Chapter – IV

Transmission electron microscopic analysis of the mechanism of secretion in the male Mullerian gland of *Uraeotyphlus narayani* (Amphibia: Gymnophiona)
INTRODUCTION

Caecilians (Amphibia: Gymnophiona) are a unique group of vertebrates retaining the Mullerian duct, the progenitor of the female duct, in the adult male as a functional gland known as Mullerian gland (Muller, 1831; Tonutti, 1931; Wake, 1970, 1977a, 1977b, 1981; Exbrayat, 1985, 1986, 1992). The Mullerian gland is formed of a large number of tubular glands lined by a pseudostratified epithelium arranged around the circumference of a central Mullerian duct (Wake, 1970, 1977a, 1977b, 1981; Exbrayat, 1985, 1986, 1992). It has been shown in the previous chapter that in *Uraeotyphlus narayani* a distinct duct connects the tubular glands and the central Mullerian duct. The tubular glands differ between the column and the base in respect of i) the basement membrane, ii) the epithelial organization, iii) the nature of the secretory material, and iv) the profile of the lumen. The basement membrane of the column of the gland is so thick that amoeboid cells do not penetrate through it, whereas the basement membrane underlying the epithelium of the base of the gland is thin and appears to allow penetration of amoeboid cells. The epithelium of the column is made up of microvillated secretory cells with the nuclei located in the basal cytoplasm and ciliated non-secretory cells with apical nuclei. On the other hand the epithelium of the base possesses amoeboid cells, in addition, which put forth filamentous and branched pseudopodia, which interdigitate with similar process put forth by the microvillated secretory cells, and make vertical migration. Histochemical tests revealed more intense reactivity in the cells of the column than at the base. The lumen of the column is a wide and straight tube whereas the lumen of the base, which is continuous with that of the column, spreads on top of the cells lining the base in such a way that the latter assumes a pyramidal shape.
The very few published reports on the role of male Mullerian gland of caecilians have shown the tubular glands to be secretory (Wake, 1981; Exbrayat, 1986, 1992). It has been hypothesized, based on certain indirect evidences, that the Mullerian gland could be the evolutionary predecessor of the prostate gland of mammals and, thus, its secretory material would be comparable to the seminal plasma in which sperm are suspended during ejaculation in the mammals (Tonutti 1931; Wake 1970; 1981; Exbrayat, 1985). In this hypothesis, it has been speculated that the Mullerian gland at the caecilian stage of evolution is an attempt to secrete a medium in which sperm are suspended during transfer from male to female in the context of one of the earliest attempts towards internal fertilization that is a prerequisite for terrestrialization. However, to date there is no report of the cellular mechanisms of formation and release of the secretory granules in the epithelial cells of the tubular glands. In the light of the aforesaid differences between the column and the base of the tubular glands in *Uraeotyphlus narayani*, it was hypothesized in the present work that the mechanism of formation and release of the secretory granules would differ between the cells lining the two parts. Based on critical transmission electron microscopic observations of the tubular glands of *U. narayani*, the present paper provides evidence of the microvillated cells, rich in secretory granules, lining the column to be truly secretory. On the other hand, the secretory cells of the base differ among themselves in respect of electron density of cytoplasm, the source of the secretory material, the nature of the secretory granules and the destination of the secretory products. The basal epithelium is further complicated by the presence of amoeboid cells in it, with an apparent role in transport of the secretory material into and out of the tubular glands.

MATERIALS AND METHODS

*Uraeotyphlus narayani*, belonging to the family Uraeotyphilidae, was chosen for the study in view of its availability in plenty in the Western Ghats of Kerala. The methodology is the same as explained earlier (Chapter III). Totally 10 animals were collected from the coconut groves and rubber plantations in and around Thodupuzha in Idukki district, Kerala, India (Lat 09° 53’ 52” N Long 76° 42’ 29” E), during the
period of active spermatogenesis (July to February), anesthetized using MS222 and dissected. Transverse slices of the Mullerian gland were fixed in 2.5% glutaraldehyde prepared in cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide and embedded in thin viscosity resin (Spurr kit, Sigma Chemical Co.). Semithin sections (1µm) obtained with Richert Jung ultramicrotome were stained with toluidine blue-O (TBO) for identification of the area for obtaining ultrathin sections. Ultrathin sections obtained with a Leica ultramicrotome were stained with uranyl acetate and lead citrate and observed under a Phillips 201C transmission electron microscope (Hess and Thurston, 1977). Micrometric measurements of size of the secretory granules were also made. The images were scanned into the computer and processed using Adobe Photoshop 6.0 software.

