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Original Article
Animal Morphophysiology

Morphological description of ovary and uterus of the nurse shark (*Ginglymostoma cirratum*) caught off at the Fortaleza coast, Northeast Brazil[1]

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ABSTRACT.- Rêgo M.G., Araujo M.L.G., Barros M.E.G., Aires, L.A., Oliveira, P.G.V., Hazin F.H.V., Fitzpatrick J.L. & Evêncio-Neto J. 2019. **Morphological description of ovary and uterus of the nurse shark (*Ginglymostoma cirratum*) caught off Fortaleza coast, Northeast Brazil**. *Pesquisa Veterinária Brasileira 39(12):997-1004.* Departamento de Morfologia e Fisiologia Animal, Área de Histologia, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: mari_rego03@hotmail.com

The nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1778) is one of the most studied species of elasmobranchs. However, the knowledge of their reproductive biology is still relatively rare, particularly in the western South Atlantic. This study aimed to describe the morphology of the uterus and the ovary of *G. cirratum*, based on specimens caught off at the Fortaleza/CE coast, northeast Brazil. Samples were collected from September 2012 to June 2013, from regular landings of artisanal fishing, which commercialize this species freely. A total of ten females were collected. The methodologies followed for analyzing the ovaries and uterus of those females included both macroscopic and histological analysis. *G. cirratum* has internal type ovary morphology, with invaginations of connective tissue, which defines compartments and separate oocyte groups in ovigerous lots. The epithelium lining the ovary changes from simple columnar ciliated in the area without ovigerous lots, which turns into a simple cubic epithelium in the coating portion of the epigonal organ where ovarian tissue is absent. The uterine mucosa has secretory cells denoted by Alcian Blue staining, indicating the production of mucopolysaccharides, even in immature individuals. This lecithotrophic shark has a uterine vascularized mucosa that is one characteristic of viviparous elasmobranch species.

INDEX TERMS: Morphology, ovary, uterus, nurse shark, *Ginglymostoma cirratum*, Fortaleza, Brazil, female, reproduction, histology, sharks.

RESUMO.- [Descrição morfológica de ovário e útero de Tubarões-lixa (*Ginglymostoma cirratum*) capturados na costa de Fortaleza, Nordeste do Brasil.] O tubarão-lixa, *Ginglymostoma cirratum* (Bonnaterre, 1778) é uma das espécies mais estudadas de elasmobrânquios. O conhecimento de sua biologia reprodutiva, no entanto, ainda é relativamente raro, particularmente no Oeste do Atlântico Sul. Este estudo objetivou a descrição morfológica do útero e ovário de *G. cirratum*, baseado em espécimes capturados na costa de Fortaleza/CE, no Nordeste do Brasil. Um total de dez fêmeas foi coletado de setembro de 2012 a junho de 2013, nos desembarques regulares da pesca artesanal, que comercializam

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essa espécie livremente. A metodologia foi de acordo com as analises macroscópica e histológica dos ovários e útero. *G. cirratum* tem o tipo de morfologia de ovário interno, com invaginações de tecido conjuntivo, que define compartimentos e separa grupos de oócitos em lotes ovígeros. O epitélio que reveste o ovário varia de epitélio simples colunar ciliado na área que não apresenta lotes ovígeros para epitélio simples cúbico na porção de revestimento do órgão epigonal, onde o tecido ovariano é ausente. A mucosa uterina possui células secretoras, evidenciadas pela coloração em Alcian Blue, indicando a produção de mucopolissacarídeos, mesmo em indivíduos imaturos. Este tubarão lecitotrófico apresenta a mucosa uterina vascularizada, sendo esta uma característica de espécies de elasmobrânquios vivíparos.

TERMOS DE INDEXAÇÃO: Morfologia, ovário, útero, tubarão-lixa, *Ginglymostoma cirratum*, Fortaleza, Brasil, fêmea, reprodução, histologia, tubarões.

INTRODUCTION

The nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1778) is an abundant species in tropical and subtropical waters of the Atlantic Ocean, being present along the entire coast of northeast Brazil. It is a coastal species, widely used in aquariums around the world, because of its docility and resistance (Cervigón & Alcalá 1999, Compagno et al. 2005, Garla et al. 2009, Santander-Neto et al. 2011). According to the International Union for Conservation of Nature's (IUCN) Red List, the nurse shark is listed as vulnerable in Brazil. It is also included in the national list of endangered species (IN445/2015 - MMA) and, therefore, its capture is prohibited in Brazil. However, there are several reports of illegal fishing and usage of these animals in aquariums without the necessary license from the responsible bodies (Arthaud 1999, Gruber & Sundström 2000, Rosa et al. 2005, Cunha et al. 2016). For instance, in the Port of Fortaleza, Ceará, Brazil, the species accounts for 15% to 20% of annual shark landings (Arthaud 1999).

According to Castro (2000), this species, which may reach up to 4m long, is viviparous lecithotrophic, with a litter size from 10 to 20 embryos per uterus and a gestation period ranging from 131 days to 240 days (Castro 2000, Carrier et al. 2004). The reproductive cycle is biennial, with an estimated vitellogenesis period of 18 months (Castro 2009).

The reproductive system of *G. cirratum* has the same anatomical features shared by other elasmobranchs, given the conservative nature of the morphology of female reproductive tract in this group (McMillan 2007). The ovaries of the nurse shark are asymmetric (Castro 2000), being only the right ovary functional. The gonad is 5% to 10% the weight of the epigonal organ in immature specimens (Fänge & Mattisson 1981). The other components of the reproductive system correspond to the pair of oviducal glands and uteri, both functional.

The nurse shark is one of the most studied species of elasmobranchs, with regard to several aspects of their biology, such as their immune system (Fidler et al. 1969, Ross & Jensen 1973, Fänge & Mattisson 1981, Rumfelt et al. 2002, Dooley & Flajnik 2005). Knowledge of their reproductive biology, however, is still relatively rare (Carrier & Luer 1990, Castro 2000, Pratt Junior & Carrier 2001, Saville et al. 2002), particularly in the western South Atlantic (Castro & Rosa 2005, Garla et al. 2015, Rêgo et al. 2015). This study, therefore, aimed to describe the morphology macroscopic and microscopic of the uterus and the ovary of *G. cirratum*, based on specimens caught off at the Fortaleza/CE coast, northeast Brazil.

