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A Novel Set of Structures Within the Elasmobranch, Ovarian Follicle

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ABSTRACT Elasmobranch fishes produce some of the largest oocytes known, exceeding 10 cm in diameter. Using various microscopy techniques we investigated the structural adaptations which facilitate the production of these large egg cells in three species of shark: the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, dusky smoothound, *Mustelus canis* and the little gulper shark, *Centrophorus uyato*. The ovarian follicle of elasmobranchs follows the typical vertebrate pattern, with one notable exception; the zona pellucida reaches extreme widths, over 70 μm, during early oogenesis. Contact between the follicle cells and the oocyte across the zona pellucida is necessary for oogenesis. We describe here a novel set of large, tube-like structures, which we named follicle cell processes that bridge this gap. The follicle cell processes are more robust than the microvilli associated with the follicle cells and the oocyte plasma membrane and much longer. During early oogenesis the follicle increases in size relatively quickly resulting in a wide zona pellucida. At this stage the follicle cell processes appear taut, uniform and radially oriented. As oogenesis continues the zona pellucida narrows and the follicle cell processes change their orientation, appearing to wrap around the oocyte. The presence of the contractile protein actin within the follicle cell processes and their change in orientation may well be an adaptation for maintaining the integrity of these large oocytes. The follicle cell processes also contain electron dense material, identical to material found within the follicle cells, suggesting a role in the transport of metabolites to the developing oocyte. J. Morphol. 272:557–565, 2011.

KEY WORDS: elasmobranch; oogenesis; follicle cell processes

INTRODUCTION

The chondrichthyan fishes contain two subclasses; the holocephali (chimeras) and the numerically larger group the elasmobranchii (sharks, skates, and rays). The chondrichthyans are the oldest extant jawed vertebrates and are among the most successful predators ever to have evolved. They first appeared in the Devonian Period some 400 million years ago. Like most apex predators, they tend to be large, long-lived animals that produce relatively few offspring (Stearns, 1976). To offset low fecundity their young are large and precocial. The production of large offspring favors a transition from the more ancestral oviparity, to viviparity. More than 50% of chondrichthyans give birth to live young, in contrast to the 2–3% of osteichthyan fishes (Wourms and Lombardi, 1992). The occurrence of live bearing in chondrichthyans has been documented as far back as the Lower Carboniferous, 355 million years ago (Lund, 1980). Chondrichthyans were among the first vertebrates to successfully make the transition to viviparity and we can follow this evolutionary transition in extant species. There are two ways of producing large offspring: by producing large oocytes that can store increased amounts of nutrients for use by the developing embryo or by evolving systems where the maternal organism supplies nutrients to the embryo throughout gestation. Both these strategies, reviewed by Wourms (1977), are found among the chondrichthyans.

Egg laying chondrichthyans typically produce relatively large oocytes contained within large, robust and often elaborately designed egg cases that provide protection and anchorage throughout development. When the oocyte and its subsequent nutrient supply are the sole source of nutrition for the developing embryo (lecithotrophy), the size of the embryo at hatching is ultimately a function of oocyte size. The oocyte is somewhat restricted in size as it has to fit within the confines of the egg case, which in turn has to pass out of the female reproductive system. Eliminating the need for protective egg cases by having the embryo develop in utero, could allow for an increase in oocyte size. Larger oocytes store more nutrients for use by the

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future embryo. This initial transition to viviparity is still lecithotrophic, thus no major physiological adaptations are needed. Neonates are streamlined and thus can afford to attain larger sizes and still pass out of the reproductive tract with relative ease. Oocytes can become extremely large, approaching 10 cm in diameter in some species, including: carpetsharks, *Ginglymostoma*, dogfish sharks, *Centrophorus*, and *Centroscymus*, and frilled shark, *Chlamydoselachus anguineus* (Breder and Rosen, 1966; Wourms, 1977; Tanaka et al., 1990). Oocytes in the 10 cm size range are not strictly confined to the chondrichthyans; they are found in at least one osteichthyan fish, the coelacanth, *Latimera chalumnae* (Smith et al., 1975).

Although producing large oocytes is clearly a successful strategy, there are limits to the size of the oocyte. Since they are a single cell they are subject to physical and physiological constraints. Two major considerations limiting the size of oocytes are: (1) maintenance of the physical integrity of the oocyte during ovulation and passage through the reproductive tract and (2) difficulties in provisioning of the oocyte i.e., in supplying the oocyte with organelles and vitellogenin. To investigate the physical properties associated with producing large oocytes, we used: light microscopy to observe the general morphology of the elasmobranch ovarian follicle, immunofluorescence to look at aspects of the cytoskeletal architecture, specifically actin distribution and transmission electron microscopy for cell ultrastructure. Our investigations revealed a novel set of structures within the ovarian follicle that may well play a significant role in the evolution of large egg cells.

MATERIAL AND METHODS

We used two local species of shark with a more moderate egg size to establish patterns of oogenesis. Specimens of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, ($n = 12$) and the dusky smoothound, *Mustelus canis*, ($n = 6$) (Carcharhiniformes) were collected off the coast of Charleston, South Carolina, during November and December 1999–2002. We were only able to obtain oocytes up to a size of ~1 cm in diameter (mature oocytes from both species are around 2 cm in diameter). Ovaries and oocytes were dissected within the hour and placed in the appropriate fixatives. For light and fluorescence microscopy, tissues were placed in 4% paraformaldehyde in a 0.1 mol l^{-1} Sorenson's phosphate buffered saline (PBS) with 0.35 mol l^{-1} sucrose, pH 7.2. For light microscopy, the tissue was embedded in Technovite 7100 (Kulzer, Wehrheim), sectioned at 1 μm and stained with methylene blue and azure II (blue) or methylene blue and acid fuchsin (pink/purple). For fluorescence microscopy, fixed tissues were placed in OCT embedding media for cryo-sectioning (Sakura Finetechnical Co., Tokyo), cut into sections ~20 μm thick, and stained with Texas Red labeled Phalloidin (Molecular Probes, Eugene, Oregon). Phalloidin is a toxin from the death cap mushroom (*Amanita phalloides*) which binds specifically to filamentous actin. Slides were photographed using a Zeiss 510 laser scanning confocal microscope.

