ALLANTOPLACENTAL ULTRASTRUCTURE OF AN ANDEAN POPULATION OF *MABUYA* (SQUAMATA, SCINCIDAE)

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RESUMEN

TÍTULO: **ULTRAESTRUCTURA DE LA ALANTOPLACENTA DE UNA POBLACIÓN ANDINA DE *MABUYA* (SQUAMATA, SCINCIDAE)**[′]

AUTOR: **JOSE FERNANDO CARREÑO ESCOBAR**[″]

PALABRAS CLAVES: **Membranas fetales; *Mabuya*; matrotrofía; placenta; reptiles; viviparidad**

Descripción

Las especies de *Mabuya* son lagartos vivíparos altamente matrotróficos con alantoplacenta epiteliocorial tipo IV.La alantoplacenta de una población andina de este genero posee especiaizaciones relacionadas con la nutrición histotrófica en el hemisferio embriónico (placentoma, paraplacentoma Y areolas corionicas); mientras que en el hemisferio abembriónico se puede observar una función combinada: Transferencia histotrófica (placas de absorción) y nutrición hemotrófica (intercambio de gas en los segmentos respiratorios). Estas especializaciones placentales fueron estudiadas usando microscopia óptica de alta resolución y microscopía electrónica de transmisión, y comparadas con las encontradas en otros reptiles escamados y mamíferos euterios.

Los rasgos citológicos del placentoma sugieren que esta es la region más importante para la provisión de nutrientes; El paraplacentoma también muestra características de transferencia de nutrientes, especialmente lípidos. Las areolas coriónicas permiten la absorción de productos glandulares, al igual que restos uterinos y coriónicos producto de la lisis de algunas células durante la formación de la areola. En las placas de absorción el epitelio uterino y coriónico esta firmemente unido a sus ápices celulares que muestran gránulos electrodensos que se pueden relacionar con funciones paracrinas y autocrinas. La distancia interhemal corta encontrada en los segmentos respiratorios confirma su error en el intercambio de gases. La complejidad placental observada en estas especies de *Mabuya* es mas alta que en cualquier otro reptil, lo cual indica una convergencia evolutiva a nivel histológico y citológico entre ambos clados. Sin embargo, ninguna especie de mamíferos euterios muestra simultáneamente todas estas especializaciones en la cámara embriónica como lo muestra *Mabuya*.

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ABSTRACT

TITTLE: **ALLANTOPLACENTAL ULTRASTRUCTURE OF AN ANDEAN POPULATION OF *MABUYA* (SQUAMATA, SCINCIDAE)** [*]

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KEY WORDS: **membranes; *Mabuya*; matrotrophy; placenta; reptiles; viviparity**

DESCRIPTION

Mabuya species are highly matrotrophic viviparous lizards with type IV epitheliochorial allantoplacenta. The allantoplacenta of an Andean population of this genus possesses specializations related to histotrophic nutrition at the embryonic hemisphere (placentome, paraplacentome and chorionic areolas); while at the abembryonic hemisphere it has a mixed function: Histotrophic transfer (absorptive plaques) and hemotrophic nutrition (gas exchange in respiratory segments). These placental specializations were studied using high resolution light microscopy and transmission electron microscopy, and compared with those found in other squamate reptiles and eutherian mammals. Cytological features of the placentome suggest that this is the most important region for nutritional provision; the paraplacentome also shows characteristics for nutrient transference, especially lipids. Chorionic areolas allow the absorption of glandular products, as well as uterine and chorionic cellular debris produced by lysis of some cells of both epithelia during the areola formation. In the absorptive plaques both uterine and chorionic epithelia are firmly attached and their cellular apexes exhibit electron dense granules that could be related to autocrine and paracrine functions. The short interhaemal distance found in the respiratory segments confirms their role in gas exchange. A common feature of all regional specializations in the *Mabuya* allantoplacenta is the presence of lipids in the interacting chorionic and uterine epithelia, suggesting that lipids are transferred throughout entire embryonic chamber; placental transfer of lipids may be the principal fetal energy and lipid source in this species. The placental complexity observed in this species of *Mabuya* is greater than in any other reptile, and resembles that of eutherian mammals: Each one of these specializations of the placental membranes in *Mabuya* is similar to those found among different eutherian mammals, indicating a very impressive evolutionary convergence at histological and cytological levels between both clades.

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INRODUCTION

The allantoplacenta or chorioallantoic placenta is the term used for placental structures in which the fetal contribution consist of the chorion, allantois, and sometimes amnion, but does not include any part of the yolk-sac endoderm (Yaron, 1985). Weekes (1935) described three types of chorioallantoic placentas in lizards based in cytological and histological features; moreover, the presence of specialized structures for maternal-fetal nutrient and gas exchange in the Neotropical *Mabuya heathi*, lead to the recognition of a new placental morphotype, the “type IV” allantoplacenta (Blackburn and Vitt, 2002).

The lizard family Scincidae (Squamata) shows the major diversity in placental morphologies among amniotes (Stewart and Thompson, 2000). Among them, *Mabuya* species have the most complex and specialized of the squamate allantoplacentae, the type IV allantoplacenta. This clade shows an evolutionary convergence with the eutherian mammals for fetal nutrition (Blackburn et al., 1984; Blackburn and Vitt, 2002), and other reproductive specializations like long gestation period, ovulation of tiny ova (microlecithic), and placental provision of virtually all of the nutrients for development (Blackburn et al., 1984). Flemming and Blackburn (2003) suggested that these features are widespread, if not universal in New World *Mabuya*, including *M. agilis*, *M. brachypoda*, *M. caissara*, *M. frenata*, *M. heathi*, *M. nigropunctata*, *M. macrorhyncha* and *M. mabouya;* however, placental morphology has been studied only for three of these species (*M. heathi* Blackburn et al., 1984, Blackburn and Vitt, 2002, *M. nigropunctata* Vitt and Blackburn, 1991, and an Andean population temporally assigned to *M. mabouya* Jerez and Ramírez-Pinilla, 2001, 2003). Recently, Mausfeld et al. (2002) divided *Mabuya* genus into four different genera, being the name *Mabuya* kept only for this American clade, which seems to constitute a monophyletic group (Greer et al., 1999; Mausfeld et al., 2002; Carranza and Arnold, 2003). Interestingly, the African skinks *Eumecia anchietae* and *Trachylepis ivensii* share reproductive specializations with New World *Mabuya* as ovulation of microlecithic ova, intimate apposition of fetal and maternal tissues after breakdown of egg shell, development of an absorptive chorioallantois, and development of an unusual yolk sac that lacks of an isolated yolk mass (Flemming and Branch, 2001; Flemming and Blackburn, 2003).

The ultrastructure of allantoplacentae in Squamata has been studied in species with relatively simple allantoplacentas (*Virginia straiatula*, Stewart and Brasch, 2003; *Eulamprus tympanum,* Hosie et al., 2003; *Thamnophis ordinoides*, *T. sirtalis*, *T. radix*, Blackburn et al., 2002, Blackburn and Lorenz, 2003a) and complex allantoplacentas (*Pseudemoia entrecasteuxii,* Adams et al., 2005); but not in placentotrophic *Mabuya*, in which are found different and highly specialized structures for placental exchange.

In the allantoplacenta of an Andean population of *Mabuya,* Jerez and Ramírez-Pinilla (2001) described several placental specializations related to hemotrophic and histotrophic transport: At the mesometrial hemisphere (placentome, paraplacentome, and chorionic areolas), and at the antimesometrial hemisphere (absorptive plaques and respiratory segments). The present study describes, using high resolution light microscopy and TEM, the cytological features of these specializations in late pregnant females from the same population of *Mabuya.* These features are compared with those known for other squamate reptiles and eutherian mammals.

1. MATERIALS AND METHODS

Mabuya pregnant females were obtained by manual collection in the Inspección de Policía de Guadualito, Municipio de Yacopí, Departamento de Cundinamarca, Colombia (N: 05° 37´, W: 74° 18´, 840 m altitude). The females were the same used in past studies (Jerez and Ramírez-Pinilla, 2001, Ramírez-Pinilla et al., 2002). Seven pregnant females with embryos of stages 39 and 40 (following the table of Dufaure and Hubert, 1961) were studied. For the study of absorptive plaques we also included embryonic chambers of one female in embryonic stage 31. The incubatory chambers were dissected to obtain small pieces of placental tissues that were fixed in 2.5% glutaraldehide in Millonig buffer (pH 7.3) and post-fixed in osmium tetroxid (1%). The tissues were embedded in Epon-Araldite, sectioned at 0.1 µm – 2 µm for semithin sections and 60 nm for ultrathin sections in a LKB 480 ultramicrotome. Semithin sections were stained with toluidine blue dye; the high resolution photomicrographs were taken using a Nikon microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate. All the allantoplacental structures (placentome, paraplacentome, chorionic areolas, absorptive plaques, and respiratory segments) were observed, described and photographed using a Jeol JEM Transmission Electron Microscope.

To describe the histology and ultrastructure features of the allantoplacenta of this population of *Mabuya* we followed the terminology employed by Blackburn (1993, 2000), Mossman (1987), Stewart and Thompson (2000) and Jerez and Ramírez-Pinilla (2001, 2003).

2. **RESULTS**

Placentome

The placentome is an area located dorsally to the embryo; its distinctive feature is the very strong interdigitation between the villous folds of uterine endometrium and chorioallantois. The interacting epithelia consist of a uterine syncytium, and a hypertrophied chorionic epithelium with giant and interstitial cells. The interdigitation between the uterine villous folds and corioallantois invaginations follows a radial pattern emerging from the embryonic pole.

The chorionic and uterine epithelia form a wide microvillar band (~ 7 µm) (Fig. 1a) that consists of straight and long microvilli protruding from both epithelial surfaces. They are closely interdigitated with no significant gaps. The uterine epithelial syncytium has a pale staining cytoplasm with fine reticular material, small lipid droplets, and small clear vesicles; the nuclei are spherical with one nucleolus and without heterochromatic material. The chorion has a basal layer of squamous cells and an external layer constituted by two cellular types: very thin cells (interstitial cells), and large and broad binuclear cells (giant cells) (Fig. 1b). The giant cells exhibit a homogeneous dark stained cytoplasm that contains small vesicles, abundant lysosomes and lipid droplets in the lateral and basal regions; their nuclei are ovoid with one or two prominent nucleoli. Occasionally, inserted into the apex of the uterine syncytium, thin cells with dense cytoplasm are found. Their ultrastructure is similar to that in the interstitial cells and they can extend as far as the uterine epithelial basement membrane (Fig. 1a).