MATERIALS AND METHODS

Ethics statement. The authorization for keeping these samples was part of our scientific collector's permit granted by ICMBio ("Instituto Chico Mendes de Conservação à Biodiversidade") number 40674-4. The method adopted in this study was approved by the Ethics Committee on Animal Experimentation (CEUA) at the "Universidade Federal Rural de Pernambuco" (UFRPE), under the license number 058/2013.

Collection area. Ten female nurse sharks were collected at Mucuripe port, Fortaleza/CE (lat: 03°43'00"S; long: 038°28'07"W) (Fig.1), from September 2012 to June 2013, from regular landings by fishing boats, which routinely sell them freely. Before being marketed, the total length (TL) and pre-caudal length (PC) were measured. After being eviscerated by the fishermen, the ovaries and uterus were collected and then fixed in 10% buffered formalin.

Macroscopic description and characterization of the ovary and uterus. The ovaries and uteri were measured in length and width (cm) and classified macroscopically, according to The International Council for the Exploration of the Sea (ICES 2013). Females were classified as: immature, developing, and capable of reproduction, in early pregnancy, in middle pregnancy, in the end of pregnancy, post-birth and in regeneration.

Histological characterization. After 24 hours of fixation, the organs were cleaved, replaced in 10% buffered formalin for 24 hours, and then transferred to 70% ethanol. Subsequently, the samples were sent to the UFRPE, were processed them using standard histological protocol. The ovary and uterus fragments were dehydrated through successive washing in alcohol solutions with gradually increased concentrations, diaphanized, impregnated in butyl alcohol and embedded in paraplast. The paraplast blocks were then cut into 5µm-thick sections, which were placed on slides, kept at 37°C for 24 hours for drying. The sections were then stained with hematoxylin eosin-phloxine (HE) and Gomori trichrome, periodic acid–Schiff (PAS) and Alcian Blue to 2.5%. A trinocular microscope NIKON 50i, equipped with an imaging system to capture microscopic images, captured the images of histological sections.

RESULTS

The nurse shark ovaries are internal and suspended by the mesentery in the anterior abdominal cavity, with only the right ovary being functional (Fig.2). Of the ten females examined, eight were immature, while the remaining two were in the development stage. Immature females varied in their total length (TL) from 85 and 135cm. The reproductive tract showed a whitish ovary with width ranging from 1.5 to 2.5cm, and the presence of undifferentiated ovarian follicles. The ovary was embedded in the epigonal organ with its cranial morphology being similar to a network (Fig.2A). At this stage, females present extremely thin and slender uteri and reduced ovidrucal glands.

Females in developing stage had a total length (TL) ranging from 152 to 164cm. The ovaries had a width ranging from 3 to 4cm, with thicker walls and more yellowish aspect (Fig.2B) than in immature females. The ovarian follicles were evident

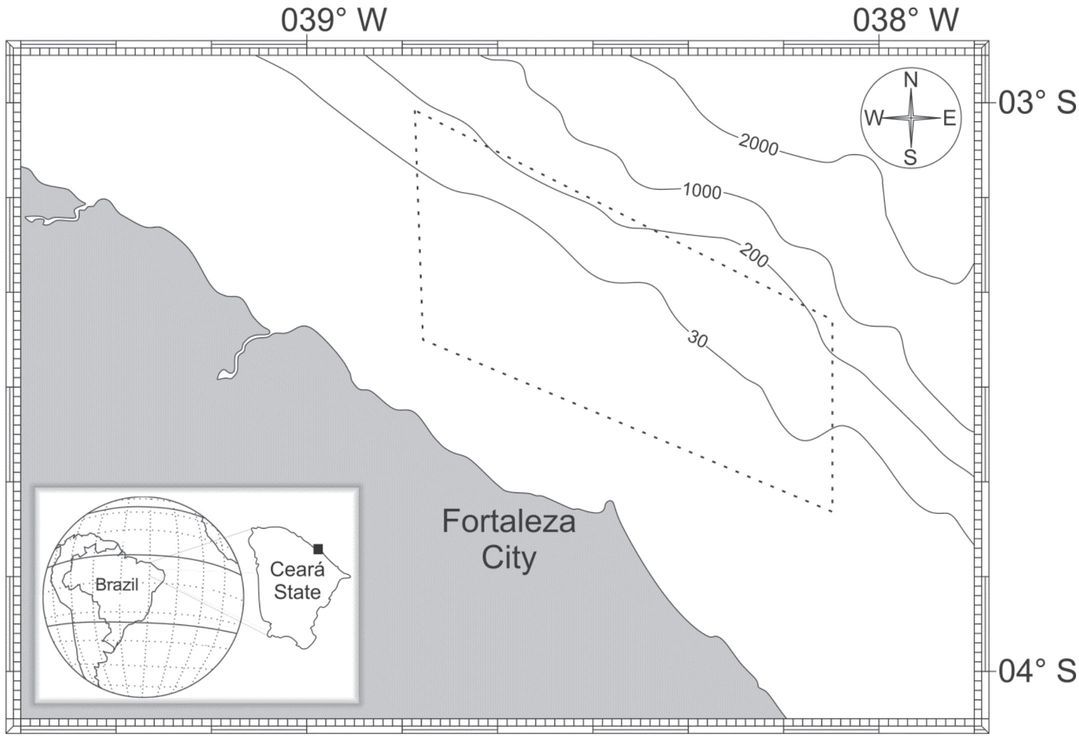

Fig.1. Map indicating the location of the sampling area, port of Mucuripe, Fortaleza/CE (Santander-Neto 2014).

A B

Fig.2. Macroscopic and microscopic photos of shark ovary evidencing (**A**) immature ovary (arrow), and (**B**) ovary in development (arrow).

with oocytes at different stages of vitellogenesis. The uteri from females in this stage had thicker walls than in immature females, and the oviducal glands were more developed as well.

