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Tutorial

Female reproductive traits of a commercially exploited skate: *Atlantoraja platana* (Günther, 1880) (Chondrichthyes, Rajidae). Ovarian morphology, gametogenesis and microscopic verification of maturity criteria

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ABSTRACT

Atlantoraja platana is an endemic species of the Southwest Atlantic Ocean, and is one of the most captured by the local bottom trawl industrial fisheries. In this work, the microscopic architecture of mature female's gonads and the dynamics of follicle development are studied as a contribution to raise awareness of reproductive biology of the species. Folliculogenesis depicts the same histologic pattern as in other Elasmobranchs. Follicles in different degrees of maturation coexist in mature animals. The oogonia were only found in immature individuals. Likewise, atretic follicles were recorded in ovaries of all sexual maturity stages. The microscopic size recorded from the beginning of yolk input is smallest than the detected with the necked eye. This study provides valuable information about female's gametogenesis that could be taken into account in the development of fisheries management.

1. Introduction

In the context of fishing pressure, variability in reproduction's temporal pattern and fitness is known (Anderson et al., 2008; Jager et al., 2008; Lowerre Barbieri et al., 2011; Winemiller, 2005; Wright and Trippel, 2009). Although the knowledge of life history is available for only some species of fish, there is a general agreement that stocks are undergoing fisheries-induced evolution (Dulvy et al., 2014; Ward Paige et al., 2012). Most Chondrichthyes are at the top of the trophic chain and exhibit a wide range of reproductive strategies (Camhi et al., 1998). These features, as well as the internal fertilization, the long gestation/incubation periods and the late sexual maturity, make them more vulnerable to overfishing than Teleosts (Stevens et al., 2000), placing this group at risk of population depletion (Kyne and Simpfendorfer, 2007). To evaluate reproductive potential and reproductive success, fish females are usually examined with macroscopic and morphometric tools, such as ovaries size and texture, oocytes color and size, uteri wide and oviductal gland development (Stehmann, 2002; Serra Pereira et al., 2011) and recently by histologic analysis. This last approach is actually considered the most accurate means to assess gonadal development in fish, by identifying the presence of vitellogenic follicles, sperm storage in oviductal gland, atresia and ovarian fecundity, among other features (Alonso Fernandez et al., 2011; Da Silva et al., 2017; Henderson et al., 2014; Moura et al., 2011; Waltrick et al., 2017).

The reproductive cycles of fish present a similar pattern of gonadal development (Brown Peterson et al., 2011) and germinal cells pass through consecutive phases leading to the formation of a mature vitellogenic oocyte (Mc Millan, 2007). The outlines of oocyte recruitment are clearly associated with the reproductive strategies (Castro, 2009; Lowerre Barbieri et al., 2011). For example, in Chondrichthyan species that show an annual cycle, with or without peaks, gametogenesis progress is continuous along sexual maturity, and different cohorts of developing follicles coexist (Serra Pereira et al., 2011; Waltrick et al., 2017). The observation of vitellogenic oocyte in the ovary has been commonly used as an indicator of maturity in females, and this is the standard for many biologic studies based on macroscopic observations (*Raja clavata*, Serra Pereira et al., 2011, 2015). Currently, these parameters have been complemented with the macroscopic general features of the reproductive tract. Likewise, different approaches as endocrinology (Awruch, 2015) and detection of vitelline precursors (*Torpedo marmorata*, Prisco et al., 2004), may supplement this

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information to accurate the stage of maturity of females.

At least, half of Chondrichthyes belong to the order Rajiformes and the Rajidae is the most abundant family within that order (Compagno, 2005). Some of them are the target of many Chondrichthyan fisheries around the world and display an increasing commercial value (Bernasconi and Navarro, 2014). All skates are oviparous, producing large oocytes with considerable yolk supplies. They wrap them in horny egg cases that are deposited in the substrate (Carrier et al., 2004). The embryo nutrition is exclusively lecithotrophic.

Atlantoraja platana (Günther, 1880, Rajidae) is a "vulnerable" (San Martín et al., 2007) endemic skate of the Southwest Atlantic Ocean, with a discontinuous distribution, showing two different geographic nuclei: Espírito Santo, Brazil (20°S) and San Matías Gulf, Argentina (45°S). This skate is one of the most captured by local bottom trawl industrial fisheries, due to its abundance (Estalles et al., 2011). There are few reports about its life history (Reproductive biology: Coller et al., 2011; Marçal, 2003; Oddone and Amorim, 2008; Phylogeny: Mc Eachran and Dunn, 1998; Distribution and Biology: Menni and Stehmann, 2000). However, there is no information about folliculogenesis or about the microscopic size of vitellogenesis.

The knowledge of the morphofunctional features of folliculogenesis, as well as the correct evaluation of reproductive parameters and the size at follicular recruitment onset, is essential for fisheries management. In most chondrichthyans, a difference of magnitude order in the oocyte size at vitelline precursors input has been found (Díaz Andrade et al., 2011; Galíndez et al., 2010; Wehitt et al., 2015). This fact involves an implicit risk of overestimating the size of the first maturity and therefore underestimating the sexually active population in which the fishing pressure is exerted.

Chondrichthyans show evolutive milestones such as internal fertilization and a wide range of reproductive modes, turning them important objects of reproductive studies. On the other hand, the entire group, and particularly the species in the present study, is subject to intensive fishing, with signs of populations declining. Taking this into account, the aim of this work is to analyze the outstanding aspects of folliculogenesis, as a contribution to the knowledge and understanding of the different aspects of the life history of *A. platana,* a species with signs of population decline (San Martín et al., 2007).

