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REGULAR ARTICLE

# Stages in follicle cell/oocyte interface during vitellogenesis in caecilians *Ichthyophis tricolor* and *Gegeneophis ramaswamii*: a transmission electron-microscopic study

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**Abstract** We describe the ultrastructural organization of the vitellogenic follicle stages in two caecilian species. Monthly samples of slices of ovary of *Ichthyophis tricolor* and *Gegeneophis ramaswamii* from the Western Ghats of India were subjected to transmission electron-microscopic analysis, with special attention to the follicle cell/oocyte interface. In order to maintain uniformity of the stages among the amphibians, all the stages in the caecilian follicles were assigned to stages I–VI, the vitellogenic and post-vitellogenic follicles being assigned to stages III–VI. Stage III commences with the appearance of precursors of vitelline envelope material in the perivitelline space. Stages IV and V have been assigned appropriate substages. During the transition of stage III to stage VI oocytes, a sequential change occurs in the manifestations of follicle cells, perivitelline space, vitelline envelope and oocyte cortex. The vitelline envelope becomes a tough coat through the tunnels of which the macrovilli pass to interdigitate between the microvilli. The oocyte surface forms pinocytic vesicles that develop into coated pits and, later, coated vesicles. Contributions of the oocyte cortex to the vitelline envelope and of the follicle cells to yolk material via synthesis within them are indicated. The follicle cell/oocyte interface of vitellogenic follicles of these two caecilians resembles that in anurans and urodeles, with certain features being unique to caecilians. Thus, this paper throws light on the possible relationships of caecilians to anurans and urodeles with special reference to ovarian follicles.

**Keywords** Vitellogenic follicle · Macrovilli · Microvilli · Vitelline envelope · Oocyte · Caecilians, *Ichthyophis tricolor, Gegeneophis ramaswamii* (Gymnophiona)

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## Introduction

The pattern of oogenesis varies in different amphibian species resulting in various sizes and numbers of eggs being formed. These variations are attributable to the adaptational requirements of the species to the diverse environments in which they lay their eggs and thus secure their development (Guraya 1976, 1979; Del Pino et al. 1986; Del Pino 1989; Villecco et al. 1996, 1999, 2000, 2002; Uribe 2001, 2003; Sanchez and Villecco 2003; Exbrayat 2006). A definite number of primordial follicles are established in the embryo (Skinner 2005). However, in lower vertebrates, including amphibians, oogonia persist in the adult ovary embark on oogenesis during the reproductive cycles and generate a new cohort of oocytes each year (Van Voorhis 1999). When the oogonia enter the first meiotic division they become primary oocytes and then undergo a complex process of cyto-differentiation and division resulting in the production of female gametes

(Dumont 1972; Holland and Dumont 1975; Brummett and Dumont 1977; Dumont and Brummett 1978; Kanamadai and Saidapur 1982; Exbrayat and Collenot 1983; Exbrayat 1986; Villecco et al. 2002; Sanchez and Villecco 2003). Oocytes derived from the oogonia are enveloped by a single layer of somatic cells (follicular epithelium) to form the ovarian follicles. After the follicular establishment by the recruitment of oocytes, they pass through a complex developmental sequence. The major events during this sequence are the accumulation of yolk in the oocyte cytoplasm and the establishment of the vitelline envelope around it. Although the role of the oocyte in the synthesis of yolk constituents is by no means small, the major contribution comes from the liver in the form of vitellogenin, which is sequestered from the blood by the follicle cells and translocated to the ooplasm through the highly complex process of vitellogenesis. The critical effector in this translocation is the follicle cell/oocyte interface (Villecco et al. 2002).

The development of the ovarian follicle from its initial establishment to ovulation in amphibians can be divided into three phases: previtellogenic, vitellogenic and post-vitellogenic (Sanchez and Villecco 2003). Previtellogenesis is the period of primary growth of the oocyte and involves the accumulation of precursors for DNA, RNA and protein synthesis (Davidson 1994). Among the several activities that occur during amphibian previtellogenesis, dramatic development of the follicle cell/oocyte interface takes place involving the appearance of a perivitelline space between the previously adherent follicle cells and the oocyte. The macrovilli from the follicle cells and microvilli from the oocyte establish membrane associations between them in the perivitelline space. This facilitates the translocation of vitellogenin to the oocyte (Sanchez and Villecco 2003).

During vitellogenesis the follicle cell/oocyte interface is intensified. This results in a complex inter-relationship between the follicle cells and the oocyte. Yolk accumulates in the oocyte and the vitelline envelope develops outside it. In the amphibian follicle the role of follicle cells in yolk accumulation in the oocyte is generally passive and the follicle cells translocate vitellogenin from the blood to the oocyte. The vitelline envelope is a product of the synthetic activity of the follicle cells. During vitellogenesis the oocyte increases in size and the follicle cell/oocyte interface is gradually transformed. The yolk platelets, consisting of vitelline, are derived from the vitellogenin, glycogen and lipid. Subsequently, the oocytes pass through the vitellogenic phase, during which the follicles are transformed into post-vitellogenic follicles, making them ready for ovulation (Sanchez and Villecco 2003).

Although Hope et al. (1963, 1964) identified nine stages in the developing oocytes of the salamander *Notophthalmus viridescens*, the consensus among several investigators is for six stages in amphibians in general (Sanchez and Villecco 2003; Uribe 2003). Stages I and II constitute the previtellogenic stages-stages III, IV and V are considered as- the vitellogenic stages. Stage VI is the post-vitellogenic stage.