At the ultrastructural level, the chorionic-uterine interface is seen to be occupied by a broad microvillar band consisting of microvilli from the uterine epithelium interdigitated closely with microvilli from giant cells of the chorionic epithelium. Between the bases of the uterine microvilli are found inpocketings of the plasmalemma budding off coated and uncoated vesicles into the uterine epithelial cytoplasm. On the chorionic side, in contrast there are narrow tubular extensions of the plasmalemma into the apical cytoplasm (Fig. 3). At lower magnification, the chorionic cytoplasm is stained homogeneously and its moderate electron density allows the observation of numerous and small mitochondria, and lysosomal bodies with heterogeneous content; some of them may be secondary lysosomes (Fig. 4). Higher magnification reveals an extensive RER with dilated cisternae that contains stored products (Fig. 5). Giant and interstitial cells have highly folded basolateral membranes with multiple projections, these defining irregular spaces loosely interlocked with each other, and close to lymphatic and blood vessels. Interstitial cells have a very slender shape, with RER surrounding the nuclei, mitochondria, and some lysosomes; the nuclei are ellipsoid, heterochromatic, and with prominent nucleoli (Fig.4).

The ultrastructural investigation confirmed the existence of a uterine syncytium since no intercellular membranes with tight junctions around their apices were found (Fig. 6a). The cytoplasm is rich in small mitochondria (0.19 ± 0.04 μm equator width), polyribosomes, and lysosomes. The cytoplasm shows cisternae of rough and smooth endoplasmic reticulum around the nucleus. At the basal region, rough endoplasmic reticule (RER) profiles (Fig. 6b) and empty vesicles predominate; some big lipid droplets are also observed in the cytoplasm. The uterine epithelium is underlain by a basement membrane below which there is a thin layer of connective tissue in which capillaries and other blood vessels are abundant and very close to the epithelium. Indications of localized invasion of the uterine syncytium by chorionic cells were observed occasionally in the ultrathin sections (Fig. 6c). The invader chorionic cell has a small nucleus, and a cytoplasm with RER cisternae and electron dense granules.

Paraplacentome

The paraplacentome is a narrow zone, peripheral to the placentome, at the dorsal hemisphere of the incubatory chamber; it is seen macroscopically like a narrow yellow band ventral to the placentome. The uterine endometrium and chorioallantois are not folded in this region. Also, there are no apical uterine or chorionic microvilli, like those observed in the placentome. Furthermore, the uterine syncytium is replaced abruptly by a columnar uterine epithelium with well defined tight junctions at their apices (Fig. 7). The cytoplasm of uterine epithelial cells is paler than those of the chorion; it contains numerous lipid droplets that almost fill the whole cell volume, and fuse forming large droplets (Fig. 8). Alveolar uterine glands can be found in the lamina propria; gland ducts or secretion granules could not be seen, although glands contain densely stained material at their lumen. The external layer of the chorionic epithelium is similar to that of the placentome, composed by giant and interstitial cells.

At the ultrastructural level, the paraplacentomal uterine and chorionic epithelia adjacent to the placentome display irregular apexes with developed interdigitant convoluted cytoplasm projections (Fig. 9); at the paraplacentomal region these cytoplasmic projections disappear and the apical surfaces of both uterine and chorionic epithelia do not interdigitate. The chorion-uterine interface is very thin and no material is observed between the epithelia. The uterine epithelium has well defined basolateral membranes; the cells have nuclei with dispersed heterochromatin or aggregated close to the nuclear envelope. Numerous small mitochondria and vesicles of heterogeneous sizes with dispersed fine material are present in the cytoplasm where short cisternae of rough endoplasmic reticulum are common (Fig. 10).

The cytoplasm of chorionic cells has numerous vesicles surrounded by membranes that correspond to lysosomal bodies that contain residual material; some of them are completely filled with this material (fig. 9). At the basal region the lysosomal bodies are fewer than in the apex, and the cytoplasm shows dilated cisterns of RER (Fig. 11); in the basolateral areas are observed abundant lipid droplets and highly convoluted membrane projections between cells. Also at this region, allantoic lymphatic and uterine blood vessels are in close proximity to one another (Fig. 12).

Chorionic Areolas

In the studied population of *Mabuya* the chorionic areolas are found at the dorsal hemisphere, out of the paraplacentomal region; few of them reach the equatorial region of the incubatory chamber. They can be recognized only at the light-microscopic level; macroscopically, they do not differ from absorptive plaques. The areola formation is initiated at stage 39 of development. The chorion proliferates toward the allantois forming a pluristratified saccular structure and the uterine epithelium exhibits a slight evagination (Fig. 13). Lipids accumulate inside chorionic cells forming large lipid droplets that make the observation of cell borders difficult. The fold of the uterine epithelium is progressively extended toward chorion (Fig. 14a), and the areolar cavity is originated by gland secretion and cellular degeneration of apical cells of chorionic epithelium (Fig. 14b). The areolar cavity is invaded by glandular secretions and vacuolated and granular rests of cytoplasm released from the endometrial and chorionic cells. Endometrial glands open short ducts to the cavity of the areola; although a mixed secretion is observed in the glandular lumen of sections stained with toluidine blue (small granules and condensed substance), intracellular granules cannot be seen (Fig.15a). The glandular content stored in the lumen is released in the areola concavity (Fig. 15b). Abundant lymphatic and blood vessels underlie uterine and chorionic epithelia.

Ultrastructurally, some luminal uterine epithelial cells have an intact apical membrane. However, in most of them the apex is broken and massively expulses cytoplasmic material in dissolution and lipid droplets of all sizes (Fig. 16). This material disperses into areola cavity, in front of chorionic epithelium. Uterine epithelial cells are joined by junctional complexes. The apical portion of the cytoplasm exhibits small and smooth vesicles and RER cisternae; abundant lipid droplets are distributed throughout all cytoplasm. Plasma membranes of neighboring cells are very close and follow a straight trajectory. Blood vessels are in tight contact to the epithelial basal lamina. The chorionic epithelium consists mainly of giant cells with irregular apexes that exhibit short and thin projections to which is adhered abundant interface material. The nucleus is euchromatic with small heterochromatin aggregates (Fig. 17). The apical region of the cell contains big lipid droplets, and abundant both spherical and irregular vesicles with granular fine content and membrane debris. Loosely interlocked intercellular convoluted membranes define the neighboring cellular spaces.

Uterine glands have columnar cells with short microvilli in the apex (Fig.15c). Few electron dense granules of heterogeneous forms and sizes are distributed in the apical region. Some isolated granules are observed also in the basal region. Some cells release secretory granules to the lumen; since both the granules and stored secretion products in the lumen have similar electron density, granule fusion process apparently occurs at the glandular lumen. At low magnification, conspicuous junctional complexes are observed and cytoplasmic membranes run parallel without an appreciable space between them. In some cells, basolateral sinuous membranes surround vesicles located laterally*;* these vesicles have irregular form and size, and a granular fine dispersed and membranous content. The cytoplasm displays elongated mitochondria and abundant RER, especially in the basal region. Uterine lumen at the areola concavity is filled by aggregations of cytoplasmic granular material, cellular debris as membranous vesicles, mitochondria, lysosomes, lipid droplets, and glandular secretion products (Fig.18).

Absorptive Plaques

The absorptive plaques are located at the abembryonic hemisphere of the incubation chamber, ventrally to the chorionic areolas area. Approximately 50-60 absorptive plaques were estimated per incubatory chamber. Hypertrophied chorionic and uterine epithelia are in close association (Fig. 19, 20); the chorion has two layers, in the very tall external layer are found both giant and interstitial cells. The cytoplasm above the nuclei of chorionic cells stains deeply. One noteworthy feature of the giant cells is the presence of one to three big lipid droplets beneath the nucleus; lipid droplets inside chorionic cells at other cell regions are numerous and small. The luminal uterine epithelial cells have basal nuclei, some of them are binucleated. Small lipid droplets practically fill the cytoplasm; some are fused forming great lipid lagoons.

At ultrastructural level, the chorionic and uterine epithelial apexes are closely adherent (Fig. 21a) displaying different junctional complexes: In certain areas appear be joined by gap junctions and others exhibit junctional complexes similar to zona adherens and desmosomes (Fig. 21b). A very close association is observed between cytoplasmic short interdigitant projections of both epithelia; focal joint points between both apical membranes are found. Uterine apical cytoplasm is rich in lipid droplets and organelles (Fig. 22); it contains small electron dense granules which cannot be easily observed at semi - thin sections (see Fig. 21b). As in other regions, uterine neighbor cells are joined by complex junctional structures in apical, lateral and basolateral regions; adjacent membranes run parallel without visible spaces between them. The apical cytoplasm of giant chorionic cells contains lysosomes, electron dense granules, dilated cisternae of RER, and abundant smooth ER (Fig. 23). The lateral region has short projections that interlock with neighbor cells; depending on level, the adjacent cells can be giant or interstitial, since interstitial cells are shorter and do not reach the apical portion of the chorionic epithelium. Nuclei of the giant cells are located centrally. Interstitial cells are very thin and elongated; their cytoplasm displays lysosomes, mitochondria, and basal lipid droplets, the euchromatic nuclei have elongated shape. Squamous cells of the chorionic basal layer have numerous small lipid droplets and are associated with others through cytoplasmic projections. Abundant allantoic capillaries are adjacent to this layer of chorionic cells (Fig. 24).

Respiratory Segments

The respiratory segments of the allantoplacenta are found out of the polar placentomal - paraplacentomal region of the incubatory chamber. A profuse subepithelial vascularization in the intimately associated chorionic and uterine epithelia characterizes the respiratory segments (Fig. 25). The height of the chorionic and uterine epithelia decreases progressively during last stages of development. This causes the uterine and chorionic blood vessels approach so closely that the interhaemal distance is approximately only 6 - 9 µm (Fig.26). The allantois is vascularized by numerous capillaries that contact with the basal lamina of the chorionic squamous cells. Lymphatic vessels are in the allantois external portion or between blood vessels; in the uterus they underlie the epithelium and exhibit several lymphocytes and neutrophiles. Active uterine glands can be also observed; however, gland ducts and openings were not observed.