For transmission electron microscopy (TEM) samples were fixed overnight in 2.5% glutaraldehyde and 3% formaldehyde in a 0.1 M Sorenson's PBS with 0.35 mol l^{-1} sucrose, pH 7.2 and postfixed in 2% osmium tetroxide in PBS. Gold sections were collected on 100 mesh copper grids then stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Sections were photographed using a Hitachi 7000 TEM.

Large oocytes were collected from the little gulper shark, *Centrophorus uyato*, (Squaliformes; $n = 4$). These were collected from St. Annes Bay, Jamaica, during the summer of 2000. Oocytes ranged in size from ~50 μm to around 10 cm in diameter. Tissue samples were processed as quickly as possible but up to 6 hours could have elapsed before they could be removed and placed in fixative. Tissues were prepared for light microscopy and TEM as previously described. The quality of fixation was better with the two local species as tissues were removed and placed in fixative extremely quickly. Therefore the majority of the images used in this study are from *R. terraenovae* and *M. canis*.

Oocytes with a diameter of ~1 mm or less were measured using calibration software associated with the microscope. For larger oocytes, the follicle diameter was measured using a 150 mm dial calliper (General).

RESULTS

Light Microscopy

In light micrographs, the shark ovary appears as a diffuse structure with follicles randomly scattered throughout the ovarian tissue (Figs. 1A and 3A). Figure 1A shows relationship between the ovary and the epigonal organ to which it is attached (Lutton and Callard, 2008). In the three species studied, the arrangement of the ovarian follicle conforms to the general vertebrate pattern. There is a centrally placed oocyte surrounded by the acellular, zona pellucida; this in turn is surrounded by the follicle cell layer. The follicle cells form a uniform, single celled layer around the oocyte. This layer does appear more random in arrangement early on in oogenesis due to the mitotic activity of the follicle cells. A basal lamina separates the outer theca from the follicle cell layer. Using light microscopy, these concentric layers of the ovarian follicle become distinct in follicles with diameters from around 200 μm. These layers can be seen in Figure 1C. There is, however, one prominent distinguishing feature; viz. during early oogenesis, in follicles with a diameter of 1–2 mm in *R. terraenovae* and *M. canis* and follicles with a diameter of ~6 mm in *C. uyato*, the zona pellucida reaches widths of 50 μm and in some areas in excess of 70 μm (Figs. 1C, 2A,B, 3C,D, and 6A). As oogenesis progresses the zona pellucida begins to narrow (Figs. 1D, 2D, and 3E,F).

Fluorescent Microscopy

We used Texas Red labeled Phalloidin to visualize the distribution of the contractile, cytoskeletal protein actin. During early oogenesis, the zona pellucida appears to swell and can reach widths in excess of 70 μm. Connecting the follicle cells to the oocyte are a spectacular array of actin-based processes that appear to radiate inwards from the follicle cells (Figs. 2A–D and 3B,D,F). The dra-

