JOURNAL OF **FISH** BIOLOGY fsbi

Observations of follicle cell processes in a holocephalan

María C. Díaz Andrade[1] (ORCID) | **Ana C. Moya[1]** | **Anahí Wehitt[1]** | **Edgardo E. Di Giácomo[2]** |
Elena J. Galíndez[1]

[1]Departamento de Biología, Bioquímica y Farmacia (DBByF, UNS)-INBIOSUR (CONICET), Bahía Blanca, Argentina

[2]Centro de Investigación Aplicada y Transferencia Tecnológica en Recursos Marinos Almirante Storni (CIMAS), San Antonio Oeste, Argentina

Correspondence

María C. Díaz Andrade, Departamento de Biología, Bioquímica y Farmacia (DBByF, UNS)-INBIOSUR (CONICET), San Juan 670, 8000, Bahía Blanca, Argentina.
Email: mcandrade@criba.edu.ar

The presence of follicular cellular processes (FCP) that cross the zona pellucida, has been recorded in the ovarian follicles of *Callorhinchus callorhynchus*. This constitutes the first report describing the presence of these structures in a species of the Holocephali. Considering that FCPs have only previously been reported in the Selachii, these findings suggest that FCPs could have been lost by the Batoidea after their divergence, around 280 M B.P.

KEYWORDS

Batoidea, *Callorhinchus callorhynchus*, divergence, ovary, selachii, zona pelucida

Cartilaginous fishes (Chondrichthyes) diverged from a common ancestor of bony vertebrates in the early Silurian, approximately 420 M B.P. (Benton *et al*., 2009). The living Chondrichthyes comprise more than 30 species of Holocephali (chimaeras) and about 1,100 species of neoselachian elasmobranchs (sharks, rays and skates; Compagno, 2002). These predatory fishes are generally characterized by their longevity, late sexual maturity and low fecundity (Bubley *et al*., 2012). As a strategy to compensate for these features, they produce relatively few active and large offspring (Moyle & Cech, 2000).

Several reproductive strategies have evolved in Chondrichthyes during their long evolutionary history, such as internal fertilization (Grogan *et al*., 2012) and two distinct parity modes: oviparity (egg-laying) and viviparity (live-bearing). Moreover, there is a wide diversity of maternal–embryonic trophic relationships, based on the method of nutrient transfer. These range from lecithotrophy (*i.e.* yolk contained within the egg being the only source of nutrition for the developing embryo) to different types of matrotrophy (*i.e.* additional nutrients produced by the mother are supplied to the embryos in utero) (Hamlett & Koob, 1999). Various combinations of these strategies result in nine distinct reproductive modes (Awruch, 2015).

Despite reproductive mode, all Chondrichthyes begin their development as lecithotrophic, so a nutrient reserve is needed to sustain initial growth. As a consequence, eggs can reach a considerable size. For example, eggs that are 10–12 cm in diameter have been reported in some shark species (Breder & Rosen, 1966; Tanaka *et al*., 1990; Wourms, 1977). Such a large volume: mass ratio is needed to meet the challenge that developing structures face to maintain cellular integrity. Davenport *et al*. (2011) described a novel set of tube-like structures, termed follicle-cell processes (FCP), that extend from the ovarian follicle cells to the developing oocyte, passing through the zona pellucida. Among other functions, the authors suggested that these projections play a role similar to the cortical ring of actin found in the cytoskeleton of somatic cells, allowing the oocyte to resist compression. They also hypothesized that FCPs were associated with elasmobranchs in general. However, Dunbar *et al*. (2017) suggested that FCPs were present in selachians but absent in batoids. On the other hand, the presence of such structures has not been reported yet for any holocephalan species.