Ovary

Histologically, the ovaries were coated by a simple columnar ciliated epithelium in the area without ovigerous lots, which turns into a simple cubic epithelium in the coating portion of the epigonal organ where ovarian tissue is absent (Fig.3A). Although it is an internal ovary, there is an area of connective tissue that makes up the ovarian stroma and separates the ovigerous portion from the limphomieloid portion of the epigonal body. The connective tissue creates compartments with isolated ovigerous lots (Fig.3B). The epithelial tissue in these places becomes pleated (Fig.3C).

In the ovary parenchyma of immature individuals, the presence of previtellogenic follicles type I and type II were identified by an increase in oocyte and by the polymorphism in the follicular layer, which consists initially of a layer of squamous cells, progressing to a layer of columnar and cuboidal cells. At this stage of follicular development, the teak and the pellucid zone are visible. The larger previtellogenic follicles show an increase in vascularization network between theca (Fig.4A,B).

Females in the developing stage had ovaries in stages I and II of vitellogenesis. Around the vitellogenic oocytes, there is the pellucid zone, the stratified follicular epithelium and the teak, with evident vascularisation. Atresia was observed in all follicular development stages (Fig.4C). In this phase, the epigonal organ is already proportionally small. The ovarian

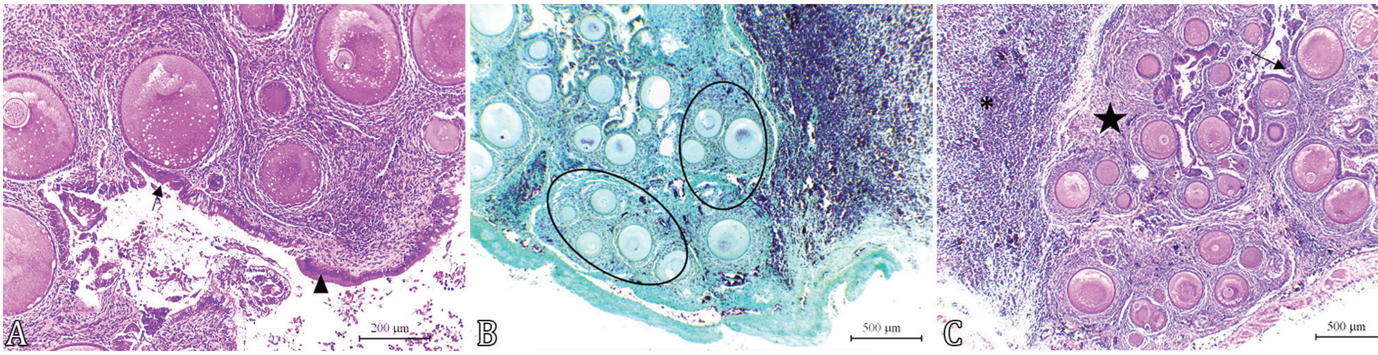

Fig.3. Nurse shark ovary evidencing (**A**) the modification of the ovarian epithelium which is simple columnar epithelium in the region without the ovigerous lots (arrow) and simple cubic epithelium in the epigonal organ lining portion (arrowhead). HE, bar = 200µm. (**B**) Different ovigerous lots (circulated). Trichrome Gomori, bar = 500µm. (**C**) Epithelial tissue at these sites becomes pleated (arrow). It is observed that there is a portion of connective tissue (star) that composes the ovarian stroma and that separates the ovum portion of the portion of the epigonal organ (asterisk). HE, bar = 500µm.