The follicles of several anuran species, such as *Xenopus laevis* (Dumont 1972), *Flectonotus pygmaeus* (Del Pino and Humphries 1978) and *Bufo arenarum* (Valdez Toledo and Pisanó 1980) have been subjected to transmission electron-microscopic (TEM) analysis. Among the urodeles the salamander *Notophthalmus viridescens* (Hope et al. 1963) and the newt *Necturus maculosus* (Kessel and Ganion 1980) are two species that have been studied in adequate detail. No reports of TEM analysis of caecilian ovarian follicles exist, except for our description of the previtellogenic follicles of *Ichthyophis tricolor* and *Gegeneophis ramaswamii* of the Western Ghats of India (Beyo et al. 2007a, 2007b). However, the light-microscopic morphology of the ovarian changes in relation to follicular dynamics is known for *Ichthyophis beddomei* (Masood-Parveez and Nadkarni 1993a, 1993b) and *Typhlonectes compressicauda* (Exbrayat 2006). The structural descriptions of the caecilian ovary and the ovarian cycle are far too limited and are all based on light-microscopic histology (Wake 1968, 1970a, 1970b, 1972, 1977, 1980, 1993; Exbrayat and Collenot 1983; Exbrayat and Laurent 1983; Exbrayat 1986; Berois and de Sa 1988; Masood-Parveez and Nadkarni 1993a, 1993b; Wake and Dickie 1998; Anjubault and Exbrayat 2004). Thus, a detailed ultrastructural analysis of the ovarian follicles of caecilians in general and the follicle cell/oocyte interface in particular during the vitellogenic phase is highly pertinent from comparative, anatomical and evolutionary perspectives. This paper describes the follicle cell/oocyte interface in two species of caecilians, *I. tricolor* and *G. ramaswamii*.

## Materials and methods

The TEM analysis of the follicle cell/oocyte interface of *I. tricolor* and *G. ramaswamii* involved the same basic methodology as described earlier (Beyo et al. 2007a, 2007b). *I. tricolor* (Ichthyophiidae) and *G. ramaswamii* (Caeciliidae) were collected from terraced plantations of mixed coconut and rubber from Thekkada (08°37′N, 76°57′E) in the Trivandrum district of Kerala and Maramalai (08°26′N, 77°24′E) in the Kanyakumari district of Tamil Nadu, Southern India, from June 2004 to June 2005. These species are relatively abundant in the Western Ghats of Kerala and Tamil Nadu (Oommen et al. 2000). Monthly samples (three animals from each species) were collected, anesthetized with MS-222 (tricaine methane sulfonate) and dissected to expose the female reproductive system.

The specimens were fixed in 10% formalin and preserved in 70% alcohol with appropriate labels for future use. Oocytes and slices of intact ovary were fixed in 2.5% glutaraldehyde in cacodylate buffer for 2–3 h, post-fixed in 1% osmium tetroxide and embedded in thin viscosity resin (Spurr mix; Sigma, St. Louis, Mo., USA). Semi-thin sections (1 μm thick) were stained with toluidine blue O (TBO) for light-microscopic observation. Ultrathin sections (60–90 nm) were stained with uranyl acetate and lead citrate and subjected to TEM analysis by using a Philips 201C transmission electron microscope (Amsterdam, Holland). Images were processed by means of Adobe Photoshop version 7.0. In addition to making observations on the status of the ovary, the diameters of vitellogenic oocytes were also measured in five randomly selected cells of each type by using a research microscope supported with Q-Win software (Leica, Jena, Germany). The classification used for assigning the follicles to different stages was that of Sanchez and Villecco (2003).