Ultrastructurally, the chorionic epithelium is low and resembles the endothelium of blood vessels; the chorionic apex display very small projections leaving a very small lumen between the epithelia (Fig.25). The uterine epithelial apex has no cytoplasmic projections. Adjacent lateral and basolateral membranes are in close contact, and have parallel and some sinuous course. The apical region shows junctional complexes. The large nuclei of uterine epithelia have an irregular shape and small nucleoli. The cytoplasm exhibits short cisternae of rough and smooth endoplasmic reticulum, dispersed small mitochondria, and small lipid droplets. At late stage 40, the uterine epithelium is cuboidal to flat; nuclei, with small nucleoli, occupy a large portion of cytoplasm volume; rough endoplasmic reticulum is observed and lipid droplets are fewer than in previous stages. At the basal region, the uterine epithelial cells are broadened, increasing the surface between them and the endothelium of blood vessels; however, there are always basal laminae between them.

3. DISCUSSION

The combination of all the allantoplacental specializations (placentome, paraplacentome, chorionic areolae, and absorptive plaques) distinguishes the American *Mabuya* from all viviparous squamates. Moreover, these morphological specializations that allow the nutrient exchange in the *Mabuya* allantoplacenta are similar to those found among placentas of eutherian mammals. The placentome has been described in ruminant mammals (Hoffman and Wooding, 1993; Wooding et al., 1996b; 1997). The chorionic areolas are present in camelids (*Lama pacos* Olivera et al., 2003a, and *Camelus dromedarius* Abd-Elnaeim et al., 2003), in equids (Wooding et al*.,* 2000; 2001), and in pigs (Bielanska-Osuchowska and Kunska, 1995). Absorptive plaques are structures associated to mammal groups that possess villous epitheliochorial and endotheliochorial labyrinthine placentas (Mossman, 1987). The respiratory segments correspond to gas exchange areas widespread in mammal groups (Enders and Blakenship, 1999). However, the variety of combined specializations observed in *Mabuya* incubatory chamber has not been described in any mammal species. Since this is a highly matrotrophic species, it is suggested that each one of these allantoplacental specializations should have specific functions in the nutrient exchange. This study provides a detailed morphological description of chorionic and uterine cellular components of each allantoplacental specialization to infere functional features, and compares them with similar structures in squamates and eutherian mammals.

Placentome

The observed morphological features in the *Mabuya* placentome suggest that this is the most important region for nutrient transference. This specialized region is observed from earlier developmental stages (from gastrula stages) with a similar morphology to the absorptive plaques (Jerez and Ramírez-Pinilla, 2003), suggesting an active and early nutrient transfer of molecules; however, its highest specialized features, as the interdigitant uterine and chorionic mucosal folds bearing long and thin microvilli, syncytial uterine epithelium, invasive chorionic cells in the uterine syncytium, and features of high metabolic activity, are observed only during the final stages of development (fetal growth stages).

The placentome of this population of *Mabuya* was considered synepitheliochorial due to the formation of a uterine epithelial syncytium in front of the chorionic epithelium (Jerez and Ramírez-Pinilla, 2001). Ruminant mammals exhibit a cotyledonary epithelichorial placenta, which shows five or eight placentomes in the embryonic chamber (Mossman, 1987); each placentome is conformed by a sincytium (Wooding et al., 1997). This syncytium is originated by the fusion of endometrium and binucleated trophoblastic cells, forming a hybrid syncytium; due to the hybrid nature of ruminant placentae this receives the name of synepitheliochorial (Hoffman and Wooding, 1993; Wooding et al., 1997). The observation in the placentome of *Mabuya* of some invasive protruding chorionic cells on the uterine syncytium could suggest a chorionic invasion that can approximate them to the endothelial endometrium; however, cellular fusion between chorionic and syncytial endometrial cells was not observed and then a hybrid nature in *Mabuya* syncytium is not suggested as in ruminant mammals. Chorionic invasion on the uterine epithelium might be a common feature in highly matrotrophic squamates since for the highly matrotrophic African lizard *Trachylepis ivensi*, Flemming and Blackburn (2005) found that giant chorionic cells eliminate the uterine epithelium constituting the only known example of endothelial placentation in reptiles. However, both observations for squamates must be best studied and analyzed.

Mabuya giant and interstitial placentomal chorionic cells are binucleated, similar to those observed in mammal trophoblast where these cells have diploid nuclei and play an important role in the lactogen and steroids production (Hoffman and Wooding, 1993). In viviparous reptiles as *Chalcides chalcides* (with type III allantoplacenta) progesterone production was detected in the maternal component of the placentome, after corpus luteum degeneration in late pregnancy (Guarino et al., 1998). The role of uterine and chorionic cells in hormone production must be evaluated in viviparous lizards and particularly in species with type IV allantoplacentation, since it has been observed that corpora lutea degenerate very early at gestation in this population of *Mabuya* (Gómez and Ramírez-Pinilla, 2004).

The *Mabuya* placentome exhibits a great increase in the exchange surface between mother and fetus. Two features increase the *Mabuya* placentomal surface area: 1) The radial development of branching endometrial folds from the incubatory embryonic pole that contact corresponding chorionic folds, and 2) the presence of long and thin apical microvilli at the surfaces of both epithelia forming an interlocked microvillar tightly joined band (interdigitant brush border) between maternal and fetal tissues. Chorionic and uterine epithelial interdigitation is also observed in the diffuse epitheliochorial placentas of Suiformes, Tragulidae and Camelidae eutherian groups (Mossman, 1987). Also, it is described in the placentome of lizards as *Pseudomoia entrecasteauxii* (with type III allantoplacenta, Adams et al., 2005), and other *Mabuya* species, as *M. heathi* (Blackburn and Vitt, 2002), *M. macrorhyncha* and *M. nigropunctata* (Flemming and Blackburn, 2003).

In *Mabuya* allantoplacenta the microvillar surfaces are observed from gastrula stages in the entire extraembryonic ectoderm and uterine epithelium surfaces; however they are especially developed in the epithelia of the dorsal absorptive plaque that will constitute the placentome during allantoplacental development (Jerez and Ramírez-Pinilla, 2003). In the mature allantoplacenta, placentomal microvilli distribution is apparently similar in both epithelia and the interface is completely filled by the microvillar interdigitation without any material. This type of interdigitant brush border provides an extensive platform for nutrient transporters and is commonly observed between epithelia with active transference of materials (i.e. the zona radiata of ovarian follicles), in placentas of mammals (i.e. the brush border membrane of the syncytiotrophoblast of the human placenta is recognized as the major site of synthetic and transport activity, Whitsett, 1980) and squamates (brush border cells of the bilaminar omphalopleure in the omphalantoic placenta of the snakes *Thamnophis radix* and *T. sirtalis,* Blackburn and Lorenz, 2003a). In mammals, the interdigitated microvillar zone between maternal and fetal epithelial apices allows accommodating the transport molecules for glucose and aminoacids (Wooding et al., 2003). Also, brush border and the mitochondrial richness of the apical cytoplasm in the chorionic and uterine epithelia of *Mabuya* placentome suggest an active transport of molecules and ions. The uterine syncytium of the placentome shows small mitochondria (0.19 ± 0.04 µm equator width) and abundant RER in the basal region, completely euchromatic nuclei, and abundant vesicles and lipids suggesting a great metabolic activity; also, the great proximity of the subjacent blood vessels to the syncytial epithelium suggests a great transference of molecules and ions from maternal blood. For mammals, Matsubara *et al.* (2001) observed that mitochondria and RER morphology in the villous syncytiotrophoblasts, leave cytotrophoblasts and villous cytotrophoblasts placentas is related with metabolic activity, suggesting that features as small mitochondria (0.22 ± 0.04 µm equator width) and abundant RER are related with a high cellular metabolism.

In the placentomal chorion of *Mabuya* the fine structure of giant cells exhibits primary and secondary lysosomes and abundant RER, features that suggest a great protein synthesis activity and an important capacity for macromolecule digestion. Cellular morphology of these cells is similar in appearance to early stage trophoblast of *Lama pacos*, where protein synthesis activity, ion transport, and a great phagocytic activity occur (Olivera et al*.,* 2003a). Immunohistochemical localization reveals the important synthetic activity of ruminant placentomal trophoblast and equine trophoblast girdle and cup cells, which synthesize hormones like lactogen and glycoproteins (Wooding et al., 1997) and chorionic gonadotropin (Wooding et al., 2001). *Mabuya* giant and interstitial cells develop lateral interdigitant processes extensively elaborated and entwined. These processes form channels and structures like gap junctions that suggest the chorionic trans-cellular transfer of substances from the uterine syncytium; once the molecules have been pumped into the chorion they need to be speeded on their way to the fetal circulation. Also, in the viviparous snake *Virginia striatula* a wide mesh of membranous channels and cytoplasmic extensions that interdigitate with adjacent chorionic cells were observed (Stewart and Brasch, 2003). The absence of immunocytochemical studies in the *Mabuya* chorion hampers a detailed comparison with mammals, since the chorion morphology suggests absorption, synthesis and transference of macromolecules; however, their nature has not been described.

Paraplacentome

The *Mabuya* paraplacentome was described as a region with histological distinctive characteristics that surrounds and limits the placentome, only located near the embryonic pole (Jerez and Ramírez-Pinilla, 2001). It is different from those described with the same nomination in other scincids (*Chalcides chalcides* Blackburn and Callard, 1997; *M. heathii* Blackburn and Vitt, 2002; *Pseudomoia entrecasteauxii*; Adams et al., 2005). Due to the apparently lesser complexity of the allantoplacenta in these species, the paraplacentome is considered as a generalized region of the embryonic chamber, adjacent to the placentome, which plays an important role in the gas exchange.

In the limit between placentome and paraplacentome there is a strong interdigitation between both epithelia mediated by convoluted processes very different from the placentomal brush border. These processes can be observed only in the limit with the placentome, and could function as seal mechanisms, similar to the border seals observed in *Camelus dromedarius* areolas that prevent the dissipation of stored secretory products into areolar cavity (Abd-Elnaeim et al., 2003).

Cellular and subcellular chorionic characteristics of paraplacentome are similar to those described in the placentome, suggesting that the great molecular transference and metabolic activity is maintained in the paraplacentomal chorion. Although lipid droplets are observed in both epithelia of the entire exchange areas from *Mabuya* embryonic chamber, one distinguishing paraplacentomal feature is the observation at macroscopic and cellular levels of abundant uterine and chorionic lipid droplets. A significant intra and trans-cellular transport of lipids toward allantoic blood and lymphatic capillaries is suggested by the proximity between the chorionic cells and allantoic blood vessels, and by the abundant lysosomes and stored lipid droplets close to the lateral and basolateral regions of giant chorionic cells. This fact can suggest that the paraplacentome is a site of great maternal-fetal lipid transference. Other materials can be also transferred by the paraplacentome: The electron-dense granules in the supra-nuclear region of uterine epithelial cells (eosinophilic in optical microscopy, Jerez and Ramírez-Pinilla, 2001) seem to be secreted and absorbed by the chorionic apical region; however, the role and nature of this secretion is unknown.