The cockfish *Callorhinchus callorhynchus* (L. 1758) is the only holocephalan species present in coastal waters of the south-western Atlantic Ocean (Cousseau & Perrota, 2004). It is an oviparous benthic species, producing large eggs protected by ornamented leathery egg cases that are *c*. 15 cm long (Di Giácomo & Perier, 1994). The aim of this work was to determine if FCPs are present in *C. callorhynchus*, to describe them and to analyze some possible evolutionary and phylogenetic implications.

To investigate FCPs in *C. callorhynchus*, sexually active females were collected for histological observation in April–June, September

and November 2014 and February, April, August and November 2015 (n = 25) from commercial fishery catches landed at La Perla del Este and Rio Salado processing plants [San Antonio Este (40.73° S; 64.93° W) and San Antonio Oeste (40.74° S; 64.97° W) ports, Argentina). For the ultrastructural study, the material was obtained by net fishing by the F.V. *Viernes Santo* in February 2015. The collected fish (n = 6) were already dead. The commercial fishing fleet operates within the San Matias Gulf (41–42° S; 64–65°W) at depths ranging from 50 to 190 m. Each animal used in this study was obtained and handled according to the Bioethics Protocol approved by DBByF - UNS (Institutional Committee for the Care and Use of Experimental Animals ICCUEA: CICUAE-Prot.069/2015, Res. CDBByF 716/15).

Ovaries were carefully extracted and the size of bigger follicles was measured. Reproductive organs were kept in ice and processed at the laboratory. Small pieces of the gonads were fixed in Bouin's solution in seawater for approximately 24 h. Afterwards, all material was dehydrated through a graded alcohol series and embedded in Paraplast (Sigma Aldrich; www.sigmaaldrich.com). Sections 5–6 μm thick were stained with Masson's trichromic stain, haematoxylin-eosin and periodic acid Schiff reaction (PAS). Selected sections were photographed using an Olympus BX51 light microscope equipped with an Olympus C-7070 digital camera (www.olympus-global.com).

