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Organization of the corpus luteum in a caecilian *Gegeneophis ramaswamii*

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Summary

Corpus luteum is a transient and well known endocrine gland that develops from the ruptured follicles in the female vertebrate ovaries, immediately after ovulation. A major source of progesterone, this gland is concerned with gestation in the viviparous animals and retention of eggs in the oviduct in the oviparous species. Caecilians are a group of amphibians with several aspects of reproductive anatomy and physiology unique. In as much as corpus lutem has been described in a few caecilian species, it has not been described from ultrastructural perspectives. Here in we describe the ultrastructural features of corpus luteum of a single specimen of *Gegeneophis ramaswamii*, which was captured immediately after it laid the eggs. The corpus luteum is formed by three types of cells, namely granulosa lutein cells, thecal lutein cells and phagocytic cells. Both the types of lutein cells are characterized by abundant mitochondria, endoplasmic reticulum and Golgi apparatus. The granulose lutein cells abound with discrete granules. The phagocytic cells engage in phagocytosing cell debris. The cellular features are discussed in relation to information from oviparous as well as viviparous vertebrates. Thus, the paper describes, for the first time the corpus luteum of a caecilian from ultrastructural perspectives.

***Key words*:** Corpus luteum, endothelium, theca, SER, follicle cell.

Introduction

Ovarian follicular development in amphibians occurs in sequential stages of differentiation beginning with the initial formation of follicles and ending with ovulation. A definite number of stages in this sequence has been identified and reviewed. Sanchez and Villecco (2003) classified these stages in the anurans as previtellogenesis, vitellogenesis and postvitellogenesis, and suggested that such a classification can serve as a framework for more studies in anuran oogenesis to compare this process in different species. Dumont (1972) divided oogenesis in *Xenopus leavis* into six stages, and each stage of oocyte development was correlated with specific histological, ultra-structural, physiological and biochemical characteristics. According to Uribe (2003), follicular maturation in *Ambystoma mexicanum*, an urodele, can be divided into six stages- previtellogenic stages 1 and 2, vitellogenic stages 3 – 5 and pre-ovulatory oocyte, stage 6, which correlate with morphological changes in the oocyte, zona pellucida, follicular cell layer and theca. Exbrayat (2006) identified six stages in the follicular development of caecilians, the first (stage A) in which oogonia are grouped into germinal nests, the second (stage B) having an early primary oocyte, which may or may not be surrounded by follicle cells, the third (stage C) consisting of previtellogenic follicles, the fourth (stage D), consisting of vitellogenic follicles, the fifth (stage E) having atretic follicles, and the sixth (stage F), consisting of corpora lutea.

The caecilians, which belong to the order Gymnophiona or Apoda, are unique in several aspects of anatomy and reproductive biology. All caecilians practice internal fertilization, making use of the eversible phallodeum as the phallus. There are species representing different grades of female reproductive patterns, namely oviparity and viviparity. Several of the oviparous species lay eggs with embryos developed to

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different stages depending upon the species, thus shortening larval life (ovo-viviparity). However, the sequential changes in the ovary towards the establishment of follicles and their development into various ovarian stages and the formation of corpus luteum in the caecilians have only been poorly studied (Wake, 1968, 1970 a ,b, 1972, 1980; Exbrayat and Sentis, 1982; Exbrayat and Collenot, 1983; Exbrayat, 1986, 2006; Masood- Parveez, 1987; Berois and De Sa, 1988; Masood- Parveez and Nadkarni, 1993a,b; Exbrayat and Anjubault, 2003).

