The ultrastructural features of the infundibulum of the green iguana, *Iguana iguana*.

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ABSTRACT

The purpose of this study is to describe, in detail, the ultrastructure of the infundibulum of the sexually mature and active female green iguana, *Iguana iguana*. The infundibulum of five iguanas was remarkably distinct from the uterus, and was also clearly demarcated into cranial (expanded v-shaped) and caudal (tubular) divisions. Tissue samples obtained from five portions (three from the cranial division and two from the caudal division) of the infundibulum were processed conventionally for light and electron microscopy. The epithelial lining of the most anterior, middle, and posterior, parts of the cranial division displayed non-ciliated cells predominantly, and occasional ciliated cells. The numerous secretory granules in non-ciliated type 1 cell found in the fimbrial aspect of the infundibulum were homogenous and deeply electron-dense, but those in the other two regions

were variants of this cell type because they contained variably electron-dense secretory granules. Two main types of non-ciliated cells (type 2 and its variant, type 3, as well as type 4) occurred in the epithelial lining of the caudal division of the infundibulum, but they, significantly, showed no dense secretory granules. Whereas the non-ciliated type 2 cell and its variant (type 3 cell) contained large glycogen deposits, the type 4 cell lacked these deposits but its apical part contained large lipid-like droplets and, remarkably, blebbed into the duct lumen. The non-ciliated cells lining the mucosal tubular glands contained highly electron-dense secretory granules, which were similar to those found in the non-ciliated type 1 cell in the epithelial lining of the fimbrial part of the cranial division of the infundibulum.

KEYWORDS

Iguana; squamate; oviparous; infundibulum; ultrastructure

INTRODUCTION

The reptilian oviduct is an intricate organ that carries out a multitude of functions, which include fertilization, sperm storage, egg shell deposition, embryo maintenance, and parturition (Blackburn, 1998, Girling, 2002). Lizards within the Squamata order exhibit a variety of reproductive patterns that include the presence

of viviparous and oviparous species (Blackburn, 1982, 2006; Shine 1985, Girling, 2002). The oviduct of reptiles comprises, cranio-caudally, the infundibulum, magnum, isthmus, uterus and vagina (Girling et al., 1998). The literature is replete with variations in the oviductal regions recognized, and the terminology used to distinguish these regions differs from one species to another (Blackburn, 1998, Girling et al., 1998, Girling, 2002). Studies by Fox (1963) and Blackburn (1998) recognize three separate regions the oviduct of lizards, cranio-caudally, as the infundibulum, uterus and vagina. Cuellar (1966), however, recognized an additional region known as the 'tube', thus dividing the oviduct into four regions (infundibulum, tube, uterus and vagina). Although it is generally agreed that the most anterior part of the oviduct is the infundibulum, there are, however, differences in the interpretation of the end of this region relative to the succeeding 'uterus'. Blackburn (1998) and Siegel (2015) report that various 'functional regions' are easily noticeable in gravid adult reptiles, although in several squamate species the infundibulum could not be differentiated from the uterus (Halpert et al., 1982; Adams and Cooper, 1988; Aldridge, 1992; Shanthakumari et al., 1992). The infundibulum in certain reptiles has been recognized to have two segments, anterior and caudal (Palmer and Guillette, 1988; Al-Amri, 2012; Siegel et al., 2015).

The green iguana, *Iguana Iguana*, is an oviparous lizard (Blackburn, 1998) within the Squamata order and family, Iguanidae (Etheridge, 1982; Frost and Etheridge, 1989; Macey et al*.,* 1997). Squamates, among reptiles, exhibit the greatest amount of variation in their life history (Manriquez-Moran et al., 2013) as well as in

their reproductive cycles (Shine, 1985), probably influenced by a number of factors such as the environment, phylogeny, and parity mode (Méndez-de la Cruz et al., 1998*)*. Green iguanas reproduce annually and typically reach the size of sexual maturity between 1.5 and 3 years of age (Rodda, 2003). Body size is thought to be a determining factor in the sexual maturity of the green iguana, and reproduction occurs when the head and trunk length reaches 224–295 mm [8.81–11.61 in] (Cole, 1966; Mason, 1992). The clutch size of the iguana is markedly variable (9 to 71 eggs) depending on body size, nutritional status, and maturity of the female (Hirth, 1963; Müller, 1972; Fitch & Henderson, 1977; Rand, 1984). The female green iguana has a functionally developed left and right ovary and oviduct, respectively, and they lie dorsally on either side of the body cavity (Blackburn, 1998; Fox, 1977).

Girling (2002) encapsulates the dilemma of biologists when she said, "Although the oviduct has long been an organ of interest, understanding of its functions is still in its infancy. This is especially apparent when compared with the advances made for other vertebrates." Reports on studies of the reptilian oviduct, grossly and histologically, are numerous (Fox, 1977; Guillette and Jones, 1985; Uribe et al., 1988; Palmer et al., 1993; Perkins and Palmer, 1996; Girling et al., 1997, 1998; Al-Kindi et al., 2006; Machado-Santos et al., 2015) but there are very few ultrastructural studies of this organ in the reproductive cycle (Girling, 1998).

The purpose of this study is to identify the relative position, gross, histological, and ultrastructural features of the infundibulum of the green iguana, with a view to

contributing to knowledge and understanding of this segment of the oviduct in this squamate reptile found commonly in the Lesser Antilles. These reptiles are kept as pets by persons or used as a source of animal protein by others. Studies of the reproductive cycle of the green iguana are planned, and detailed structural features of the regions of the oviduct are necessary and invaluable in pursuance of this objective.

MATERIALS AND METHODS

The animal procedures used in this study were approved by the Institutional Animal Care and Use Committee (Approval # IACUC18005-R) of the St. George's University. In addition, the Ministry of Agriculture of Grenada, granted approval for the trapping and removal of female green iguanas, *Iguana iguana* from their natural habitat. Tissues from the oviduct of five sexually mature, healthy, and active female iguanas were humanely caught, using nets and ropes by local hunters, on the island of Carriacou. A physical examination was performed on each female iguana to ensure only healthy iguanas were used in the study. Thereafter, the iguanas were euthanized by an overdose of ketamine infused into the coelomic cavity, and subsequently decapitated, using a guillotine. The abdominal cavity was quickly opened through a midventral incision, grossly assessed *in situ* and the oviduct was excised. Tissues were taken from animals that were gravid or in which the ovary had Graafian follicles ready to ovulate. This was done to confirm that the oviduct was in the most active phase of the reproductive cycle. Tissues from both the fimbrial and tubular parts of the infundibulum (as shown in Fig.1) were cut into

millimeter squares and quickly fixed by immersion in 3% glutaraldehyde solution buffered in 1.0 M phosphate buffer at pH 7.4. The tissue blocks were subsequently post-fixed in similarly buffered 1% Osmium tetroxide for 2 h, dehydrated in a series of graded ethanol concentrations, and embedded in epoxy-resin at a ratio of 1:2 for 1 h, 1:1 for 2 h, and 100% resin overnight. Semi-thin sections, of 1 μm thickness, were cut with a diamond knife and stained with toluidine blue. Ultra-thin (50–90 nm) sections of selected areas were cut on a Reichert-Jung Ultracut (C. Reichart AG, Vienna, Austria) using a diamond knife, collected onto copper grids, and stained with Reynold's lead acetate and counterstained by using an aqueous saturated solution of uranyl citrate (Ayache et al. 2010). The sections were examined and photographed in a Phillip CM 10 transmission electron-microscope-TEM (Phillips Electron Optical Division, Eindhoven, Netherlands), operated at 80 kV.

Tissues obtained from various parts of the infundibulum for histological studies were, respectively, fixed in Bouin's fluid and buffered formalin for, at least, 24h, and thereafter dehydrated in ascending grades of ethanol, embedded in paraffin, sectioned at 5μm, and stained with hematoxylin and eosin (H&E). The sections were subsequently examined under an Olympus BX 63® (Olympus Corporation, Tokyo, Japan) microscope, and appropriate photographs taken.