2. Materials and methods

2.1. Sampling

A total of 142 females (43 immature, 46 maturing and 53 mature) were collected from landings of the commercial fleet operating in San Matías Gulf (41°42–42°41′S; 63°45–65°09′W), Río Negro, Argentina. Samples were taken seasonally over 2012–2015. The animals used in this study were obtained and handled according to the Bioethics Protocol approved by the DBByF – UNS (CICUAE, Prot. 069/2015, Res. CDBByF 716/15). The maturity stage of fish was determined as immature, maturing and mature according to Stehmann (2002) and Serra Pereira et al. (2011) classifications, based on the macroscopic and microscopic aspect of the internal reproductive organs.

2.2. Histological and ultrastructural studies

For microscopic analysis, small pieces of the ovaries were immersed in seawater's Bouin fixative. The samples were dehydrated through a graded alcohol series and embedded in Paraplast®. Microtome sections of 3–5 µm-thick were collected and stained with Massońs trichromic and hematoxylin-eosin stains for general observation. The periodic acid Schiff reaction (PAS) and Alcian blue (AB) pH 2.5 techniques, as well as the Sudan Black protocol coloring fat, were employed to detect glycoproteins and to visualize the yolk precursors, respectively. For electron microscopy, tissue samples from five mature females were used. Small pieces of the ovary were fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer with 12% sucrose (Hyder et al., 1983), for 12 h at 4 °C and post-fixed in 1% osmium tetroxide in the same buffer for 90 min at 4 °C. They were dehydrated in graded acetone and embedded in Spurr's low-density resin. Semithin sections were stained with methylene blue-azure II-fuchsine and ultrafine sections were contrasted with uranyl acetate and lead citrate.

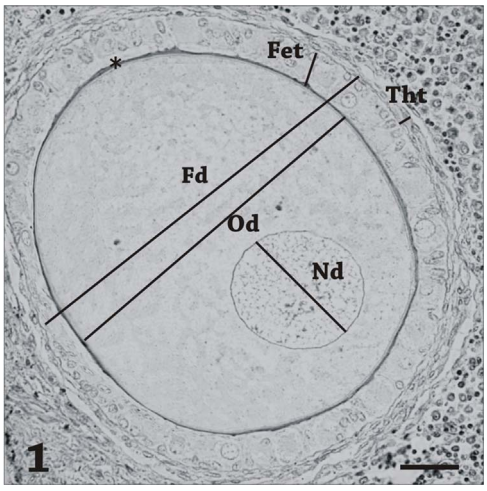


Fig. 1. Light microscopy image of an ovarian follicle. The figure depicts the measures taken for morphometric analysis. Nd: nucleus diameter; Od: oocyte diameter; Fd: follicular diameter; Fet: Follicular epithelium thickness; Tht: theca thickness; *: zona pellucida. PAS reaction. Scale bar: 300 µm.

2.3. Follicular morphometry

One slide from each 53 mature females was selected for analysis. All present follicles, as well as their layers, were recorded if the nucleus was visible. The follicular, oocyte and nuclear diameters, as well as the zona pellucida and thecae thickness, were measured with a micrometric ocular (0.1-micrometer accuracy) (Fig. 1).

The follicular spawning size was settled as the largest follicles present inside the ovary of mature females carrying egg cases. Graphics and statistical analyses were performed with InfoStat® (Di Rienzo et al., 2011).

3. Results

3.1. General morphology

The reproductive system of *A. platana* was located in the peritoneal cavity suspended by mesenteries. Both ovaries were functional, elongated, dorsoventrally compressed, and similarly sized. They were associated with the epigonal organ, which extended beyond the ovarian length (Fig. 2) and retracts to the caudal zone as maturation proceeds. Microscopically, the ovary was lined by a simple ciliated columnar epithelium, supported by a thin vascularized connective tissue (Fig. 3). The developmental wave of the gonad occurred from cranial to caudal and from ventral to dorsal. Ovarian parenchyma comprised oogonia and follicles in different maturity stages, immersed in a loose connective dense network. Follicles internalized as diameter increased (Fig. 4). Follicles were classified in 5 stages. 1: primordial; 2: primary; 3: developing without yolk accumulation; 4: developing with yolk accumulation; 5: with yolk plates (vitellogenic).

3.2. Folliculogenesis

3.2.1. Oogonia

They were only found in immature females, in scarce number and isolated (Fig. 5a). These cells were characterized by a conspicuous,

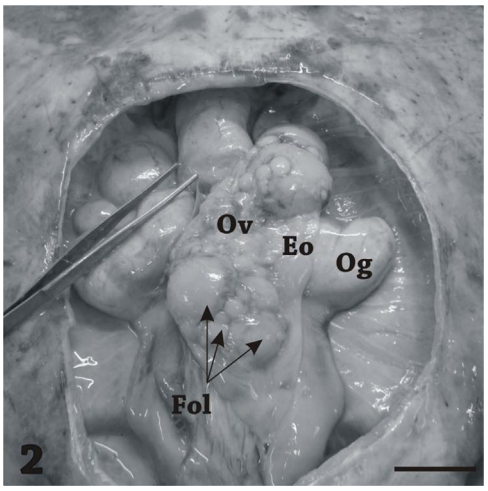


Fig. 2. Macroscopic view of the female's reproductive system. Arrows indicate follicles in different degrees of development (Fol); Eo: epigonal organ; Og: oviductal gland; Ov: ovary. Scale bar: 4 cm.