All vertebrates develop a transient endocrine gland, the corpus luteum from the post-ovulatory follicles and/or from the atretic follicles (Browning, 1973; Xavier, 1987; Powell et al., 2006). It secretes mainly progesterone, and its development in mammals is associated with viviparity (Corner and Allen, 1929: Yamada and Ishikawa, 1960; Bjersing, 1967; Adams and Hertig, 1969; Blanchette, 1966; Basom et al., 1971; Juengal et al., 1999). In non-mammalian vertebrates, including amphibians, this hormone is believed to be involved in retention of eggs in the oviduct (Beall and Reichsten, 1938; Chieffi and Chieffi-Bacceri, 1999). All amphibians, including caecilians, develop this gland (Tonutti, 1931; Vilter and Vilter, 1960, 1962; Joly, 1964, 1965, 1971; Joly and Picheral, 1972; Browning, 1973; Wake, 1968 a, b; Exbrayat and Collenot, 1983; Exbrayat, 2006). However, ultrastructural description of caecilian corpus luteum has not been attempted so far. In our endeavor to describe the histo-physiology and ultrastructure of the ovary of chosen caecilians of the Western Ghats of India (Beyo et al., 2007 a, b, 2008a, b, 2009) we attempted to locate the corpus luteum in *Ichthyophis tricolor* and *Gegeneophis ramaswamii* but were not successful until now, perhaps because we did not adopt serial sectioning since we practiced resin-embedding and semi-thin sectioning. This prompted us to locate the corpus luteum in a caecilian. Since we deal with oviparous caecilians, it was hypothesized that if an animal at laying is captured (thereafter, until the young hatch out, since caecilians guard the eggs) the ovary should contain corpus luteum. We were fortunate to locate a gravid female *G. ramaswamii* laying the eggs. Corpora lutea were located in this animal. Thus, we describe the corpus luteum of this animal from light microscopic and ultra-structural perspectives.

Materials and Methods

The single animal in this study, *Gegeneophis ramaswamii* (Caecilidae), was collected from terraced plantations of mixed coconut and rubber from Thekkada (08^0 37’N, 76 ^{0} 57’ E) in Trivandrum district of Kerala in June 2004. The animal was dissected under mild sodium pentobarbital anesthesia and the ovaries were fixed in 2.5 % glutaraldehyde prepared in cacodylate buffer, post-fixed in 1 % osmium tetroxide and embedded in methacrylate resin (Sigma, USA). Semi-thin sections (1 µM thick) were stained in toluidine blue O (TBO) and observed in a Carl Zeiss research microscope (Jena, Germany). Ultrathin sections were stained with uranyl acetate and lead citrate and subjected to transmission electron microscopic analysis using a Philips 201C transmission electron microscope (Amsterdam, Holland). The images were processed using Adobe Photoshop version 7.0.

Results

Light microscopic structure of corpus luteum

The corpus luteum, at the time of egg laying, in *G. ramaswamii* is a compact structure with an outer boundary formed of cells with acidophilic cytoplasm and the spherical to elongated nuclei located at the outer boundary of the cells (Fig. 1A). However, a few of the cells were found in a deeper location as well. These are the thecal lutein cells (TLCs). Underlying the TLCs is a dense mass of cells with slightly basophilic cytoplasm and large irregular nuclei invariably possessing a nucleolus along the outer boundary. Blood capillaries bounded by endothelium are present amongst the cells. These are the granulosa lutein cells (GLCs), as inferred from the dense granules (Fig. 1A). A few irregularly shaped cells, dispersed among the TLCs and GLCs, possess smaller and densely heterochromatic nuclei, and these are the phagocytic cells. Deeper in the corpus luteum the gland is filled with a stroma with fewer cells (Fig. 1B).

Ultrastructural organization of corpus luteum

Thecal lutein cells

The TLCs are elongated cells, with the nuclei lying close to the outer boundary. Thus, in the transverse section of this cell, the nucleus occupies almost two thirds the size (Fig. 2A). The nucleus has dense heterochromatin underneath the nuclear envelope and dark dense patches of heterochromatin distributed throughout. There is a prominent nucleolus with several discrete pars amorpha surrounded by the dense nucleolenema (Fig. 2B). The cytoplasm contains prominent mitochondria, saccular smooth endoplasmic reticulum (SER), fenestrated rough endoplasmic reticulum (RER), a prominent Golgi apparatus (GA) and lysosomes.