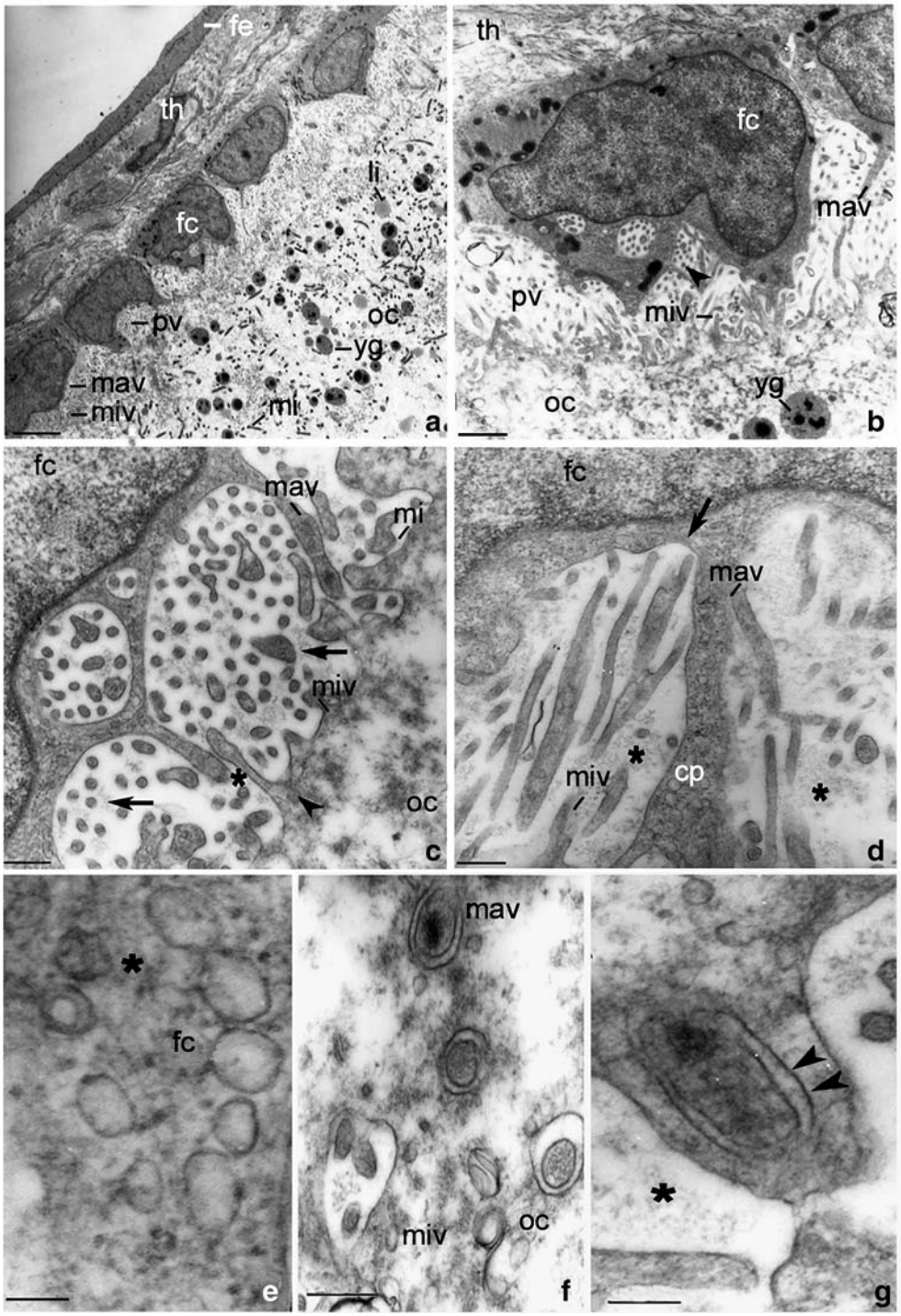
## Results

The earliest vitellogenic follicles (1.0–1.5 mm diameter) are invested with a few fibroblasts and a dense layer of fibrils (Fig. 1a). The follicle cells are tall and cuboidal and are arranged in a compact layer with an irregular profile towards the perivitelline space. All the cells are of the dark type (stage III of Sanchez and Villecco 2003) and possess large nuclei surrounded by a thin layer of cytoplasm containing abundant mitochondria and an accumulation of dense material (Fig. 1a,b). Towards the perivitelline space, these cells produce large cytoplasmic processes and the macrovilli among which microvilli of the oocyte are present (Fig. 1b,c). The macrovilli and the cytoplasmic processes can be distinguished based on the nature of their cytoplasm. In the macrovilli, the cytoplasm contains a few organelles, whereas in the cytoplasmic processes, rich saccular rough endoplasmic reticulum is present (Fig. 1d,e). The macrovilli are limited to the perivitelline space, whereas the cytoplasmic processes extend into invaginations of the oocyte cortex. The oolemma has a fuzzy material forming the coated pits (Fig. 1c) where the cytoplasmic processes and the macrovilli of the follicle cell surround the microvilli. Both these structures have a clathrin coat, as seen in transverse (Fig. 1f) and longitudinal (Fig. 1g) sections. The perivitelline space, excluding the cytoplasmic processes, the macrovilli of the follicular cells and microvilli of the oocyte, contains a diffuse material, the precursor of the vitelline envelope (Fig. 1c,d,g). The cortical cytoplasm of the oocyte contains yolk precursor material in addition to abundant mitochondria. The yolk precursor material undergoes condensation into one or more dark yolk granules (Fig. 1a,b). The macrovilli and cytoplasmic processes of the follicle cells in section appear to be discontinuous, reflecting pinocytic vesicle formation (Fig. 1c).

We identified oocytes with the vitelline envelope material undergoing condensation as being at stage IV (Sanchez and Villecco 2003). In stage IVA (1.6–2.0 mm diameter), the theca and the follicle cells mostly have a structural organization similar to that of the earlier stage. However, the follicle cells are not as irregular as in stage III. Fewer cytoplasmic processes of the follicle cells are present (Fig. 2a–d). The perivitelline space has increased and the precursor material of the vitelline envelope is condensed. However, the extent of this condensation does not appear to affect the macro- and microvilli passing through it. The peripheral cortical cytoplasm of the oocyte is also condensed and appears to be free of mitochondria, which are abundant in the cortical cytoplasm interior to the condensed portion (Fig. 2a,b). Dense yolk granules lie among the uncondensed yolk precursor vesicles deeper in the cortical cytoplasm (Fig. 2a). The follicle cell cytoplasm is rich in mitochondria and has a dark dense material in membrane-bound vesicles (Fig. 2c).

In stage IVB (2.1–2.5 mm diameter), the material of the vitelline envelope extends closer to the oolemma resulting in a condensed material in two layers. The layer closer to the follicle cells has only the macrovilli penetrating through it, whereas the other layer closer to the oolemma has more microvilli (Fig. 3a,b). The oocyte surface forms deep invaginations, the coated pits, into which macrovilli extend (Fig. 3a) and form pinocytic vesicles. During this stage, the follicle cell cytoplasm is rich in rough endoplasmic reticulum, mitochondria and vesicles containing a material in different phases of condensation (Fig. 3c). The intercellular space between the follicle cells appears almost continuous (Fig. 3a), although gap junctions and desmosomes are found between the follicle cells.

During subsequent development (stage IVC; 2.6–3.0 mm diameter), the vitelline envelope material becomes further condensed, with fewer macrovilli penetrating through it (Fig. 4a,b). The zonation of the vitelline envelope material of the early stages is no longer discernible. The oocyte surface is extended into the microvilli with deeper invaginations forming the coated pits. The macrovilli, intercalating between the microvilli, extend into these invaginations. Some uncondensed vitelline envelope material is still present. The cortical cytoplasm of the oocyte contains cortical vesicles enclosing uncondensed material. Some yolk granules have a partially condensed content (Fig. 4a). The follicle cell cytoplasm is rich in mitochondria and also in vesicles containing a dense material. Adjacent follicle cells possess gap junctions except for nearer the oocyte where a desmosomal junction is always found (Fig. 4c).




When the vitelline envelope material is almost condensed and the space containing the macro- and microvilli is delimited, the follicle is identifiable as being at stage VA (3.1 mm–3.5 mm diameter; Sanchez and Villecco 2003). With the growth of the oocyte, the follicle cells become flat (Fig. 5a). Tunnels appear in the vitelline envelope through which a few macrovilli reach the oocyte surface (Fig. 5b). The cortical cytoplasm of the oocyte possesses yolk granules and large vesicles containing an amorphous material. This, in all probability, is comparable to the lipid vesicles of stage VA of Sanchez and Villecco (2003).