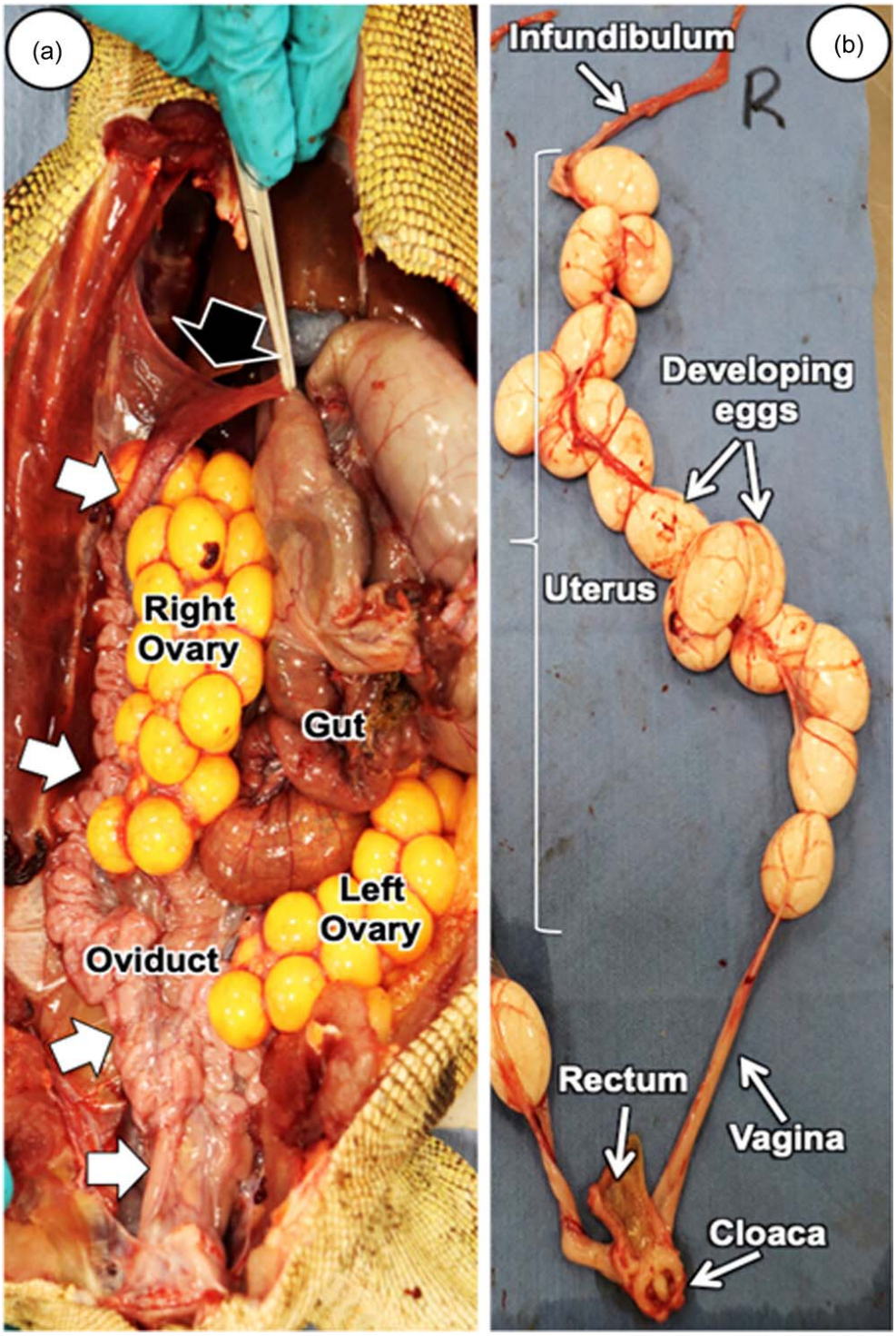

Fig. 1: **A** -- an adult sexually mature and active female iguana. The ovary is filled with ready-to-ovulate follicles covered by a thin, translucent membrane. The oviduct extends from the fimbrial end (broad black arrow) to the vagina (last white broad arrow). **B**: the right oviduct contains several developing eggs between the end of the infundibulum and the beginning of the vagina.

RESULTS

Prior to ovulation, the ovaries of the sexually mature and active female green iguana displays Graafian follicles of similar sizes and colour, all of which are covered with a transparent membranous covering (Fig. 1 A). The oviduct extends from an easily identifiable anterior, expanded fimbrial end of the infundibulum through the uterus to the vagina that opens into the cloaca, posteriorly (Figs. 1 and 2). At ovulation, the oviduct picks up the ova that are linearly arranged, antero-posteriorly along the length of the oviduct (Fig. 1 B).

Grossly, the infundibulum of the green iguana is funnel-shaped (Fig. 2), with the anterior/cranial v-shaped or fimbrial part being wide, almost transparent, and opening through the ostium into the coelomic cavity in the region of the ovary, and the caudal part, which is narrow, straw-colored and tubular, and is continued posteriorly with the rest of the oviduct, at an easily recognized junction with the uterus (Fig. 2A, B).

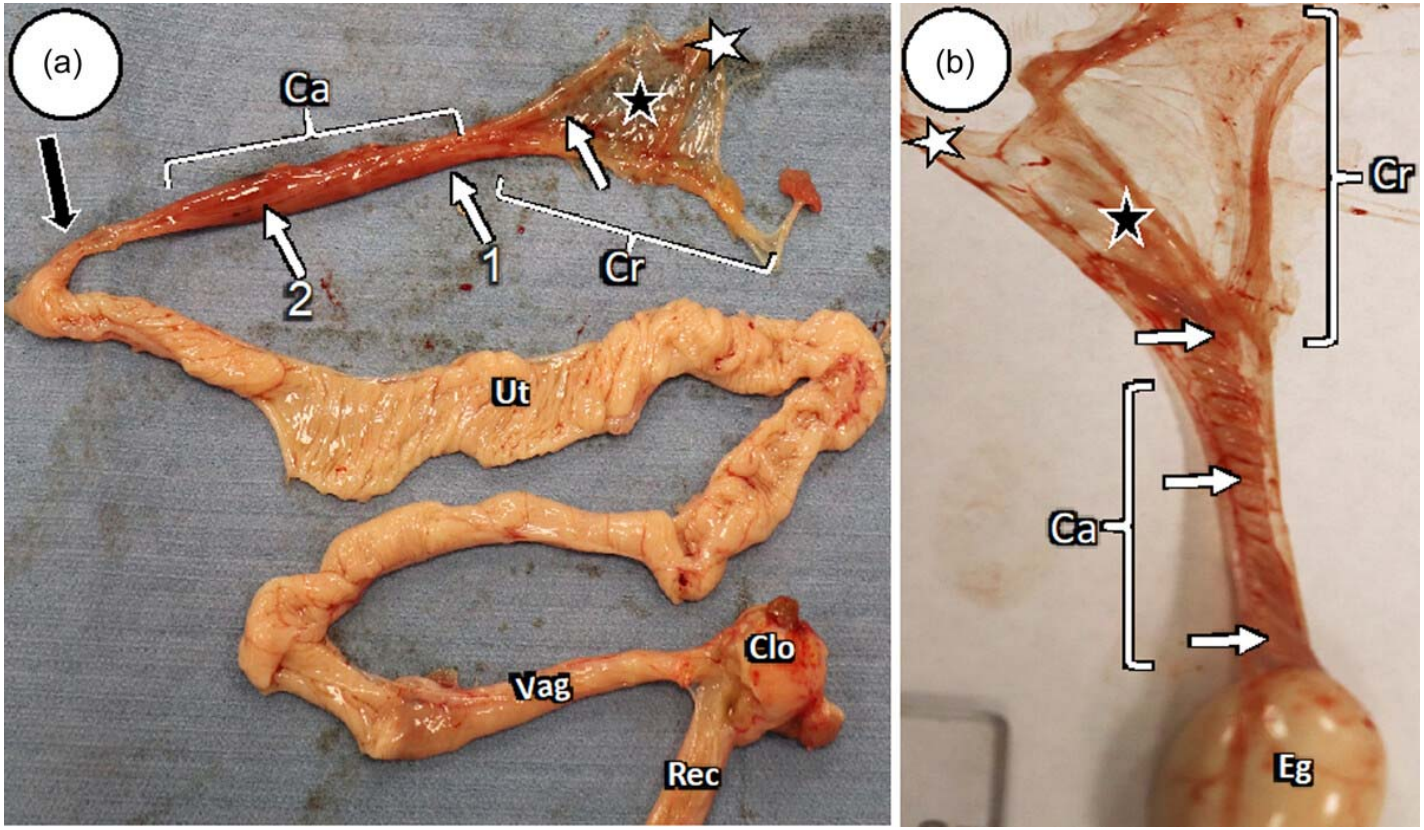

Fig. 2: A (with no developing eggs) and **B** (with developing eggs) show gross features of the oviducts of sexually mature and active iguanas. Cr and Ca areas constitute the cranial and caudal divisions of the infundibulum, respectfully. Tissues were obtained from the most proximal part (white star), middle (black star), and distal (white arrow) of the fimbrial or cranial division (Cr) of the infundibulum. In the caudal division, tissues were obtained from the regions of white arrows, numbers 1 and 2. Black arrow = junction between the infundibulum and uterus (Ut) of the oviduct. In B, a developing egg (Eg) is in the proximal part of the uterus. Vg = vagina; Rec = rectum; Clo = cloaca.

Histologically, three main areas are recognized based on their cell content and characteristics. The epithelial lining of the most anterior aspect of the fimbrial portion of the infundibulum is generally smooth-surfaced in profile (Fig. 3 A). The lamina propria is relatively thin, displays prominent blood vessels (Fig. 3), and is surrounded by thin layers of smooth muscle. The epithelial lining extends onto/over the outer wall of the fimbrial region for some length along that surface before yielding to the serosa (Fig. 3 A). The cuboidal to low columnar epithelium comprises mainly non-ciliated cells, with a few ciliated cells interspersed between them. The nuclei are irregular in shape and occupy the basal two-thirds of the cytoplasm (Fig. 4). The middle to posterior end of the infundibulum has relatively thicker walls, displaying mucosal folds with the epithelium containing more ciliated cells than in the most anterior part of the infundibulum. The thicker lamina propria is better defined and surrounded by well-developed smooth muscle layers (Fig. 3 B, D). Posterior to the expanded fimbrial part of the anterior segment, the mucosal folds are higher, and the grooves between adjacent folds are narrow and deep (Fig. 3 B, C**).** These folds yield to lower and broader mucosal folds that contain simple tubular or mucosal glands arranged in a single row within the sub-epithelial region of the lamina propria (Fig. 3 D). The structural features and variations in these segments of the infundibulum are best illustrated at the ultrastructural level. There was little connective tissue in the lamina propria, blood vessels are well developed, but the muscle layer was very thin (Fig. 3).