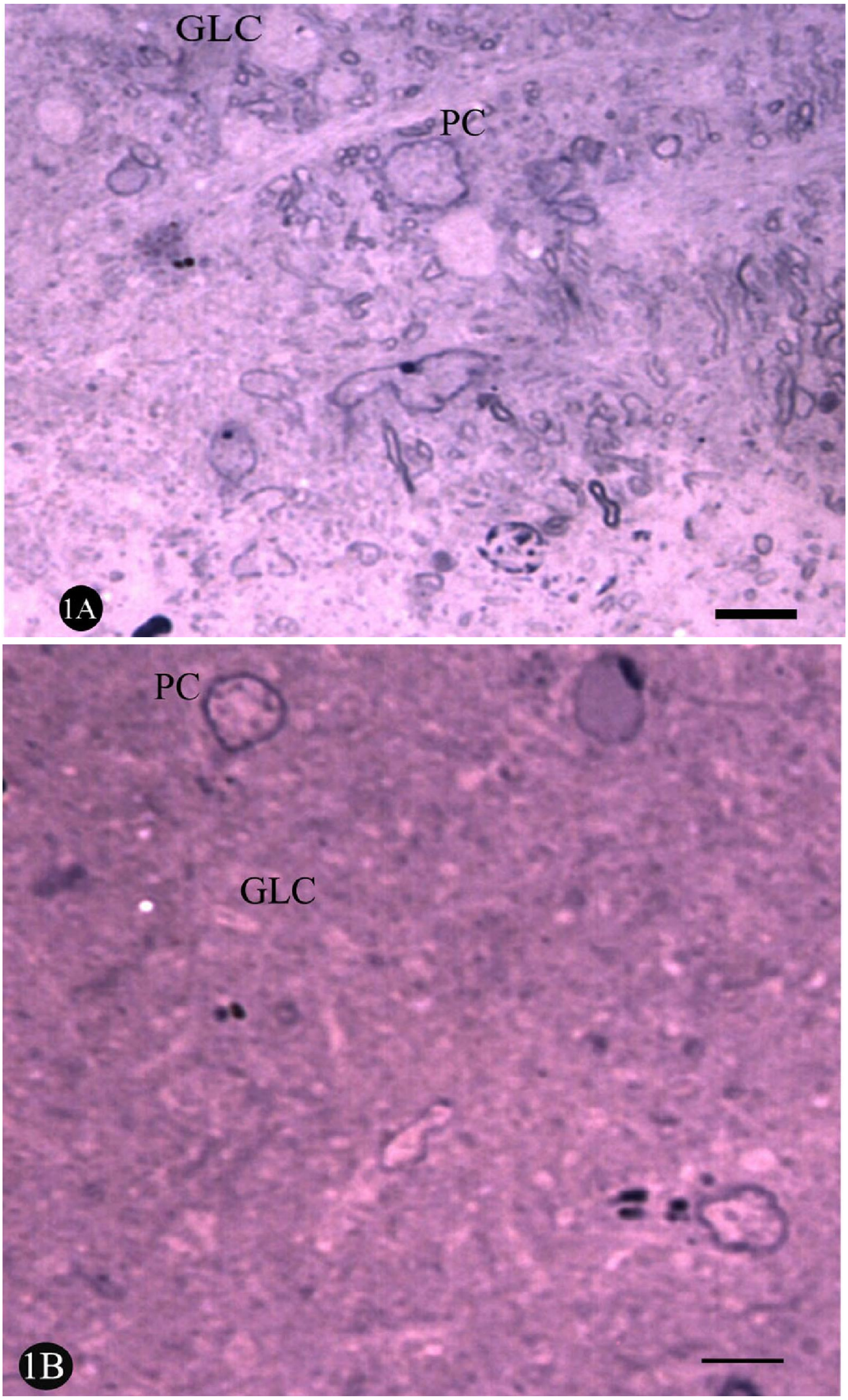

Fig. 1. *Gegeneophis ramaswamii* corpus luteum. Light micrographs of TBO-stained sections. A, closer to the outer boundary; granulosa lutein cell (GLC) and phagocytic cell (PC) are abundant; B, deeper in the gland; few granulosa lutein cells (GLC) phagocytic cells (PC) are present in an abundant stroma. Scale bar = 20µm.