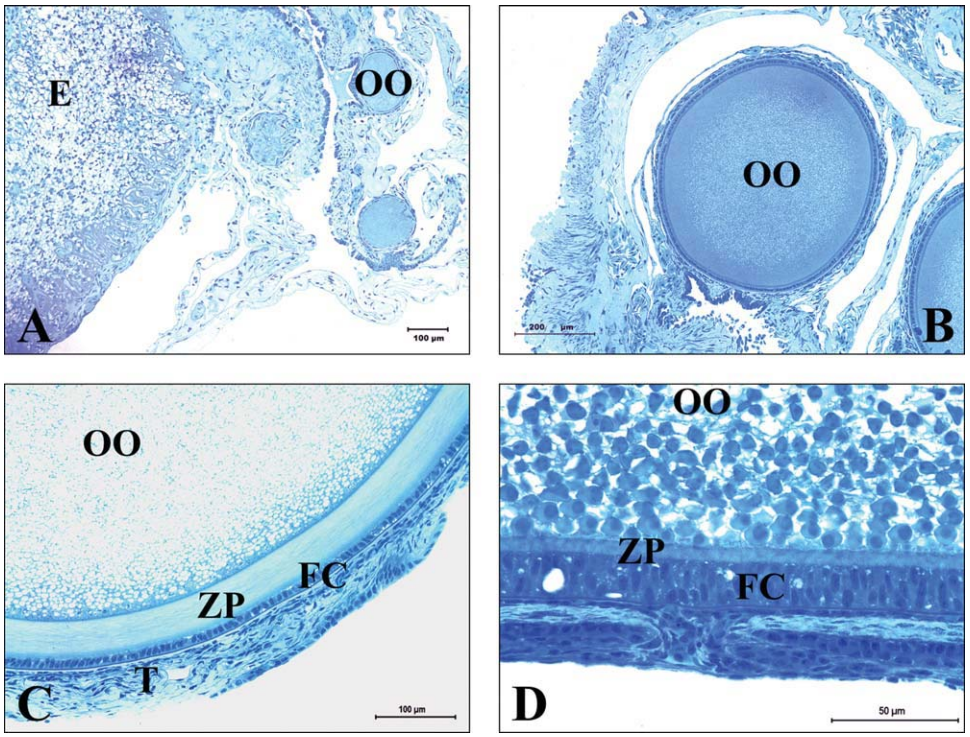

Fig. 1. Light micrographs of *M. canis* stained with methylene blue and azure II. (**A**) Section through ovarian tissue (right) and associated epigonal organ (E). The ovary is a diffuse structure with follicles that appear randomly scattered throughout, small oocytes can be seen (OO). (**B**) A section through a developing follicle with a diameter of ~500 µm. The distinct layers of the follicle are becoming apparent. (**C**) The follicle diameter here is around 2 mm. The concentric layers of the ovarian follicle are evident here. A centrally placed oocyte surrounded by the zona pellucida (ZP), which in turn is surrounded by the follicle cell layer (FC) and finally the theca (T). The extreme width of the ZP is apparent. The FC are cuboidal and loosely arranged. (**D**) Is from a 6 mm diameter follicle, the ZP has narrowed considerably. The FC are still loosely aligned but are becoming more elongated. Note that yolk platelets are visible at this stage. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

matic increase in the width of the zona pellucida during early oogenesis appears to pull the follicle cell processes (FCP) into a taut, organized, radial array (Fig. 2A). This orientation is maintained until the maximum width of the zona pellucida is reached. As oogenesis continues and the zona pellucida begins to narrow, the orientation of FCP also changes. They start to appear loose and less organized in their arrangement (Figs. 2B,C and 3B,D). As the zona pellucida continues to narrow, the FCP take on a more mesh-like configuration and become increasingly circumferential in their orientation (Figs. 2D and 3F). No actin based structures are apparent within the cortical region of the developing oocyte that could provide the physical support needed during ovulation; although extensions of the FCP penetrate deep into the oocyte cortex during previtellogenic and vitellogenic stages (Figs. 2D and 3D).

Transmission Electron Microscopy

Rhizoprionodon terraenovae. The oocyte plasma membrane exhibits a border of microvilli (Fig. 4A–D). The individual microvilli do not span the extreme width of the zona pellucida allowing for connections between the follicle cells and the oocyte. This is where the follicle cell processes play a role. This can be seen in Figure 4A, a low magnification image through a 2 mm diameter follicle. The lower region of the micrograph depicts the microvilli of the oolema and the upper region the follicle cells; their loose configuration is indicative of mitosis during early, rapid growth of the follicle. The middle region of the micrograph is the zona pellucida with many FCP passing through it. In *R. terraenovae*, the FCP often exhibit a light and dark, banded appearance (Fig. 4B–D). The robust nature of some of the FCP can clearly be seen in Figure 4B. The FCP penetrate deep into the cortex of the oocyte (Fig. 4C,D). At this point direct cytoplasmic continuity between the follicle cell and the oocyte has not been established in *R. terraenovae*. Their possible role in transport is highlighted in Figure 4E,F. In Figure 4E a large FCP can be seen leaving the follicle cell and entering the zona pellucida. Note that the sizes of adjacent mitochondria are well within the dimensions of the FCP. The presence of electron dense material within the follicle cells appears identical to that found within the FCP (Fig. 4F).

As oogenesis progresses and the width of the zona pellucida begins to narrow, the orientation of