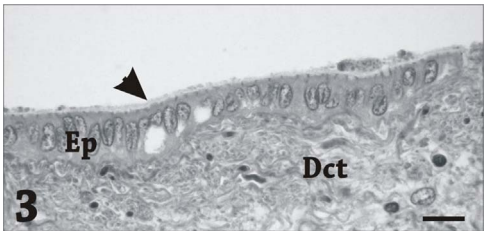


Fig. 3. Detail of the ovary epithelium. Arrowhead points to cilia; Ep: epithelium; Dct: dense connective tissue. Masson's trichromic stain. Scale bar: 15 μm.

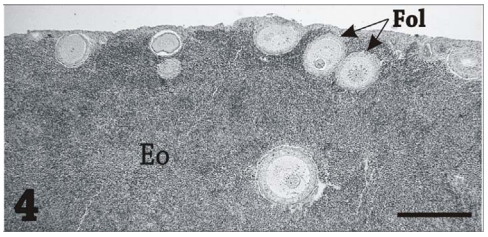


Fig. 4. General image of the ovary of an immature female. Fol: Primary follicles; Eo: epigonal organ. Masson's trichromic stain. Scale bar: 300 μm.

euchromatic nucleus and slightly basophil cytoplasm. They were in close association with pre-follicular like cells (Fig. 5b). Follicles formed when an oogonium was completely surrounded by pre-follicular like cells.

3.2.2. Stage 1: primordial follicles

The oocyte was surrounded by a simple layer of squamous follicular cells. The nucleus showed a single nucleolus and "lampbrush chromosomes" (Fig. 6 insert). The cytoplasm was slightly acidophil with Balbiani's bodies. The zona pellucida (or vitelline envelope) appeared as a diffuse and discontinuous PAS and AB pH 2.5 (+) structure in early primordial follicles and ended up completely surrounding the oocyte as development continued (Fig. 6). The thecae were undifferentiated.

3.2.3. Stage 2: primary follicles

At this stage, follicular cells remained unilayered and became cuboidal. Several globed shaped cells appeared between them (Fig. 7). Cuboidal cells were eosinophilic with heterochromatic nucleus, while enlarged global shaped cells were basophilic with conspicuous euchromatic nucleus. The zona pellucida was a continuous homogenous

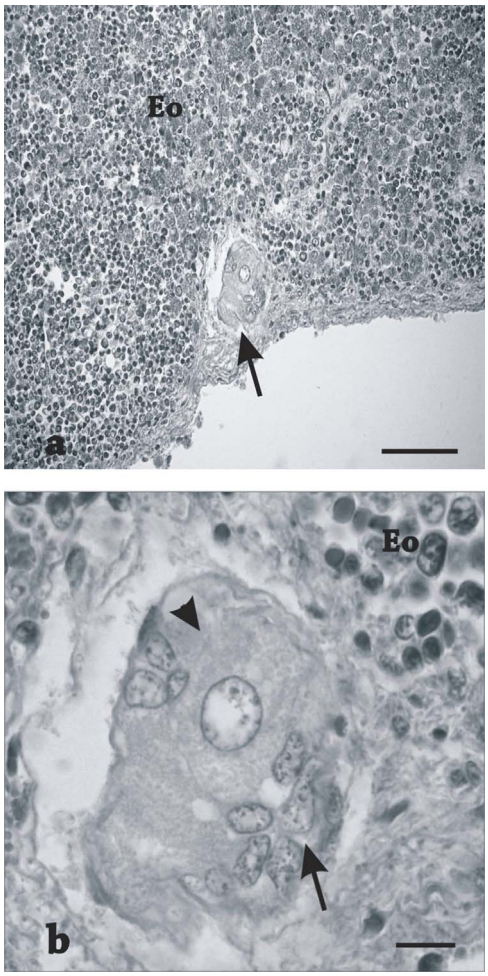


Fig. 5. a General view of the gonad showing the localization of an oogonium associated with pre-follicular cells (arrow). Note the immunocompetent tissue (Eo: epigonal organ) closely associated with the ovary tissue. Masson's trichromic stain. Scale bar: 40 μm. b Detail of an oogonium (arrowhead) with associated pre-follicular cells (arrow). Eo: epigonal organ. Masson's trichromic stain. Scale bar: 20 μm.

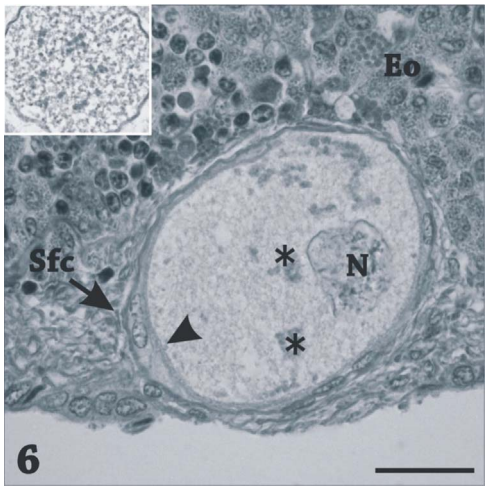


Fig. 6. Primordial follicle. Note the incipient zona pellucida (arrowhead) and the Balbiani's bodies (asterisks). Sfc: squamous follicular cell; N: nucleus; Eo: epigonal organ. Masson's trichromic stain. Scale bar: 40 μm. Insert: Detail of the nucleus showing "lampbrush chromosomes". Masson's trichromic stain. Scale bar: 10 μm.