For transmission electron microscopy, small pieces of follicles of different sizes were fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer with 12% sucrose (Hyder *et al.*, 1983), for 12 h at
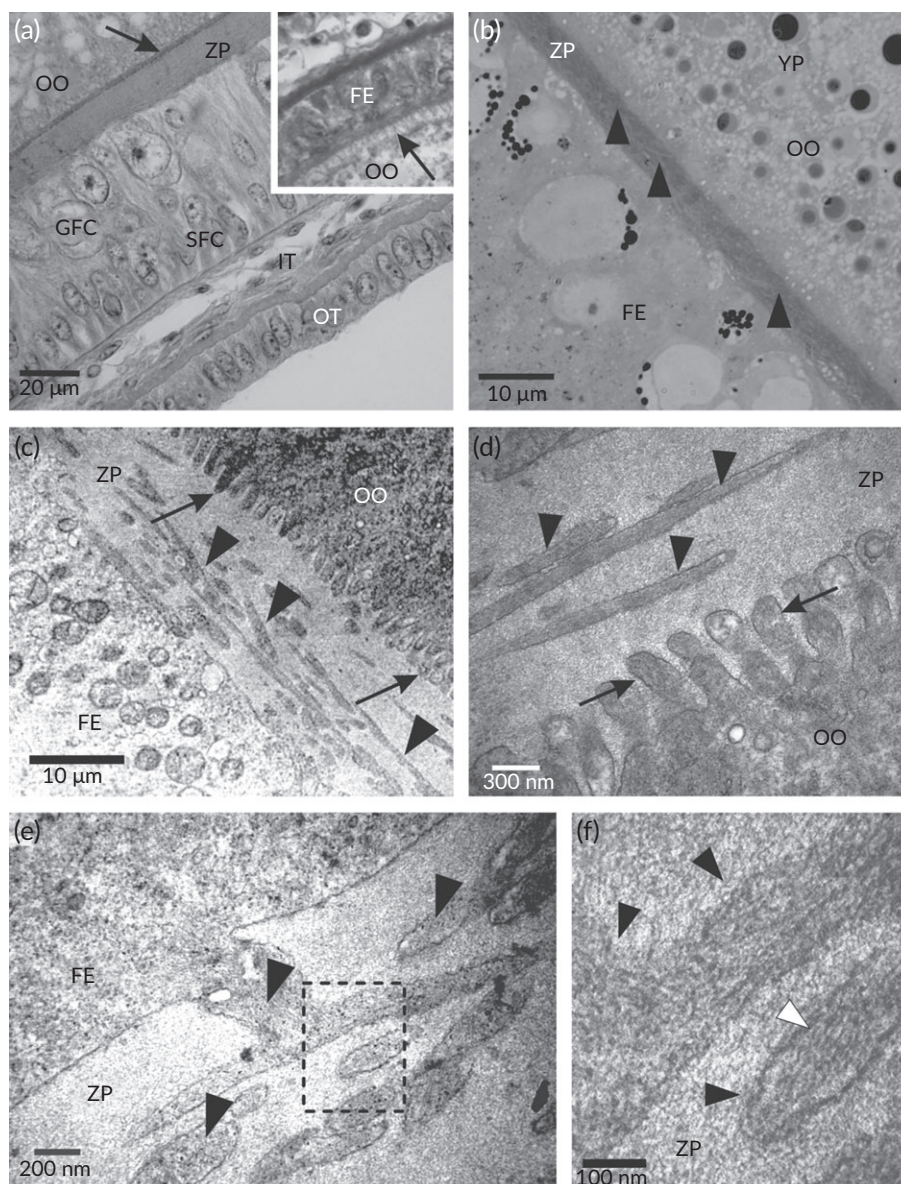
FIGURE 1 Ovarian follicle wall of mature female *Callorhinchus callorhynchus*. (a) Light micrograph (Masson's trichromic stain) showing the general structure of an ovarian follicle with (insert) detail of the follicle wall, showing the folded oolema (⇵). (b) Semi-thin section (methylene blue-azure II-fuchsine stain) showing follicular cell projections (▲). (c)–(f) Transmission electron microscopy images showing (c), (d) the disposition and (e), (f) internal structure of the follicular cellular processes (▲). The area delimited in (e) is enlarged in (f) to show actin-like filaments (△). FE, follicular epithelium; GFC, globous follicular cell; IT, inner theca; OO, oocyte; OT, outer theca; SFC, small follicular cells; white arrowheads: Actine-like filaments; YP, yolk platelets; ZP, zona pellucida

4°C and post-fixed in 1% osmium tetroxide in the same buffer for 90 min at 4°C. Samples were washed in buffer, dehydrated in graded acetone and infiltrated in low-density epoxy resin (Spurr; www.sigmaaldrich.com). Semi-thin sections (0.5–1 μm) were colored with methylene blue-azure II-basic fuchsine stain and ultra-thin sections on grids (70–90 nm) were contrasted with uranyl acetate and lead citrate. Specimens were examined using a Jeol CXII electron transmission microscope (www.jeol.co.uk).

In general, ovaries of *C. callorhynchus* exhibited the same features as other Chondrichthyes. As in other Holocephali, there was no evidence of an epigonal organ in *C. callorhynchus* (Bone & Moore, 2008; Fänge & Sundell, 1969). The maximum recorded follicle diameter was 49.3 mm. This size is, to a greater or lesser extent, larger than those recorded for other Holocephali species and for the same species in other locations (Alarcón *et al.*, 2011; Barnett *et al.*, 2009; Chierichetti *et al.*, 2017; Finucci *et al.*, 2017; Malagrino *et al.*, 1981; Márquez Farías & Lara Mendoza, 2014; Moura *et al.*, 2004), but was similar to sizes registered for this species from the same area by Di Giácomo and Perier (1994).