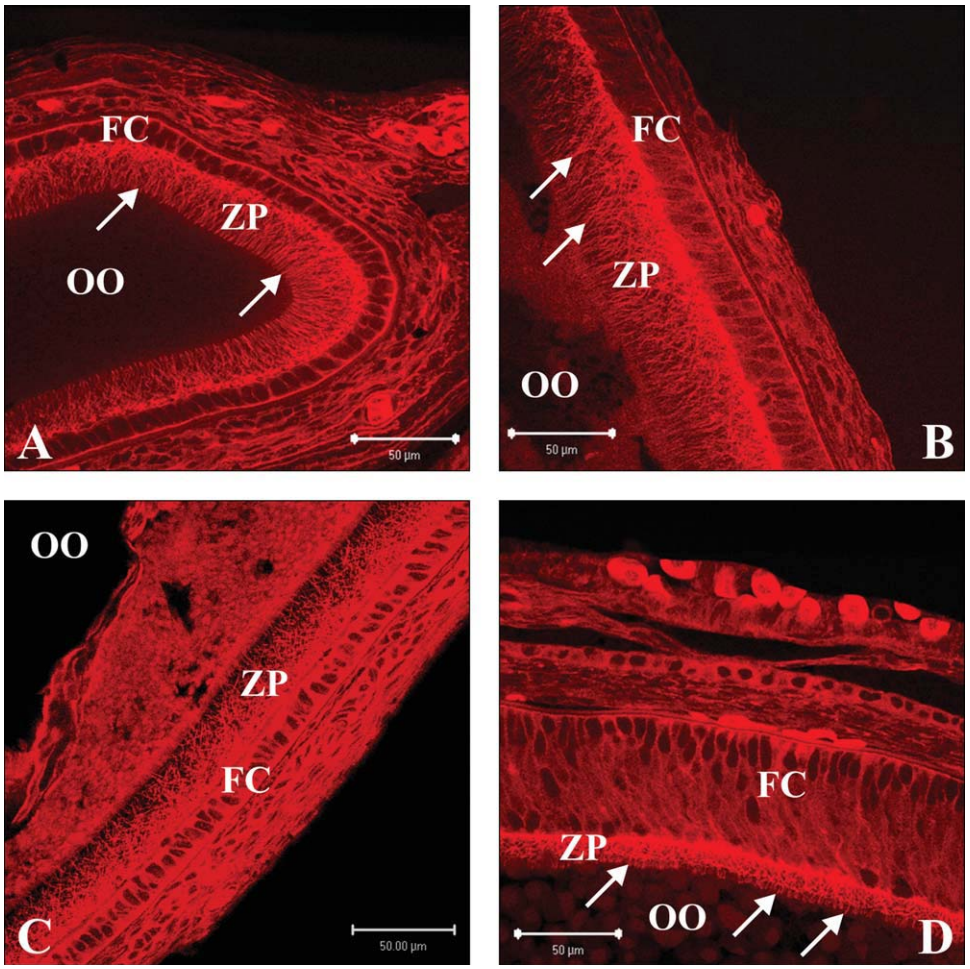

Fig. 2. Fluorescent micrographs of *M. canis* stained with Texas Red labeled Phalloidin. (**A**) Section through a 1 mm follicle. The region of the zona pellucida (ZP) which appears translucent in light micrographs is in fact packed with a spectacular array of follicle cell processes (FCP). The FCP contain the cytoskeletal protein actin and hence appear as the red structures embedded within the acellular ZP (arrows). The FCP connect the follicle cells (FC) with the oocyte (OO). (**B**) Section though a 2 mm follicle. At this stage the ZP has reached its maximum width and the FCP appear slightly less taut (arrows). (**C**) Section through a follicle ~4 mm in diameter. As the ZP narrows the FCP start to appear more mesh-like in appearance. (**D**) Section from a follicle 6 mm in diameter. The ZP is relatively narrow and FCP form a quite dense, mesh-like framework embedded within the ZP. Arrows indicate the regions of the FCP that penetrate the oocyte cortex. Yolk platelets can be seen within the oocyte. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the FCP changes from radial to a more mesh-like configuration. The FCP also appear to assume an orthogonal pattern, i.e. "a plywood type" configuration (Fig. 4G,H). The substantial, tube-like nature of the FCP is evident again in Figure 4G. Here, a sagittal view along the FCP can be seen as it leaves the follicle cell and enters the zona pellucida. There are also many FCP visible in transverse section, reiterating the plywood type configuration. The circumferential arrangement of the FCP can be seen in Figure 4H, where they appear to wrap around the zona pellucida in a fashion similar to the steel, radial bands in a car tire.

Mustelus canis. The FCP of *M. canis* can exhibit sac-like dilations (Fig. 5A,B). The extended length of the FCP in relation to the uniform length of the oocytes border of microvilli is evident (Fig. 5A,B). The FCP again appear to pass deep into the cortex of the oocyte (Fig. 5C,D). In Figure 5C,D the FCP are not totally in the plane of section, but their presence deep into the oocyte cortex is clearly visible. The robust nature of some of the FCP can be seen in Figure 5E. Their role in the transportation of materials from the follicle cells to the developing oocyte is again indicated in Figure 5F.

Centrophorus uyato. Follicle cell processes were also observed in *C. uyato*, both in light and electron micrographs (Fig. 6). Most light micrographs do not tend to show the FCP, but here they can clearly be seen traversing the zona pellucida (Fig. 6A). They may well be visible in this light