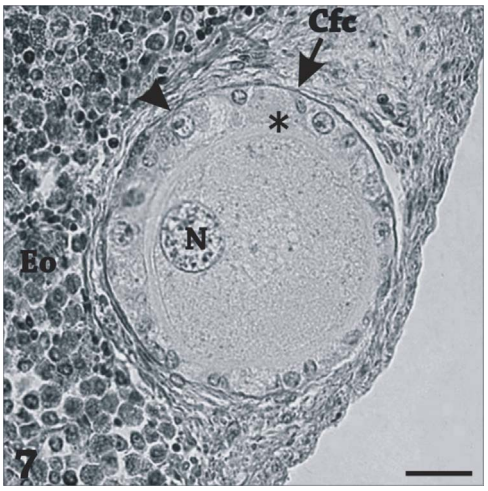


Fig. 7. Primary follicle. N: nucleus; Cfc: cuboidal follicular cell; arrowhead: globed shaped cell; asterisk: zona pellucida; Eo: epigonal organ. Masson's trichromic stain. Scale bar: 50 μm.

structure that reacted to PAS and AB pH 2.5. These affinities were maintained throughout the follicular maturation. Its thickness became maximum at this stage. The theca was evident.

3.2.4. Stage 3: developing follicles without yolk cumulation

As the oocyte grew, the follicular cells became columnar. When follicles reached about 600 μm of diameter, the granulose stratified. Between cylindrical cells, there were some globed basophilic cells interspersed, with a euchromatic nucleus (Fig. 8).The oolema folded tightly (Fig. 9), and was evident at optical level as a refringent edge. The theca was evident, but still undifferentiated.

3.2.5. Stage 4: developing follicles with yolk cumulation

When follicles reached about 2 mm in average, yolk cumulation was evident by the presence of suboolemic acidophilic droplets (Fig. 10). Granulosa cells remained stratified and they emitted apical projections that stretched out through the zona pellucida toward the oocyte (Fig. 11). Surrounding follicles there was an enlarged perifollicular vessel network (Fig. 12). The thecae were fully differentiated. The inner one was composed of connective fibers and vascularized. The outer thecae had a glandular-like aspect, with low cubic cells with

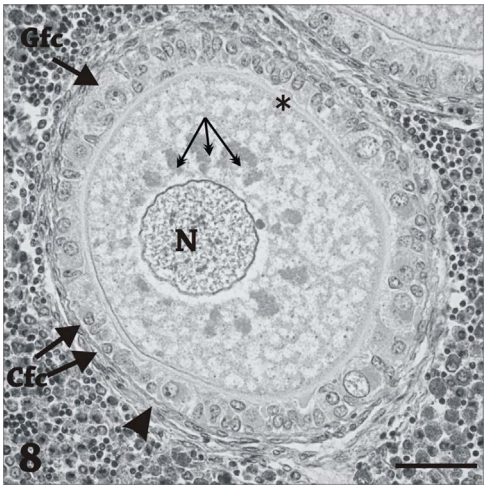


Fig. 8. Developing follicle without yolk cumulation. The granulosa stratifies and the theca is evident. N: nucleus; thin arrows: Balbiani's bodies; *: zona pellucida; Gfc: globed follicular cells; Cfc: cubic follicular cells; arrowhead: theca. Masson's trichromic stain. Scale bar: 75 μm.

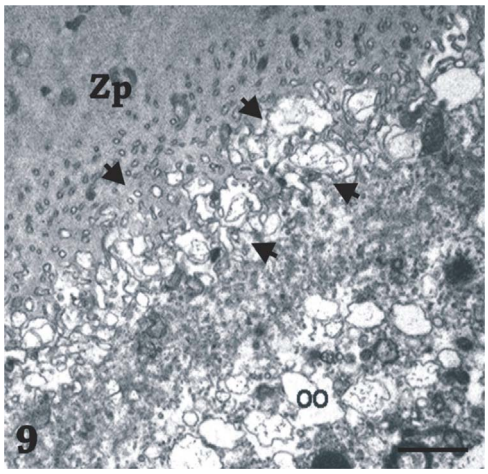


Fig. 9. Microscopic electron image of the oocyte membrane in a developing follicle without yolk. Note oocyte projections (arrows) reaching the zona pellucida (ZP). oo: oocyte. Scale bar: 1 μm.

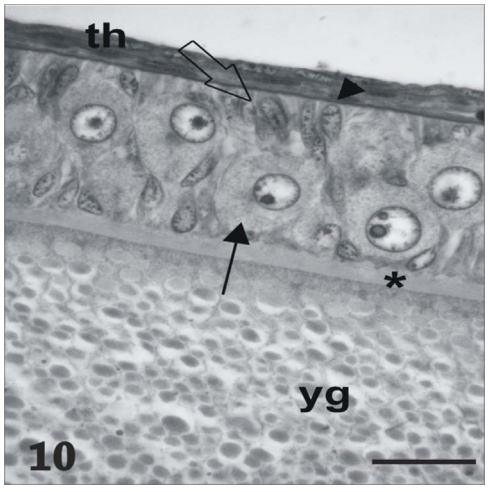


Fig. 10. Follicle with early yolk accumulation. Black arrow shows globed cell while empty arrow depicts the nucleus of prismatic follicular cells; arrowhead: basal membrane; Yg: yolk granules; Th: thecae; *: zona pellucida. Sudan B stain. Scale bar: 60 μm.