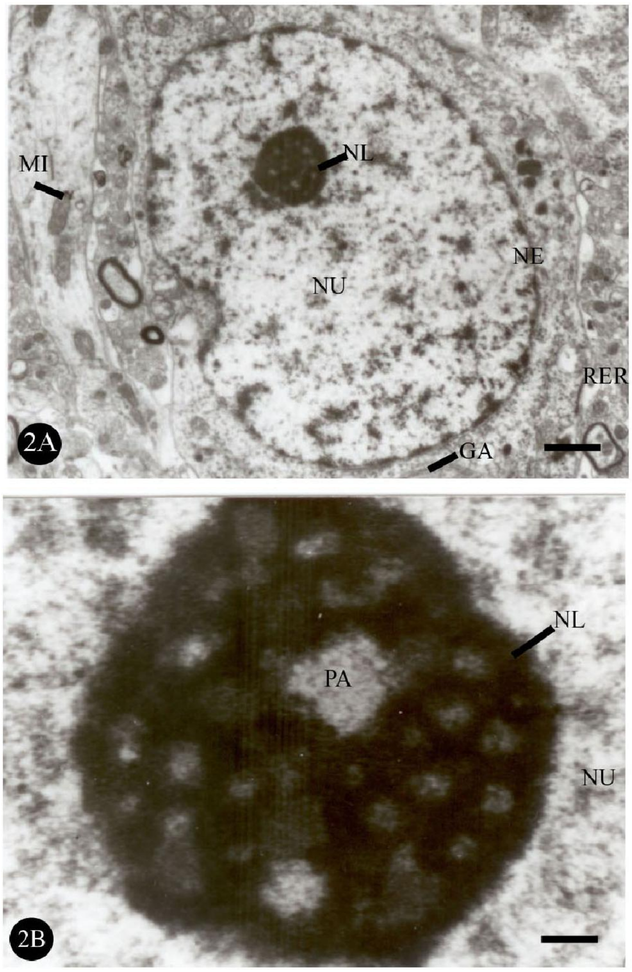

Fig. 2. TEM of a theca lutein cell. The nucleus occupies two thirds the size of the cell. A, the cell (TLC) possesses Golgi apparatus (GA), rough endoplasmic reticulum (RER) and mitochondria (MI); the nucleus (NU) has nuclear envelope (NE) and nucleoli (NL); B, a magnified portion of figure (A) showing the prominent nucleolus in the nucleus (NU); the nucleolus has pars amorpha (PA) and nucleolonema (NL). Scale bar = 1.5 µm in A; 0.3 µm in B.

Granulosa lutein cells

These are the most abundant cells in the corpus luteum (Fig. 3A). They are loosely scattered throughout the corpus luteum. The darkly stained nucleus is in general irregularly shaped and densely heterochromatic. There is a prominent nucleolus not differentiated as pars amorpha and nucleolenema (Fig. 3B). There are large intercellular spaces containing debris between the cells in an irregular pattern (Fig. 3B). The darkly stained cytoplasm contains mitochondria with plicate cristae (Fig. 3C), large saccules containing an amorphous material (Fig. 3C, D), a prominent GA (Fig. 3D), dense, small and large granules (Fig. 3E), and SER (Fig. 3F, G). Some of the mitochondria are entrapped in an amorphous material (Fig. 3 F, G) contained in a vesicular structure with double membrane. The central stroma, poorly differentiated when observed in the light microscope, is also formed of highly elongated cells but the nuclei are rarely to be seen (Fig. 4A, B). Trabeculae of connective tissue separate the blocks of the cells (Fig. 4A). At higher magnifications these cells possess organization as the GLCs (Fig. 4C, D). The outer mitochondrial membrane is continuous with SER (Fig. 4C). The RER is also prominent (Fig. 4D).

Phagocytic cells

Phagocytic cells are irregularly shaped migratory cells found among the granulosa lutein cells (Fig. 3A). They possess a densely heterochromatin nucleus. The cell possesses pseudopodial protuberances (Fig. 5A). The cytoplasm contains abundant mitochondria, RER and lysosomes (Fig. 5B). These cells are active in phagocytosis of debris from the intercellular spaces (Fig. 5C).

Blood capillaries

Blood capillaries are abundantly present in the corpus luteum. They possess the characteristic organization, with an endothelial lining (Fig. 5D).

Discussion

Reproductive cycles are indicative of the reproductive strategy of the species. Within amphibians, the reproductive activity is correlated with any number of environmental cues. Several factors, including temperature, photoperiod and food availability are important as regulators of amphibian reproductive activity (Saidapur, 1989). The paired ovaries of the caecilian are elongated sac-like structures, with pre-vitellogenic, vitellogenic and post-vitellogenic follicles present like a string of beads. The neighbouring follicles are connected by inter-follicular stromal tissue (Beyo et al., 2007a). The ovary, having formed and discharged the egg, which is its contribution to the next generation, continues to function as an organ of internal secretion, the corpus luteum. In the presence of fertilized ova, it undergoes changes which, in general, define its character for the rest of the gestation period (Wake, 1993). After discharge of the ova from the ovary, the wall of the ruptured follicle undergoes a series of changes, which transforms it into a compact, transient structure, the corpus luteum.