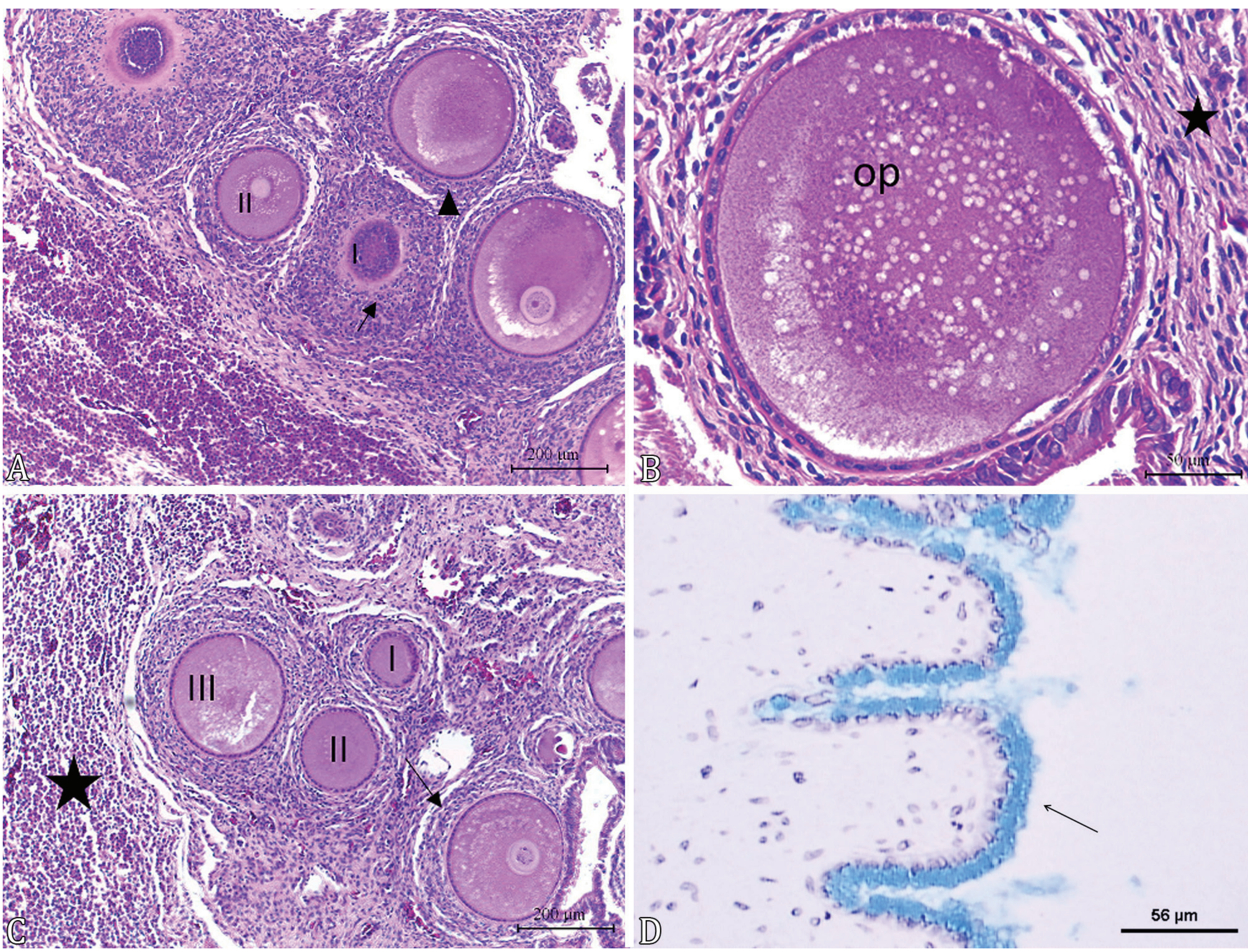

Fig.4. Nurse shark ovary. (**A**) In the immature ovary, the presence of type I and type II pre-vitellogenic follicles was observed, identified by the oocyte enlargement and polymorphism of the follicular layer, which initially consists of a layer of pavement cells (arrow), and progressing to a layer of cubic and columnar cells (arrowhead). HE, bar = 200µm. (**B**) Major pre-vitellogenic follicles present a layer of polymorphic stratified cells (star). HE, bar = 50µm. (**C**) The ovary of females in the developing stage had follicles in stages I and II of vitellogenesis. Around the vitellogenic oocytes, the zona pellucida, stratified follicular epithelium (arrow), and teak with evident vascularization are observed. The epigonal organ is already in small proportion (star). HE, bar = 200µm. (**D**) The columnar ovarian epithelium presented secretory cells with reactivity to Alcian Blue 2.5% (arrow). Alcian Blue 2.5%, bar = 56µm.

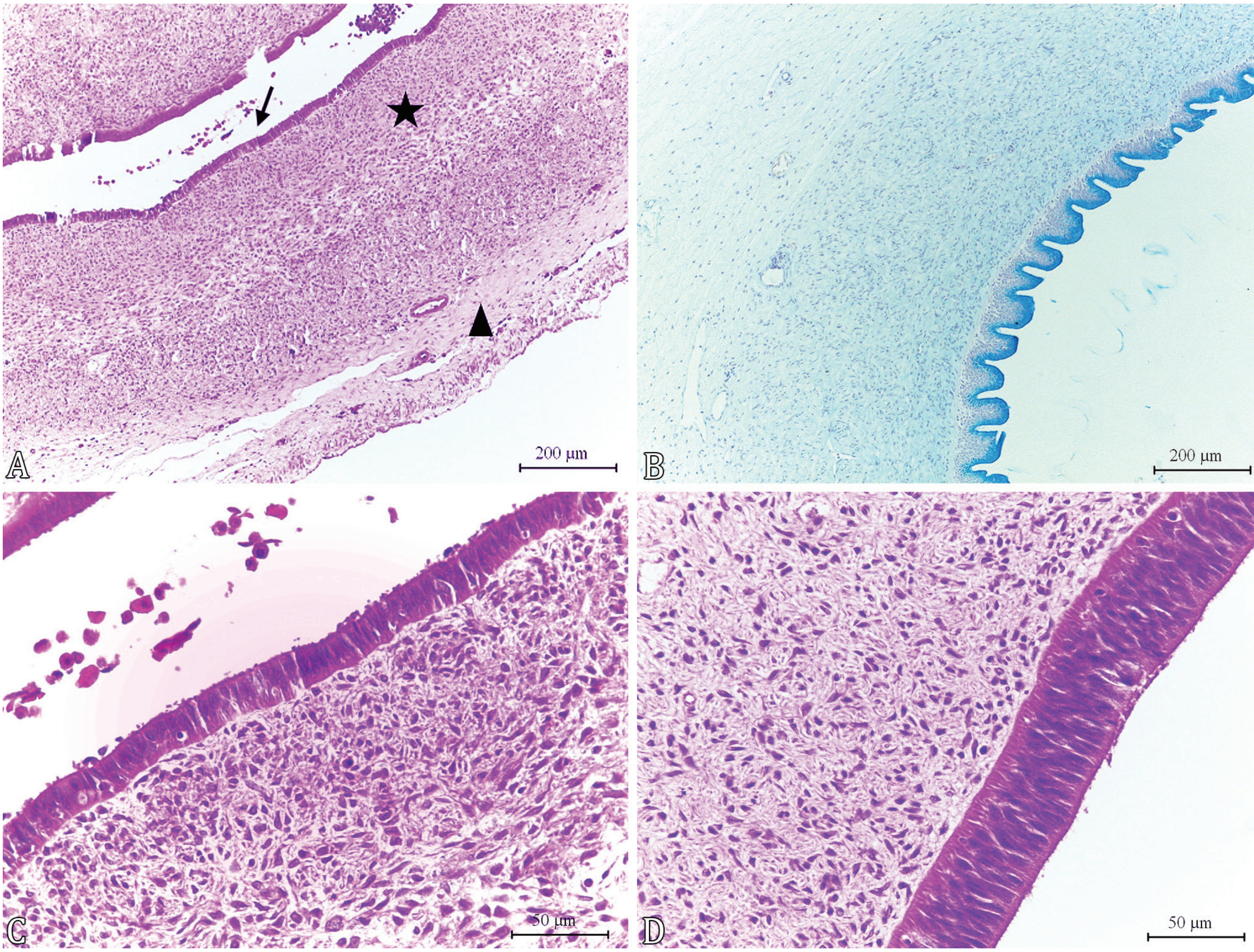

Fig.5. Nurse shark uterus. (**A**) Showed that the nurse shark uterus is constituted by three (3) layers: mucous (star), muscular (arrowhead) and serosa (arrow). HE, bar = 200μm. (**B**) It was evident an acellular layer that was positive to trichromatic Gomori and negative AB to 2.5% between the mucosa and submucosa suggesting that the formed layer of collagen. Alcian Blue, bar = 200μm. (**C**) In the middle region, a decrease in cell layer of the epithelium and the basal portion was observed with invagination of connective blood vessels into the columnar epithelium (arrow). HE, bar = 50μm. (**D**) The distal segment of uterus is lining with the epithelium stratified prismatic ciliary. HE, bar = 50μm.

developmental stage of the columnar epithelium showed the presence of secreting cells with reactivity to Alcian Blue 2.5% (Fig.4D).