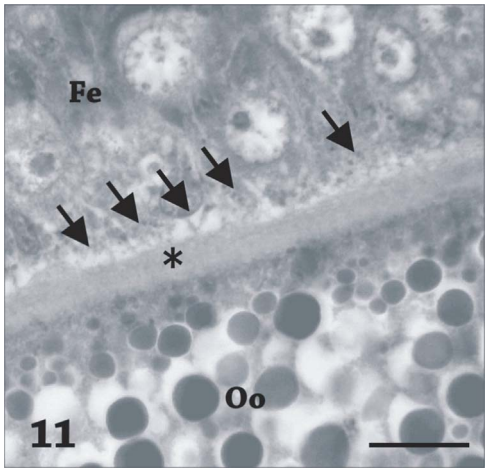


Fig. 11. Detail of the apical surface of the follicular cells showing the apical projections stretching out through the zona pellucida, toward the oocyte. Fe: follicular epithelium; arrows: projections; *: zona pellucida; Oo: oocite. Sudan B stain. Scale bar: 25 μm.

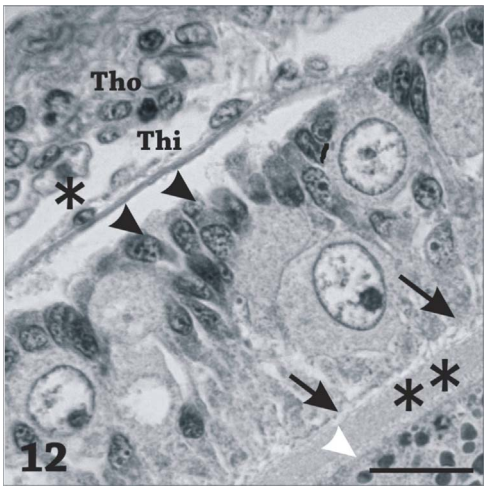


Fig. 12. Advanced yolk comulation.*: vascular sinus **: zona pellucida; black arrowheads: prismatic cells; Thi: inner theca; Tho: outher theca; arrows: apical surface of granulosa cells; white arrowhead: oolema folds. Masson's trichromic stain. Scale bar: 25 μm.

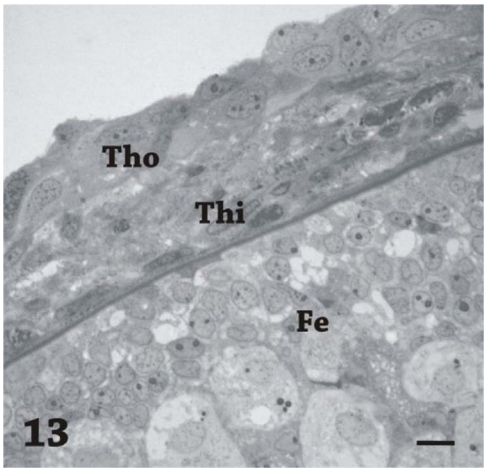


Fig. 13. Semithin section showing the thecae in detail. Arrow: Glandular-like thecal cell. Fe: follicular epithelium; Tho: outer theca; Thi: inner theca. Methylene-blue-azure II-fuchsine stain. Scale bar: 50 μm.

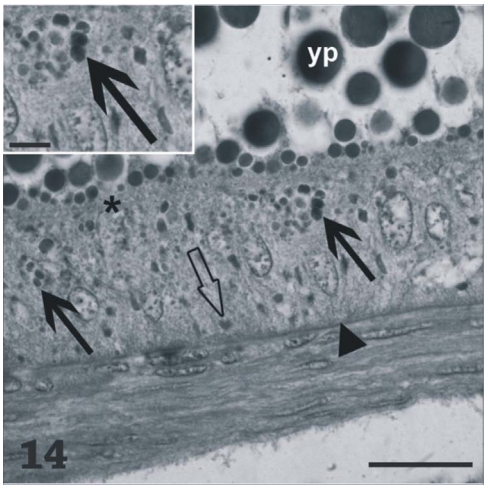
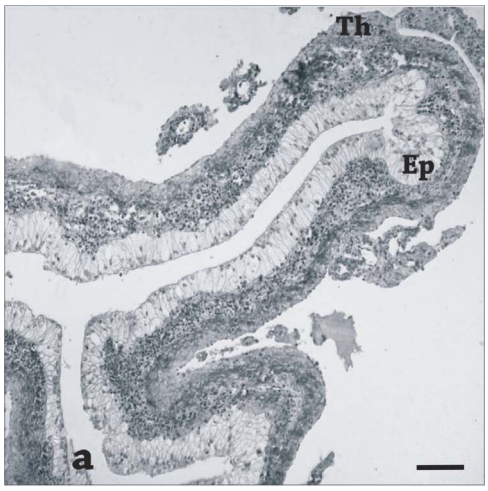


Fig. 14. Yolked follicle. Lipidic droplets (vitelline precursors) located both inside the cytoplasm of follicular cells (empty arrow) and at the intercellular spaces (arrows). Arrowhead: basement membrane; Yp: yolk plates. Sudan B stain. Scale bar: 25 μm. Insert: Detail of lipidic droplets. Sudan B stain. Scale bar: 10 μm.