The above trend is continued during subsequent development, which we identify as stage VB (3.6 mm–4.5 mm diameter). In this stage, the vitelline envelope material is further condensed and the space for intercalation of the macro- and microvilli is reduced (Fig. 6a,b). The oocyte

◀ **Fig. 1** Transmission electron micrographs of the follicle cell/oocyte interface of a stage III oocyte of *Gegeneophis ramaswamii*. **a** Micrograph showing the theca (*th*), follicular epithelium (*fe*), follicle cell (*fc*), perivitelline space (*pv*) with macrovilli (*mav*) and microvilli (*miv*) and the oocyte cortex (*oc*) containing yolk vesicles undergoing condensation into yolk granules (*yg*), lipid inclusions (*li*) and mitochondria (*mi*). *Bar* 10 μm. **b** Part of **a** enlarged to show the theca (*th*), follicular cell (*fc*), perivitelline space (*pv*) containing macrovilli (*mav* and microvilli (*miv*) and the oocyte cortex (*oc*) with yolk vesicles condensing into yolk granules (*yg*). Note sectioned microvilli in the interstices of cytoplasmic process of the follicle cells (*arrowhead*). *Bar* 2 μm. **c** Part of **a** enlarged to show the follicle cell (*fc*), oocyte cortex (*oc*), mitochondria (*mi*), macrovilli (*mav*) and microvilli (*miv*) in the interstices of the cytoplasmic process of follicle cells (*asterisk*). Coated pits are associated with pinocytic vesicles (*arrowheads*). Note the material of the vitelline envelope in the perivitelline space (*arrows*). *Bar* 1.5 μm. **d** Cytoplasmic process (*cp*) of follicle cells (*fc*) containing saccular rough endoplasmic reticulum (*arrow*). Macrovilli (*mav*) and microvilli (*miv*) are also shown. *Bar* 0.7 μm. **e** Part of **d** at higher magnification showing the saccular nature of the rough endoplasmic reticulum in a cytoplasmic process (*asterisk*) of a follicle cell (*fc*). *Bar* 0.15 μm. **f** Transection showing macrovilli (*mav*) and microvilli (*miv*) surrounded by the cytoplasmic processes of a follicle cell in the oocyte (*oc*). *Bar* 0.5 μm. **g** Longitudinal section of the interface showing the cytoplasmic processes of the follicle cells surrounding the microvillus (*arrowheads*). *Bar* 0.5 μm. The *asterisks* in **d** and **g** indicate a diffuse material in the perivitelline space

cortical cytoplasm is rich in mitochondria and in membrane-bound vesicles containing lipid and a partially condensed material. The constituent material of the yolk granules exists in two distinct zones: a dark central and a light peripheral zone. One interesting observation is the presence of some dark dense granules in the macrovilli and in the cortical cytoplasm of the oocyte (Fig. 6b). The tunnels in the vitelline envelope are prominent (Fig. 6c). The cytoplasm of the follicle cells has a few mitochondria and membrane-bound vesicles.

In the next stage (stage VI), the vitelline envelope with its characteristic tunnels is fully formed with less elaborate macro- and microvilli (Fig. 7a,b). The follicle cell cytoplasm is highly limited and contains few organelles. The cortical cytoplasm of the oocyte possesses biphasic yolk granules