Uterus

The results showed that the uterus of the nurse shark is constituted by three layers: mucosa, muscular and serosa (Fig.5A). The mucosa consists of epithelium, which varies according to the uterine region. Just below the epithelium coating is the undeveloped lamina propria, composed of loose connective tissue with blood and lymphatic vessels. In this area, the uterine glands are not visible. The muscle layer consists of two layers of well-developed muscular tissue. The serosa is a thin layer formed of loose connective tissue, covered externally by a single layer of squamous epithelial tissue (Fig.5A). An acellular layer that was positive to trichrome Gomori and negative Alcian Blue to 2.5% between the mucosa and connective tissue was evident, suggesting a collagenous nature (Fig.5B).

The epithelium of uterine mucosa ranged from simple to stratified columnar. In the cranial region of the uterus, the epithelium has stratified columnar appearance with the mucosal cells Alcian Blue 2.5% reactive (Fig.5B). In the middle region, occurs a decrease in cell layer of the epithelium. In the basal portion, an invagination of connective blood vessels into the columnar epithelium was observed (Fig.5C). Stratified prismatic ciliary epithelium was present in the distal segment of uterus (Fig.5D). In the uterine body region, vascularization of the mucosa was further developed. The oviduct mucosa had a well-developed invagination covered by stratified epithelium, with superficial secreting cells with reactive secretion to the Alcian Blue to 2.5% (Fig.6A,B).

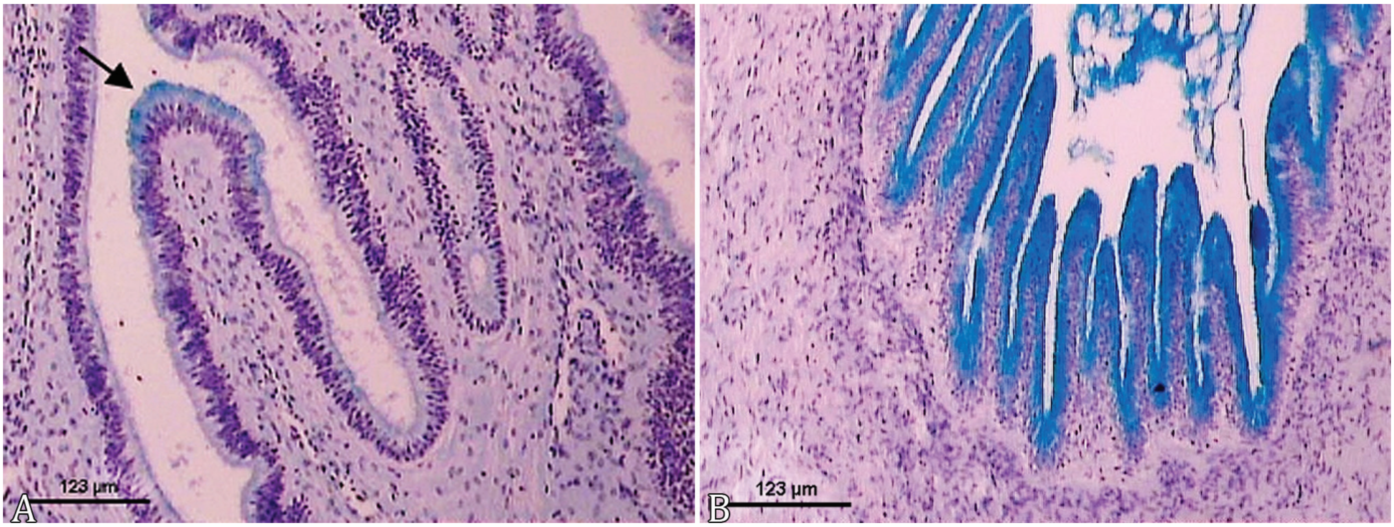

Fig.6. Nurse shark uterus. (**A**,**B**) The oviductal featured by presenting a mucosa with well developed invagination covered by stratified epithelium, with apical portion of superficial secreting cells reactive to the AB 2.5%. (**A**) Trichrome Gomori, bar = 123µm. (**B**) Alcian Blue, bar = 123µm.

DISCUSSION

According to Carrier et al. (2004) and Hamlett (2007), sharks have two ovaries that are paired and located at the front end of the abdominal cavity. Typically, both ovaries are functional, though in some species of sharks (e.g. members of the genera *Scyliorhinus*, *Carcharhinus*, *Mustelus* and *Sphyrna*) only the right ovary is functional, and the left ovary is absent or non-functional (Pratt Junior 1988). In *Ginglymostoma cirratum* only the right ovary is functional, with a structure similar to that described for *Cetorhinus maximus* (Matthews 1950) and *Rhincodon typus* (Nozu et al. 2015).

Pratt Junior (1988) classified the ovaries of sharks as internal and external, based on the morphological interactions between the ovary and the epigonal body. Nurse sharks have the same ovarian characteristic as the Lamnidae family (Pratt Junior 1988) and Cetorhinidae family (Matthews 1950), which have an internal ovary. This type of ovary is embedded in the epigonal body, where invaginations of connective tissue define separate groups of oocyte compartments, as found here for *G. cirratum* (Fig.3C).

The lining of the ovary identified in the nurse shark (Fig.3A) has the same morphological characteristics of the epithelium described for the ovary of the *Zearaja chilensis* (Wehitt et al. 2015) and *Sympterygia acuta* (Díaz-Andrade et al. 2009). Both species are oviparous and have the ciliated epithelium lining the ovary.

Chatchavalvanich & Visuttipat (1997) described the ovary of *Dasyatis bleekeri* as a simple epithelium composed of cells varying from squamous to cubic, without the presence of cilia. However, Hamlett et al. (1999) described the *Stingaree jamaicensis* ovary and identified epithelial cells lining the organ with apical microvilli by using electron microscopy. Considering the conservative profile of elasmobranch ovaries (McMillan 2007), we can infer that the epithelium of *G. cirratum* ovarian coating consists of epithelial cells with apical microvilli and no ciliated cells. The elucidation of this question, however, depends on future studies of the ultrastructure of the ovary of that species.