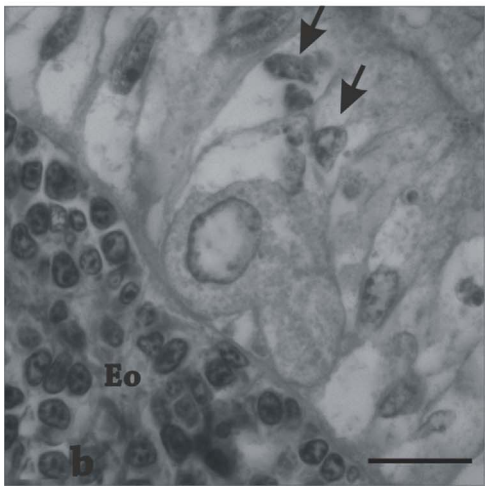


Fig. 15. a Corpora lutea. Look at the folded epithelium (Ep). Th: thecae. Masson's trichromic stain. Scale bar: 100 μm. b Corpora lutea. Detail of luteinic cells. Arrows depict apoptotic nucleus. Eo: epigonal organ. Masson's trichromic stain. Scale bar: 25 μm.

euchromatic nucleus (Fig. 13).

3.2.6. Stage 5: yolked follicles (yolk in plates)

At this stage, granulosa cells depicted Sudan B (+) granules. These small vesicles were located both in the cytoplasm and inside the enlarged intercellular spaces (Fig. 14). As development continued, the granulosa cells adopted a new distribution. While prismatic cells located close to the follicular basal membrane, the others accommodated adjacent to the oocyte.

The largest follicle measured was 44 mm of diameter.

3.2.7. Corpora lutea

When the oocyte leaved the ovary, the remainder follicular cells transformed in a *corpora lutea* (Fig. 15a). Luteinic tall cells were eosinophilic, with a fringed apical surface. Between them there were few enlarged cells and cells depicting the first signs of apoptosis (Fig. 15b). Finally, the epithelium disorganized and the remaining structure was invaded by dense connective tissue. In some cases, the *corpora lutea* showed lymphocytic infiltration.

3.2.8. Corpora atretica

Atresia occurred in all stages of follicular development. Likewise, atretic follicles were present in the ovaries of immature, maturing and mature females. During this process, cells completely lost their architecture and dense connective tissue infiltrated the whole structure, transforming it into a dense scare-like image (Fig. 16).

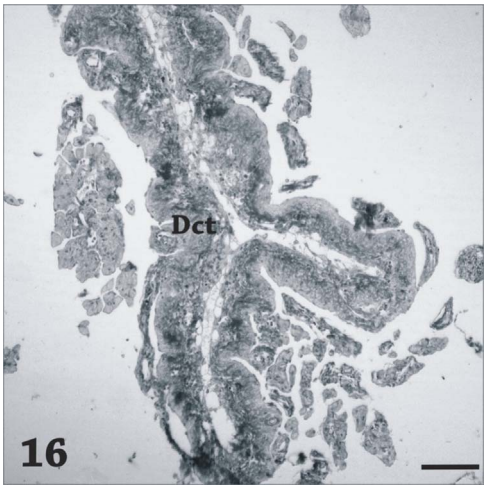


Fig. 16. General view of an atretic body. Dct: dense connective tissue. Masson's trichromic stain. Scale bar: 100 µm.

3.3. Follicular sizes at the start of vitellogenesis

The average diameter of the start of yolk accumulation measured microscopically was 1.9 mm (0.85-3.2 mm). On the other hand, the macroscopic average diameter of follicles that showed signs of yolk accumulation (yellow coloration) was 8.9 mm (3.5–20.57 mm).

3.4. Morphometric dynamic of folliculogenesis

As the follicular development advanced, the proportion of each of its components varied (Fig. 17). All follicular layers lost representativeness against the increase of oocyte diameter. Especially, the zona pellucida thickness was greatly increased, quite along previtelline stages, until stage 3. This growth was correlated with those of the granulosa (Pearson correlation coefficient 0.592, $p < 0.0001$). Since then, and throughout the follicles development, it behaved in a similar way to the other follicular layers.

4. Discussion

Cartilaginous fish have shown a highly conserved model throughout more than 400 million years (Mc Eachran and Dunn, 1998). In that context, the general morphology of the reproductive system of *A. platana* is similar to that seen in other Elasmobranchs, as well as the basic organization of the ovary and its relationship with the epigonal organ (*Sympterygia acuta* and *S. bonapartii,* Díaz Andrade et al., 2009; 2011; *Mustelus schmitti,* Galíndez and Aggio, 2002; *Raja clavata,* Serra Pereira et al., 2011; *Zearaja chilensis,* Wehitt et al., 2015). Although a detailed study of ovarian symmetry was not performed, the observation of two ovaries that are both functional and similarly sized suggests that there are no differences in laterality. This feature is common on skates but different from most Selachians, where one of the ovaries could be undeveloped or even completely absent (*M. schmitti,* Galíndez et al., 2014; *Rhizoprinodon taylori*, Waltrick et al., 2017).

