ULTRASTRUCTURAL FEATURES OF FOLLICLE CELL-OOCYTE INTERFACE DURING DIFFERENT STAGES OF FOLLICLES IN THE CAECILIAN *ICHTHYOPHIS TRICOLOR*

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SUMMARY

This study is the first to describe the major stages of follicles of a caecilian from ultrastructural perspectives. The female reproductive system of *Ichthyophis tricolor* is described. Assembly of the follicles involves the origin of primary oocytes from oogonia and the pre-granulosa cells from ovarian stroma, each from a separate nest, and the arrival of pre-granulosa cells around the meiotic oocyte to become granulosa cells. Appearance of a perivitelline space between the oocyte and the granulosa cells marks the beginning of pre-vitellogenic follicles. Macrovilli arise from the granulosa cells and ramify in the perivitelline space. In the vitellogenic follicles the macrovilli are further more prominent, and microvilli arising from the oolemma intercalate between the macrovilli. Yolk platelets appear in the cortical ooplasm, which are initially small and scattered but later the smaller ones fuse, and lipid and glycogen are added on resulting in large yolk platelets. The material of the vitelline envelope appears in the perivitelline space, which later condenses into the vitelline envelope. Some of the follicles undergo atresia when the granulosa cells become atrophied, and amoeboid cells from the theca enter the ooplasm and phagocytose the yolk.

Key words: Caecilian, follicle, granulosa cells, *Ichthyophis tricolor*, oocyte, ovary.

INTRODUCTION

Caecilians, the limbless amphibians, are an unique group of amphibians with considerable terrestrialization. Most of the species live in moist soil and are subterranean. This has necessitated evolution of strategies in reproduction different from the other two groups of amphibians, anurans and urodeles. Caecilians practice internal fertilization and the male animal ejaculates the sperm into the female cloaca through the eversible phallodaeum. The female caecilians have evolved reproductive anatomy and physiology accordingly, resulting in the ovaries becoming a pair of elongated structures containing the oocytes in a longitudinal row. On ovulation, the ova measuring 1-7 mm, depending on the species, are acquired by the oviduct on each side. Fertilization occurs in the oviduct. The fertilized ova are retained in the oviduct when the embryo starts developing. Quite a few species are viviparous where as several are oviparous and lay embryonated eggs in the moist soil, which is practically ovo-viviparity. Histological changes in the ovary during the reproductive cycle are known only for a few species of caecilians, *Chthonerpeton indistinctum, Typhlonectes compressicuda* and *Ichthyophis beddomei* (for review, see 1), and the observations are based only on light microscopy. Ultrastructural description of caecilian ovary in relation to the reproductive cycle has not yet been attempted. In the present report we briefly describe the ultrastructural changes in the ovary of *Ichthyophis tricolor* of the Western Ghats of India, with special reference to the follicle cell-oocyte interface.

MATERIALS AND METHODS

Ichthyophis tricolor (Amphibia, Gymnophiona, Ichthyophidae) were collected from the terraced mixed coconut plantations of the Western Ghats of Kerala. Female specimens, totally 18 during the study period of two years (2004-2005), were sacrificed using MS-222 anaesthesia and dissected. Slices of ovary were fixed in Bouin's fluid or neutral-buffered formalin for paraffin wax embedding followed by staining with haematoxylin and eosin or 2.5% glutaraldehyde followed by 1% osmium tetroxide for resin-embedding, semithin sectioning and toluidine blue O staining for light microscopic observation and ultra-thin sectioning and uranyl acetate and lead citrate staining for transmission electron microscopy.

RESULTS

The female reproductive system of *Ichthyophis tricolor* consists of a pair of elongated ovaries with oocytes arranged like beads in a string (Fig1). The prominence of the ovary and the size of the oocytes vary in relation to the stage in the follicle development. The colour of the oocytes ranges from creamy white (Fig. 1) to deep yellow. The oviducts, extending from the oesophagus to the cloaca, are found along the inner phase of the ovaries and attached to the kidneys. The fat bodies also occur like strings of beads, one on each side, outside the ovary (Fig. 1). The oviducts connect separately to the cloaca. Though several stages were identified in the ovarian cycle, for convenience four critical stages are described.