Chorionic Areolas

The process of formation of the chorionic areolas in *Mabuya* is similar to that observed in the areola of *Camelus dromedarius*. Abd-Elnaeim et al. (2003) described an initial proliferation of trophoblast (mitosis) followed by cellular degeneration creating the areolar cavity, and producing material for later absorption by the non-degenerated trophoblast. Nonetheless, the formation process of chorionic areolas in *C. dromedarius*, does not show the development of the big lipid droplets found in the areolas of *Mabuya*, before and after the degeneration stage.

The *Mabuya* chorionic areolas exhibit three types of secretory products that seem to be absorbed by the chorion: 1) Products from luminal uterine cells; 2) Products from chorionic cell lysis during the formation of the areolar cavity; 3) Products from endometrial glands. The first type is characterized by an amorphous secretion of eosinophilic nature (Jerez and Ramírez-Pinilla, 2001) produced by an apocrine mechanism suggesting the extrusion of soluble and membrane-associated proteins and cytoplasm remnants. The second type of secretory product is characterized by the presence of big lipid droplets and chorionic cellular debris produced by degeneration of the apical chorionic cells (holocrine products); these secretory materials are absorbed by the basal chorionic cells of the areola concavity. The third type has ultra-structural features that seem to be of enzymatic nature and its function is unkown; this glandular secretion could mediate in the formation of the chorionic cavity of the areola, in the extrusion and lysis of the apocrine and holocrine secretory products or in the digestion and absorption of chorionic and uterine cellular products. The areolar glands of *Mabuya* have characteristics that differ to those described in the areola of mammals (in *Lama pacos* Olivera et al., 2003a, and in *Camelus dromedarius* Abd-Elnaeim et al., 2003), particularly in the appearance of glandular secretions and the baso-lateral membranes arrangement.

In mammals most placental types use histotrophic production by gland to chorionic areola. Iron deposits and acid phosphatase activity, is observed in alpaca areolar cavities (Olivera et al. 2003a), and equid areolar cells allow the iron and calcium transference (Wooding et al., 2001). In pigs two types of chorionic areolas are described: 1) irregular, with sulphomucin vacuoles characterized by the resistance to trypsin digestion, and 2) regular, with PAS positive glycoproteins, associated to uteroferrine absorption (Bielanska-Osuchowska and Kunska, 1995). It is possible that in *Mabuya* some of these molecules and ions are transferred via chorionic areolas. Transplacental movements of Ca^{++} in *Mabuya* are increased dramatically during the last stages of gestation, when fetal skeletal mineralization reaches its maximum and when the placentome is mature and chorionic areolas occur (Ramírez-Pinilla et al*.,* 2005). Morphological evidence suggests lipid transfer and exchange of other molecules as proteins and other nutrients; histochemical studies would identify these substances.

Absorptive Plaques

The original descriptions of type IV allantoplacentas, restricted to the embryonic hemisphere and on formalin fixed material, indicate the presence of a highly specialized placentome and chorionic areolas related to the histotrophic transfer of nutrients (Vitt and Blackburn, 1991; Blackburn and Vitt, 1992, 2002; Blackburn, 1993; Flemming and Blackburn, 2003). However, for this population of Andean *Mabuya* Jerez and Ramírez-Pinilla (2001) described and nominated for first time the presence of absorptive plaques. These flat round areas located at the antimesometrial hemisphere, immediately ventral to the zone of chorionic areolas, have histological characteristics that correspond to specialized structures for transfer of nutrients. Thus, the antimesometrial hemisphere has two placental functions (gas exchange and nutrient transfer), whereas the mesometrial hemisphere functions predominantly in histotrophic transfer.

The origin and nature of transferred products in *Mabuya* absorptive plaques are different from mammals. In the eutherian mammals the absorptive plaques are found near to one or more endometrial glands and allow the transference of maternal products by phagocytosis (Mossman, 1987). In *Mabuya* absorptive plaques, the endometrial glands are not specially developed and their ducts do not open to the interface of the absorptive plaque; thus, a significant histotroph uptake by the chorion can not be suggested. The absorptive plaques have closely apposed chorionic and uterine epithelia without microvillar surfaces between them; then, membrane transport of ions and molecules would not be especially enhanced. However, absorptive plaques exhibit a complex system of interwoven apical membrane projections. *Lama pacos* has also points of attachment (Olivera et al. 2003b) morphologically similar to these observed in *Mabuya* absorptive plaques; they are glycan-glycan interactions, at maternal fetal interface that improve the adhesion of the placenta (Olivera et al. 2003a). For *Mabuya* can be suggested that these adhesion areas also can function as a mechanism to prevent the material dispersion to respiratory segments, and to improve the interaction between the two epithelia.

Similar to the other specializations described in the embryonic pole of the *Mabuya* allantoplacenta, in the absorptive plaques the numerous lipid droplets in the uterine epithelium and in the chorionic cytoplasm, and in the intercellular and basal spaces between chorionic cells suggest the transference of lipids from uterus to chorioallantois. Moreover, the presence of small lipids droplets near to the endothelium of allantoic blood capillaries, suggests the lipids transference from uterus to allantoic blood vessels. Additionally to the lipids, giant cells of absorptive plaques are very tall and have a great amount of smooth ER in the chorionic apical region. This feature might be parallel with the giant multinucleate cells in camel which produce steroids (Wooding et al., 2003). Also, in the absorptive plaques numerous electron dense granules are observed in the chorionic apex and their size suggests an autocrine or paracrine function. In the human placenta the chorion plays an important role in the implantation, fetal development, and parturition through peptides that function like endocrine and paracrine markers (Petraglia et al., 1996). However, to determine the exact nature of these materials and speculate more accurately concerning their function in *Mabuya* absorptive plaques, further immunocitochemical studies are required.

Respiratory Segments

In the respiratory segments of *Mabuya* allantoplacenta, the uterine and chorionic epithelia show close apposition, and the diffusion distance between uterine and allantoic vessels is highly diminished, since both epithelia reduced their thickness and epithelial projections or microvilli are not observed. The respiratory segments alternate with absorptive plaques in the subparaplacentomal area of the embryonic chamber exhibiting a small interhaemal distance. A small thickness and a wide surface area allow an easy transference of oxygen, carbon monoxide, and other substances (Schröder, 1995).

The type IV allantoplacenta of *Mabuya heathi* also exhibits attenuated chorionic and uterine epithelia (Blackburn and Vitt, 2002). In the allantoplacenta of the snakes *Virginia striatula* (Stewart and Brasch, 2003), *Thamnophis sirtalis* and *T. radix* (Blackburn and Lorenz, 2003a) uterine epithelia is thinned by overlie blood vessels that create a tightly folded area, caused by the increasing pressure of expanding blood vessels which displace the cytoplasm attenuating the overlaying epithelial cells. In the paraplacentome of *Pseudemoia entrecasteauxii*, the extremely attenuated cytoplasm and the loss of cellular organelles suggest a variant of epitheliochorial placenta called cyto-epitheliochorial (Adams et al., 2005). Differently, in mature placenta from eutherian mammals as guinea pig (Enders and Blakenship, 1999) the interhaemal distance is reduced by the loss of trophoblast layers. In the *Mabuya* respiratory segments, loss of chorionic layers was not observed and the epithelia attenuation is smaller than that observed in *Pseudemoia entrecasteauxii*.

Lipids in the chorionic and endometrial cells found in the respiratory segments are remnants of their transfer in early developmental stages (at gastrula and neurula stages, Jerez y Ramírez-Pinilla, 2003) when the oxygen transference was not highly required. Due to oxygen demand is highest during preparturition stages, as is observed in other squamates (i.e. *Eulamprus tympanum* Robert and Thompson, 2000) and in eutherian mammals (i.e. human primates Burton et al., 2001), some regions among the absorptive plaques mainly in the abembryonic hemisphere constitute the respiratory segments specialized in gas exchange.

A common feature of all structural specializations of the allantoplacenta of *Mabuya* is the transfer of lipids, which supply the metabolic and energetic necessities for embryo development since the egg is microlecithal. In eutherian mammals, glucose is the principal fetal and placental energy source (Smith et al., 1992; Takata et al*.,* 1997; Burton et al*.,* 2001; Ward et al., 2003; Fuchs and Ellinger, 2004; Wooding et al*.,* 2005) while lipids and proteins are the main energy sources in reptiles (Stewart and Thompson, 1993; Speake and Thompson, 1999; Thompson *et al*., 1999a, b, c). A great lipid transference is observed in *Mabuya* embryonic chamber from the earliest stages of embryo development (Jerez and Ramírez-Pinilla, 2003) and throughout allantoplacentation; in *Mabuya* the necessity of lipids for embryonic development must be supplied by the placental transference due to the almost complete suppression of the vitellogenesis (Gómez and Ramírez-Pinilla, 2004). However, in the lipid transfer from uterus to chorion, each lipid must be metabolized to its constituent molecules, and each type of molecule (i.e. fatty acids, cholesteryl esters) must have their own membrane transport systems. Lipid droplets must be reformed in the fetus with a different composition as has been found in other placentotrophic lizards (Speake et al., 2004). Studies by Speake et al. (2004) revealed that the evolution of placentotrophy in *Pseudemoia entrecasteauxii* requires the expression of molecular mechanisms to accomplish the transference of lipids from maternal circulation to fetus during gestation. This assertion suggest that in the highly matrotrophic type IV allantoplacentas, these transport systems and molecular mechanisms must be highly developed.

In this Andean population of *Mabuya* each specialized area of the allantoplacenta has different histological and cellular features related to: 1) the maternal-fetal apical interactions between epithelia, 2) the morphology of endometrial and choriallantoic tissues and cells and 3) the material stored, secreted and absorbed in the uterine and chorionic cells. These characteristics suggest that each specialization of *Mabuya* allantoplacenta has particular functions in the transference of nutrients as ions, lipids, proteins, amino acids, sugar, water and gas exchange, and in the possible synthesis of hormones and proteins. Different studies show that the placenta of eutherian mammals has also regional functional specializations, as the cotyledonary and inter-cotyledonary regions in ruminants, which show different synthesis capacity (Wooding et al. 1996b), or areolas and microcotyledons in the equids placenta, which allow the transfer of different substances (Wooding et al., 2000). It is possible that the complex regionalization of the *Mabuya* allantoplacenta follows a similar eutherian mammal pattern in such a way the molecular transference and synthesis of particular molecules can be regionally specialized. The nature of the molecules as the cellular and molecular mechanisms that allow the function of each specialization should be determined.