The general structure of the ovarian follicles in *C. callorhynchus* agrees with that reported for other Chondrichthyes and vertebrates in general (Guraya, 1978; Hamlett & Koob, 1999) (Figure 1(a)). Follicles are formed by an oocyte, surrounded by follicular cells and thecae. Between the follicular cells and the oolema, there is an acellular, PAS (+), zona pellucida that envelopes the oocyte (Figure 1). This zona pellucida thickens as follicular development progresses, reaching a maximum width of about 12 μm (7–12 μm; Figure 1(a)). As follicles grow, the entire oolema folds tightly (Figure 1(a), (c), (d)), to the point that it is visible even under light microscopy, as a bright striated band (Figure 1(a)). The follicular epithelium is composed of two types of cells: small, columnar cells and large, globous cells (Figure 1(a)). These cells emit long projections, of about 200–250 nm in diameter, that penetrate the zona pellucida (Figure 1(b)–(f)). In nonvitellated follicles *c.* 1 mm in diameter, as well as in larger vitellate follicles, these projections are arranged circumferentially (Figure 1(c), (d)). Some of these projections were ramified close to their base (Figure 1(e)). Inside these ridges, actin-like microfilaments of 5–7 nm in diameter align parallel to the ridges' longitudinal axis (Figure 1(f)). These filaments correspond with the actin filaments described by Davenport *et al.* (2011) in three species of sharks. The features observed in the follicular projections in *C. callorhynchus*, as well as their location and disposition, suggest that these structures are analogous to the FCPs described by Davenport *et al.* (2011).

Dunbar *et al.* (2017) studied these projections in sharks and batoids and suggested that FCPs occurred exclusively in selachians. According to these authors, an explanation for this could be that batoids, because of their flattened body, have a reduced internal cavity, would produce smaller eggs than sharks and would therefore not require special structures to maintain cellular integrity. Taking this into account, Dunbar *et al.* (2017) proposed two possible evolutionary scenarios to explain the absence of FCPs in batoids. First, FCPs could have been present in the common ancestor of batoids, selachians and the holocephalans, but lost in batoids as a consequence of reduced egg size. Alternatively, FCPs could be an evolutionary novelty confined to selachians as a response to selective pressure for producing

larger offspring before matrotrophy. Holocephalans could help resolve these alternative hypotheses. Presence in *C. callorhynchus* of structures similar to those described by Davenport *et al.* (2011) and Dunbar *et al.* (2017) in selachians would support the first hypothesis, suggesting that batoids lost FCPs during evolution. However, some ovarian features make *C. callorhynchus* more similar to skates than to sharks. For example, *C. callorhynchus* is oviparous, with an ovulating follicle size of nearly 5 cm diameter. *Callorhinchus callorhynchus* also has a heterogenic follicular epithelium and a narrower zona pellucida than sharks: 12 *v.* 70 μm (Dunbar *et al.*, 2017).

Exclusively lecithotrophic embryonic development would be a primary determinant, in terms of the amount of vitelline reserves accumulated within the oocyte. Amount of space available in the abdominal cavity might be a secondary limiting factor. However, other variables probably influence ovulating oocyte dimensions, such as hatching size, frequency of oviposition, length of the laying period and embryonic development time. Thus, the components that influence the ovulating follicle size are likely multifactorial and possibly even variable between populations. On the other hand, Prisco *et al.* (2002) and Hamlett *et al.* (1999) found projections of follicular cells in *Torpedo marmorata* Risso 1810 and *Urolophus jamaicensis* (Cuvier 1816). However, those structures were, apparently, of a different nature to those found by Davenport *et al.* (2011) and Dunbar *et al.* (2017) and they were only seen in pre-vitellogenic follicles. Prisco *et al.* (2002) and Hamlett *et al.* (1999) also observed that those projections participated in the accumulation of reserve substances in the oocyte. Based on this, more in-depth studies in other batoid species should be conducted to understand the phylogenetic distribution and correlation of related follicular characters throughout Batoidea.