RESULTS

Cells of the column

The epithelium of the column is formed of microvillated secretory cells and ciliated non-secretory cells. The secretory cells are tall cuboidal, broad at the base and narrow towards the lumen. The nucleus is basally located. The cytoplasm apical to the nucleus, and rarely elsewhere, abounds with dark secretory granules of varying electron densities. The granules measure 0.5 to 3.0µm dia. The mature granules are confined to the medullary portion of the cytoplasm, the cortical cytoplasm being generally free from the granules (Figs.1, 2). The perinuclear and basal cytoplasm is invariably free from such granules, but rich in mitochondria, endoplasmic reticulum and polyribosomes. In fact, mitochondria are confined to the perinuclear and basal cytoplasm (Fig. 3). The prominent Golgi apparatus is supranuclear and present as two or more vertical or horizontal stacks (Fig. 4). The Golgi cisternae are fenestrated and the Golgi vesicles are prominent (Fig. 5). The cisternae of rough endoplasmic reticulum associate with the Golgi cisternae at their cis-phase. The secretory material appears first at the trans-phase of the Golgi apparatus i.e., between the Golgi apparatus and the nucleus. At the earliest appearance the secretory material appears as fine granules or as an amorphous material in large membrane-bound vesicles (Fig. 5). Small vesicles containing the amorphous secretory material fuse with the large
ones, as a result of which the vesicles increase in size (Figs. 6, 7). Once formed, the content of the vesicles undergoes condensation resulting in the secretory granules (Figs. 6-9). The vesicles are clearly free from ribosomes and, hence, not rough endoplasmic reticulum (Fig. 9). The granules present even in the apical cytoplasm are in different phases of condensation (Fig. 10). The mature granules are released into the lumen of the tubular glands in an apocrine mechanism when the membrane surrounding the granules fuses with the apical plasma membrane of the cell and the content is released as a structured granule (Figs. 11, 12).

**Cells of the base**

As described in the Chapter III, three types of cells are present in the epithelium lining the base of the tubular glands viz., microvillated secretory cells, ciliated non-secretory cells and amoeboid cells which put forth filamentous and branching pseudopodial protuberances. Pseudopodial processes of the amoeboid cells associate with similar processes of the secretory cells, and the amoeboid cells make vertical migration in the intercellular spaces between the secretory cells and the ciliated cells. Depending upon the abundance of secretory granules in the cytoplasm and the electron density of the granules as well as the cytoplasm, at least three stages of maturation of the secretory cells or three variants of the cell type are identified. In the first, the clear cytoplasm containing a few secretory granules abounds with mitochondria and endoplasmic reticulum. In the second, the cytoplasm is lightly staining and electron-lucent but densely packed with highly electron-dense secretory granules, almost always spherical, measuring 0.2–1.0μm dia. In the third, the cytoplasm is darkly staining and electron-dense and the secretory granules, many of which are irregularly shaped, measure 0.5–2.0μm dia (Figs. 13, 14). In all the three variants / functional stages of the cells the Golgi apparatus is located in the cytoplasm basal to the nucleus, in a position little eccentric, and is more prominent than in the secretory cells of the column (Figs. 15-18). The cytoplasm possesses prominent sac-like rough endoplasmic reticulum all around the nucleus (Figs. 15-18). The cis-phase of the Golgi apparatus, as seen from the wells, lies towards the basal aspect of the cell and the trans-phase faces the nucleus. The mitochondria are more abundant in the Golgi area than elsewhere.
Small membrane-bound vesicles are budded off from parts of the endoplasmic reticulum, which are free from ribosomes (Fig. 15). These vesicles associate with the Golgi apparatus at the cis-phase (Fig. 15). The secretory granules emerge out from the trans-phase of the Golgi apparatus, more prominently along the edges of the Golgi stacks (Fig. 16). High power TEM pictures reveal that the cells that do not contain any granules in the cytoplasm are incipient secretory cells. In these cells the Golgi apparatus is surrounded by only a few mitochondria (Fig. 15). The cells with electron-lucent cytoplasm and highly electron-dense granules are active in manifestations of the secretory granules (Fig. 16). In these cells the Golgi apparatus is surrounded by abundant mitochondria. In the cells with highly electron-dense cytoplasm, the mitochondria accumulate as dense aggregations around the nascent secretory granules (Fig. 17). In the process of maturation of the secretory granules, small membrane-bound vesicles containing a moderate electron-dense content fuse with the growing secretory granules (Fig. 18). An intimate association between the mitochondria and the growing secretory granules is indicated in a few TEM pictures (Fig. 19).