Corpus luteum is a ubiquitous endocrine gland developing in all vertebrate species (Browning, 1973; Xavier, 1987; Powell et al., 2006). The only group of vertebrates about which there has been an ambiguity regarding the existence of functional corpus luteum is cyclostomes. A recent report has proved its presence in the cyclostome, the Atlantic hag fish, Myxine (Powell et

PLATE 3

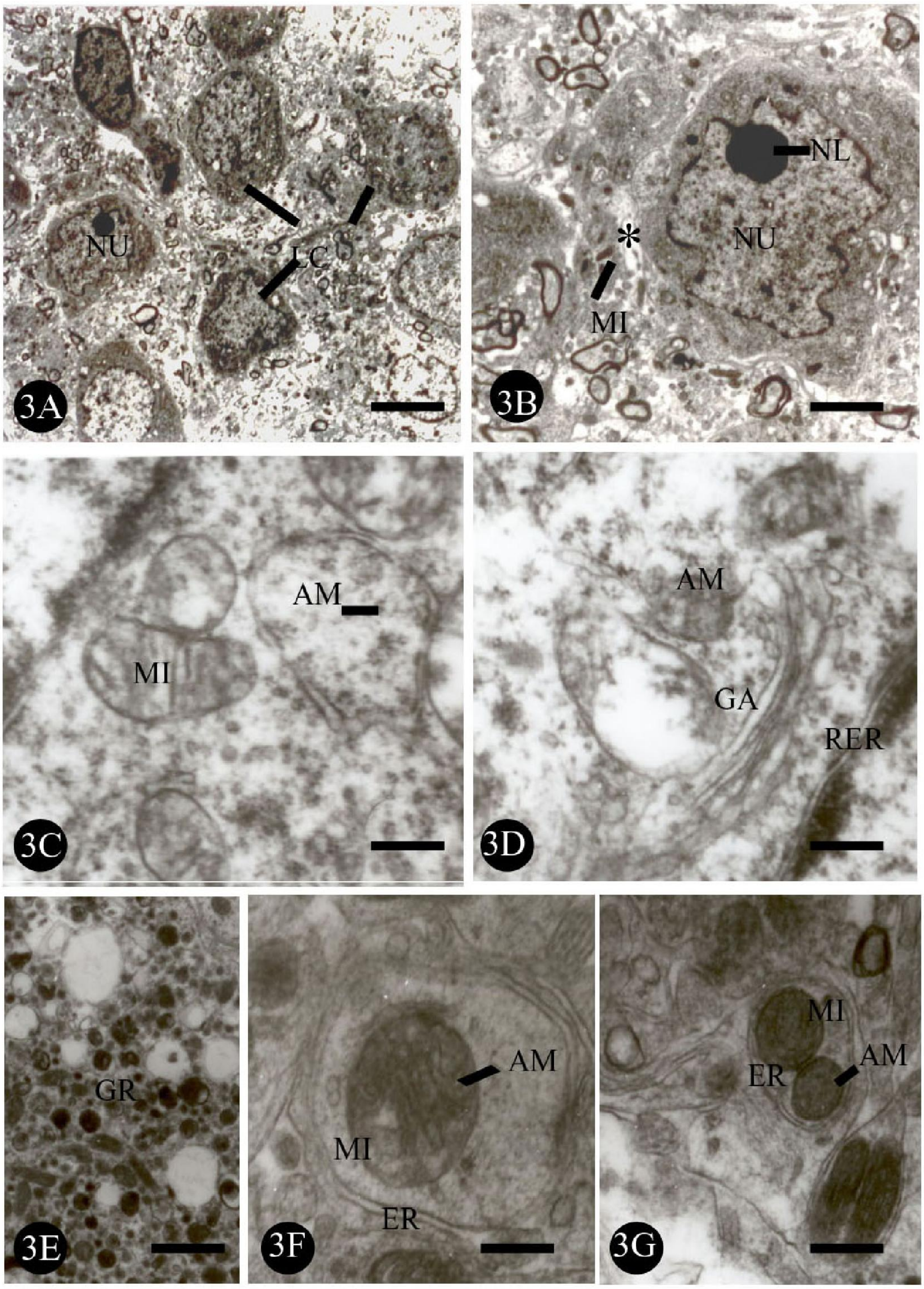

Fig. 3. A: Granulosa lutein cells (LC); the nucleus (NU) is indented and densely heterochromatic. B: One GLC is blown up, showing the nucleus (NU) containing a nucleolus (NL), which is not differentiated as the pars amorpha and nucleolenema; this cell has an irregular pattern with intercellular spaces (asterisk). C, D: Portions of GLC further blown up; mitochondria (MI), rough endoplasmic reticulum (RER), Golgi apparatus (GA), and membranes of large saccules containing an amorphous material (AM) are highlighted. E: A portion of GLC containing abundant dense granules (GR). F, G: Well developed smooth endoplasmic reticulum (ER) in this GLC surrounds the mitochondria (MI) which lie in an amorphous material (AM). Scale bar = 4µm in A; 2µm in B; 0.3µm in C, D; 0.4 µm in E; 0.2 µm in F, G.