ORCID

María C. Díaz Andrade 🄳 http://orcid.org/0000-0002-0374-3972

REFERENCES

Alarcón, C., Cubillos, L. A., & Acuña, E. (2011). Length-based growth, maturity and natural mortality of the cockfish *Callorhinchus callorhynchus* (Linnaeus, 1758) off Coquimbo, Chile. *Environmental Biology of Fishes*, *92*, 65–78. https://doi.org/10.1007/s10641-011-9816-0

Awruch, C. (2015). Reproduction strategies. In R. Shadwick, A. Farrell, & C. Brauner (Eds.), *Physiology of elasmobranch fishes: Structure and interaction with environment* (Vol. 34A, 1st ed., pp. 255–310). London, England: Academic Press. https://doi.org/10.1016/B978-0-12-801289-5.00007-9

Barnett, L. A. K., Earley, R. L., Ebert, D. A., & Cailliet, G. M. (2009). Maturity, fecundity and reproductive cycle of the spotted ratfish, *Hydrolagus colliei*. *Marine Biology*, *156*, 301–316. https://doi.org/10.1007/s00227-008-1084-y

Benton, M. J., Donoghue, P. C. J., & Asher, R. A. (2009). Calibrating and constraining molecular clocks. In S. B. Hedges & S. Kumar (Eds.), *The timetree of life* (pp. 35–86). Oxford, England: Oxford University Press.

Bone, Q., & Moore, R. H. (2008). *Biology of fishes* (3rd ed.). Abingdon, England: Taylor & Francis Group.

Breder, C. M., & Rosen, D. E. (1966). *Modes of reproduction in fishes*. Garden City, NY: Natural History Press.

Bubley, W. B., Kneebone, J., Sulikowski, J. A., & Tsang, P. C. W. (2012). Reassessment of spiny dogfish *Squalus acanthias* age and growth using vertebrate and dorsal-fin spines. *Journal of Fish Biology*, *80*, 1300–1319. https://doi.org/10.1111/j.1095-8649.2011.03171.x

Chierichetti, M. A., Scenna, L. B., Di Giácomo, E. E., Ondarza, P. M., Figueroa, D. E., & Miglioranza, K. S. B. (2017). Reproductive biology of the cockfish, *Callorhinchus callorynchus* (Chondrichthyes: Callorhinchidae), in coastal waters of the northern Argentinean Sea. *Neotropical Ichthyology*, *15*, 1, e160137–10. https://doi.org/10.1590/1982-0224-20160137

Compagno, L. J. V. (2002). Sharks. In K. E. Carpenter (Ed.), *The living marine resources of the western central Atlantica: Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes and chimaeras, Volume 1 FAO Special Publication* (Vol. 5, pp. 357–505). Rome, Italy: FAO. Retrieved from www.fao.org/docrep/009/y4160e/y4160e00.htm

Cousseau, M. B., & Perrota, R. G. (2004). *Peces marinos de la Argentina. Biología, distribución, pesca*. Mar del Plata, Argentina: Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP).

Davenport, I. R., Weaver, A. L., & Wourms, J. P. (2011). A novel set of structures within the elasmobranch ovarian follicle. *Journal of Morphology*, *272*, 557–565. https://doi.org/10.1002/jmor.10932

Di Giácomo, E. E., & Perier, M. R. (1994). Reproductive biology of the cockfish, *Callorhinchus callorynchus* (Holocephali: Callorhynchidae), in Patagonian waters (Argentina). *Fishery Bulletin*, *92*, 531–539. Retrieved from www.st.nmfs.noaa.gov/spo/FishBull/923/digiacomo.pdf

Dunbar, M., Onuora, C., Morgan, S., Stone, F. E., Huckaba, T. M., & Davenport, I. R. (2017). Follicle cell processes: A shark thing? *Journal of Fish Biology*, *90*, 1031–1036. https://doi.org/10.1111/jfb.13196