The amoeboid cells of the base of the tubular glands appear to be of extra-tubular gland origin. They arrive at the epithelium from the peritubular connective tissue, penetrating the basement membrane. This inference is based purely on histological and ultrastructural evidences. The connective tissue around the basal aspect of the tubular glands contains irregularly-shaped cells possessing elongated, irregular and highly heterochromatic nuclei (Fig. 20). They are tentatively identified as fibroblasts. The amoeboid cells at the early stage of establishment in the epithelium resemble the peritubular fibroblasts in respect of the morphology and the densely heterochromatic organization of the nucleus. Such cells penetrate the basement membrane and arrive at the opposite side, i.e., the epithelium, where they establish on the top of the basement membrane as pyramidal cells with nuclei also of similar shape (Figs. 21-23). The amoeboid cells put forth pseudopodial protuberances and the secretory cells also produce similar processes. The two kinds of cells appear to interact through these processes (Fig. 24). Subsequently, the amoeboid cells appear to move away from the basement membrane into the deeper parts of the epithelium, confining their distribution to the base of the tubular glands (Figs. 25, 26). On being established
in the epithelium, the amoeboid cells appear to increase in the amount of cytoplasm. A prominent Golgi apparatus and abundant mitochondria appear. In the mean time granules, comparable in size to those present in the cytoplasm of secretory cells, appear to reach the cytoplasm of the amoeboid cells (Figs. 27, 28). In another variant, in addition to granules comparable in size to those in the secretory cells, minute granules measuring 0.2-0.5μm dia accumulate in small membrane-bound vesicles in the cytoplasm which may in turn aggregate as large membrane-bound vesicles measuring 8-10μm dia. The minute granules appear to be acquired from outside or, alternatively, these granules are in the process of exocytotic release (Fig. 29). Occasionally, such minute granules are present in the cytoplasm of cells that possess large membrane-bound vesicles containing a homogeneous to heterogeneous, electron-lucent to electron-dense content (Fig. 30).

In an animal collected during the month of February, the histological organization of the epithelium, particularly of the base of the tubular glands, is indicative of extensive accumulation of amoeboid cells, and atypical aggregation of secretory material in the cytoplasm of the secretory cells. Ultrastructural analysis revealed that these cells are indeed amoeboid cells as they are of irregular shape and put forth pseudopodial processes. The nucleus occupies almost the entire cell, densely heterochromatic and the cells reflect high nucleo-cytoplasmic ratio. The cytoplasm is poor in organelle content and, invariably, the cells lack granules comparable to those in the secretory cells (Figs. 31, 32). Some of the secretory cells surrounding the amoeboid cells have their secretory material organized into small to big aggregations (Figs. 33-35). In certain parts of the Mullerian gland the amoeboid cells accumulate the granules, acquiring them from the neighboring secretory cells. The granules accumulate as dense aggregations, and measure 7-10μm dia. The content of such aggregations is indeed the granules acquired from the secretory cells, but highly heterogeneous in terms of electron density. The nuclei of the cells are altered in morphology so as to accommodate such large aggregations. The maximum number of aggregations in a cell in section is only two (Fig. 36). The cells possessing such aggregations appear to leave the epithelium, penetrating through the basement membrane, to reach the peritubular tissue (Figs. 37, 38).
The present study clearly shows that the microvillated tall columnar cells of the column and the base of the tubular glands of the caecilian under investigation are concerned with secretion of fairly large-sized secretory granules. The secretory cells of the column match in organization the generalized glandular epithelial cells concerned with synthesis of secretory material and fabrication of secretory granules. It is an established fact that in the glandular epithelial cells the secretory product first appears in the polysomes attached to endoplasmic reticulum and pass through the latter's membrane into its lumen (Waters and Hughson, 2000). Vesicular or tubular structures transport proteins from there to the cis-Golgi region and the proteins travel through the stacks of the Golgi complex to the trans-Golgi side (Gu et al., 2001). At this point, several kinds of sorting into different vesicles occur, such as separation of lysosomal proteins from those bound for the cell surface (Castle, 1990; Arvan and Castle, 1998; Thiele and Huttner, 1998). Thus, the secretory granules bud from the trans-Golgi network and the difference between the trans-Golgi network, and immature secretory granules is a quantitative one depending on the amount of membrane removal, rather than a qualitative one. Such a mechanism clearly occurs in the mammary gland cells (Dannies, 1999). The secretory granules are formed at the trans-Golgi network as short-lived vesicular intermediates termed immature secretory granules (Tooze et al., 1991). Immature secretory granules undergo a complex and poorly understood maturation process resulting in mature secretory granules, which are primed and docked presumably in proximity to the plasma membrane (Robinson and Martin, 1998).