Gračan et al. (2013) observed a predominance of smaller previtellogenic (type I) and few average previtellogenic follicles (type II) in the ovaries of immature *Squalus acanthias*. The same was observed for *G. cirratum* here, which had a higher incidence of follicles type I in immature females and an increase of type II in the ovaries of developing females. The presence of oocytes in the beginning of vitellogenesis occurred in developing ovaries of *R. typus* (Nozu et al. 2015) and of *S. acanthias* (Gračan et al. 2013). Like in the present study, Castro (2000) also reported vitellogenesis occurrence in immature and developing female ovaries.

The variation in shape and number of layers of follicle cells, based on follicular development, was recorded for various elasmobranch species (Guraya 1986, Pratt Junior 1988, Díaz-Andrade et al. 2009, Gračan et al. 2013). As recorded by Lutton et al. (2005), granulosa cells of immature batoid *Leucoraja erinacea* may vary from simple to cubic form and while follicular genesis and oogenesis take progress the cells develop into a columnar structure. In *G. cirratum*, the morphology of vitellogenic follicles is similar to that described for *S. acanthias* (Gračan et al. 2013), but different from *Z. chilensis* (Wehitt et al. 2015) and *D. bleekeri* (Chatchavalvanich & Visuttipat 1997). These discrepancies can be attributed to the existing differences between sharks and rays (Dodd 1983).

The uterus of *G. cirratum* agrees with the general pattern described by Hamlett et al. (1998) for elasmobranch species. The morphological constitution of the uterine lining presents variations depending on the reproduction mode of elasmobranchs (Matthews 1950, Storrie et al. 2009, Wehitt et al. 2015). *G. cirratum* has secretory cells in the endometrium denoted by Alcian Blue staining (Fig.5B), indicating the production of mucopolysaccharides, even in immature individuals. This is also observed in other lecitotrophic species, such as *Mustelus antarcticus* (Storrie et al. 2009).

The presence of an underlying laminate layer containing vessels of different dimensions (Fig.5C) reinforces the vascularization role of the uterus mucosa in viviparous species (Hamlett & Koob 1998).

Recently, a study on the shark *R. typus* showed that there is an increase in the uterine vasculature to assist in gas exchange with embryos (Joung et al. 1996). In aplacentary viviparous elasmobranchs, it has been assumed that the endometrium is not specialized to secrete nutrients to the embryo, participating only in the breathing process and osmoregulation (Hamlett et al. 1999). However, various evidences have indicated that some lecitotrophic species, such as *Centrophorus granulosus* (Cotton et al. 2015), *M. antarcticus* (Storrie et al. 2009) and *Galeocerdo cuvier* (Castro et al. 2016) do present some level of matrotrophy.

The term mucoid histotrophy (Hamlett et al. 2005, Musick & Ellis 2005) indicates a form of matrotrophy, where the embryo nourishment is complemented with the mucus produced by the uterine epithelium. The distinction between lecitotrophy and mucoid histotrophy is given by the weight ratio between the newly fertilized egg and the embryo brought to term. In *S. acanthias* there is a considerable loss (15-55%) in organic matter content, from egg to embryo (Ranzi 1932, 1934, Needham 1942), indicating a lecithotrophic species. *C. granulosus* (Cotton et al. 2015) and *M. antarcticus* (Storrie et al. 2009) has a loss of less than 20%, which suggests maternal nutritional contribution to the developing embryo (Hamlett et al. 2005).

Due to the presence of structures in the uterine epithelium with high secretory activity in the early and late gestation, additional differences between these reproduction modes become evident by histological analysis (Hamlett et al. 2005).

Castro (2000) observed a 42% loss in weight during embryonic development in *G. cirratum*, indicating that it is a lecitotrophic species. The presence of AB + secreting cells in the uterine epithelium of the specimens studied in this work is, therefore, likely related to the secretion production to the uterine epithelium lubrication and not for embryo nutrition, as also identified in *M. antarcticus* (Storrie et al. 2009). However, further studies are needed to prove this statement. The acellular layer shown between the mucosa and submucosa, with a positive reactivity to Gomori Trichrome, and negative for Alcian Blue, indicates the presence of collagen and fibroblasts bundles. A single layer of squamous epithelium constituting the serosa layer delimits a layer of smooth muscle in *Mustelus schmitti* (Galíndez et al. 2010). The same morphology was observed here in the nurse sharks examined.

CONCLUSIONS

This study demonstrates how necessary the understanding of mucoid histotrophy contribution is for embryonary development of elasmobranch species which has the lecithotrophy as the mode of reproduction.

The next step seems to be identifying and examine the presence of blood vessel in uterine epithelium and the advantage of having ovigerous lots to reproductive potential of *Ginglymostoma cirratum*.

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Conflict of interest.- The authors declare no conflicts of interest.

REFERENCES

Arthaud I.D.B. 1999. Fauna de tubarões alvo da pesca artesanal na praia de Mucuripe, Fortaleza-CE (Chondrichthyes, Elasmobranchii). Monografia, Universidade Federal do Ceará, Fortaleza. 338p.

Carrier J.C. & Luer C.A. 1990. Growth rates in the nurse shark, *Ginglymostoma cirratum.* Copeia 63(3):686-692. <http://dx.doi.org/10.2307/1446435>

Carrier J.C., Pratt Junior H.L. & Castro J.I. 2004. Elasmobranch reproduction, p.269-286. In: Carrier J.C., Musick J.A. & Heithaus M.R. (Eds), Biology of Sharks and Their Relatives. CRC Press, Boca Raton. <http://dx.doi.org/10.1201/9780203491317.ch10>

Castro J.I. 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida East Coast and the Bahama Islands. Environ. Biol. Fish. 58(1):1-22. <http://dx.doi.org/10.1023/A:1007698017645>

Castro J.I. 2009. Observations of the reproductive cycles of some viviparous North American sharks. Aqua 15:205-222.