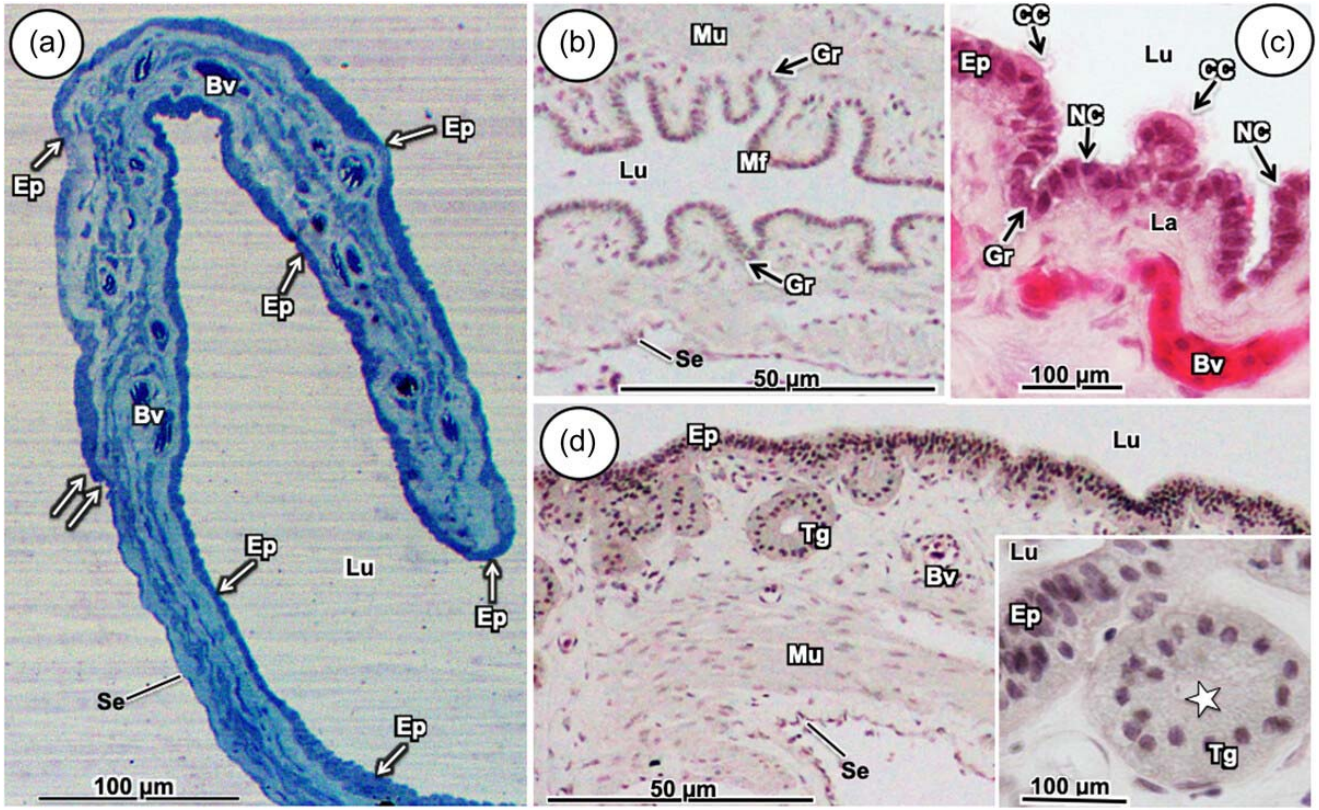

Fig. 3: A is a thick plastic section of the fimbrial part of the infundibulum stained with toluidine blue. Ep = epithelial lining, white double arrows = junction between the epithelium and serosa (Se) covering the fimbrial part of the infundibulum, Bv = blood vessels. **B** Middle to distal parts of the infundibulum. Mf = fold of mucosa, Gr = pits/grooves of the folds, Se = serosa, Mu = muscle layer. **C** shows a few ciliated cells (CC) interspersed between preponderant non-ciliated cells (NC) in the epithelial lining (Ep). Bv = blood vessel, La = lamina propria, Gr = pit/groove of mucosal fold. **D** shows wall of the tubular (caudal division) of the infundibulum. Ep = epithelial lining, Tg = tubular or mucosal gland, Bv = blood vessel, Mu = muscle layer, Se = serosa. **Inset** is a higher magnification of the epithelium (Ep) and tubular gland (Tg). Lu = lumen, white star = lumen of tubular gland.

Ultrastructurally, the fimbrial part of the epithelial lining is columnar with its luminal surface being a slightly irregular in outline due to some cells protruding moderately into the lumen compared to others (Fig. 4). The epithelium comprises non-ciliated cells mainly, and very few, randomly, interspersed ciliated cells (Fig. 4). The non-ciliated cells in this zone of the infundibulum are named non-ciliated type 1 cell because they are different from those in the more posterior zones of the infundibulum. The apical plasma membrane of the non-ciliated, but not of the ciliated cells, tended to protrude slightly into the ductal lumen and is thrown into numerous short microvilli (Fig. 4). The cytoplasm of this cell is moderately electron-dense and houses a highly irregularly shaped, elongated nucleus occupying up to half of the length of the cell (Fig. 4). The nuclei, which appear lobulated, are generally euchromatic, with chromatin margination and interspersed, small, irregularly shaped clumps of chromatin in the nucleoplasm (Figs. 4 B and 5 A). The supranuclear region of the cell contains numerous dense secretory granules of varying, but mostly oval shapes, and secretory granules may be seen being released into the lumen (Fig. 4 B). The infranuclear region contains numerous lipid droplets (Figs. 4 A and 5 A). The mitochondria are uniformly scattered within the cytoplasm, and mostly linearly arranged along the long axis of the cell. A few vacuoles are seen among the secretory granules. The ciliated cells are similar to those in the more posterior part of the infundibulum, where they will be described fully. The epithelium lies on an irregularly shaped basal lamina (Fig. 4 A) supported by bundles of collagen fibers. The muscular coat is thin.

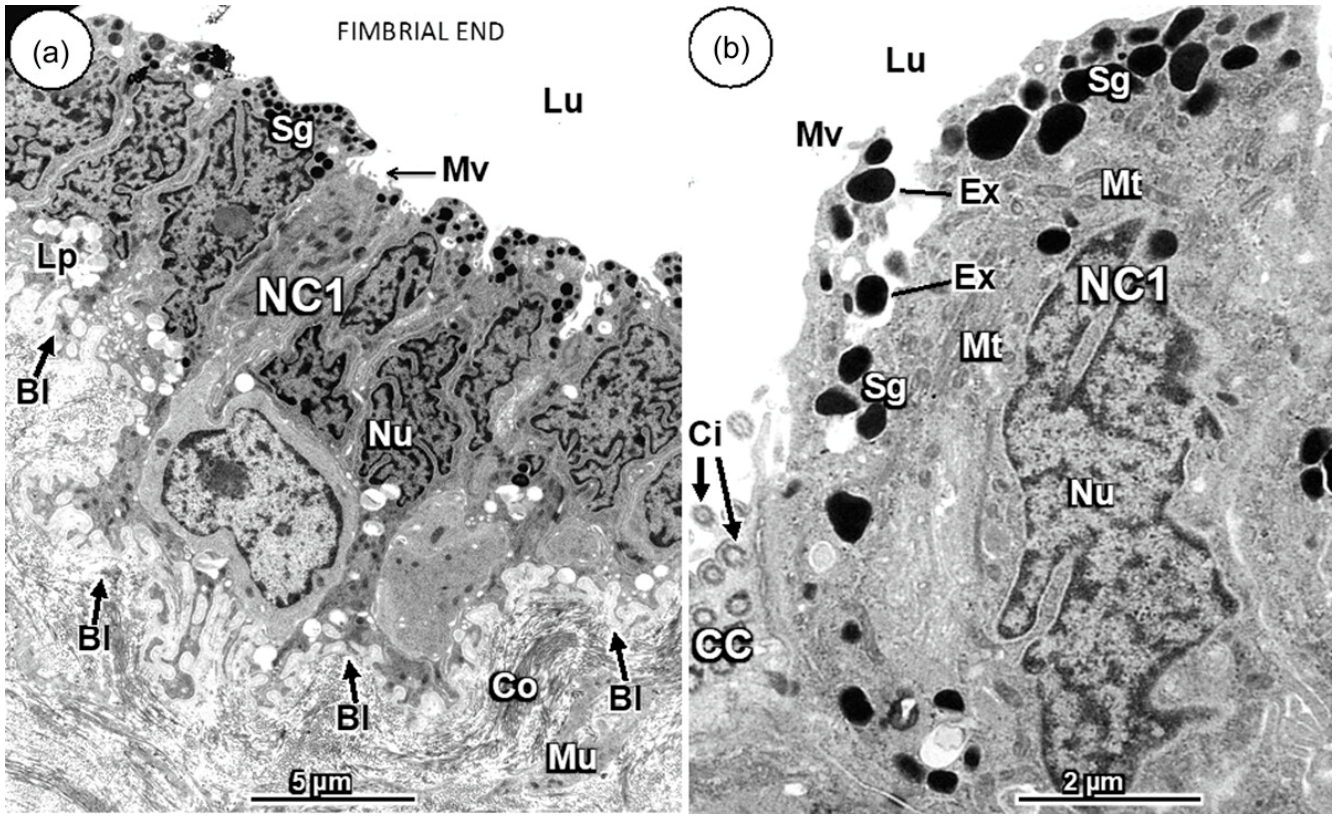

Fig. 4: In **A**, the epithelium of the most proximal aspect, or ostial end, of the fimbrial portion of the infundibulum displays only non-ciliated type 1 cells (NC1) with elongated and irregularly shaped nuclei (Nu) and dense secretory granules (Sg), Lp = lipid droplets, Co = collagen bundles, Mu = muscle layers, Bl = basal lamina. **B** shows epithelium of the middle part of the cranial division of the infundibulum, displaying an occasional ciliated cell (CC) with cilia (Ci) and the predominant non-ciliated cells type 1 (NC1). Mv = microvillus, Ex = secretory granules being exteriorized by apocrine secretion process, Mt = mitochondria.

The middle part of the fimbrial region gradually narrows as it progresses toward the tubular part, the non-ciliated cells in this region are a variant of the non-ciliated type 1 cell because they contain more compactly laden with secretory granules of varying electron-density, which may be due to varying stages of formation (Fig. 5). In addition, lipid droplets also appear, interspersed among the secretory granules and mainly in the infranuclear region of the cell (Fig. 5 A). The lamina propria is thicker than in the anterior part of the infundibulum, and the muscle layers are moderately developed (Fig. 3).

As the fimbrial part narrows further, caudally, the mucosa forms prominent folds, the crests of which are made up mainly of ciliated cells, with the grooves between the folds being occupied exclusively by non-ciliated type 1 cells, that are of similar composition as those described for the fimbrial part of the duct (Fig. 6). The cytoplasm of the ciliated cell is less electron-dense than that of the non-ciliated type 1 cell, but the nuclei are similar to those of the non-ciliated type 1 cells in being irregularly-shaped, and with chromatin margination and scattered clumps of dense chromatin in the nucleoplasm (Fig. 6). In the subapical or supranuclear region of the cytoplasm of the ciliated cell, there is an aggregation of mitochondria, which are mostly linearly arranged along the long axis of the cell (Fig. 7). The mitochondria have electron-dense matrix and well-formed cristae (Fig. 7 B). A few bundles or strands of intermediate filaments may be seen in the cytoplasm (Fig. 7 B).