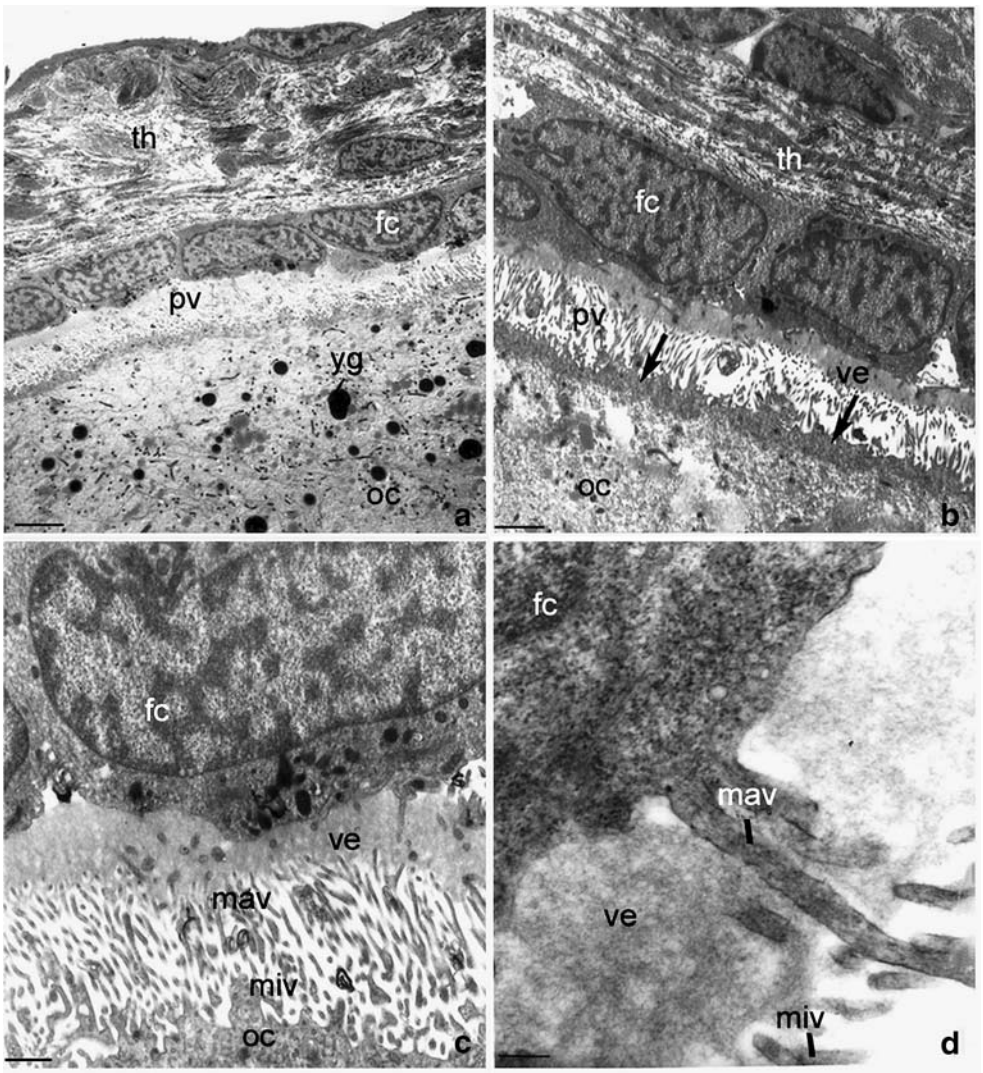



**Fig. 2** Transmission electron micrographs of the follicle cell/oocyte interface of stage IVA follicles of *Ichthyophis tricolor*. **a** Low power micrograph showing the theca (*th*), follicle cells (*fc*), perivitelline space (*pv*), yolk granules (*yg*) and oocyte cortex (*oc*). *Bar* 10 μm. **b** Similar region at higher magnification. Note the appearance of material in the vitelline envelope (*ve*) subtending the follicle cell layer (*fc*). Note also the dense material in the oocyte cortex (*arrows*) suggesting a contribution of the oocyte to the vitelline envelope. *Bar* 5 μm. **c** Higher magnification showing the ultrastructural organization of follicle cells (*fc*), vitelline envelope material (*ve*), macrovilli (*mav*), microvilli (*miv*) and oocyte cortex (*oc*). *Bar* 2.5 μm. **d** Further magnification showing the perivitelline space containing the material of the vitelline envelope (*ve*). *Bar* 0.4 μm

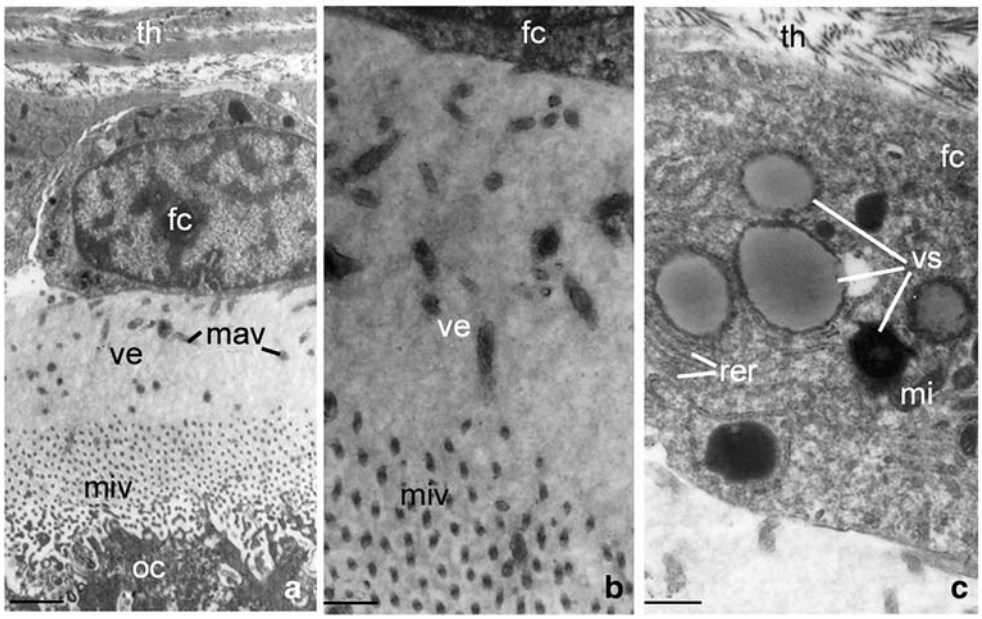



**Fig. 3** Transmission electron micrographs of the follicle cell/oocyte interface of stage IVB follicles of *G. ramaswamii*. **a** Low power micrograph showing the theca (*th*), follicle cells (*fc*), vitelline envelope (*ve*), macrovilli (*mav*), microvilli (*miv*), oocyte cortex (*oc*) and the intensification of vitelline envelope material. *Bar* 3.5 μm. **b** Higher magnification showing the microvilli (*miv*) passing through the condensed vitelline envelope (*ve*) material. *Bar* 1 μm. **c** Part of a follicle cell (*fc*) showing the theca (*th*), mitochondria (*mi*), vesicles (*vs*), rough endoplasmic reticulum (*rer*) and other ultrastructural features, all providing evidence for synthetic activity (perhaps for yolk material). *Bar* 1.7 μm

and exhibits lipid accumulation (Fig. 7a). Dark dense granules lie in the cortical cytoplasm of the oocyte (Fig. 7a). With further growth (4.60–6.0 mm in diameter), the follicle cells become flatter. The vitelline envelope also becomes flat, although macro- and microvilli are still present. Both the lipid vesicles and yolk granules of the oocyte cytoplasm increase in size (Fig. 7c).

## Discussion

The staging of oocytes and follicles facilitates studies of the cellular events during oogenesis. It serves as the basis for experimentation and comparison with other species. Exbrayat (2006) has identified six stages during follicular development in the caecilian *Typhlonectes compressicauda*: stage A involving the grouping of oogonia into germinal nests-stage B, in which the primary oocyte may or may not be surrounded by follicle cells-stage C, the previtellogenic follicle-stage D, the vitellogenic follicle; stage E, the atretic follicle-stage F, the corpora lutea. This classification is based on light-microscopic analysis and is defined by convenience. There is a need for consistency in the use of various stage designations and in the application of these stages to different animals (Begovac and Wallace 1988). Therefore, in the present ultrastructural descriptions, we