The *Mabuya* allantoplacenta exhibits a great morphological complexity related to its high level of placentotrophy; this complexity is observed not only in the remarkable regionalization of specializations for nutrient transfer, but also in the histological and cytological features of interacting chorionic and maternal tissues in the incubatory chamber. All of these allantoplacental features and other found in the analysis of extraembryonic membrane development (Jerez and Ramírez-Pinilla, 2003) as the fact that the yolk sac does not develop according to the standard squamate pattern, revealed strong differences from the generalized condition of other viviparous squamates, including placentotrophic species. The morphological differences observed between *M. heathi* and our population of *Mabuya* suggests that more detailed morphological and physiological studies on extraembryonic membranes and in their development must be done in this lineage of *Mabuya* species, and also in the highly placentotrophic African skinks *Eumecia anchiatae* and *Trachylepis ivensi*. On the other hand, several of the described features are very similar to those found in the placentas of eutherian mammals, constituting a clear evidence of evolutionary convergence at histological and cytological levels between both clades, which evolved the highest levels of placentotrophy among vertebrates and developed a complex chorioallantoic placenta that provides all the nutrients for fetal development.

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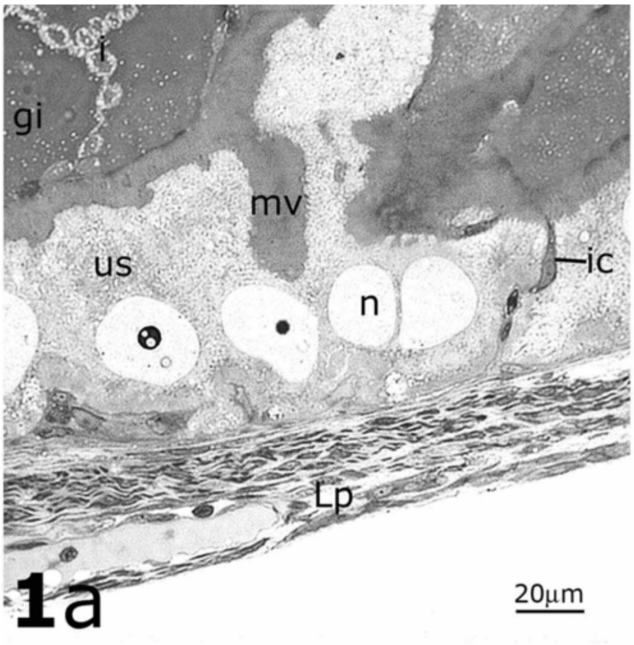
FIGURE LEGENDS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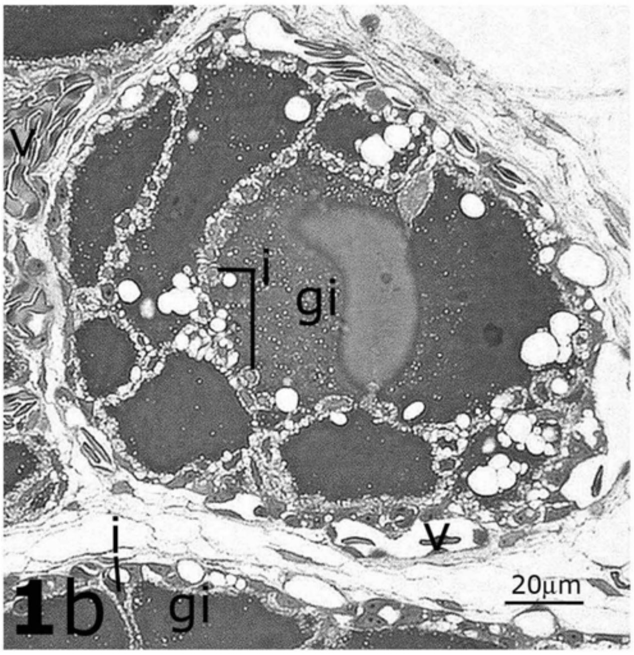
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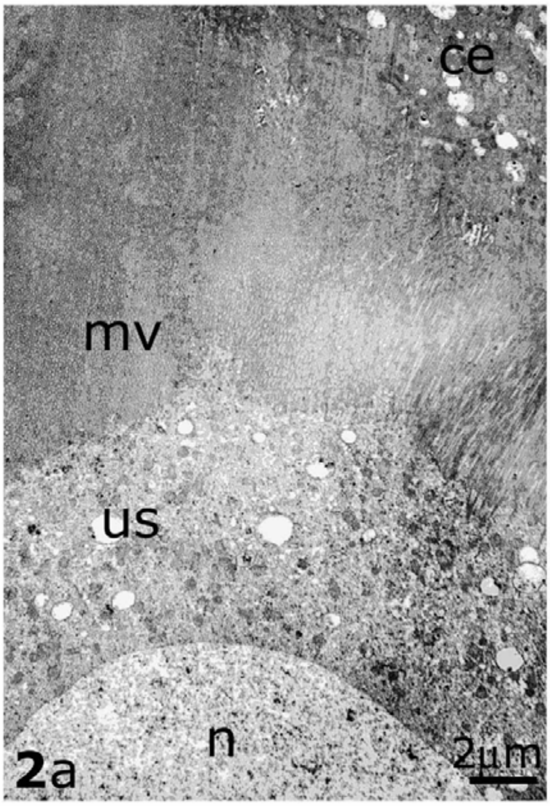
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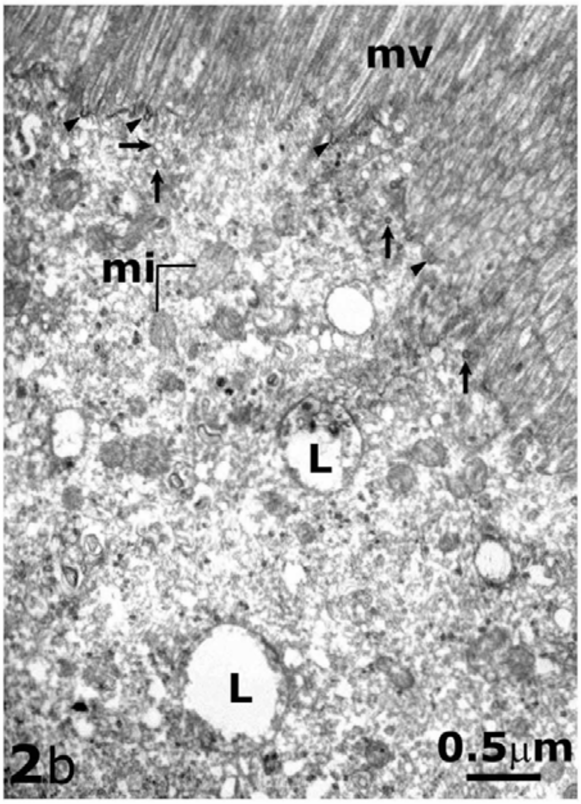
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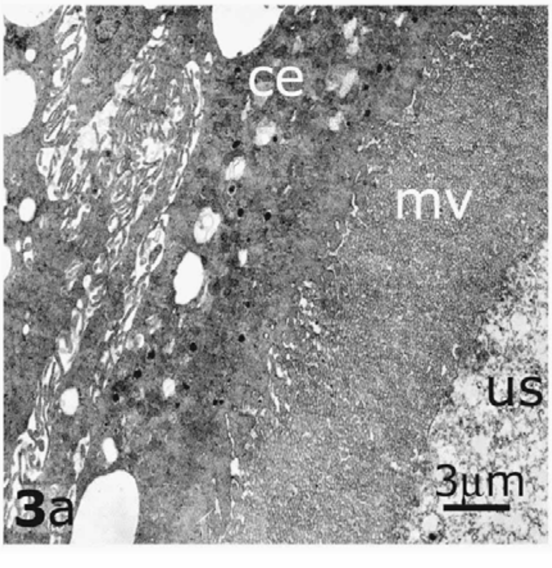
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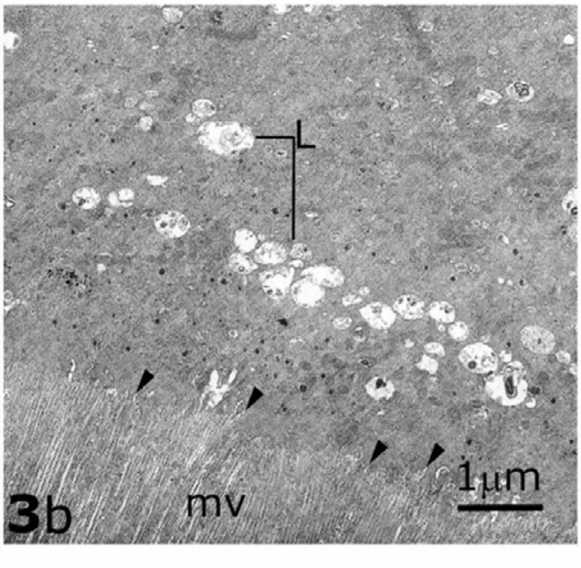
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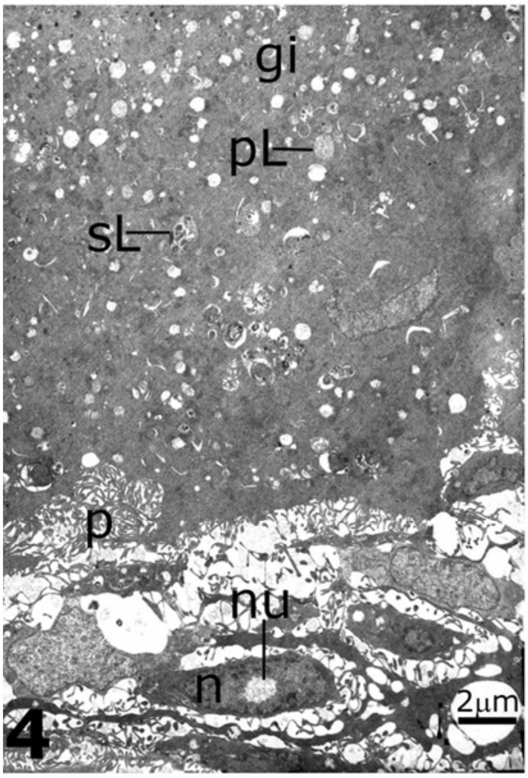
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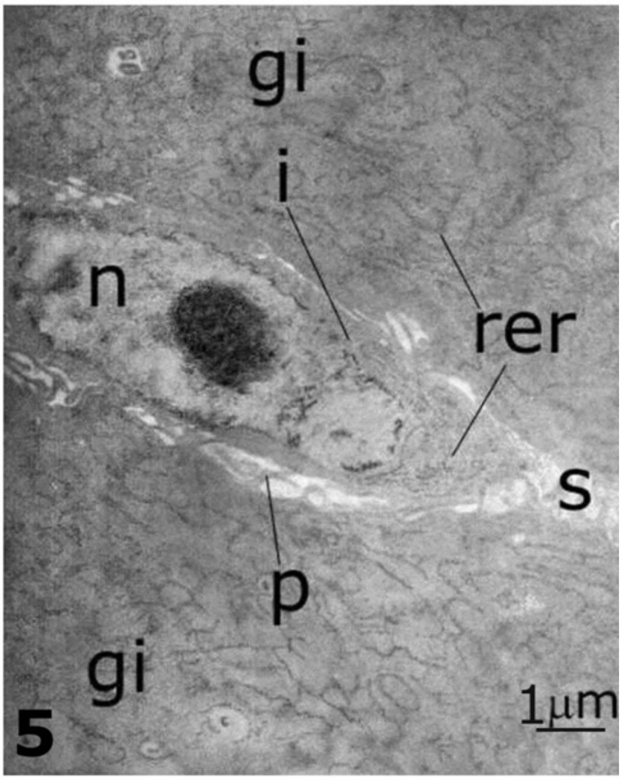
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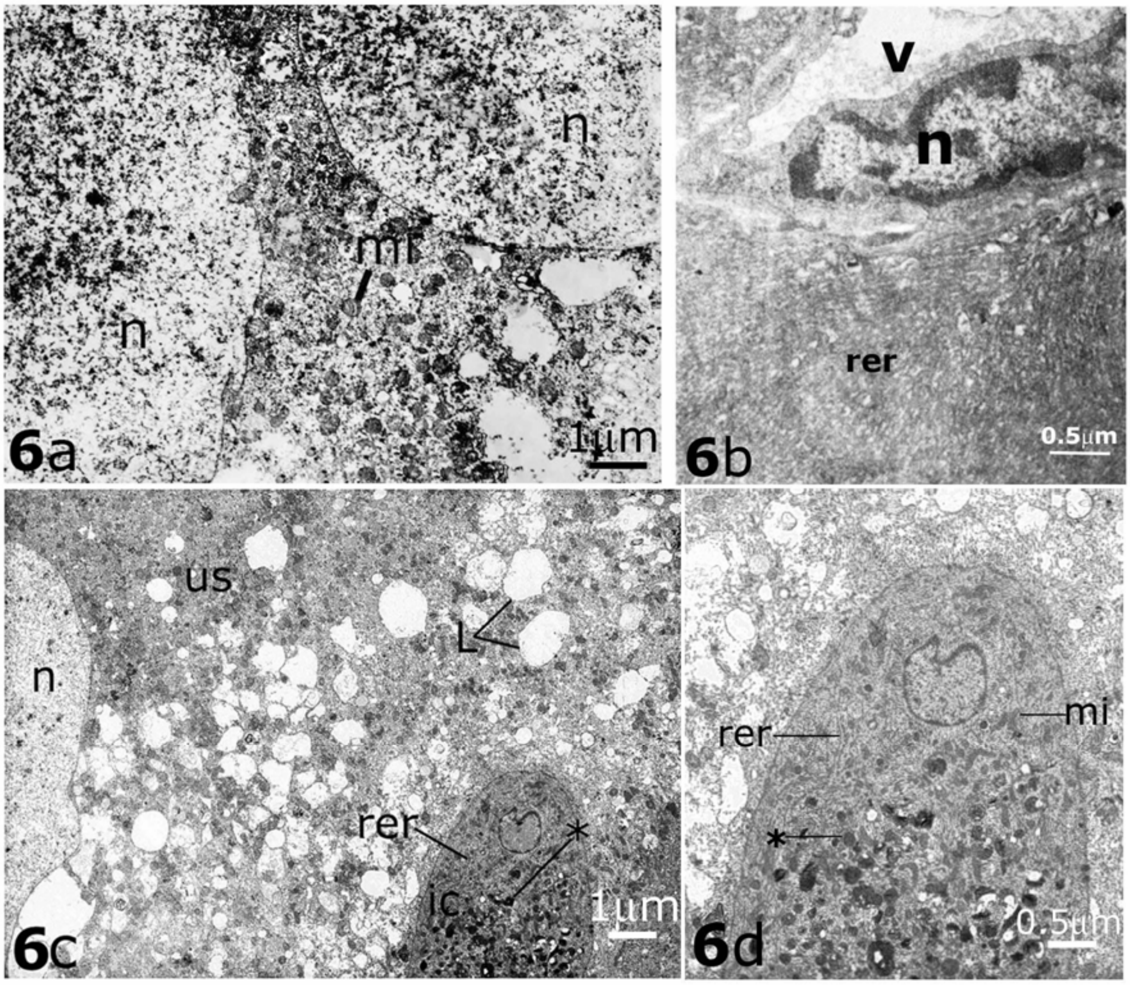