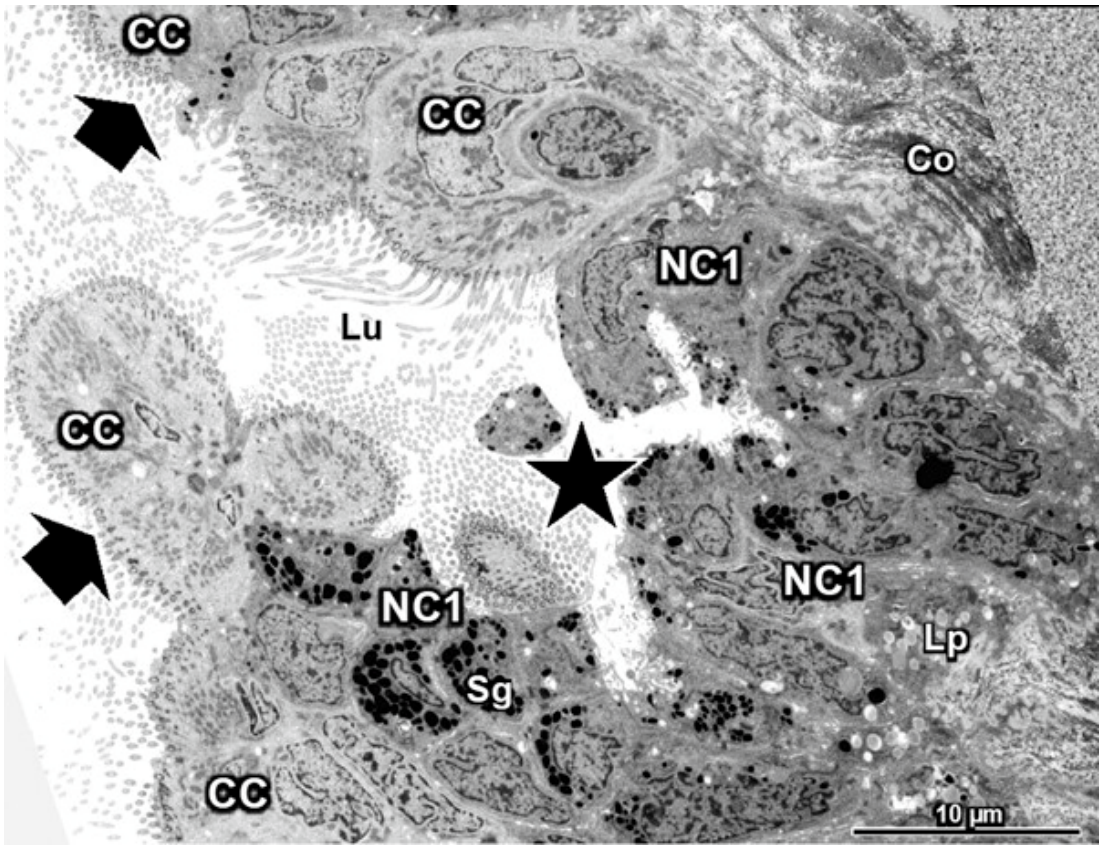

Fig. 6: At the most distal part of the cranial division of the infundibulum (i.e., the junction between the cranial and caudal divisions of the infundibulum), the grooves (black star) between the mucosal folds (broad block arrows) are deep and contain only non-ciliated type 1 cells (NC1), while the crests of the folds are made up of mostly ciliated cells (CC), Sg = dense secretory granules, Lp = lipid droplets, Lu = lumen, Co = collagen bundles.

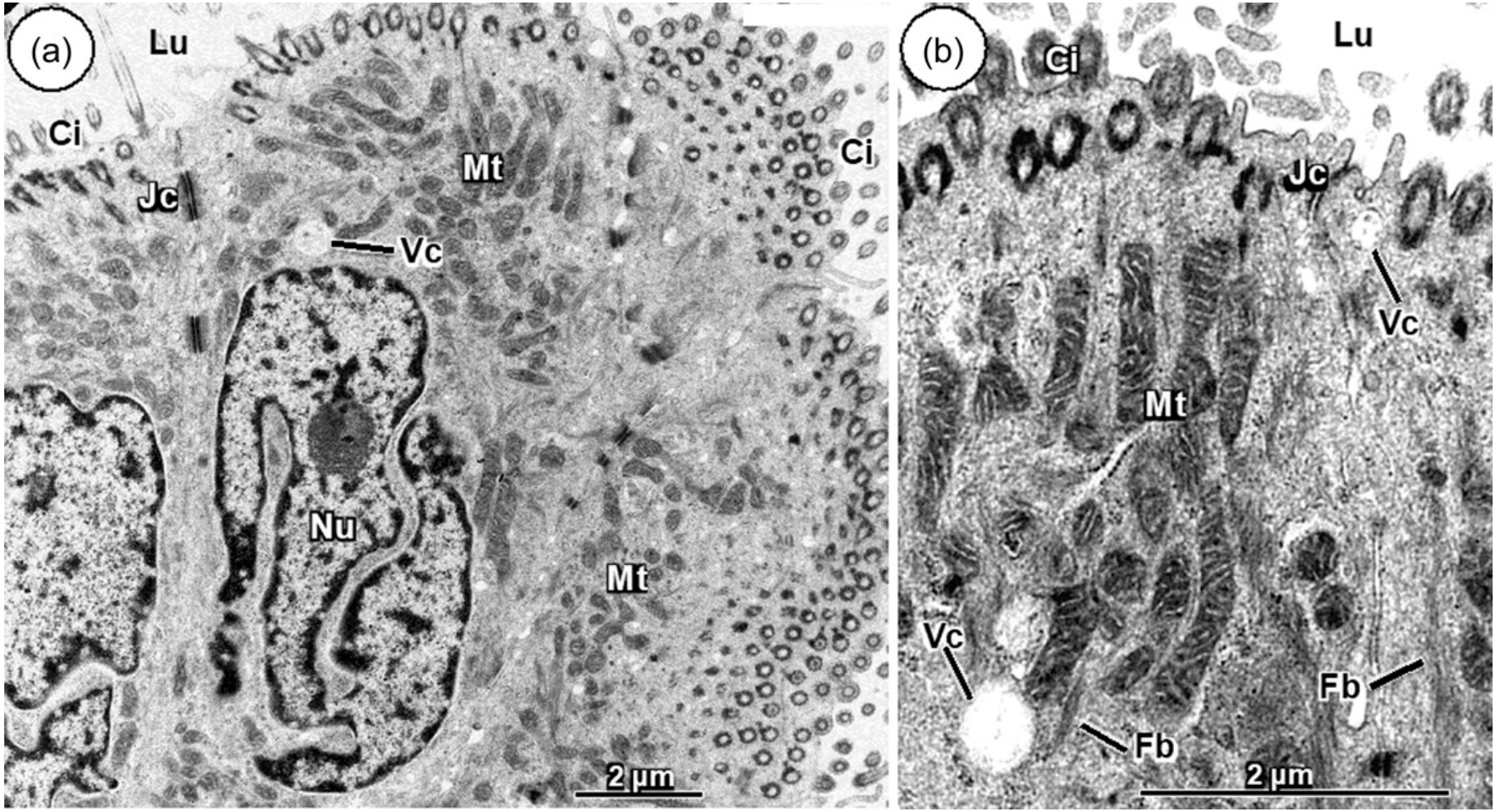

Fig. 7: A exhibits ciliated cells in the mucosal folds of the distal part of the cranial division of the infundibulum displaying linearly elongated, irregularly shaped, nuclei (Nu), aggregations of supranuclear mitochondria (Mt), Ci = cilia, Vc = multivesicular body. **B** is an enlarged subapical part of the CC, Lu = lumen, Mt = mitochondria, Jc = elements of the junctional complex, Vc = multivesicular body or vacuole, Fb = microfibrillar bundles.

Prior to the tubular part proper or caudal segment of the infundibulum, the epithelium of the duct displays fewer and lower mucosal folds, which comprise both non-ciliated and ciliated cells, with the latter cell type predominating (Fig. 8 A]. The non-ciliated cells are variants of non-ciliated type 1 cell found in the more anterior parts of the epithelial lining of the infundibulum, in being laden with secretory granules of varying electron-density in the supranuclear region and mostly lipid droplets in the infranuclear region (Fig. 8 A, B), the ciliated cells display deposits of glycogen granules surrounding the nucleus (Fig. 8 A, B).

The caudal, narrow, tubular zone of the infundibulum displays a columnar epithelial lining of the duct lumen and mucosal or tubular glands in the lamina propria (Fig. 9). The epithelial lining comprises alternating non-ciliated and ciliated cells, but with the ciliated cells predominating (Fig. 9). In the luminal epithelium, three types of non-ciliated (types 2, 3 and 4 cells, based on their content and disposition, are present. The cytoplasm of the non-ciliated type 2 cell is more electron-lucent than that of the non-ciliated type 3 cell, and its subapical cytoplasm contains numerous small, clear vacuole-like spaces and one or two large lipid droplets (Fig. 10). The euchromatic nucleus shows only a slight chromatin margination and a few, small clumps scattered in the nucleoplasm (Fig. 10). Glycogen deposits usually surround the nucleus or are concentrated in the infranuclear region of the cell (Figs. 9 and 10 A, C). Although mitochondria are numerous in the cytoplasm, dorsal and lateral to the nucleus (Fig.10), they are remarkably and tightly packed in the infranuclear region (Fig. 9 and 10 B). The non-ciliated type 3 cell has an electron-dense