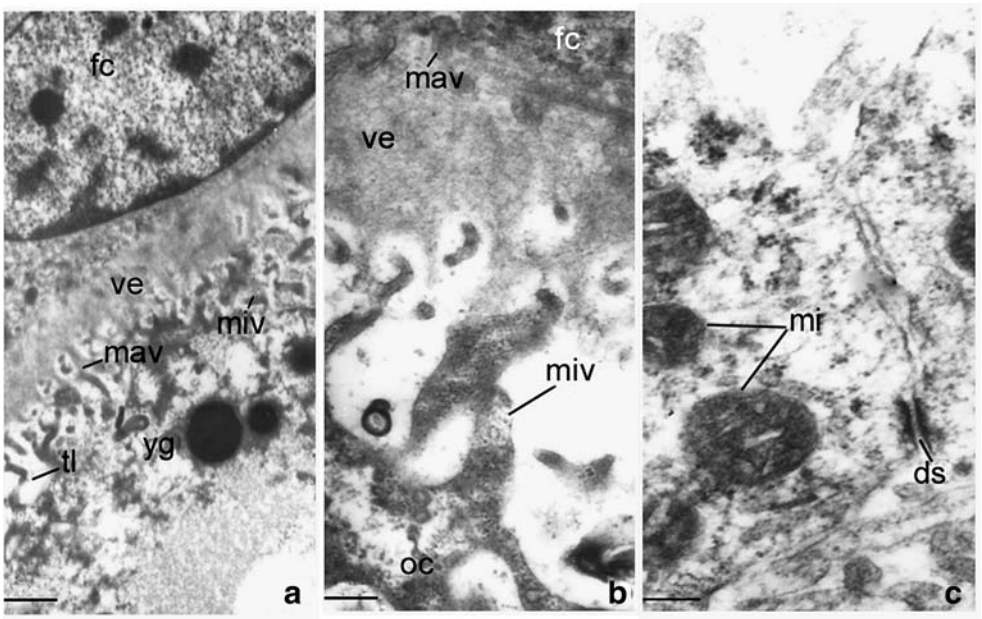



**Fig. 4** Transmission electron micrographs of the follicle cell/oocyte interface of a stage IVC oocyte of *I. tricolor*. **a** Low power micrograph showing a follicle cell (*fc*), macrovilli (*mav*), microvilli (*miv*), yolk granules (*yg*), condensation of the vitelline envelope (*ve*) and formation of tunnels (*tl*) within it and the narrowing of the perivitelline space. *Bar* 3 μm. **b** Higher magnification of a similar region. *Bar* 1 μm. **c** Intercellular junctions between two follicle cells showing a desmosome (*ds*) and mitochondria (*mi*). *Bar* 1.1 μm

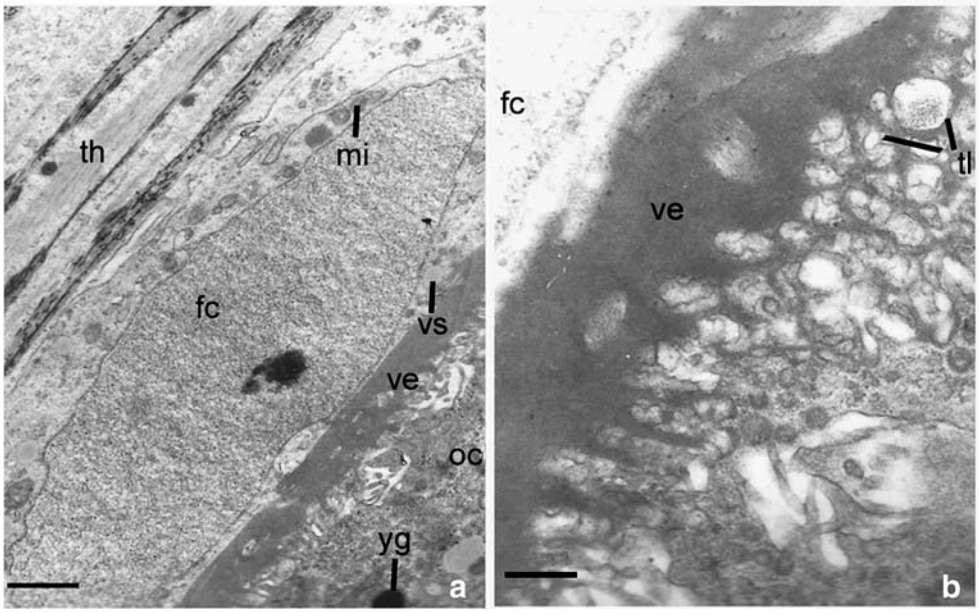



**Fig. 5** Transmission electron micrographs of the follicle cell/oocyte interface of a stage VA oocyte of *G. ramaswamii* showing further condensation of the vitelline envelope material and a reduction in the width of the perivitelline space. **a** Low power micrograph showing cytological features (*th* theca, *mi* mitochondria, *vs* vesicles, *yg* yolk granules) of the follicle cells (*fc*) in the oocyte cortex (*oc*). Note the condensation of the vitelline envelope (*ve*) material. *Bar* 2.1 μm. **b** Higher magnification showing the extreme condensation of the vitelline envelope (*ve*) material and the appearance of tunnels (*tl*) in it (*fc* follicle cell). Note the highly delimited perivitelline space. *Bar* 0.5 μm

have adopted the classification of Sanchez and Villecco (2003) and Uribe (2003) for the anuran species *Ceratophrys cranwelli* and urodelan species *Ambystoma dumerilii* and *Ambystoma mexicanum*, respectively. This classification was first introduced by Dumont (1972) for *Xenopus laevis* based on the cytological characteristics and physiological parameters of the developing oocytes.

The ultrastructural organization of the follicle cell/oocyte interface of the caecilians in this study mostly matches that in the several anuran species and the few urodelan species studied so far. The two caecilians, which overlap in their distribution in the Western Ghats of Southern India, differ little in their reproductive cyclicity, structural changes in the ovarian follicles and dependence on environmental factors (Beyo et al. 2007a, 2007b). Hence, we have traced the developmental sequence of the follicle cell/oocyte interface for these two species together.