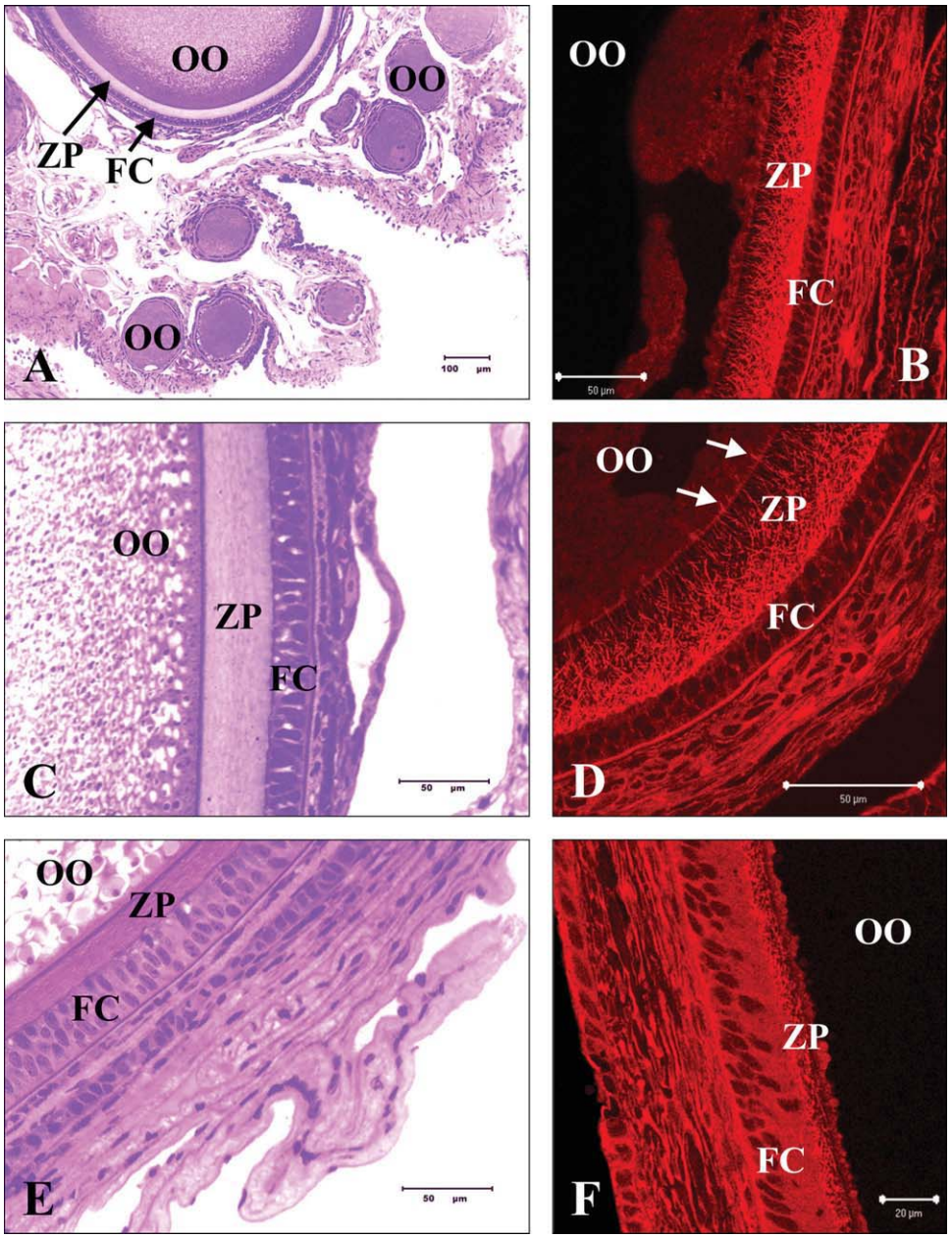

Fig. 3. Light micrographs of *R. terraenovae* stained with methylene blue and acid fuchsin (A,C,E) and stained with Texas Red labeled Phalloidin (B,D,F). (**A**) Section through ovarian tissue with several small oocytes visible (OO). As noted before, the ovary is a diffuse structure with follicles scattered throughout. Toward the top there is an oocyte with a diameter of ~1 mm, where the zona pellucida (ZP) can be clearly seen as can the follicle cells (FC). (**B**) Section through a 1 mm follicle highlighting the distribution of filamentous actin within the FCP. The follicle cell processes can be seen crossing the ZP, connecting the FC to the OO. (**C**) Section through a 2 mm diameter follicle, the extreme width of the ZP is evident. The gaps between individual FC and their uneven appearance are due to them being mitotically active. (**D**) Section through a 2 mm diameter follicle. The FCP can again be seen embedded within the ZP, connecting the FC to the OO. Arrows indicate areas where the FCP penetrate into the oocyte cortex. (**E**) Section through a 6 mm diameter follicle. Yolk platelets can be seen forming in the OO; they appear as pink oval shapes. The ZP has narrowed to ~18 µm. (**F**) A section through a 6 mm diameter follicle. The orientation of the FCP changes coinciding with the narrowing of the ZP. They take on a more mesh-like appearance within the ZP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

micrograph due to their particularly robust nature and accumulation of more stain. Figure 6B is a low magnification TEM image of a FCP traversing the zona pellucida. Apart from a very small section of the FCP, it appears that the oocyte does have direct cytoplasmic continuity with the follicle cells.