Fänge, R., & Sundell, G. (1969). Lymphomyeloid tissues, blood cells and plasma proteins in *Chimaera monstrosa* (Pisces, Holocephali). *Acta Zoologica*, *50*, 155–168. https://doi.org/10.1111/j.1463-6395.1969.tb00537.x

Finucci, B., Dunn, M. R., Jones, E. G., & Anderson, J. (2017). Reproductive biology of the two deep-sea chimaerids, longnose spookfish (*Harriotta raleighana*) and Pacific spookfish (*Rhinochimaera pacifica*). *Deep-Sea Research Part I*, *120*, 76–87. https://doi.org/10.1016/j.dsr.2016.11.008

Grogan, D. E., Lund, R., & Greenfest-Allen, E. (2012). The origin and relationships of early chondrichthyans. In J. C. Carrier, J. A. Musick, & M. R. Heithausm (Eds.), *Biology of sharks and their relatives* (pp. 1–29). Boca Raton, FL: CRC Press.

Guraya, S. S. (1978). Maturation of the follicular wall of nonmammalian vertebrates. In R. E. Jones (Ed.), *The vertebrate ovary: Comparative biology and evolution* (pp. 261–329). London, England: Plenum Press.

Hamlett, W. C., Jezior, M., & Spieler, R. (1999). Ultrastructural analysis of folliculogenesis in the ovary of the yellow-spotted stingray, *Urolophus jamaicensis. Annals of Anatomy*, *181*, 159–172. https://doi.org/10.1016/S0940-9602(99)80003-X

Hamlett, W. C., & Koob, T. J. (1999). Female reproductive system. In W. C. Hamlett (Ed.), *Sharks, skates and rays. The biology of elasmobranch fishes* (pp. 398–443). Baltimore, MD: The John Hopkins University Press.

Hyder, S. L., Cayer, M. L., & Pettey, C. L. (1983). Cell types in peripheral blood of the nurse shark: An approach to structure and function. *Tissue and Cell*, *15*, 437–455. https://doi.org/10.1016/0040-8166(83)90075-7

Malagrino, G., Takemura, A., & Mizue, K. (1981). Studies on Holocephali II. On the reproduction of *Chimaera phantasma* Jordan et Snyder caught in the coastal waters of Nagasaki. *Bulletin of the Faculty of Fisheries Nagasaki University*, *51*, 1–7. doi: 10.069/30505.

Márquez Farías, J. F., & Lara Mendoza, R. E. (2014). Notas sobre la morfología del aparato reproductor de la quimera, *Hydrolagus melanophasma* (Chondrichthyes, Holocephali), de la costa oeste de Baja California, México. *Hidrobiológica*, *24*, 151–158.

Moura, T., Figueiredo, I., Bordalo Machado, P., & Serrano Gordo, L. (2004). Growth pattern and reproductive strategy of the holocephalan *Chimaera monstrosa* L. along the Portuguese continental slope. *Journal of the Marine Biological Association of the United Kingdom*, *84*, 801–804. https://doi.org/10.1017/S002531540400997Xh

Moyle, P. B., & Cech, J. J. (2000). *Fishes: An introduction to ichthyology* (4th ed.). Upper Saddle River, NJ: Prentice-Hall.

Prisco, M., Ricchiari, L., & Andreuccetti, P. (2002). Ultrastructural studies on developing follicles of the spotted ray *Torpedo marmorata. Molecular Reproduction and Development*, *61*, 78–86. https://doi.org/10.1002/mrd.1133

Tanaka, S., Shiobara, Y., Hioki, S., Abe, H., Nishi, G., Yano, K., & Suzuki, K. (1990). The reproductive biology of the frilled shark, *Chalamydoselachus anguineus*, from Suruga Bay, Japan. *Japanese Journal of Ichthyology*, *37*, 273–290. https://doi.org/10.11369/jji1950.37.273

Wourms, J. P. (1977). Reproduction and development in chondrichthyan fishes. *American Zoologist*, *17*, 379–410. https://doi.org/10.1093/icb/17.2.379