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Fig. 1. Dissection of the female reproductive system of *Ichthyophis tricolor*. FB, fat body; OD, oviduct; OV, ovary.

Assembly of follicle

Germinal nests are found in a segmental fashion in between advanced stages of the follicles. Oogonia present in germinal nests proliferate and differentiate as pre-meiotic oocytes. The latter increase in size and enter the meiotic prophase. The oocyte nuclei are initially small and irregular in shape. With the growth of the oocyte, the nuclear diameter also increases. A mitochondrial cloud appears at one pole of the cell. Pre-granulosa cells, with intensely heterochromatic nuclei, appear in the ovarian stroma in the form of separate nests. From leptotene to pachytene of first meiotic division the oocyte nuclei further increase in size and become spherical. By late pachytene there is amplification of nucleoli resulting in several nucleoli in each oocyte nucleus. By early diplotene the pre-granulosa cells migrate towards the oocytes and initially form a discontinuous row of granulosa cells. Soon these cells become continuous and wrap the oocyte tightly without an intercellular space between them and the oocyte. The mitochondrial cloud fragments and become dispersed in the oocyte cytoplasm. The oocyte is arrested in first meiotic division during the diplotene stage and, thus, the follicle is assembled and established (Fig. 2). The interface between the granulosa cells and oocyte is such that the membranes of the somatic granulosa and the germinal oocyte in the follicle are held through tight and/or gap junctions (Fig. 3).

Pre-vitellogenic follicle

Due to the extensive synthetic activity, the oocyte increases in size. The granulosa cells wrapping the oocyte, initially tall cuboidal, become flat and the oocyte and the follicle cells are almost closely applied (Fig. 4) but soon a perivitelline space appears into which microvilli produced by the oolemma project (Fig. 5). The theca appears initially as a simple structure but later becomes established into theca externa and theca interna.

Vitellogenic follicle

When the follicle enters the vitellogenic phase the oolemma produces microvilli when the macrovilli of the granulosa cells increase in abundance. The oocyte further increases in size due to accumulation of yolk precursors. Small to large yolk platelets appear in the cortical cytoplasm of the oocyte (Fig. 6). Large membrane-bound vesicles appear in the medullary cytoplasm also. The macrovillli of granulosa cells and microvilli of the oocyte establish different levels of intimacy between themselves providing for sequestration of yolk precursors into the oocyte. A vitelline envelope, formed of a dense fibrillar material, appears around the oocyte but the macro- and microvilli associate with each other in spite of the developing vitelline envelope (Fig. 7). With the completion of yolk accumulation, the