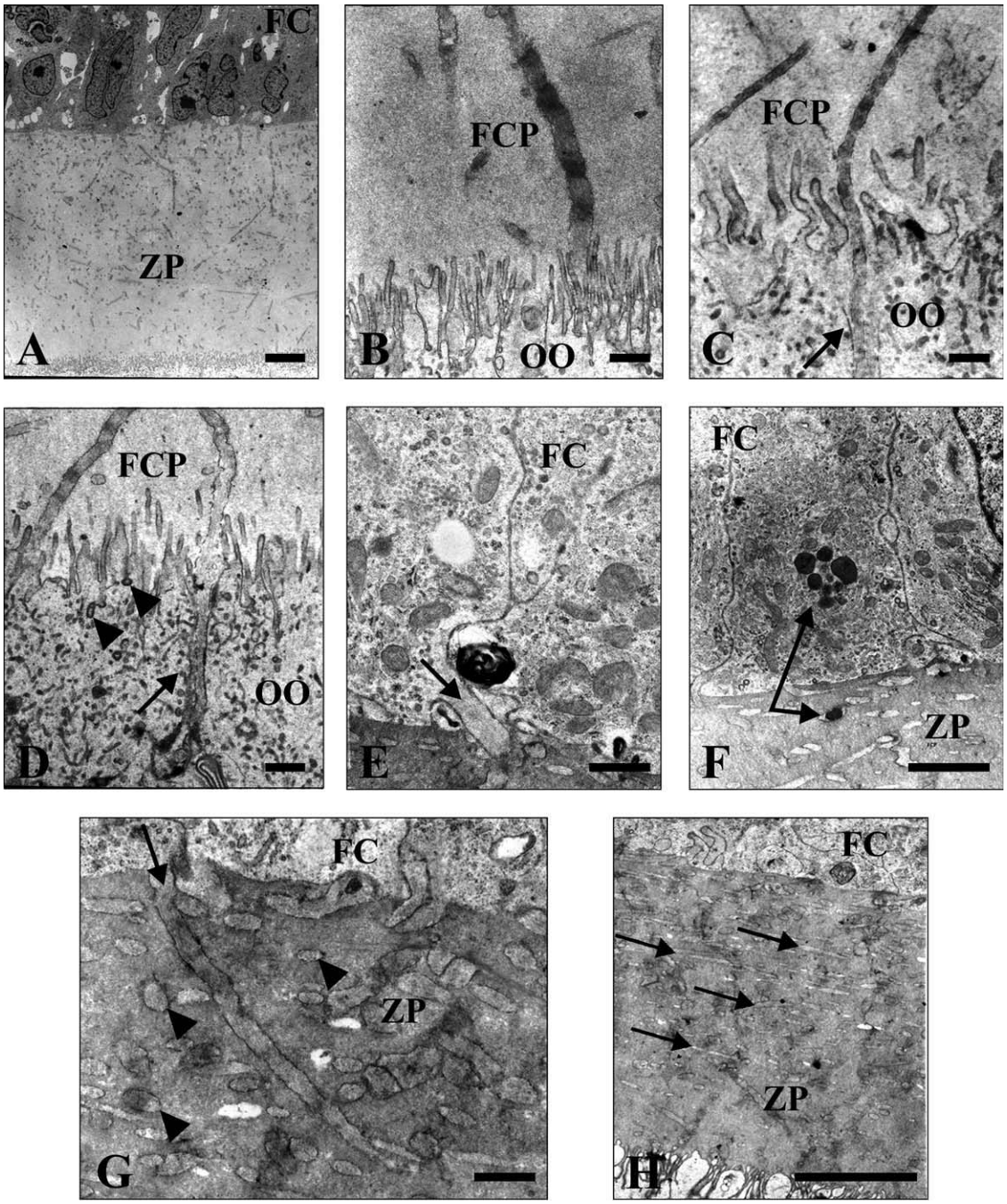

Fig. 4. Transmission electron micrographs of *R. terraenovae*. All images are oriented in the same plane i.e. the follicle cells are towards the top and the oocyte towards the bottom. (**A**) Low magnification through a follicle ~2 mm in diameter. The bulk of the image is through the zona pellucida (ZP). In the lower part of the image is the border of microvilli of the oolemma. Follicle cell processes can be seen crossing the ZP. (Scale bar = 10 μm). (**B**) Higher magnification image of one of the follicle cell processes (FCP) in a follicle ~2 mm in diameter. The FCP can be quite substantial structures when compared to the oocyte microvilli. Note the dark and light banding pattern within the FCP. (Scale bar = 1 μm). (**C**) Section from a 2 mm diameter follicle. The FCP appears to penetrate into the cortical region of the oocyte (OO) (arrow). (Scale bar = 1 μm). (**D**) Section from a follicle about 4 mm in diameter. Although the FCP fades from the plane of view near the OO the depth of penetration of the FCP into the cortex is still visible (arrow). Arrowheads indicate vesicles entering the OO via endocytosis. (Scale bar = 1μm). (**E**) Section from a follicle with a diameter of 10 mm. The FCP is leaving the follicle cell (FC) and entering the ZP (arrow). Note the relative size of the mitochondria near the entrance to the FCP. They are well within the diameter of the FCP. (Scale bar = 1 μm). (**F**) Section from a follicle 10 mm in diameter. The electron dense material seen within the FC appears identical to material found with the FCP (arrows), seen in cross section here. (Scale bar = 10 μm). (**G**) Section from a follicle 10 mm in diameter. This reiterates the substantial nature of the FCP. A large FCP can be seen in sagittal section leaving the FC (arrow), many others can be seen in cross section (arrow heads), forming a meshwork within the ZP. (Scale bar = 1 μm). (**H**) Section from a follicle 10 mm in diameter. The orientation of the FCP are now more circumferential, they appear to wrap around the OO embedded in the ZP (arrows, also indicate direction of travel). (Bar 10 μm).