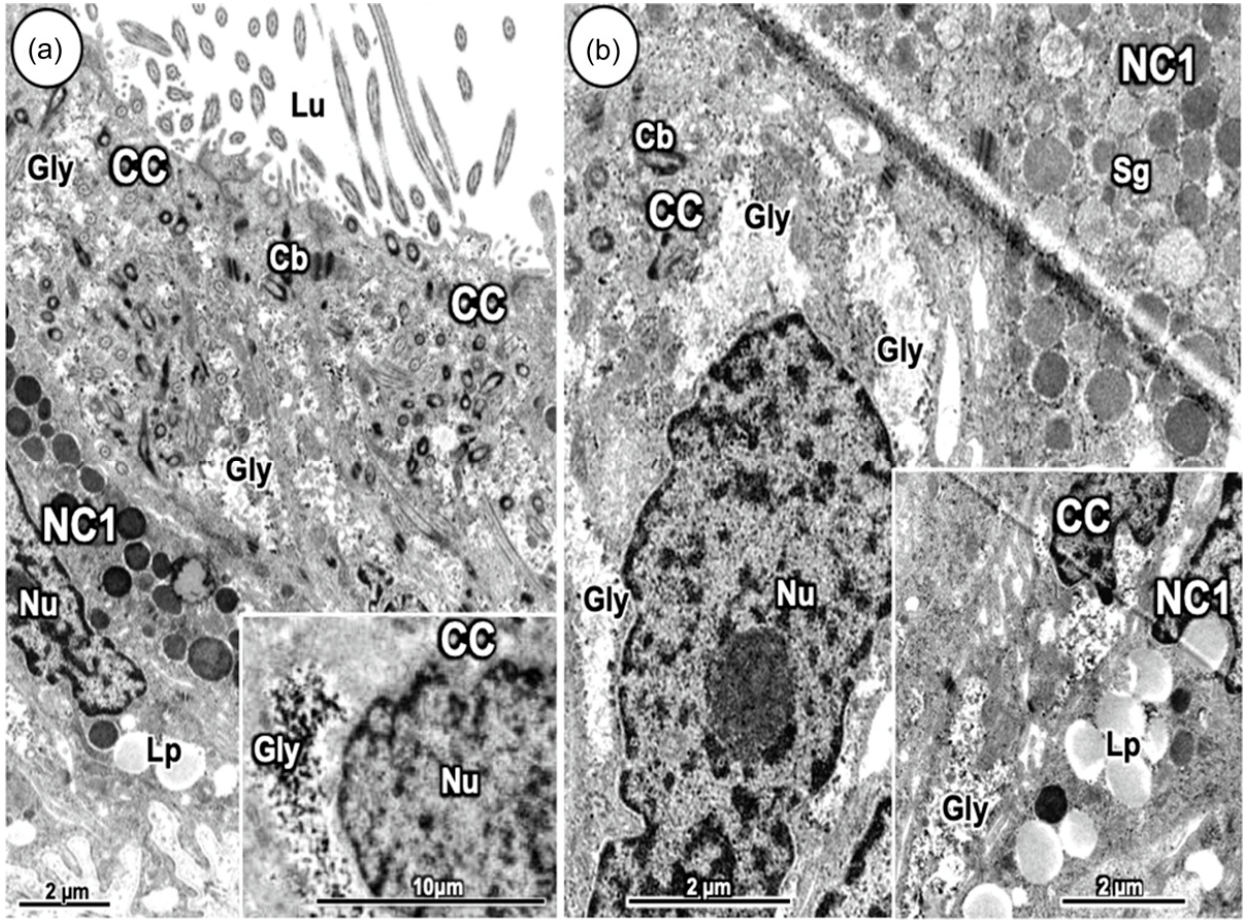

Fig. 8: A shows epithelial lining of the most distal part of the cranial (fimbrial) division of the infundibulum. Both NC1 and CC appear alternately, but with a preponderance of CC. NC1 exhibits dense secretory granules (Sg) of varying electron-density and glycogen deposits (Gly), magnified in the inset. In **B**, which is a higher view of the NC and CC, the CC display glycogen deposits, the NC1 shows secretory granules of varying electron-density, mostly in the supranuclear region, and numerous lipid droplets (Lp) in the infranuclear zone. Nu = nucleus, Cb = ciliary body.

cytoplasm relative to other non-ciliated type cells, and the sub-apical cytoplasm lacks obvious structural features. However, the supranuclear, perinuclear, and, in particular, the infranuclear regions contain large amounts of glycogen deposits (Fig. 10 A, C), making such cells appear to bulge out basally. The non-ciliated type 4 cell has its apical part protruding into the duct lumen (Figs. 10 C and 11 A). The protruded part of cytoplasm has an irregular outline, and contains an aggregation of large, secretory granules that may be of varying content and density. Long, electron-dense mitochondria may lie on either side of these secretory granules (Fig. 11 B). The nuclei have irregular outlines and are linearly arranged in the middle of the cell. The infranulear region contains lipid-like aggregations or secretory granules (Fig. 11 A). The nucleus of the ciliated cell is similar in configuration to that of the non-ciliated type cell, and is also surrounded by deposits of glycogen (Fig. 9 and 10 A). Numerous mitochondria, that are much smaller in diameter than those of the non-ciliated type cells, occur in the supranuclear region of the cytoplasm (Figs.10 C).

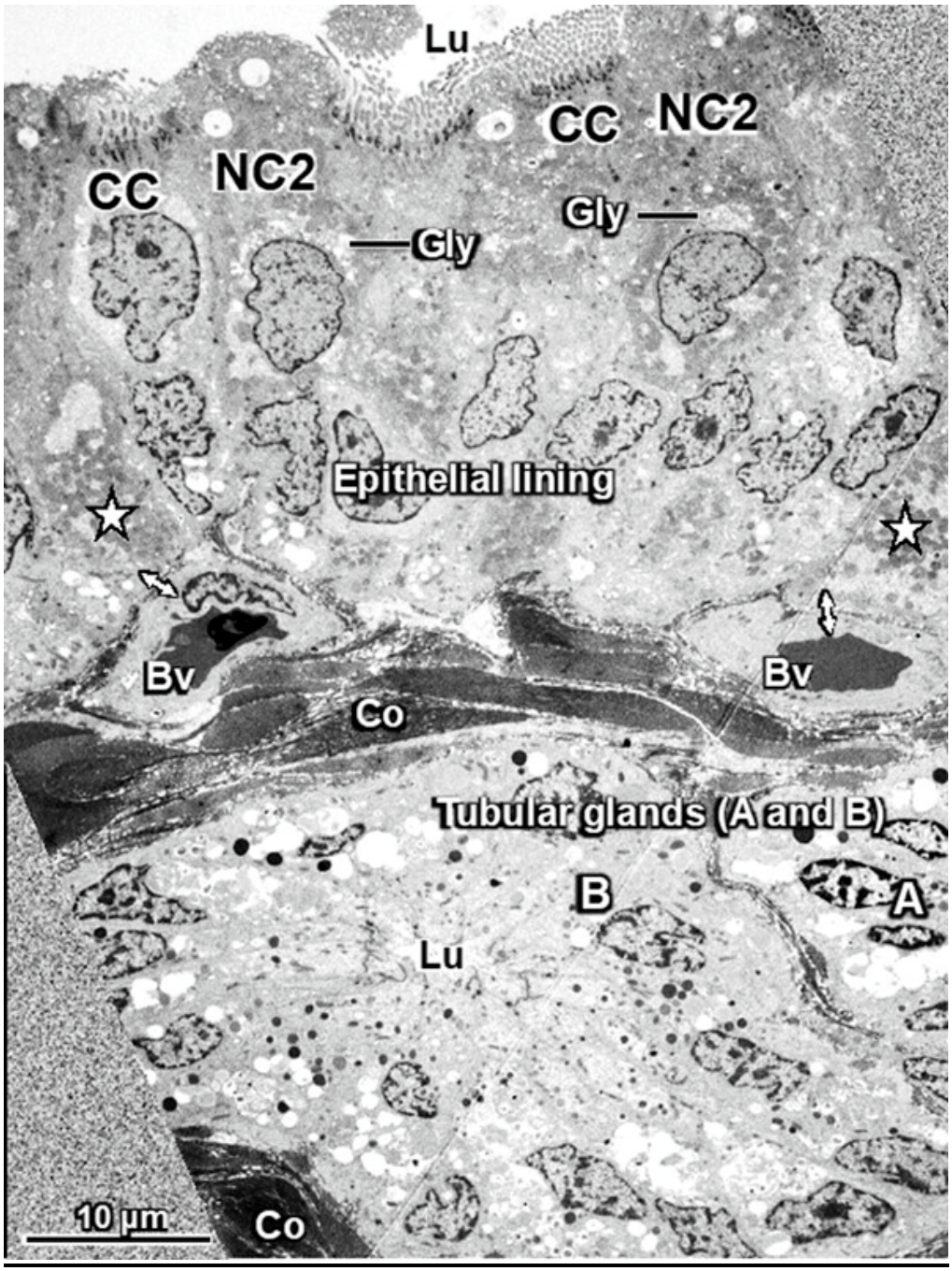

Fig. 9: A survey micrograph of the epithelial lining and lamina propria of the tubular or caudal division of the infundibulum. The luminal epithelial lining of the organ displays two types of cells, NC (type 2) and CC, glycogen deposits (arrow), infranuclear mitochondrial aggregation (star), Bv = blood vessel, up-down and left-right arrows = show thin barrier between the epithelial lining and the subepithelial blood vessels. Co = thick collagen bundles separating the epithelial lining of the segment from the tubular or mucosal glands in the lamina propria, Lu = the oviduct lumen or tubular gland lumen.