The process of development and maturation of the follicles is called folliculogenesis. According follicles mature, they increase in size and structural complexity. In *A. platana*, as in another oviparous elasmobranch, small and white previtellogenic follicles persist in the ovaries along all reproductive phases, probably acting as a reservoir for future spawning episodes (Serra Pereira et al., 2011). The recruitment of follicles is modulated by the relationships established between the oogonia with the pre-follicular cells and the production of local growth factors (Hutt and Albertini, 2007; Oktem and Oktay, 2008). During embryonic development, primordial germ cells originate at the endodermic layer of the yolk sac and migrate to the germinal ridge, where they associate with pre-follicular like cells to finally be transformed into oogonia (Motova and Cooley, 2001; Prisco et al., 2001). In *A. platana*, these cells were only present in immature animals. The occurrence of oogonia is notably variable in the Class. Prisco et al. (2001) found clusters of oogonia in newborn specimens of *Torpedo marmorata* as well as Díaz Andrade et al. (2009) in *S. acuta.* However, Díaz Andrade et al. (2011) found isolated oogonia in immature and maturing exemplars of *S. bonapartii*. On the other hand, Serra Pereira et al. (2011) did not find oogonia in immature specimens of *R. clavata*. Conversely, Galíndez et al. (2014) found oogonia in all maturative stages of the narrow nose smooth hound *M. schmitti*, even in pregnant females. These differences could be associated with the reproductive performance of each species. The presence of oogonia only in newborn specimens suggests that oogenesis occurs early in development and it continues after birth for a short time (Prisco et al., 2001). Likewise, Díaz Andrade et al. (2009) postulated that the presence of oogonia exclusively in immature specimens in *S. acuta* was related to a low fecundity and a slow population recovery after overfishing exploitation. The existence of primordial reproductive cells in all maturing stages was in line with higher values of abundance and this point of view has been confirmed by Cortés and Massa (2006) for *M. schmitti*. In this way, the oogonial dynamics in *A. platana* suggests a slight flexibility in the response to fishing

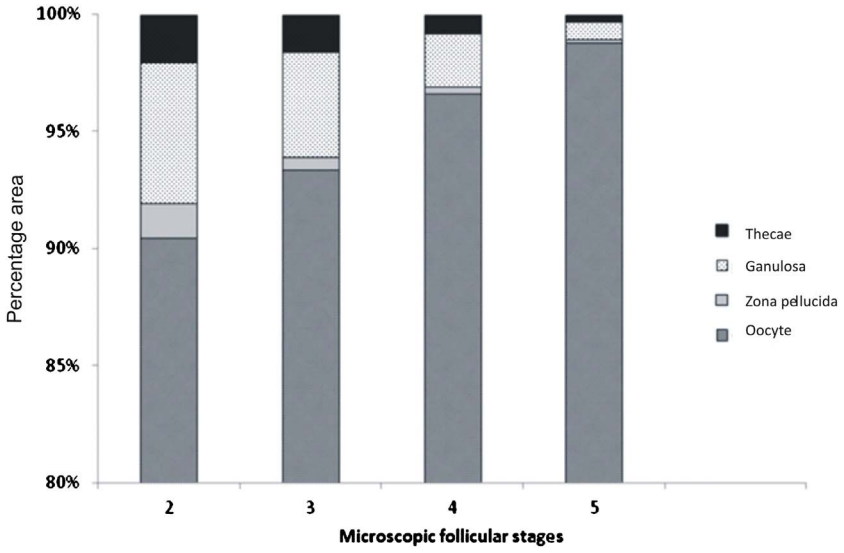


Fig. 17. Stacked bars graphic depicting the proportionality of follicular components across the maturative process.

exploitation and supports its classification as a vulnerable species provided by the IUCN.

The cytological features of early folliculogenesis show some indicators common to other Vertebrates (Mc Millan, 2007). The visualization of "lampbrush chromosomes" highlights the metabolic activity of DNA. This nuclear feature has already been described in other Chondrichthyes (*T. marmorata*, Prisco et al., 2002a,b; *S. acuta* and *S. bonapartii* Díaz Andrade et al., 2009, 2011 *Etmopterus spinax*, Porcu et al., 2014). On the other hand, the presence of Balbiani's bodies could be associated with protein biosynthesis, which precedes the yolk build-up (Brusle et al., 1989).

The zona pellucida is an acellular layer that surrounds the vertebrate oocyte. In Chondrichthyes it shows an atypical dynamics, increasing its thickness reaching the most widths within Vertebrates during previtelline phases (Davenport et al., 2011). In *A. platana* its deposition begins in primordial follicles as in *T. marmorata* (Prisco et al., 2002a). On the other hand, the glycoprotein composition of the zona pellucida comprises both acidic and neutral components. Mammals show a change from neutral to acidic nature of glycoproteins, from growing to ovulating follicles (Delgado and Zoller, 1987). However, in *A. platana*, as in *S. acuta a*nd *S. bonapartii* (Diaz Andrade et al., 2009, 2011), both components are simultaneously detected from early development. Prisco et al. (2009) described the composition of the vitelline envelope in *T. marmorata* and they concluded that its macromolecular components are similar to that reported in bony fish and amphibians.

A very close relationship between growing oocytes and its surrounding follicular cells has been reported in all Vertebrate's ovaries (Mc Millan, 2007). The follicular granulosa of Chondrichthyes is composed of a single layer of cells which can show changes in form, size, and microarchitecture along the follicular growth and maturation (Gračan et al., 2013; Prisco et al., 2007). While in some species follicular cells remain unilayered and monotypic, they can also stratify and differentiate in several cellular types (Guraya, 1986; McMillan, 2007). In *A. platana*, follicular epithelium begins as a simple layer, and then stratifies and finally, it becomes monolayered again. In other Batoids follicular cells stratify, but they never return to an exclusive simple layer (Barone et al., 2007; Prisco et al., 2002a). Considering the synthetic properties of the follicular cells (Del Giudice et al., 2011; Prisco et al., 2002b, 2004, 2009), the increase in number of cell layers found in the granulosa could be attributed to the intrinsic vitellogenesis (Prisco et al., 2004) and some components of the zona pellucida (Prisco et al., 2009).