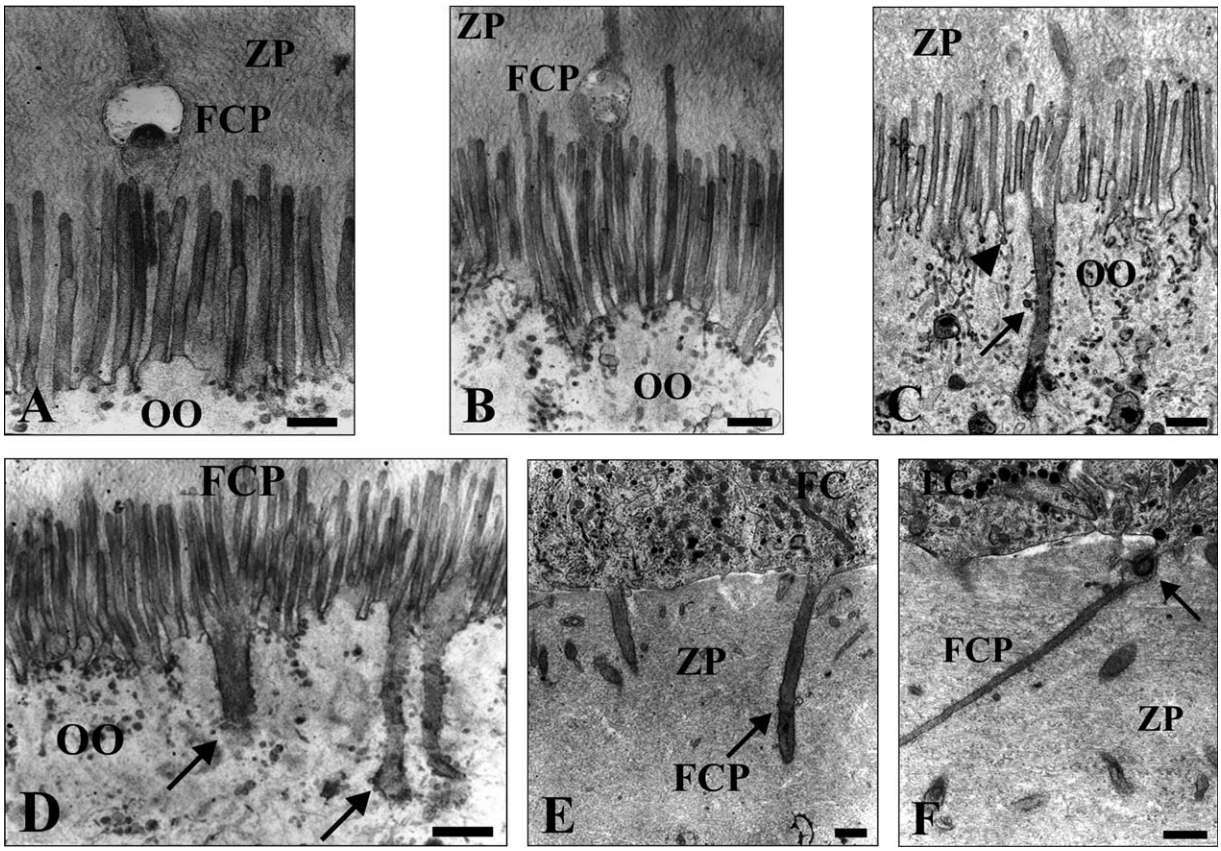

Fig. 5. Transmission electron micrographs of *M. canis*. All images are orientated in the same plane i.e. the follicle cells are towards the top and the oocyte towards the bottom. (**A**), (**B**) Sections through a follicle ~2 mm in diameter. In this species the follicle cell processes (FCP) are sometimes associated with large, swollen appearing dilations, which can clearly be seen here. These two micrographs each show a FCP approaching the oocyte (OO) and the border of microvilli of the oolemma. (Scale bar = 1 μm). (**C**) Section from a follicle with a diameter of 3 mm. Although the FCP is not fully in the plane of section, its plane of travel deep into the oocyte cortex is discernable (arrow). Endocytotic vesicles can also be seen at the oolemma (arrowhead). (Scale bar = 1 μm). (**D**) Section from another follicle 3 mm in diameter. The FCP are again out of the plane of section, but their substantial nature and the depth of penetration can be seen as they terminate in the cortical region of the oocyte. (Scale bar = 1 μm). (**E**) Section from a follicle 10 mm in diameter. Some of the FCP reach substantial dimensions in both length and girth (arrow). (Scale bar = 1 μm). (**F**) Section from a follicle 10 mm in diameter. Note that material appears to be entering the FCP and that electron dense material is also present well within the FCP (arrow). (Scale bar = 1 μm).

DISCUSSION

Results presented in this article indicate the presence of an elaborate array of tube-like structures within the zona pellucida of three species of sharks. These structures may be multifunctional in regards to the production of large oocytes, especially with respect to the provisioning of these eggs and to maintaining structural integrity during ovulation.

For oogenesis to occur there needs to be contact between the follicle cells and the oocyte (Buccione et al., 1990). This is normally achieved via the interdigitation of the microvilli of the oocyte and the follicle cells, which extend across the zona pellucida. The actual connections are established via gap junctions. This type of connection is possible when the zona pellucida remains narrow. In birds, which characteristically produce large eggs, the zona pellucida does not exceed more than 3–4 μm (Bellairs, 1965). Some reptiles also produce large oocytes. For example, in the American alligator, *Alligator mississippiensis*, oocytes can reach sizes approaching 4 cm with the zona pellucida reaching widths of 18–20 μm (Uribe and Guillette, 2000). How the follicle cells maintain contact with the oocyte in alligators is unknown. In the three elasmobranch species studied here, the zona pellucida reaches extreme widths, in excess of 70 μm. This distance appears too great a span for microvilli to cross. Instead, contact between the follicle cells and the oocyte is maintained by the FCP.

Follicle cells transport nutrients from the blood vascular system to the oocyte, as well as directly supplying other metabolites and organelles (Buccione et al., 1990). Transport across the zona pellucida is thought to occur via diffusion, with metabolites taken into the oocyte via endocytosis. Vesicles can clearly be seen entering the oocyte via endocy-