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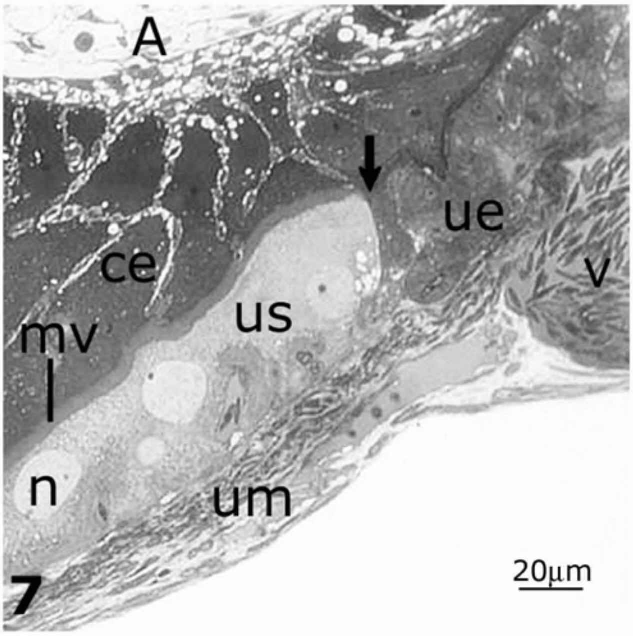
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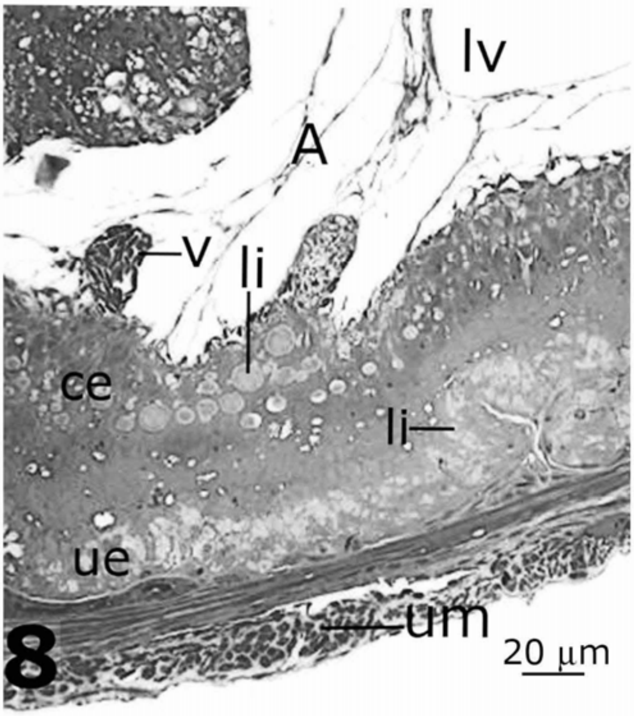
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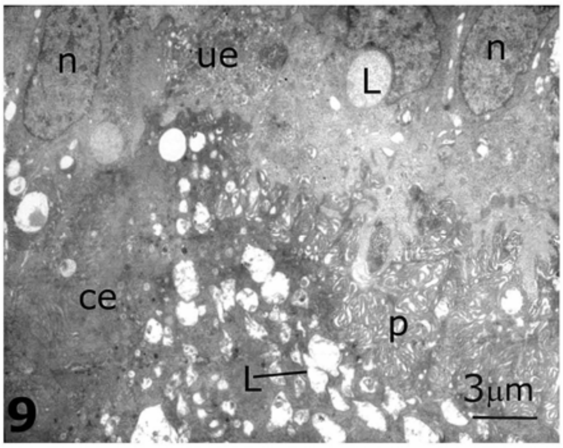
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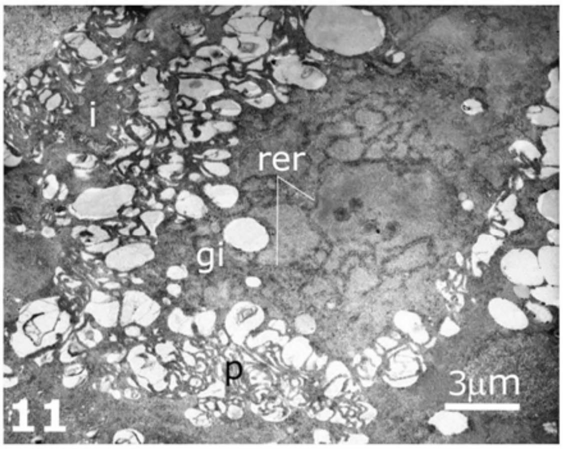
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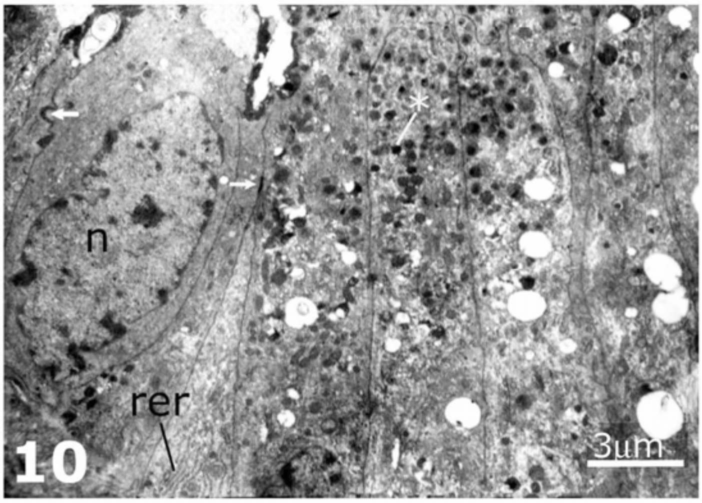
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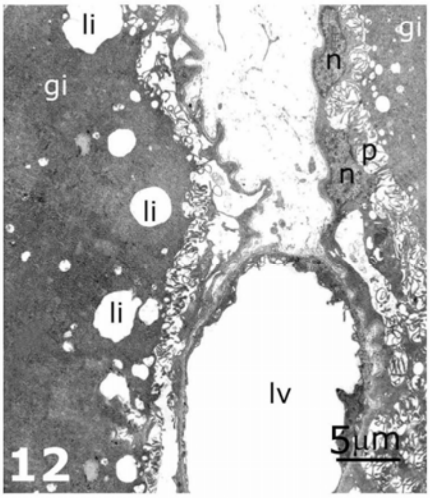
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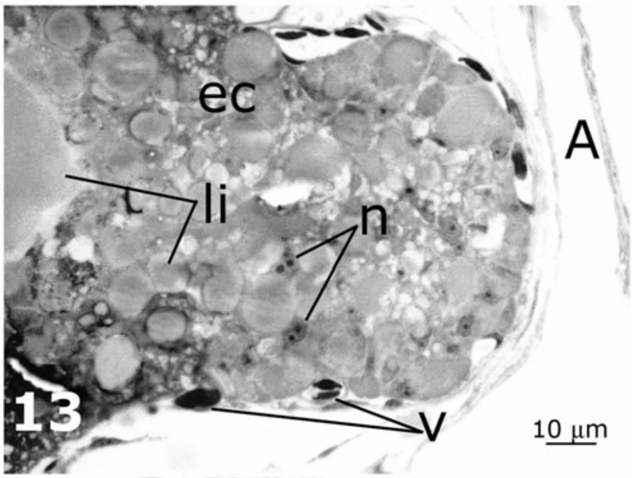
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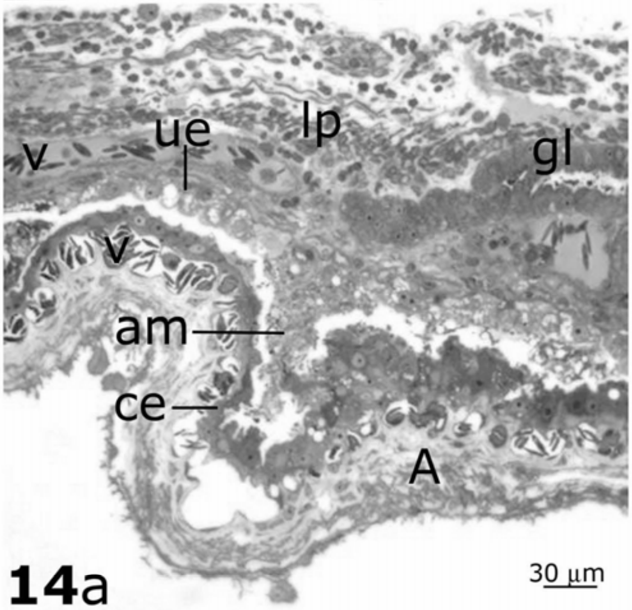
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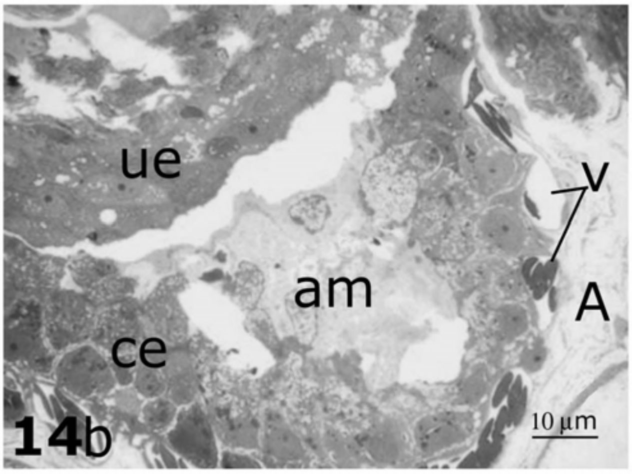
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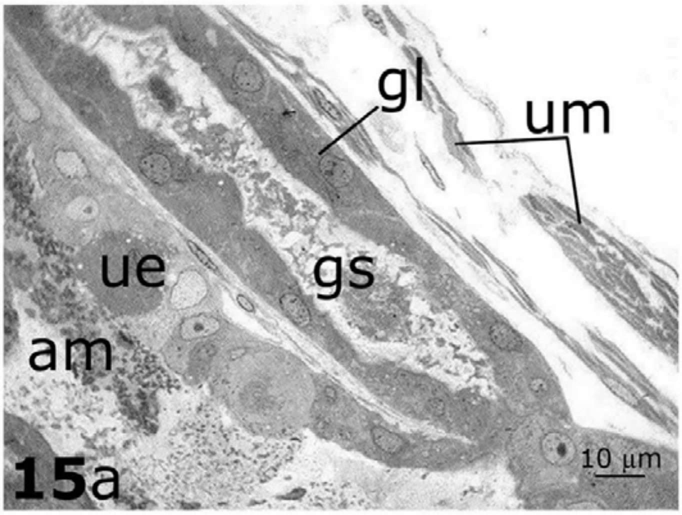
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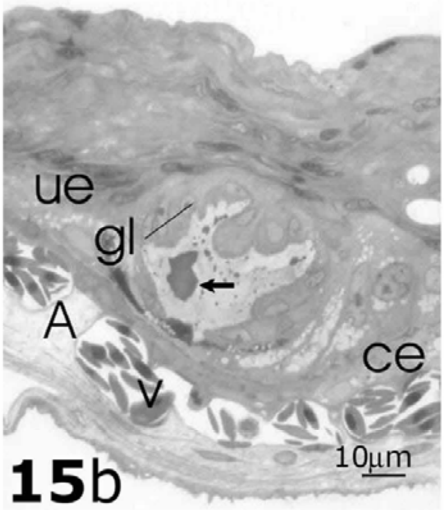
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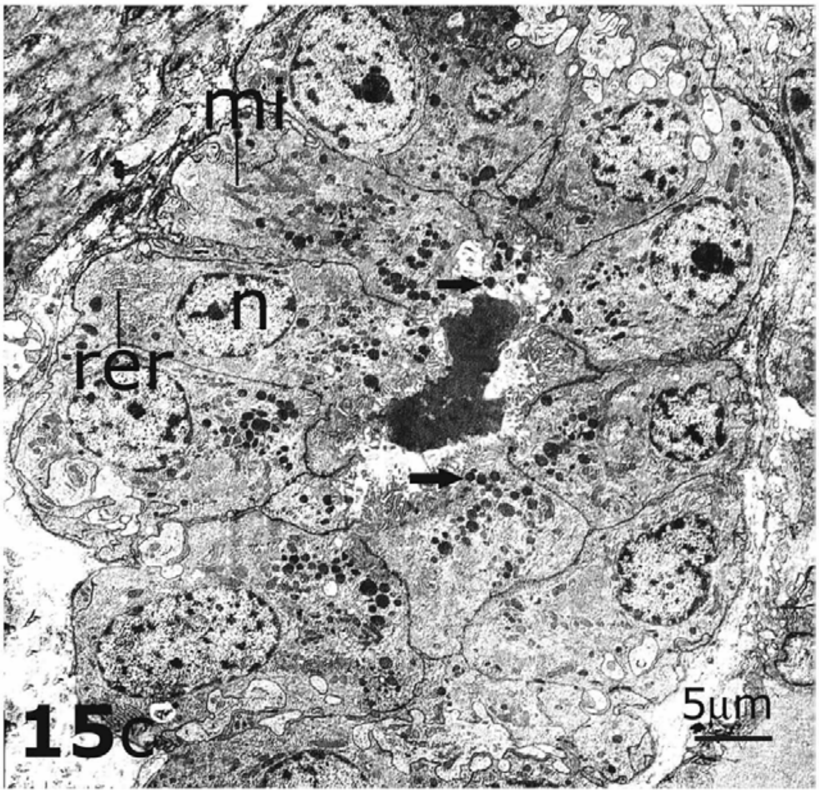
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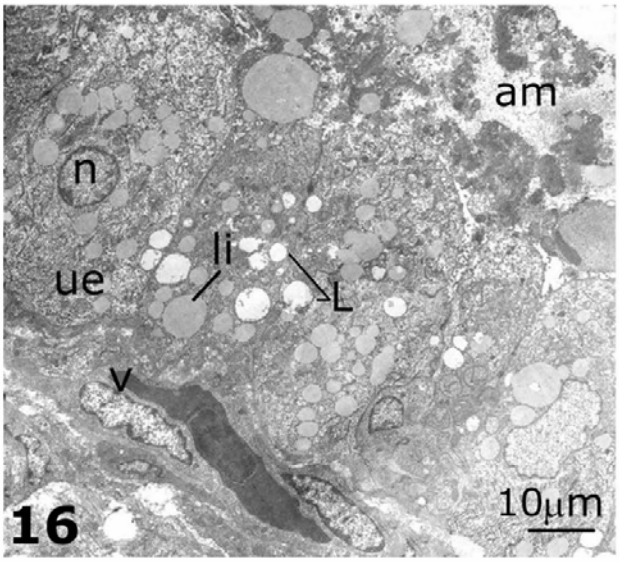