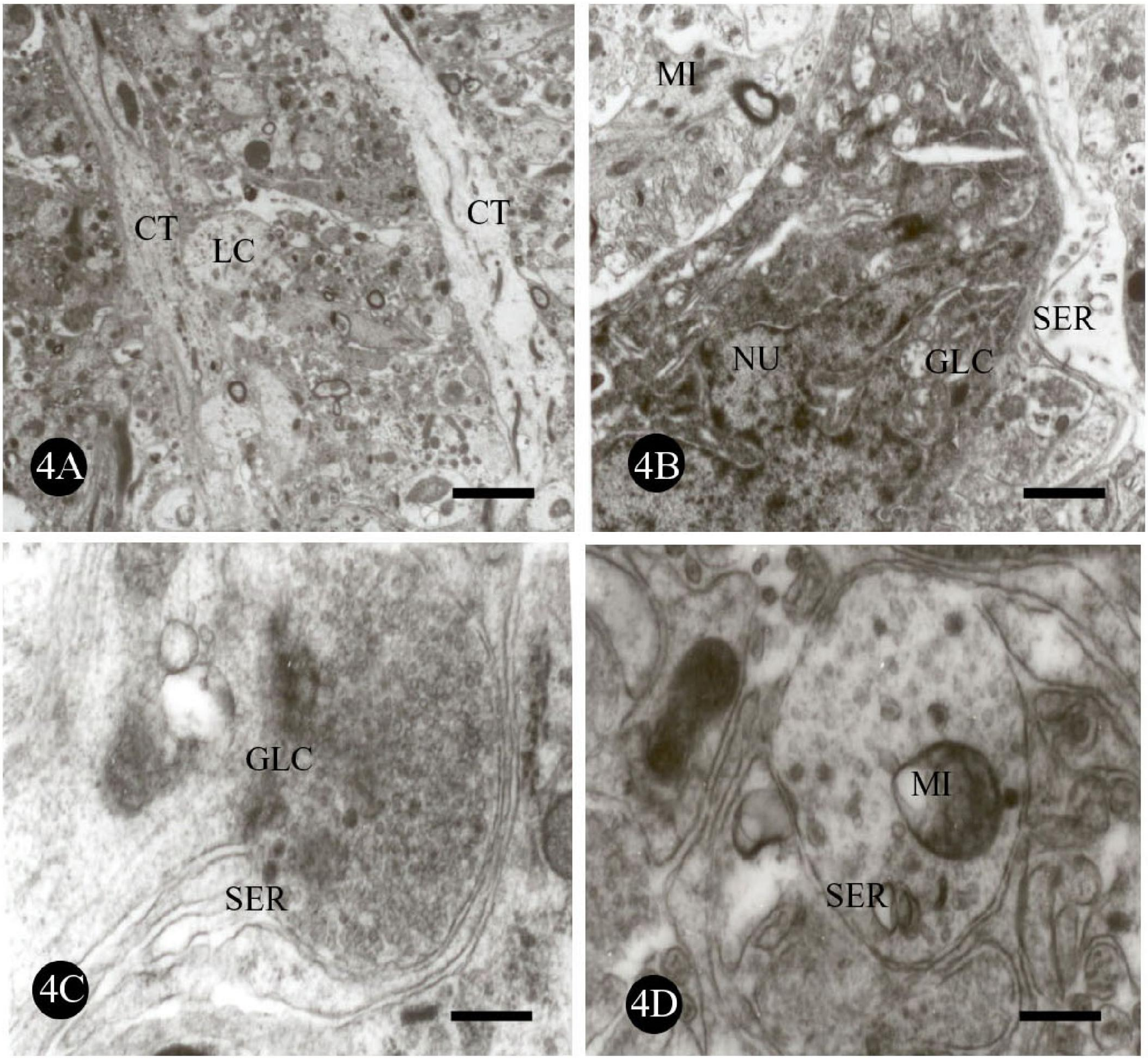

Fig. 4. A, B: TEM of GLCs (LC, GLC) which are highly elongated, and are surrounded by connective tissue strands (CT); mitochondria and SER are highlighted. C, D: In these GLCs the outer mitochondrial membrane (MI) appears to be forming association with the saccules of SER. Scale bar = 4µm in A; 1µm in B; 0.2µm in C, D.

al., 2006). Corpora lutea have been described in all groups of amphibians (Vilter and Vilter, 1960, 1962; Joly, 1964, 1965, 1971; Joly and Picheral, 1972; Ozon and Xavier, 1968; Lamotte et al., 1964; Xavier, 1970; Xavier and Ozon, 1971; Saidapur, 1982), including the caecilians (Exbrayat, 2006). Fairly detailed description of corpora lutea of caecilians is available only for *Typhlonectes compressicauda* (Exbrayat, 1983, 1986, 1992; Exbrayat and Collenot, 1983). It is a viviparous species, and the description is based on light microscopic observation. In this species, the corpora lutea possess well-developed cavities during the proliferation of granulosa cells and thecal cells. They then become vascularised, providing a means of driving the hormonal secretions into the general blood circulation. At the end of pregnancy, the corpora lutea degenerate, in correlation with the birth of offspring. These observations suggest that corpora lutea are active in the maintenance of pregnancy. At the beginning of gestation, the corpora lutea are relatively inactive but they become steroidogenic during the later part of gestation.