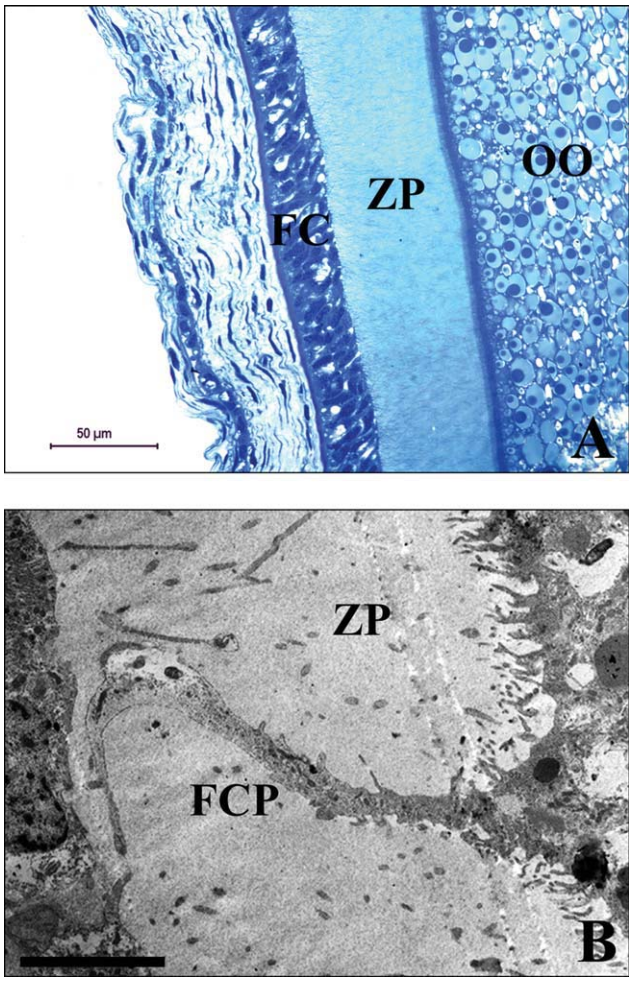

Fig. 6. Micrographs from *C. uyato*. In both micrographs the oocyte (OO) is to the right of the image and the follicle layers moving out to the left. (**A**) Light micrograph of a section through a follicle 6 mm in diameter. The follicle cell processes (FCP) can clearly be seen passing through the zona pellucida (ZP). Note the many yolk platelets within the oocyte. (**B**) Transmission electron micrograph from a follicle 23 mm in diameter. Note the substantial size to which FCP can attain. Apart from one small area of the FCP which is out of the plane of section, there appears to be direct cytoplasmic continuity between the follicle cells and the oocyte in a vitellogenic egg. (Scale bar = 10 µm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tosis in the species studied here. But our findings also indicate transport of material via the FCP. Follicle cell processes can clearly be seen branching out from the follicle cells and entering the zona pellucida; they appear to contain material which is also present within the follicle cell. This would indicate a direct role in the transport of material to the oocyte. The micrographs do not indicate a directional flow of material, although logic would dictate that as the egg increases in size there would be a net flow of material from the follicle cells to the oocyte. Our findings also indicate direct cytoplasmic continuity between the follicle cells and the oocyte at vitellogenic stages, as seen in the low magnification transmission electron micrographs from *C. uyato*. This is the first time such a connection has been reported in vertebrates during vitellogenesis. Processes extending from the follicle cells are not unique to the chondrichthyans. Similar structures have been observed "indenting" the oocyte plasma membrane of the coturnix quail and leghorn chicken (Press, 1964). These extensions were closely associated with membrane-bound vesicles, which Press termed transosomes. Similar observations were made in the domestic chicken by Bellairs (1965) who referred to these vesicles as lining bodies. In both cases, no direct cytoplasmic bridges were observed and the follicle cell extensions traversed a zona pellucida with a maximum width of ~4 µm. Direct cytoplasmic continuity between follicle cells and the oocyte has been described in a few other vertebrates but only during previtellogenic stages. In elasmobranchs, intercellular bridges have been described in the yellow spotted stingray, *Urolophus jamaicensis* (Hamlett et al., 1999), the starry ray, *Raya asterias* (Andreuccetti et al., 1999), and the spotted ray, *Torpedo marmorata* (Prisco et al. 2002), but only in previtellogenic stages. Intercellular bridges are also found during early, previtellogenic stages of some reptiles, including the lizard *Lacerta sicula* (Andreuccetti et al., 1978 and 1992) and *Podarcis sicula* (Motta et al., 1995, 1996). The FCP we described here are much larger than any of the previously reported structures and maintain contact during both the previtelloginic and vitellogenic stages. They also show direct cytoplasmic continuity between the follicle cells and the oocyte. The role of the FCP in metabolite transport that we are suggesting would be a great benefit in the provisioning of these large oocytes.

The FCP may also play a role in maintaining the physical integrity of large oocytes during ovulation and the subsequent journey through the reproductive tract. In somatic cells, actin filaments or microfilaments, form the tensile part of the cytoskeleton. Although they are distributed throughout the cell, they are particularly concentrated in the cortical region of the cell and effectively pull the membrane inwards. Resisting this inward pull are the compression resistant components of the cytoskeleton, namely, microtubules and intermediate filaments. The same combination of tension and compression resistance (Tensegrity) has been used in recent years to explain the physical properties of molecules, cells, and bodies (Ingber, 1998). We looked at actin distribution within the developing follicle to see if a novel application of the cytoskeleton or the tensegrity principle helped maintain the integrity of these large oocytes.

During oogenesis the oocyte is physically supported by the follicle. At this stage, the theca, which is heavily invested with collagen fibers,

plays a large part in maintaining the physical integrity of the oocyte. At the time of ovulation the oocyte loses this physical support and is released into the body cavity with only the zona pellucida surrounding it. This is where the FCP embedded in the zona pellucida may well play a significant role. First, from the immunofluorescent studies we know the FCP contain filamentous actin. In addition, during oogenesis the orientation of the FCP changes. Initially, the FCP form a taut, extremely uniform system of processes projecting radially inwards from the follicle cells to the oocyte. Later in oogenesis, as the zona pellucida narrows, the FCP begin to take on a more circumferential orientation, wrapping around the outside of the oocyte in a manner analogous to the radials in a car tire, applying tension around the outer surface of the oocyte. The end result suggests a mechanical system similar to that found in somatic cells. In somatic cells there is a cortical region just below the cell membrane containing a band of actin filaments exerting an inward pull. This inward force is resisted by a framework of microtubules and intermediate filaments and by the hydrostatic pressure of the cell's cytoplasm. The combination of forces results in the cell's characteristic shape (Ingber, 1993, 1998). Our observations of elasmobranch oocytes indicate the presence of a tensile region of filamentous actin external to the cell. Actin filaments are contained within the FCP, which themselves are embedded within the zona pellucida and subsequently wrapped around the outside of the oocyte. In this case, the dense, noncompressible, yolk-filled body of the oocyte itself would resist compression by the actin network, similar to how the hydrostatic skeleton is used by many aquatic invertebrates. In other words, the actin filaments within the FCP play a role similar to the cortical ring of actin found in the cytoskeleton of somatic cells. Only now the actin is found in an extracellular environment, embedded in tube-like structures within the acellular zona pellucida. This represents a novel reworking of the tensegrity principle used to maintain animal cell integrity applied to extremes in cell size.

The aims of this study were to look for structures that would facilitate the production of large egg cells. While a definitive solution to the physical problems associated with the production of large eggs has not been fully resolved, there are some tantalizing clues as to novel structures that may play a significant role in the evolution of these extremely large eggs.

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