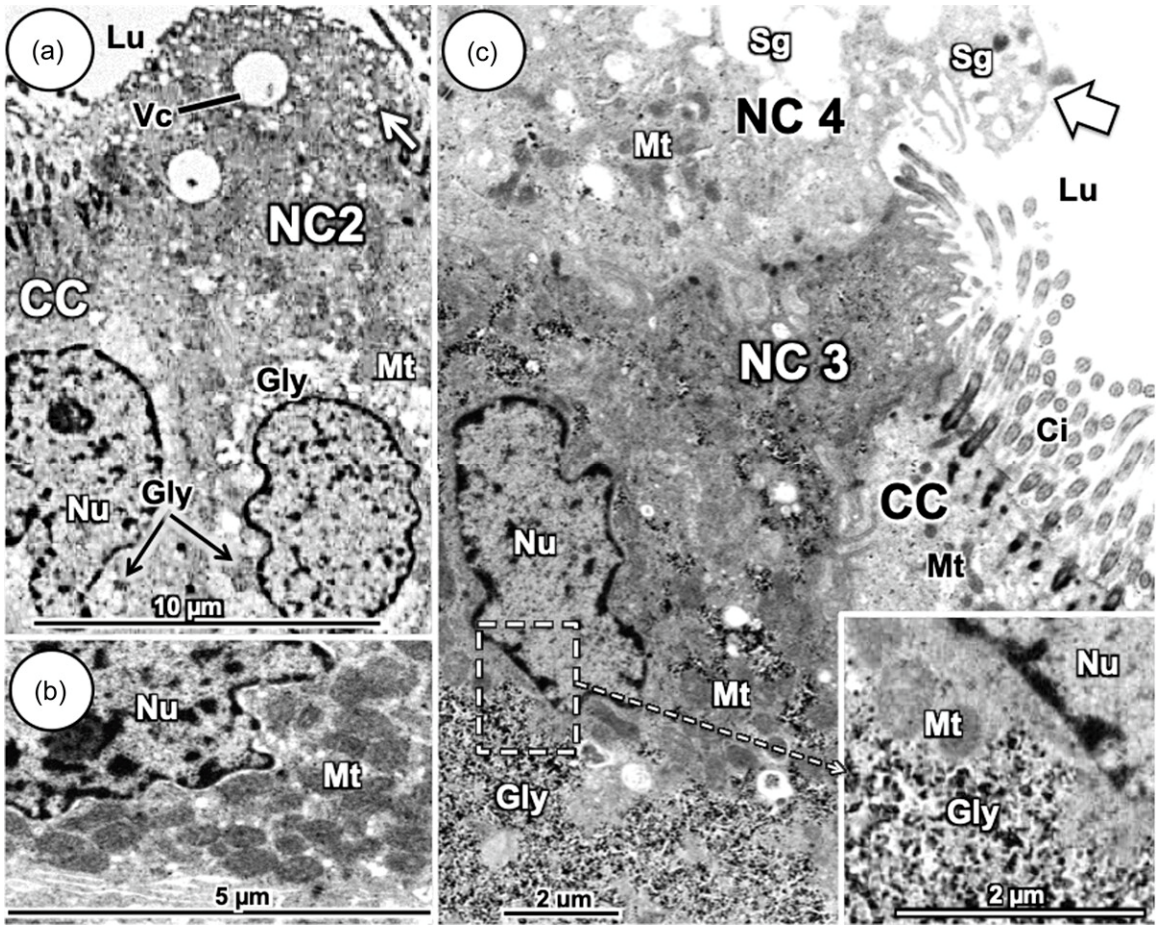

Fig. 10: Caudal division or tubular part of infundibulum of the oviduct. **A** shows a variant of non-ciliated cell, NC2, displaying a few, large vacuoles (Vc) and numerous small vacuoles (white arrow) in the subapical zone and numerous, short mitochondria (Mt) in the immediate supranuclear region of the cytoplasm. The linearly aligned nuclei (Nu) have irregular outlines and are surrounded by deposits of glycogen (Gly). CC = ciliated cell. Lu = oviduct lumen. **B** shows tightly packed mitochondria (star) in the infranuclear region of the NC2. **C** shows NC3 and NC4 and CC in the epithelium lining the tubular part or caudal division of the oviduct. NC3 has an electron-dense cytoplasm, expanded base, and a horizontally elongated nucleus with an uneven surface. Inset is a higher magnification of the part of NC 3 (rectangle) displaying glycogen accumulation. Mt = short, aggregated mitochondria, Gly = a large infranuclear deposit of glycogen. NC4 shows a bleb (white block arrow) into the duct lumen, Sg = large subapical secretory vacuoles of varying sizes and electron-density.

The tubular or mucosal glands are few, oval in shape, and form only a row of glands beneath the epithelium (Figs. 3 D, and 9). The epithelium of the tubular glands is tall columnar and comprises only non-ciliated cells surrounding a common, central lumen (Figs. 3 D, 9, and 12). The individual tubular glands are surrounded by compact bundles of collagen fibers, and unlike the lining of the duct segment, blood vessels are not found close to the glands (Fig. 9). The nucleus of the cell varies in shape, from oval to elongated (Figs. 3 D, 9, and 12 A) in shape, and is situated basally in the cell. It is generally euchromatic with a little chromatin margination and small, scattered, clumps of heterochromatin in the nucleoplasm. The supranuclear region of the gland cell is laden with highly electron-dense secretory granules (Fig. 12), with interspersed lipid droplets (Fig. 12). The immediate sub-apical zone of the cell displays a number of small, clear, foam-like spaces, which appear as extracted lipid droplets (Fig. 12 B). The infranuclear region of the cell (Fig. 13 A, B) contains scattered mitochondria, a few dense granules and numerous lipid droplets. Numerous, small, electron-lucent spaces are often seen to coalesce with the large lipid droplets (Fig. 13 B).

The lamina propria overlies the circular and longitudinal smooth muscles, which are very thin and poorly differentiated, especially in the more anterior parts of the infundibulum (Figs. 3 D and 13 C), and which are much better developed in the caudal segment of the infundibulum. Collagen bundles abound in the lamina propria, and lie between the serosa and deeper structures, as well as between muscle layers. Thick and compact bundles of collagen lie between the tubular or

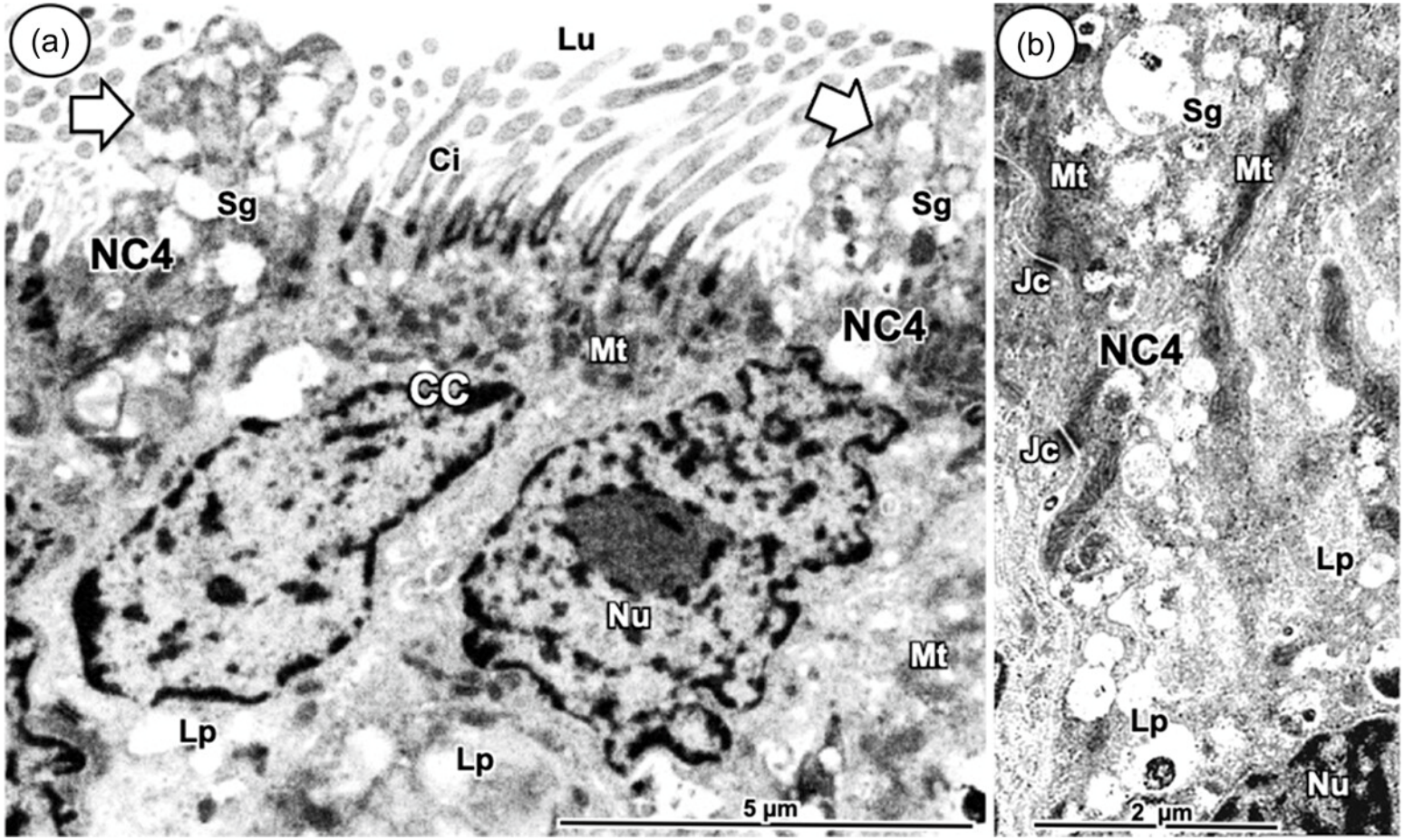

Fig. 11: A -- NC4 with blebs (white block arrows) protruding into the duct lumen. The supranuclear cytoplasm contains numerous secretory granules (Sg) of varying content and electron-density. The nucleus is vertically oriented within the cytoplasm, with an irregular outline. The infranuclear zone contains numerous lipid droplets (Lp). Lu = lumen of the duct, Nu = nucleus, Mt = mitochondria; CC = ciliated cell. **B** – supranuclear region of the NC4 containing compactly packed vacuole-like secretory granules of varying electron-density (Sg) surrounded by elongated mitochondria with electron-dense matrix (Mt), Jc = junctional complex.

