, A.A. (1997). The endocrine apparatus of the epithelium of the gastric mucosa in the steppe turtle. Morfologiia, 1, 85-90

Madrid, J.F., Ballesta, J., Pastor, L.M., Perez-Tomaz,R., & Hernandez, F.(1989) Distribution of mucins in the mucosa of the digestive tract of reptiles: a histochemical study. Acta Histochemica, 85, 117-129. doi: 10.1016/S0065-1281(89)80053-4.

Pereira, J.G.(2000).Estudos histológico e histoquímico do tubo digestivo e do pâncreas do *Kinosternon scorpioides Linnaeus, 1766* (Reptilia, Chelonia, kinosternidae), mudturtle. Dissertation (Master's in Veterinary Medicine).

Rhoten, W. (1987). Co-localization of glucagon and pancreatic polypeptide in testudine pâncreas. Experientia, 43, 28-430. doi:10.1007/BF01940439.

Tafuri, W.L., Santos, R.D.E.L.,Arantes, R.M., Goncalves, R., Melo, M.N., Michalick, M.S., & Tafuri, W.L. (2004). An alternative immunohistochemical method for detecting *Leishmania* amastigotes in paraffin-embedded canine tissues. Journal of Immunological Methods, 292, 17-23. doi: 10.1016/j.jim.2004.05.009.

Sousa, A.L., Campos-Junior, P.H.A., Costa, G.M.J., & França, L.R. (2014) Spermatogenic cycle length and sperm production in the Freshwater turtle *Kinosternon scorpioides*. Biology of Reproduction,90, 1-10. doi: 10.1095/biolreprod.113.112391.

Viana, D.C., Anunciação, A.R.A., Santos, A.C., Oliveira, C.A., Miglino, M.A., Assis-Neto, A.C., Oliveira, A.S., & Sousa, A.L. (2014). Plasma Testosterone and Seasonal Reproductive Changes in the Scorpion Mud Turtle. Pakistan journal of zoology, 3, 620-28

Chandavar, V.R., & Naik, P.R. (2008). Immunocytochemical detection of glucagon and insulin cells in endocrine pancreas and cyclic disparity of plasma glucose in the turtle *Melanochelystrijuga*. Journal of Biosciences, 2, 239-247. doi: 10.1007/s12038-008-0041-8.