During the annual reproductive cycle, oogonia derived from the oogonial nest and the follicle cells derived from the ovarian stroma associate to form the follicle (folliculogenesis). The follicles, thus established, undergo a transformation involving changes in the oocyte, follicle cells and theca to become the previtellogenic follicles. The follicle cell/oocyte interface of the fully established follicles exhibits a perivitelline space occupied by macrovilli of the follicle cells and microvilli of the oocyte. The follicles enter the vitellogenic phase with the accumulation of yolk precursors, i.e. the primordial yolk platelets. This marks

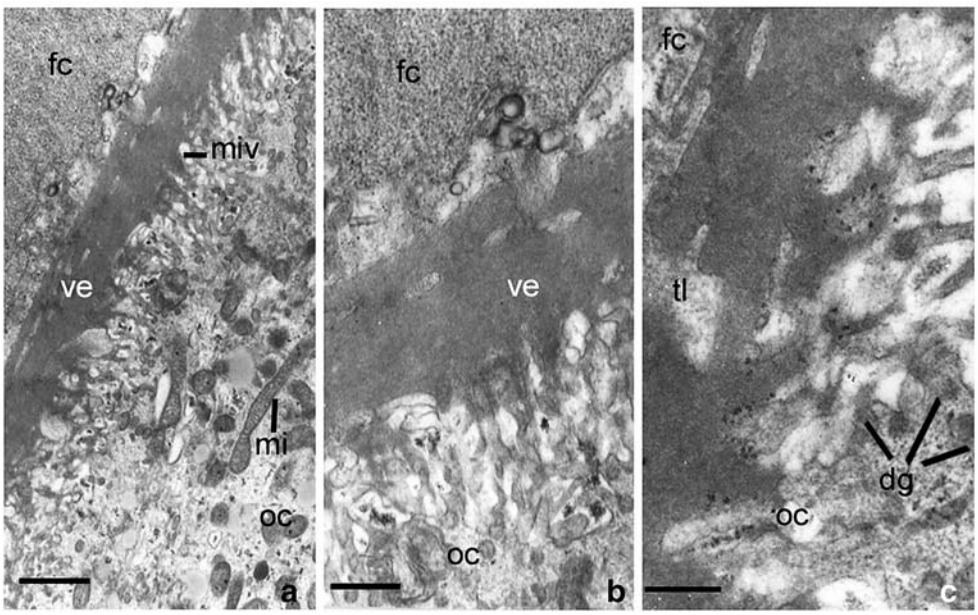



**Fig. 6** Transmission electron micrographs of the follicle cell/oocyte interface of a stage VB oocyte of *G. ramaswamii*. **a** Low power micrograph showing the ultrastructural features of the follicle cell (*fc*), vitelline envelope (*ve*), microvilli (*miv*), mitochondria (*mi*) and oocyte cortex (*oc*). *Bar* 2.1 μm. **b** Higher magnification showing the tough vitelline envelope (*ve*), follicle cell (*fc*) and oocyte cortex (*oc*). *Bar* 0.5 μm. **c** Dense granules (*dg*), oocyte cortex (*oc*) and extension of the follicle cell (*fc*) cytoplasm into the tunnel (*tl*) of the vitelline envelope. *Bar* 0.31 μm

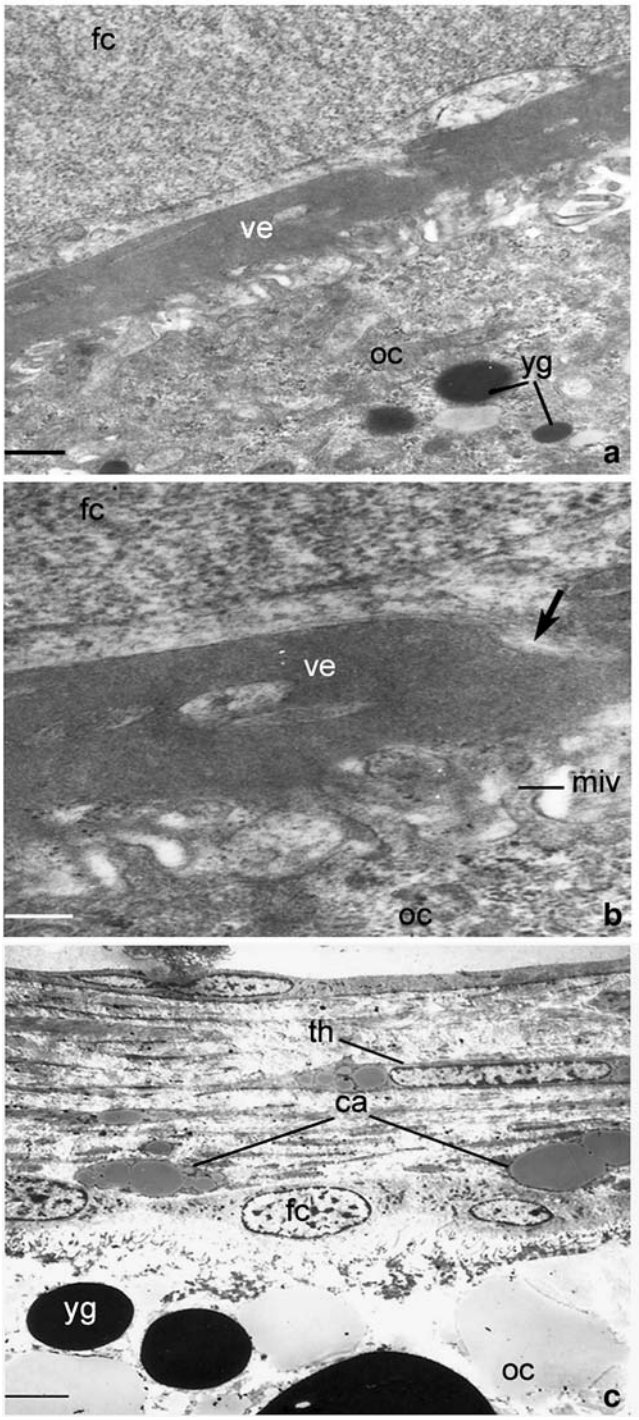



**Fig. 7** Transmission electron micrographs of the follicle cell/oocyte interface of a stage VI oocyte of *G. ramaswamii*. **a** Low power micrograph showing various ultrastructural features (*ve* vitelline envelope, *yg* yolk globules) of the follicle cell (*fc*) and oocyte cortex (*oc*). *Bar* 2.1 μm. **b** Higher magnification showing the tough vitelline envelope (*ve*) and the extension of follicle cell (*fc*) cytoplasm into a tunnel in the vitelline envelope (*arrow*). Note the almost complete absence of perivitelline space (*oc* oocyte cortex, *miv* microvilli). *Bar* 0.7 μm. **c** A fully established vitellogenic follicle showing the theca (*th*), follicle cell (*fc*), capillary (*ca*) and dense yolk granules (*yg*) in the oocyte cortex (*oc*). *Bar* 10 μm

the first arrival of the material of the vitelline envelope in the perivitelline space (Beyo et al. 2007a, 2007b).