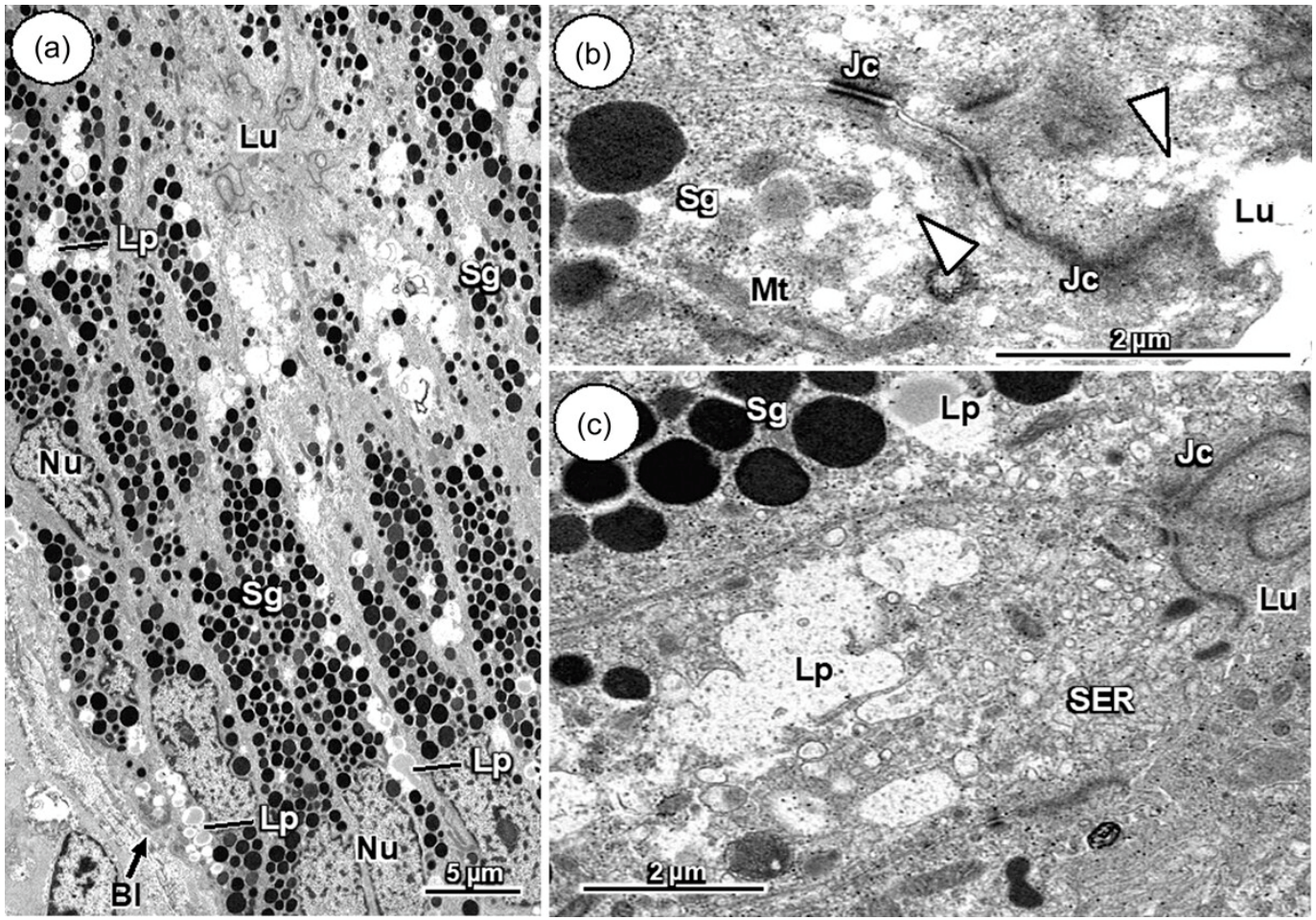

Fig. 12: A is a survey micrograph of a tubular or mucosal gland displaying cells laden with electron-dense secretory granules (Sg), Bl = basal lamina, Lp = lipid droplets. **B** and **C** are apical parts of the cytoplasm. White arrowheads = small electron-lucent vacuoles, Sg = moderately electron-dense secretory vacuoles, SER = smooth endoplasmic reticulum, Jc = junctional complexes, Lu = duct lumen, Lp = lipid droplets.

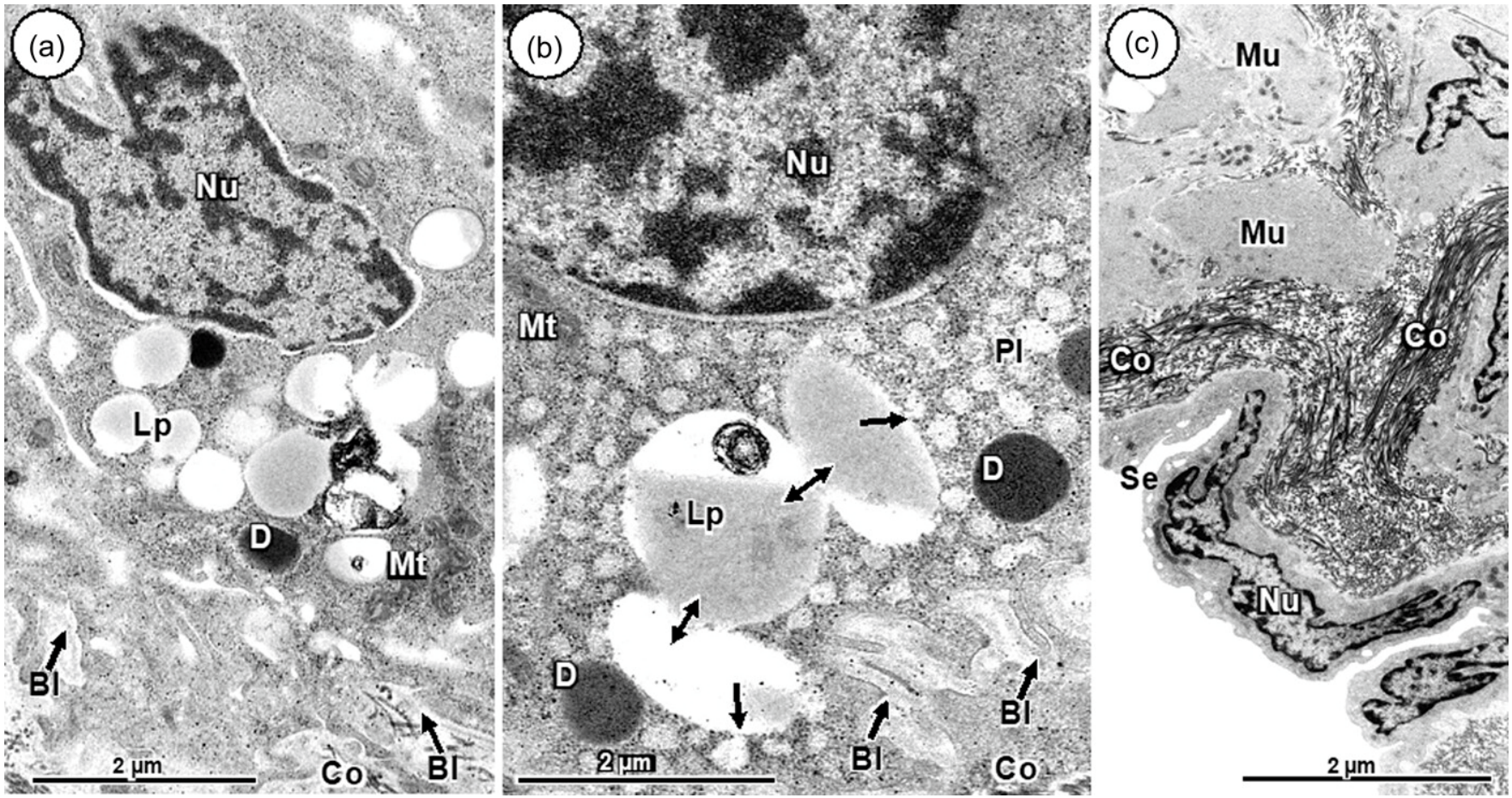

Fig. 13: Basal part of the tubular or mucosal gland epithelium. In **A**, numerous lipid droplets (Lp) in the infranuclear region, D = dense granule, Mt = mitochondria, Bl = basal lamina. In **B**, numerous, small droplets (Pl) abound in the infranuclear region and are seen to contribute to the formation of large lipid droplets (Lp). Black arrows = fusion of small lipid droplets with the large ones, up-down arrows = fusion of large lipid droplets. Bl = basal lamina, Co = collagen bundles. In **C,** Mu = smooth muscle bundles, irregular outline of serosa (Se) with its elongated and irregularly shaped nuclei (Nu).

mucosal gland and the ductal epithelial lining (Fig. 9). The serosa is generally irregular in outline (Fig.13 C), and contains irregularly elongated, euchromatic, nuclei with moderate chromatin margination.

DISCUSSION

The infundibulum was clearly and easily distinguished from the uterus, based upon their gross features and colour differences in the sexually mature and active green iguana. The gross features of the infundibulum of the green iguana are similar to those reported for most reptiles in terms of position and general structure. According to Girling (2002), changes in the mucosa of the infundibulum in certain reptilian species were sufficient to divide this segment of the oviduct into anterior and posterior regions. The ostium is wide and has fimbria-like processes that can surround the ovary or ovum. The lining epithelium of the lumen runs over the lateral aspect of the fimbriae up to a certain level before terminating and yielding to the serosa, as has been reported in the gecko (Girling, 1998). The significance of this is unknown.

As has been observed in the green iguana, Siegel et al. (2015), in their review, show that several taxa of squamates and lizards display two main portions of the infundibulum: the cranial division and the caudal division. They state that glandular-like invaginations, given varying names, occur at the boundary or junction between these two divisions, as has also been found in the iguana. The invaginations have been referred to as ‘crypts’ or ‘sacs’ in various lizards, for

example, in the *Podarcis sicula* (synonym: *Lacerta sicula*) (Botte 1973b), *Sceloporus bicanthalis* (Guillette and Jones 1985; Villagrán-Santacruz et al., 2017), *Sceloporus aeneus* (Guillette and Jones 1985), Keeled Earless Lizard (*Holbrookia propinqua*; Adams and Cooper 1988), and *Lepidodactylus lugubris* (Saint-Girons and Ineich 1992). Variants of these grooves or crypts have been variously described and referred to as branched tubular glands in *Hemidactylus mabouia* (Nogueira et al*.* 2011), alveolar glands in *Hoplodactylus* maculatus (Girling et al*.* 1997), or plain "glands" in *H. duvaucelii*, *Hemidactylus turcicus*, *Saltuarius wyberba*, in the black swamp snake, Seminatrix pygaea (Sever and Ryan, 1999), Sphenomorphus *fragilis* (Guillette 1992), in the Fossorial Snake, *Apostolepis gaboi* (Braz et al., 2019). Amerotyphlops brongersmianus (Khouri et al., 2020), and the Amazonian lancehead, Bothrops atrox (Silva, et al., 2020). It is generally agreed that the epithelium of these crypts is columnar and comprises ciliated and non-ciliated cells, as found in the lining epithelium of the oviduct.