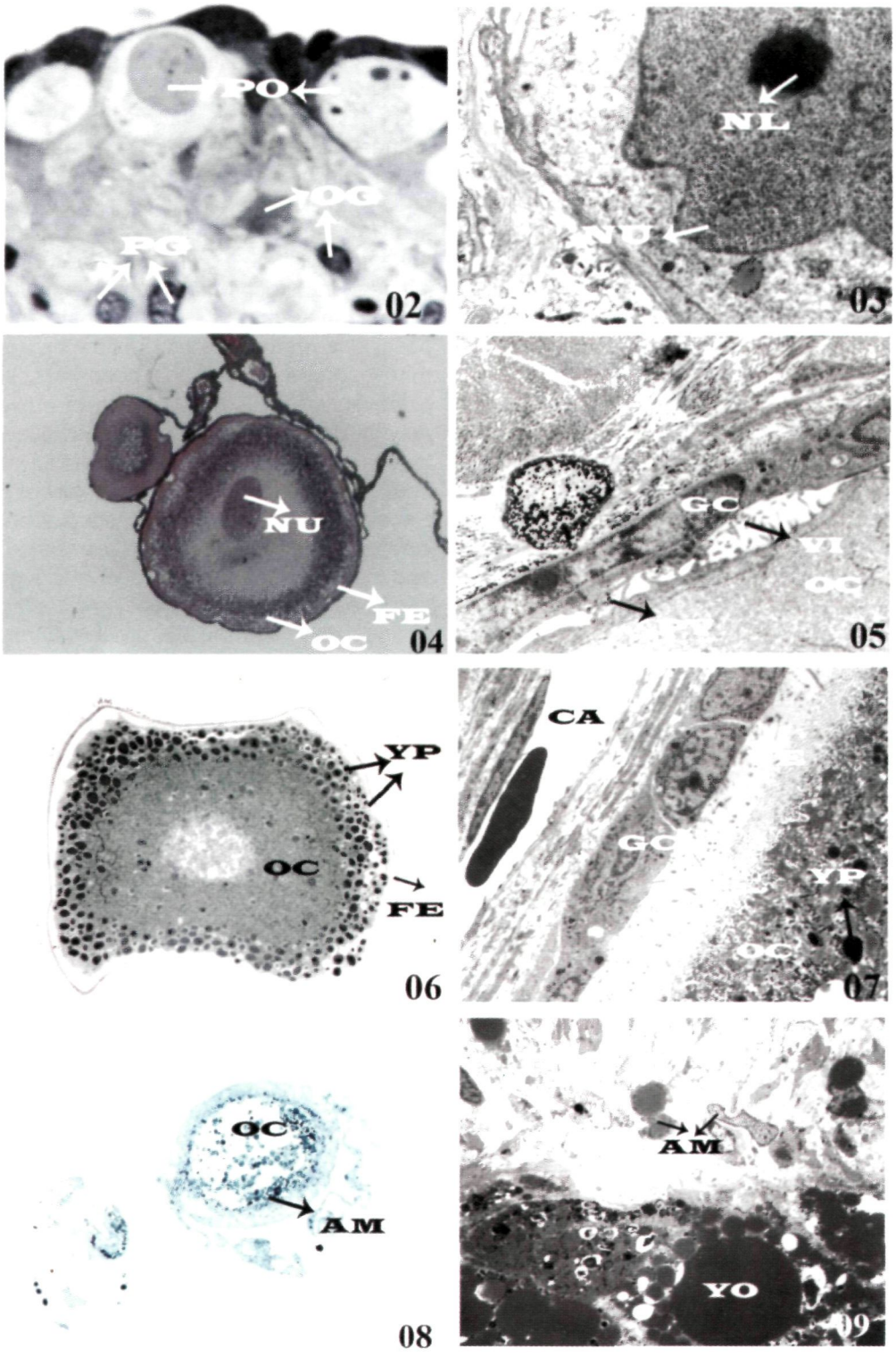

Fig. 2. Light micrograph of TBO-stained semi-thin section of ovary of *I. tricolor* showing oogonia (OG), primary oocytes (PO) and pre-granulosa cells (PG) (x1000). Fig. 3. TEM of premeiotic oocyte showing ooplasm (OO), nucleus (NU) and nucleolus (NL) (x8,400). Fig. 4. Light micrograph of H & E-stained paraffin section of a previtellogenic follicle. (x100). Fig. 5. TEM of a portion of a previtellogenic follicle. (x4500). Fig. 6. Light micrograph of TBO-stained semi-thin section of a vitellogenic follicle. (x40). Fig. 7. TEM of a portion of a vitellogenic follicle. (x4500). Fig. 8. Light micrograph of TBO-stained semi-thin section of an atretic follicle. (x40). Fig. 9. TEM of a portion of an atretic follicle. (x1500). AM, amoeboid cells; CA, capillary; FE, follicular epithelium; GC, granulosa cell; NU, nucleus; OC, oocyte; PV, perivitelline space; VE, vitelline envelope material; VI, micro- and macrovilli; YP, yolk platelets.

macro- and microvilli become less prominent and the vitelline envelope becomes fully established (not shown).

Atretic follicle

Some of the follicles, at some stage in the development, undergo atresia resulting in the theca becoming hypertrophied and thick. The follicle cells disintegrate, and amoeboid cells from the theca invade the ooplasm so as to phagocytose the yolk (Fig. 8, 9).

DISCUSSION

This paper describes for the first time the follicle cell-oocyte interface in a caecilian ovary from transmission electron microscopic perspective in relation to the histological changes during the reproductive cycle. By and large, the structure of the oocyte and the follicular epithelium and the interface between the two conform to the pattern in the anurans and urodeles (for reviews, see 2, 3), though there are certain features unique to the caecilians in view of the large amount of yolk that is accumulated.

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