Castro A.L.F. & Rosa R.S. 2005. Use of natural marks on population estimates of nurse shark, *Ginglymostoma cirratum*, at Atol das Rocas Biological Reserve, Brazil. Environ. Biol. Fish. 72(2):213-221. <http://dx.doi.org/10.1007/s10641-004-1479-7>

Castro J.I., Sato K. & Bodine A.B. 2016. A novel mode of embryonic nutrition in the tiger shark, *Galeocerdo cuvier.* Mar. Biol. Res. 12(2):200-205. <http://dx.doi.org/10.1080/17451000.2015.1099677>

Cervigón F. & Alcalá A. 1999. Los Peces Marinos de Venezuela: tiburones y rayas. Vol.5. Fundación Museo del Mar, Nueva Esparta, Venezuela. 230p.

Chatchavalvanich K. & Visuttipat R. 1997. Gross and microscopic structures of the female reproductive system in the whip-tail stingray (*Dasyatis bleekeri*). Thammasat Int. J. Sc. Tech. 2:47-55.

Compagno L.J.V., Ando M. & Fowler S. 2005. Sharks of the World. Princeton University Press, New Jersey. 496p.

Cotton C.F., Grubbs R.D., Dyb J.E., Fossen I. & Musick J.A. 2015. Reproduction and embryonic development in two species of squaliform sharks, *Centrophorus granulosus* and *Etmopterus princeps*: evidence of matrotrophy? Deep Sea Res. Part II, Top. Stud. Oceanogr. 115:41-54. <http://dx.doi.org/10.1016/j.dsr2.2014.10.009>

Cunha C.M., Oliveira L.E. & Kfoury Junior J.R. 2016. Comparative anatomy of the extraocular muscles in four Myliobatoidei rays (Batoidea, Myliobatiformes). J. Anat. 228(5):877-886. <http://dx.doi.org/10.1111/joa.12438> <PMid:26853799>

Díaz-Andrade M.C., Galíndez E. & Estecondo S. 2009. The ovary of the bignose fanskate *Sympterygia acuta* Garman, 1877 (Chondrichthyes, Rajidae) in the Bahía Blanca estuary, Argentina: morphology and reproductive features. Braz. J. Biol. 69(2):405-413. <http://dx.doi.org/10.1590/S1519-69842009000200025> <PMid:19675946>

Dodd J.M. 1983. Reproduction in cartilaginous fishes (Chondrichthyes), p.31-96. In: Hoar W.S., Randall D.J. & Donaldson E.M. (Eds), Fish Physiology. Vol.9A. Academic Press, New York.

Dooley H. & Flajnik F. 2005. Shark immunity bites back: affinity maturation and memory response in the nurse shark, *Ginglymostoma cirratum.* Eur. J. Immunol. 35(3):936-945. <http://dx.doi.org/10.1002/eji.200425760> <PMid:15688348>

Fänge R. & Mattisson A. 1981. The lymphomyeloid (hemopoietic) system of the atlantic nurse shark, *Ginglymostoma cirratum.* Biol. Bull. 160(2):240-249. <http://dx.doi.org/10.2307/1540884>

Fidler J.E., Clem L.W. & Small Junior P.A. 1969. Immunoglobulin synthesis in neonatal nurse sharks (*Ginglymostoma cirratum*). Comp. Biochem. Physiol. 31(2):365-366. <http://dx.doi.org/10.1016/0010-406X(69)91660-0> <PMid:4982087>

Galíndez E.J., Díaz-Andrade M.C., Moya A.C. & Estecondo S. 2010. Morphological changes in the pregnant uterus of the smooth hound dogfish *Mustelus schmitti* Springer, 1939 (Gatuzo)(Condrichthyes, Triakidae): microscopic study and phylogenetic reproductive implications. Int. J. Morphol. 28(4):1003-1010. <http://dx.doi.org/10.4067/S0717-95022010000400004>

Garla R.C., Garcia Junior J., Veras L.B. & Lopes N.P. 2009. Fernando de Noronha as an insular nursery area for lemon sharks, *Negaprion brevirostris*, and nurse sharks, *Ginglymostoma cirratum*, in the equatorial western Atlantic Ocean. Mar. Biodivers. Rec. 2:1-4. <http://dx.doi.org/10.1017/S1755267209000670>

Garla R.C., Freitas R.H.A., Calado J.F., Paterno G.B.C. & Carvalho A.R. 2015. Public awareness of the economic potential and threats to sharks of a tropical oceanic archipelago in the western South Atlantic. Mar. Policy 60:128-133. <http://dx.doi.org/10.1016/j.marpol.2015.06.012>

Gračan R., Lazar B., Posavec I., Gregorović G. & Lacković G. 2013. Maturation, fecundity and reproductive cycle of spiny dogfish, *Squalus acanthias*, in the Adriatic Sea. J. Mar. Biol. Res. 9(2):198-207. <http://dx.doi.org/10.1080/17451000.2012.729956>

Gruber S. & Sundström L.F. 2000. *Negaprion brevirostris*. The IUCN Red List of Threatened Species. Available at <http://iucnredlist.org> Access on Dec. 14, 2007.

Guraya S. 1986. The cell and molecular biology of fish oogenesis, p.111-163. In: Sauer H. (Ed.), Monographs in Developmental Biology. Vol.18. Karger, Basel.

Hamlett W.C. 2007. The testis and spermatogenesis, p.171-200. In: Engel K.B. & Callard G.V. (Eds), Reproductive Biology and Phylogeny of Chondrichthyes: sharks, batoids, and chimaeras. 3rd ed. Science Publishers Inc., New Hampshire.

Hamlett W.C. & Koob T. 1998. Female reproductive system, p.398-443. In: Hamlett W.C. (Ed.), Sharks, Skates and Rays: the biology of elasmobranchs fishes. Johns Hopkins University Press, Maryland.

Hamlett W.C., Jezior M. & Spieler R. 1999. Ultrastructural analysis of folliculogenesis in the ovary of the yellow spotted stingray *Urolophus jamaicensis.* Ann. Anat. 181(2):159-172. <http://dx.doi.org/10.1016/S0940-9602(99)80003-X> <PMid:10332520>

Hamlett W.C., Knight D.P., Pereira F.T.V., Steele J. & Sever D.M. 2005. Oviducal glands in chondrichthyans, p.301-335. In: Hamlett W.C. (Ed.), Reproductive Biology and Phylogeny of Chondrichthyes: sharks, batoids and chimaeras. Science Publishers Inc., New Hampshire.