The observations in this study pertain to the development of the follicle cell/oocyte interface, emphasizing the establishment of the vitelline envelope and the yolk granules/platelets in the oocyte. In both these processes, the follicle cells and the macro- and microvilli interact. Moreover, these processes occur in a sequential manner during stages III–V resulting in the mature follicles of post-vitellogenic stage VI. The sequential cytological changes in the two caecilians from these perspectives are believed to be comparable with those known for the anurans *X. laevis* (Dumont 1972; Wallace and Bergink 1974; Wallace 1985; Wallace and Selman 1990), *Rana esculenta* (Wartenberg and Gusek 1960), *Rana pipiens* (Ward 1962) and *Rana tigrina* (Sretarugsa et al. 2001) and the urodeles *Notophthalmus viridescens* (Hope et al. 1963) and *Necturus maculosus* (Kessel and Ganion 1980). The formation of the vitelline envelope in *X. laevis* (Dumont 1972) and *R. tigrina* (Sretarugsa et al. 2001) is first detectable in stage II oocytes as is the case in the caecilians (Beyo et al. 2007a, 2007b). However, a study in *X. laevis* (Yamaguchi et al. 1989) has indicated that the cytoplasm of oocytes as early as stage I contains components reacting to anti-vitelline coat antibodies, suggesting that the oocyte plays a role in synthesizing its own vitelline envelope. We have noted the dense accumulation of material underneath the oolemma concomitant with the condensation of vitelline envelope material subtending the inner phase of follicle cells (Fig. 2a,b). This presumably represents the contribution from the oocyte to the vitelline envelope, as seen in the cytological manifestation of the accumulation of dense vesicles in its cytoplasm, as suggested to occur in *Bufo arenarum* by Cabada et al. (1996). Participation of the follicular epithelium in the formation of vitelline envelope has been suggested by Wallace and Jared (1976) and Dumont and Brummett (1978) in *X. laevis*, by Cabada et al. (1996) in *B. arenarum*, by Villecco et al. (2000) in *C. cranwelli* and by Sretarugsa et al. (2001) in *R. tigrina*.

The microvilli extend from the oocyte surface and gradually increase in number and length particularly in oocytes at stages III and IV. The increased number and length of the microvilli are thought to be a provision to increase the oocyte surface area during development. Since the amphibian oocyte must store nutrients in the form of large yolk platelets, it needs a large surface area for the uptake of substances necessary for yolk formation. Corresponding to this demand, stage III and IV oocytes exhibit extensive pinocytic activity, which may reflect the mechanism by which the materials enter the cytoplasm through the endocytic pathway (Wallace and Selman 1990; Sretarugsa et al. 2001). Vitellogenesis involves the accumulation of exogenously derived yolk protein precursors

into the oocyte and, is responsible for the growth of the oocyte (Wallace 1985). This mechanism is involved in the uptake of vitellogenin that binds with the specific receptors in the coated pits, which in turn give rise to the coated vesicles of the oocyte surface (Dumont 1978). Coated pits are abundantly present on the caecilian oocyte surface suggesting that the mechanism of vitellogenin uptake by the oocyte is similar to that in the anurans (Dumont 1978; Wallace and Selman 1990; Sretarugsa et al. 2001) and urodeles (Hope et al. 1963; Kessel and Ganion 1980).

The follicle cell/oocyte interface of *Ceratophrys cranwelli* forms with a fibrillar pattern, a precursor of the vitelline envelope into which macro- and microvilli penetrate (Villecco et al. 2002). This fibrillar pattern is arranged in four-walled tunnels and sectioned macro- and microvilli lie inside. A similar pattern has been observed in the caecilians in this study. Cortical vesicles are a feature of fish vitellogenic follicles, e.g. in *Fundulus heteroclitus* (Selman et al. 1986, 1988), *Syngnathus scovelli* (Begovac and Wallace 1988), *Torpedo marmorata* (Prisco et al. 2002) and the toad *C. cranwelli* (Villecco et al. 2002) but have not been observed in *X.laevis* (Dumont 1972), *Flectonotus pygmaeus* (Del Pino and Humphries 1978), *Bufo arenarum* (Valdez Toledo and Pisanó 1980) and *Gastrotheca riobambae* (Del Pino et al. 1986). They are present in oocytes at stage IVC in the examined caecilians. Intensive interaction is thought to take place through the interface which allows the passage of particles as large as ribosomes. These particles are presumably involved in the control of oogenesis (Inoue and Inoue 1986; Kitajima et al. 1986; Selman et al. 1986, 1988; Carotenuto et al. 2000). Thus intense follicle cell-oocyte interaction occurs at the interface during vitellogenesis at all vitellogenic stages in *I. tricolor* and *G. ramaswamii*. This takes place through the macro- and microvilli passing through the condensed region of the vitelline envelope. Finally, the perivitelline space is reduced, the vitelline envelope is fully formed, the follicle cells become flatter and the oocyte matures and is ovulated.

In conclusion, the paper describes the follicle cell/oocyte interface during the vitellogenic stages (stages III–VI), providing for the passage of yolk precursor material through the follicle cells into the oocyte to establish the vitelline envelope, in two species of caecilians: *I. tricolor* and *G. ramaswamii*. The follicle cell/oocyte interface of caecilian ovarian follicles shares several ultrastructural features in common with those of the species of Anura and Urodela so far studied. However, this study in the caecilians has revealed that (1) the follicle cells produce cytoplasmic processes rich in saccular endoplasmic reticulum, in addition to producing macrovilli, (2) the oocyte may have a share in producing material for the vitelline envelope and (3) the follicle cells themselves produce some material for the yolk.

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