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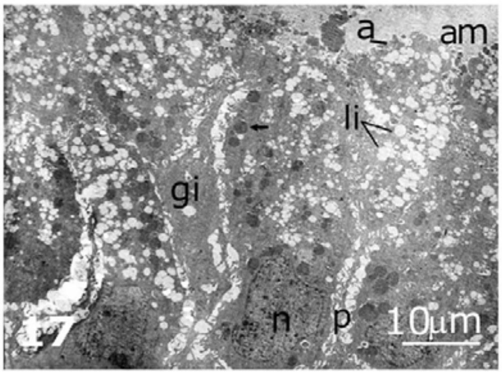
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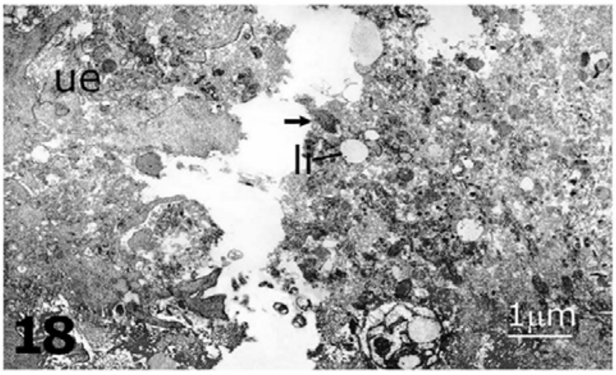
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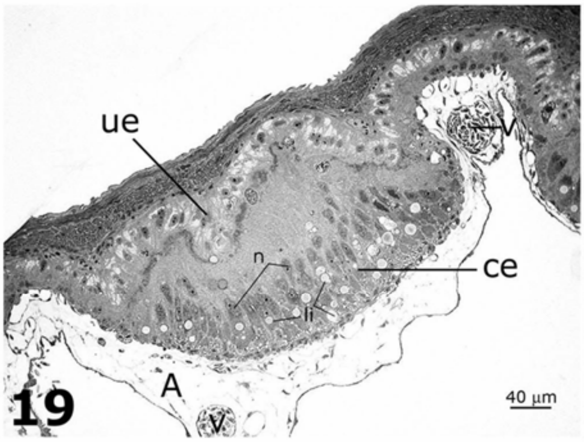
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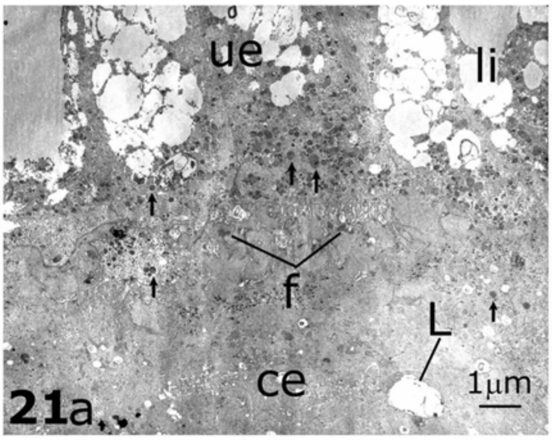
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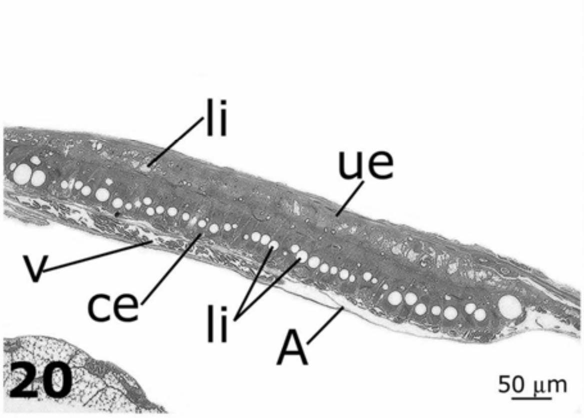
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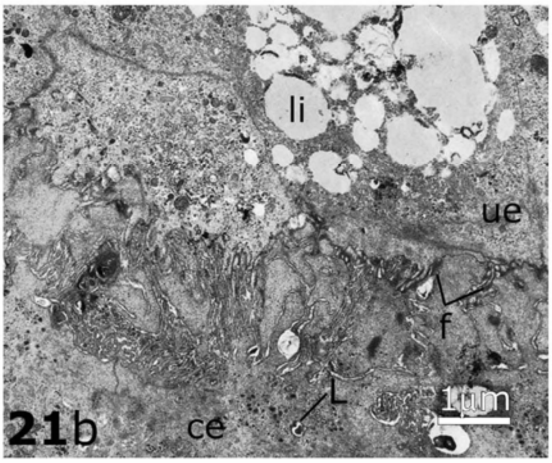
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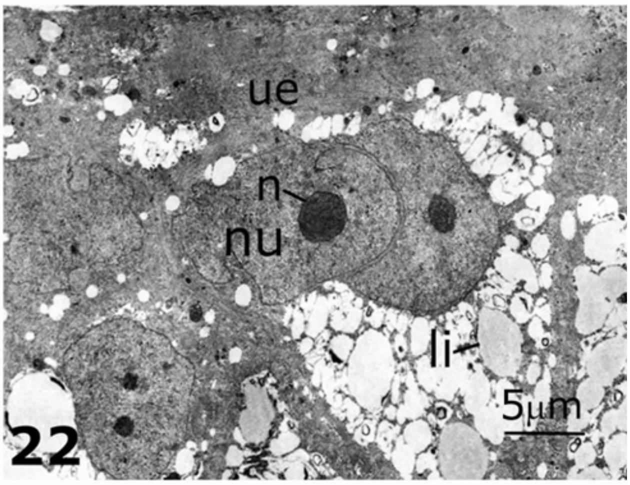
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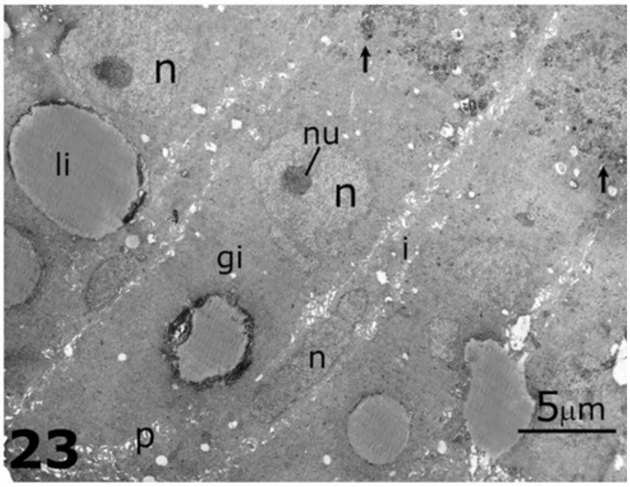
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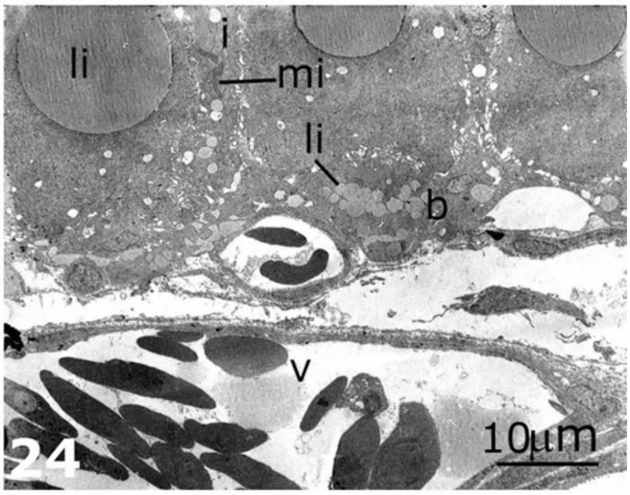
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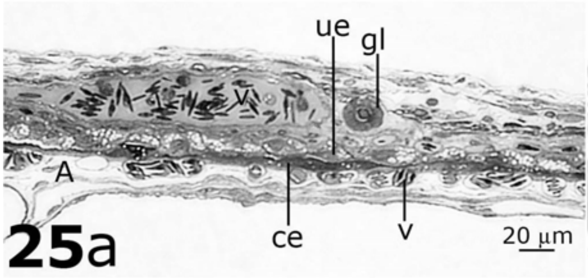
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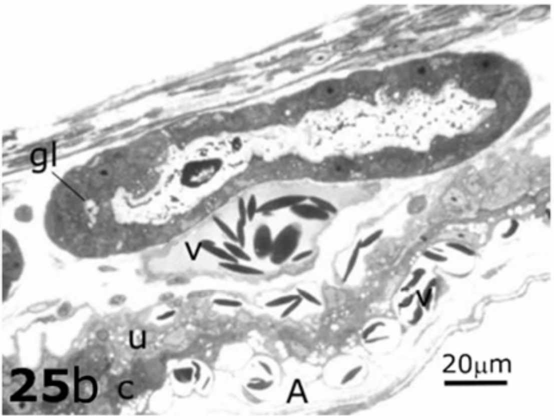
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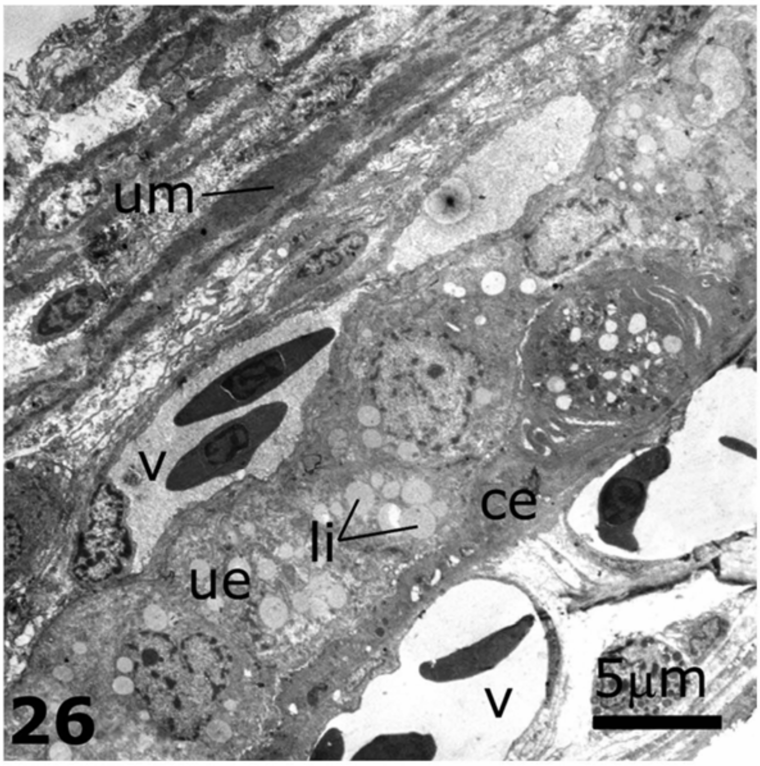
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