Hamlett W.C., Knight D.P., Koob T.J., Jezior M., Luong T., Rozycki T., Brunette N. & Hysell M.K. 1998. Survey of oviducal gland structure and function in elasmobranchs. J. Exp. Zool. 282(4-5):399-420. <http://dx.doi.org/10.1002/(SICI)1097-010X(199811/12)282:4/5<399::AID-JEZ2>3.0.CO;2-6>

ICES 2013. Report of the workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL). International Council for the Exploration of the Sea, Lisbon, Portugal. 66p.

Joung S.-J., Chen C.-T., Clark E., Uchida S. & Huang W.Y.P. 1996. The whale shark, *Rhicodon typus*, is a livebearer: 300 embryos found in a 'megamamma' supreme. Environ. Biol. Fishes 46(3):219-223. <http://dx.doi.org/10.1007/BF00004997>

Lutton B.V., George J.S., Murrin C.R., Fileti L.A., Callard I.P. & Hamlett W.C. 2005. The elasmobranch ovary, p.237-281. In: Hamlett W.C. (Ed.), Reproductive Biology and Phylogeny of Chondrichthyes: sharks, batoids and chimaeras. Science Publishers Inc., New Hampshire.

Matthews L.H. 1950. Reproduction in the basking shark, *Cetorhinus maximus* (Gunner). Philos. Trans. R. Soc. Lond. B, Biol. Sci. 234(612):247-316. <http://dx.doi.org/10.1098/rstb.1950.0003> <PMid:24537280>

McMillan D. 2007. Fish Histology: female reproductive system. Springer Netherlands, Dordrecht. 598p. <http://dx.doi.org/10.1007/978-1-4020-5715-1>.

Musick J.A. & Ellis J.K. 2005. Reproductive evolution of chondrichthyans, p.45-79. In: Hamlett W.C. (Ed.), Reproductive Biology and Phylogeny of Chondrichthyes: sharks, rays and chimaeras. 3rd ed. Science Publishers Inc., New Hampshire.

Needham J. 1942. Biochemistry and Morphogenesis. Cambridge University Press, Cambridge. 785p.

Nozu R., Murakumo K., Matsumoto R., Nakamura M., Ueda K. & Sato K. 2015. Gonadal morphology, histology, and endocrinological characteristics of immature female whale sharks, *Rhincodon typus.* Zoolog. Sci. 32(5):455-458. <http://dx.doi.org/10.2108/zs150040> <PMid:26428723>

Pratt Junior H.L. 1988. Elasmobranch gonad structure a description and survey. Copeia 3(3):719-729. <http://dx.doi.org/10.2307/1445394>

Pratt Junior H.L. & Carrier J.C. 2001. A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum.* Environ. Biol. Fishes 60(1/3):157-188. <http://dx.doi.org/10.1023/A:1007656126281>

Ranzi S. 1932. Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci: parte I. Pubbl. Staz. Zool. Napoli 12:209-290.

Ranzi S. 1934. Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci: parte II e III. Pubbl. Staz. Zool. Napoli 13:331-437.

Rêgo M.G., Fitzpatrick J., Hazin F.H.V., Araújo M.L.G., Silveira L.M., Oliveira P.G.V. & Evêncio-Neto J. 2015. Characterization of testicular morphology and spermatogenesis in the nurse sharks *Ginglymostoma cirratum* (Bonnaterre, 1788). Zoomorphology 134(1):117-123. <http://dx.doi.org/10.1007/s00435-014-0240-9>

Rosa R.S., Castro A.L.F., Furtado M., Monzini J. & Grubbs R.D. 2005. *Ginglymostoma cirratum*. The IUCN Red List of Threatened Species. Available at <http://www.iucnredlist.org> Accessed on Dec. 14, 2017.

Ross G.D. & Jensen J.A. 1973. The first component (C1n) of the complement system of the nurse shark (*Ginglymostoma cirratum*). I. Hemolytic characteristics of partially purified C1n. J. Immunol. 110(1):175-182. <PMid:4685400>

Rumfelt L.L., Mckinney E.C.M., Taylor E. & Flajnik M.F. 2002. The development of primary and secondary lymphoid tissues in the nurse shark *Ginglymostoma cirratum*: B-cell zones precede dendritic cell immigration and T-cell zone formation during ontogeny of the spleen. Scand. J. Immunol. 56(2):130-148. <http://dx.doi.org/10.1046/j.1365-3083.2002.01116.x> <PMid:12121433>

Santander-Neto J., Shinozaki-Mendes R.A., Silveira L.M., Jucá-Queiroz B., Furtado-Neto M.A.A. & Faria V.V. 2011. Population structure of nurse sharks, *Ginglymostoma cirratum* (Orectolobiformes), caught off Ceará State, Brazil, south-western Equatorial Atlantic. J. Mar. Biol. Assoc. U.K. 91(6):1193-1196. <http://dx.doi.org/10.1017/S0025315410001293>

Saville K.J., Lindley A.M., Maries E.G., Carrier J.C. & Pratt Junior H.L. 2002. Multiple paternity in the nurse shark, *Ginglymostoma cirratum.* Environ. Biol. Fishes 63(3):347-351. <http://dx.doi.org/10.1023/A:1014369011709>

Storrie M.T., Walker T.I., Laurenson L.J. & Hamlett W.C. 2009. Gestational morphogenesis of the uterine epithelium of the gummy shark (*Mustelus antarcticus*). J. Morphol. 270(3):319-336. <http://dx.doi.org/10.1002/jmor.10693> <PMid:19117062>

Wehitt A., Di Giacomo E.E. & Galíndez E.J. 2015. The female reproductive system of *Zearaja chilensis* (Guichenot, 1848) (Chondrichthyes, Rajidae): gametogenesis and microscopic validation of maturity criteria. Int. J. Morphol. 33(1):309-317. <http://dx.doi.org/10.4067/S0717-95022015000100049>