Although spermatozoa were not seen in these deep grooves or crypts in the iguana studied, several authors regard them as sperm storage sites. We agree with this assertion because the grooves were similar, structurally, to those found in birds (Van Drimmelen, 1946; Fujii and Tamura, 1963; Bakst and Bird, 1987), which act as temporary storage sites for spermatozoa. Robbins et al. (2021) noted that seminal receptacles or crypts in squamates are generally found in the vagina (non-glandular uterus) or anterior oviducts (posterior uterine tube and infundibulum), with considerable variation in both number and placement (Cuellar 1966; Girling 2002; Sever and Hamlett 2002; Eckstut et al. 2009; Siegel et al. 2015).

In this study, the caudal division of the infundibulum of the green iguana displayed simple tubular glands in the lamina propria. The glands in the posterior infundibulum and vagina of *Seminatrix pygaea* and *Agkistrodon piscivorus* have been described as simple or compound tubular, whereas glands in the uterus were simple tubular (Sever and Ryan, 1999).

It must be stressed that the mucosal tubular glands of the iguana comprised only non-ciliated cells surrounding a small lumen. With regard to sperm storage sites in the entire oviduct, Siegel et al. (2015) has observed correctly that ‘a considerable amount of variation has been found in the ultrastructure of female sperm storage in the few lizards that have been examined and the data from light microscopy on other lizards hint that the full panoply of variation has yet to unfold.’

Cytologically, the luminal epithelium of the fimbrial part comprises mainly non-ciliated type 1 cells, with few interspersed ciliated cells. Girling (1997, 1998), Guillette et al. (1989), Uribe et al. (1988), Perkins and Palmer (1996), Nogueira et al. (2011), Siegel et al. (2015) indicate that the epithelium of the fimbrial end of the infundibulum in several reptilian species comprises ciliated cells, predominantly. In their study of the oviduct of the green iguana, Tsuruno et al. (2011) observed, *inter alia,* that ‘almost none of the epithelial cells had cilia, although a small number of epithelial cells with poorly developed cilia were present’, as corroborated in the present study. Therefore, it is clear that there is either a discrepancy in specific observations or that there is a great deal of variation in cellular content of the epithelium of the infundibulum of the reptilian oviduct.

There are no marked structural differences in the non-ciliated type cells in the

anterior part of the infundibulum but those in the more posterior parts have secretory granules that are variably electron-dense. All the non-ciliated type 1 cells are laden with secretory granules, some of which can be seen undergoing exocytosis. This indicates that the non-ciliated cells in this part of the infundibulum employ merocrine type of secretory activity. No other form of secretory activity has been observed. It is not inconceivable that the fimbrial part of the infundibulum does secrete some substance that is incorporated in the ovum as it passes through. It is noteworthy that the infundibulum is ideally placed to secrete materials directly onto the ovulated egg prior to albumen and shell secretion (Girling, 2002). Palmer et al. (1993) consider that secretory material is deposited around the ovum as soon as it enters the fimbrial part of the infundibulum in oviparous lizards. The nature of the secretions added to the ovum are not known to-date, but it is speculated that they may be albumen proteins (Palmer et al., 1993) or just mucus for the lubrication of the oviduct in facilitation of egg passage (Botte, 1973; Aitken and Solomon, 1976; Girling et al., 1997, 1998). In the lizard, *Podarcis sicula*, alkaline and acid phosphatase was observed along the cells of the epithelium in the infundibulum. Botte (1973b) speculated that these cells are involved in secretory activities especially because of the presence of the enzyme alkaline phosphatase along the walls of blood vessels and capillaries.

The distal part of the cranial division of the infundibulum first displays mucosal folds, the crests of which are mainly lined by ciliated cells, with non-ciliated type 1 cells occupying the grooves. As this portion runs more distally, the mucosa transforms into low, broad folds, which, along with the grooves between them, are

lined by a similar epithelium containing both ciliated and non-ciliated cells. However, the latter cells, which are variants of the non-ciliated type 1 cells exhibit secretory granules of varying electron-density. This might indicate differences in the formative process of the granules or in their respective constitution. Remarkably, the ciliated cells contain aggregations or deposits of glycogen. The staining of the tissue sections with lead citrate is a pre-requisite for the identification of glycogen granules or deposits in electron microscopy (Revel et al., 1960; Revel, 1963; Lafontaine & Allard, 1964; Fawcett, 1981). Although very little is known of the nature of secretions produced by the infundibulum (Girling, 2002), the non-ciliated cells of the infundibulum of three species of the gecko were positive for carbohydrate that occurred as numerous secretory granules of varying electron density in the apical regions of cells (Girling et al., 1998). However, in the present study and in this part of the infundibulum, the non-ciliated type 1 cell and its variant did not show glycogen deposits.

In the caudal division of the infundibulum or tubular part of the infundibulum, not only did the luminal epithelium contain three types of non-ciliated cells that were different from the non-ciliated type 1 cell in the cranial division of the infundibulum with regard to content, but also, this portion of the oviduct displayed a row of sub-epithelial, simple, non-branched, tubular or mucosal glands in the lamina propria. Whereas the epithelial lining had a number of sub-epithelial blood capillaries, they were not found around the tubular glands, probably indicating that the epithelial lining was a very active part of the duct. There were three types of non-ciliated

cells (types 2, 3, and 4) in the epithelial lining of the tubular part of the infundibulum. The non-ciliated type 2 cells in the epithelial lining of the infundibulum contained dense aggregations of mitochondria in the infranuclear region of the cell, and glycogen deposits in that part of the cytoplasm surrounding the nucleus. It is interesting that the non-ciliated types of cells in the tubular part of the infundibulum did not display any obvious secretory granules that were seen in the cranial division of the infundibulum, a possible reflection of differences in content or composition of their secretion(s), too. It is noteworthy that this is the first time that glycogen deposits have been described in the oviduct of any reptile, ultrastructurally. The apical end of a variant of one of the non-ciliated type 2 cell, i.e. the non-ciliated type 3 cell, did not project into the duct lumen, but its infranuclear region was filled with large accumulations of glycogen. Although the apical portions of the non-ciliated type 2 protruded slightly into the duct lumen, there was no evidence of apocrine or holocrine activity. The presence of a large number of lipid droplets in both the supranuclear and infranuclear regions of the non-ciliated type 2 cytoplasm may be an evidence of lipid secretion into the lumen of the duct by these cells. The |non-ciliated type 4 cells in the tubular part of the infundibulum are probably similar to the so-called ‘bleb cells’ described by several authors in the infundibulum of certain other reptiles (Palmer & Guillette, 1988; Girling et al., 1997, 1998; Girling, 2002; AlKindi et al., 2006). It is also noteworthy that the non-ciliated type 4 cells did not contain glycogen deposits but their supranuclear and subnuclear zones were filled with lipid droplets.

Although PAS-positive staining of the epithelial lining of the infundibulum of reptiles

has been reported in several species (Botte,1973a; Guillette et al., 1989; Picariello et al., 1989; Kumari et al., 1990; Girling et al., 1997, 1998; Girling, 2002; Machado-Santos et al., 2015), glycogen deposits have not been confirmed ultrastructurally. The significance of the large glycogen deposits in both ciliated and non-ciliated cells of the epithelial lining of the infundibulum of the green iguana is not known. Glycogen is a storage version of glucose in cells and tissues, and it is readily converted to glucose, which is easily released in tissues (Roach et al., 2001; Nielsen & Ortenblad, 2013; Prats et al., 2018). Could these vast amounts of glycogen be reserves in parts of the oviduct where a rapid release of glucose is periodically necessary? In male animals, glycogen accumulations have been demonstrated in epithelia of mesonephric tubules and ductules (de Martino & Zamboni, 1966; Tiedemann, 1971; Pelliniemi et al., 1983), and, in particular, in the reproductive organs, such as in the tubulus rectus of the guinea pig testis (Fawcett & Dym, 1974) and the efferent and closely related ducts of the golden hamster (Ford et al., 2014). Their functions in these tissues and cells are unknown, although Ford et al. (2014) consider that these deposits may be associated with the potential for hibernation in hamsters and subsequent reproductive tract regression and recrudescence. In female mammals, Dean (2019) observed that glycogen in the endometrium of the uterus and Fallopian tube was an essential source of glucose during the peri-implantation period. In the domestic fowl, Gilbert et al. (1968) observed glycogen deposits in the host cells of the utero-vaginal region, but not in the general surface epithelium. It is intriguing/instructive to learn that they found considerable amounts of glycogen in the gland lumen of a bird

killed shortly after oviposition.

The tubular/mucosal glands comprised only non-ciliated (could be classified as non-ciliated type 5) cells, which had a similar content (dense secretory granules and numerous lipid droplets) as the non-ciliated type 1 cell in the epithelial lining of the cranial part of the infundibulum.

CONCLUSION

Cytologically, non-ciliated type 1 cell and its variant were the predominant cell types in the cranial division of the infundibulum and three (types 2, 3, and 4) in the caudal, tubular division. The epithelial lining of the posterior aspect of the cranial division displayed grooves or crypts while the caudal division contained tubular mucosal glands. Remarkably, a hitherto unreported observation was the presence of glycogen accumulations in both non-ciliated and ciliated cells in parts of the epithelial lining of this duct. It is therefore evident that the infundibulum does not act as a passageway only for the ovum, but also contributes to the formation of the developing egg during the passage of the ovum through this segment of the oviduct. Based on current knowledge and understanding, it is suggested that further studies, including immunohistochemical investigations, are necessary to gain further insight into the function of the infundibulum in the Iguanidae family of squamate reptiles.

ACKNOWLEDGEMENT

The authors thank the Ministry of Agriculture, Lands and Forestry of Grenada for giving permission for the use of the animals and St. Georges University Institutional Animal Care and Use Committee for approving the animal procedures used in this study. Funding was provided by St. Georges University Small Research Grant Initiative and we are grateful to the University of Pretoria, South Africa that kindly provided facilities for electron microscopy.

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CONFLICT OF INTEREST DISCLOSURE

There are no conflict of interest

AUTHORSHIP CONTRIBUTIONS:

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C-A. Harrylal, S. K. Gupta, T. A. Aire: Funding acquisition

C-A. Harrylal, A. V. Lensik , S. K. Gupta, T. A. Aire: Investigation

C-A. Harrylal, S. K. Gupta, T. A. Aire: Methodology

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