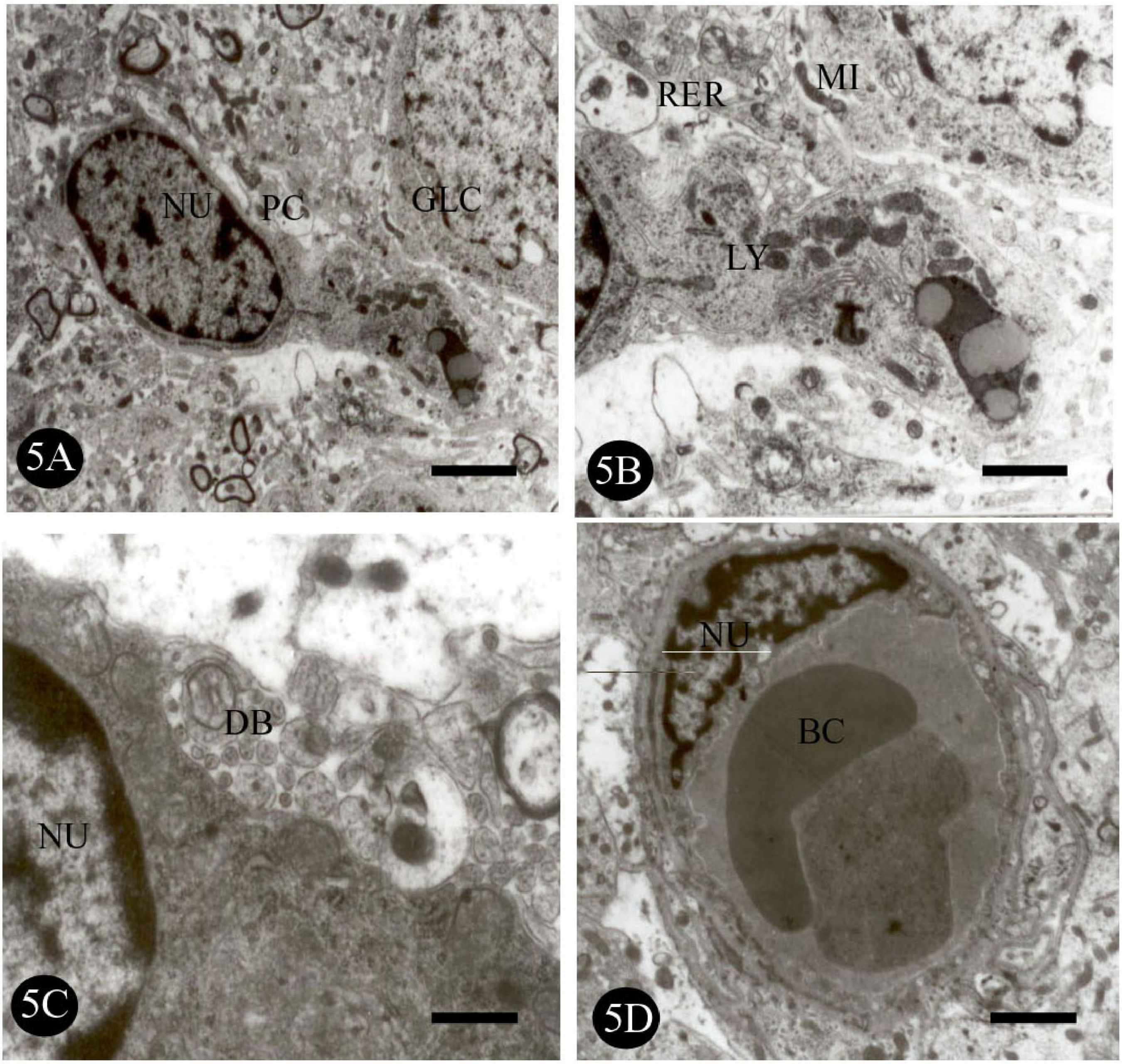

Fig. 5A: TEM of a phagocytic cell (PC) in the vicinity of a GLC; the phagocytic cell possesses a pseudopodial protuberance. B: The pseudopodial protuberance in Fig. 5A has abundant lysosomes; the mitochondria (MI) and RER belong to the neighbouring lutein cells. C: A phagocytic cell in the process of phagocytosing debris (DB). D: TEM of a blood capillary; an erythrocyte (BC) and an endothelial cell with its nucleus (NU) are shown. Scale bar = 3µm in A; 1µm in B; 0.2µm in C; 4 µm in D.

At the end, the number of active corpora lutea decreases and the hormones become less and less abundant (Exbrayat, 2006).

Since our report is basically an ultrastructural description of the corpora lutea at the time of egg laying in *G. ramaswamii*, we relied on corresponding literature pertaining to vertebrates other than amphibians. Further, due to limitation in the sample size and the stage in the reproductive cycle, we were unable to trace the sequential changes in the organization of corpus luteum from establishment to regression. For reasons mentioned, we believe that the gland described was in the early regression phase. In the corpus luteum formation, the role of the granulosa cells (follicular epithelial cells) has been described in our earlier papers (Beyo et al., 2007 a, b; 2008a, b; 2009). These have been referred to respectively as GLCs and the TLCs, and a third cell type that has been described is migratory/amoeboid cells (Bulmer, 1964;

Paavola, 1977; Page et al., 1978; Krisch et al., 1981). In this respect, *G. ramaswamii* corpus luteum has the cell types known for most of the vertebrate species including human (Long and Jones, 1967a, b).