Yolk provides energetic sustenance for embryonic development and is the main responsible process for the strong growth of follicles (Davenport et al., 2011). At the beginning, follicles show small yolk globules in the peripheric cytoplasm, while larger follicles exhibit elliptical yolk platelets of various sizes, packaged in the ooplasm (az Andrade et al., 2009, 2011; Prisco et al., 2002b).

In *A. platana*, as well as in other elasmobranchs (Galíndez et al., 2014), the granulosa cells of yolking follicles show intercellular spaces filled by Sudan B (+) globules. In part, yolk precursors are synthesized in the liver and transported to the oocyte. This movement occurs through the follicular intercellular spaces (Prisco et al., 2002b) and across the zona pellucida, by the interconnection between the oolema and follicular cells. Even though the role of follicular cells in the production of yolk precursors has been established (Del Giudice et al., 2011; Prisco et al., 2004), the existence of vitelline precursors at the intercellular follicular spaces also supports the hypothesis of uptake from the maternal circulation. On the other hand, the increase of perifollicular vascularization observed in vitellogenic follicles in *A. platana*, may constitute an adaptation to increase the flow of nutrients and delivery products to the oocyte. La Plata skate has differentiated inner and outer thecae. There are many studies about the steroidogenic capacity of this tissue and there are differences among species referring to which thecal layer has this capacity (Cook and Callard, 1999). In *A. platana* the outer theca has features consistent with synthetical activity. This has also been observed in other species such as *Squalus acanthias* (Guraya, 1978), *T. marmorata* (Prisco et al., 2002a), *S. acuta*, *S. bonapartii* (Díaz Andrade et al., 2009, 2011), *Z. chilensis* (Wehitt et al., 2015) and *M. schmitti* (Galíndez et al., 2014).

In Elasmobranchs, corpora lutea are formed as a result of two different processes: ovulation, and follicular atresia and could occur in oviparous species as *A. platana*, and in viviparous ones, as *M. schmitti* and *M. canis* (Galíndez et al., 2010; Hisaw and Hisaw, 1959). Thus, their presence is not necessarily associated with the gestation (Waltrick et al., 2017). In females, 17 ß- estradiol and testosterone are mainly produced by the granulose and the theca cells of ovarian follicles, and while follicular cells are also capable of producing progesterone, the corpora lutea is the primary source of this gonadal steroid (Awruch, 2015). In some species, accessory corpora lutea form by luteinization of follicles which are not ovulated and are called "corpora lutea atretica" (Callard et al., 1992). The lymphocyte infiltration of corpora lutea observed agrees with these structures. However, more studies are required to elucidate the participation of immunocompetent cells and to confirm if they are related to an inflammatory response.

It is known that atresia is a highly frequent event in Chondrichthyes and it can be triggered at any stage of follicular development (Hamlett and Koob, 1999; Hisaw and Hisaw, 1959; Waltrick et al., 2017). In this skate, atresia was observed in follicles of any developing degree and in animals of all maturative stages. The follicular recruitment and the regulation of the number of follicles ready to ovulate (ovarian fecundity) would be modulated by atresia, even at early maturative stages. This could be considered as an adaptive regulatory mechanism to the environmental conditions prevailing in the breeding season.

Different methods are used to determinate the sexual maturity in cartilaginous fish, but the external appearance of the ovary and gonadal indices are the most commonly used (Serra Pereira et al., 2011; Stehmann, 2002). In *A. platana* there is a significant difference between the microscopic and macroscopic follicular size at the start of vitellogenesis (1.9 and 8.9 mm, respectively). For the Brazil's population, Oddone and Amorim (2008), estimated the macroscopic size in which vitellogenesis started as 7 mm and this value agrees with the results shown in this work using the same methodology. The fact that follicles start to accumulate yolk at a smaller size admits the possibility of redefining the size at sexual maturity. This is an important issue to evaluate, especially if we consider that approximately 65% of the oocytes that were analyzed as previtellogenic following the macroscopic criteria were already committed to its reproductive function. If we take this into account, it seems clear that considering only macroscopic criteria could lead to an overestimation of the size at first sexual maturity, and therefore to underestimate the potential of the resource. On the other hand, the values registered in this work for the follicles ready for ovulation (44 mm), differ from those reported by Oddone and Amorim (2008) (25–30 mm) but they are close to those found by Coller (2012) (47 mm) for the same population studied in this work. It is known that there may be differences in various life history parameters between different populations of the same species, indicating that there are diverse factors that may affect populations locally (Díaz Andrade et al., 2011). The variation in ovulation size could be explained by the latitudinal difference between both populations (about 20°). On the other hand, the fish here studied come from the San Matias Gulf, a more protected area, whereas Brazilian animals were sampled at open sea.

Knowledge and understanding of different aspects of elasmobranch reproduction is a clue factor for the elaboration of sustainable fisheries management and conservation policies. Bearing this in mind, in the last years, the histological tools have been recognized as crucial for reproductive studies, allowing improvements in the efficacy of management options (Alonso Fernández et al., 2011). In this sense, the microscopic analysis performed in the ovary of *A. platana* in this work provides not only a more accurate information on the female's gonad morphology and dynamics but also has proven to be a complementary

approach that may contribute to a better knowledge and care of this species.

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