Though the light microscopic disposition and the ultrastructural organization of these three cell types in the corpus luteum of *G. ramaswamii* generally matches with the other vertebrates including humans (Green and Macqueo, 1965; Van Lennep and Maddan, 1965), the striking difference lies in the paucity of SER in TLCs. The SER and mitochondria are the organelles particularly concerned with steroidogenesis (Enders, 1962; Christensen, 1965; Long and Jones, 1967 a, b; Belt and Cavazos, 1967). Another perplexing observation in this study is the presence of well developed GA in both the lutein cells. The GA is always concerned with packaging of proteinaceous secretory material (Enders, 1962; Enders and Lyons, 1964; Rennels, 1966; Flaks and Bresloff, 1966; Long, 1973; Warshawsky et al., 1963; Caro and Palade, 1964). Interestingly, well developed GA has been reported in the lutein cells of the other vertebrates studied (Long and Jones, 1967a). Presumably, we have dealt with a gland which has ceased steroidogenesis, concomitant with the completion of retention of eggs in the oviducts and their laying. However, the features like presence large-vesicles containing dense accumulations, prominent GA, differentiation of nucleolus into pars amorpha and nucleolenema, phagocytic activity of the amoeboid cell, the abundant blood capillaries, etc., typically identify this organ as corpus luteum. Thus, this paper describes, for the first time, the ultrastructural organization of the corpora lutea in a caecilian.

Acknowledgments

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