One of the findings in the present study is that the secretory granules in the microvillated secretory cells of the column, and not in the secretory cells of the base, since their origin from the trans-Golgi network, are invariably confined to the medullary region of the cytoplasm. This is in contravention to the observation that in the secretory cells most of the secretory granules get dispersed to the cortex during the first 3 hr after biogenesis (Rudolf et al., 2001). The major difference between the cortical and medullary cytoplasm lies in the richness of F-actin in the cortex. The characteristic increase in the density of granules during the time span has been
proposed to indicate that maturation of immature secretory granules takes place in the F-actin rich place in the cortex. The cortical restriction of immature secretion granules is achieved by microtubules and F-actin. Therefore, the significance of confinement of the secretory granules to the medullary cytoplasm in the secretory cells of the column of the Mullerian gland is worth further investigation.

The secretory cells of the column and the base of the tubular glands differ in several respects. First, the secretory cells of the column do not differ among themselves in regard to organelle content and electron density of the cytoplasm and the profile of the secretory granules, whereas such differences could be found between the different secretory cells of the base. This has prompted us to identify three functional stages/structural variants of the secretory cells of the base. Second, in the secretory cells of the column the granules are confined to the cortical cytoplasm whereas in the secretory cells of the base a cortico-medullary differentiation of either the cytoplasm or distribution of the granules is not discernable. Third, unlike in the secretory cells of the column, in the base the secretory granules occur as different variants, 0.2-1.0 μm dia, 0.5-2.0 μm dia, 1.0-3.0 μm dia and 8.0-10 μm dia. Fourth, the mitochondria appear to play an intimate role in the fabrication of secretory granules in the cells of the base of the tubular glands which is not indicated in the cells of the column. These various differences between the cells of the column and the base suggest different roles in secretion to these cells. The ultrastructural organization of the secretory cells of the column clearly indicates auto-synthesis of the secretory material, its fabrication into secretory granules and their release through an apocrine mechanism into the lumen.

On the other hand the variation in the ultrastructural organization, combined with the already reported differences in the histochemical nature of the secretory material (Exbrayat, 1986; also, Chapter III of the thesis) tempt us to propose a variety of possibilities. i) Like the secretory cells of the column, the secretory cells of the base are also auto-synthetic and release the secretory material into the lumen on top of the base as structured granules. ii) The second possibility is that the secretory material of the cells of the base arrive from elsewhere, particularly the liver through the blood,
sequestered at the base of the cells into the cytoplasm, accumulated in saccules of endoplasmic reticulum, glycosylated during passage through the endoplasmic reticulum and Golgi apparatus and formed into secretory granules for release into the lumen of the tubular glands. Such a mechanism has been reported in the extra-ovarian synthesis of yolk proteins in arthropods and vertebrates (Don-Wheeler and Engelmann, 1997; Tseng et al., 2001; Lattier et al., 2002).

The presence of composite and heterogeneous granules and the individual granules with homogeneous content in the amoeboid cells of the base of the tubular glands makes an interesting observation. Though it would require confirmation, the granules in the amoeboid cells appear to be acquired from the epithelial cells. It is possible that the amoeboid cells perform a physiological role of acquiring the granules from the epithelial cells and functioning as carriers of the granules to elsewhere. We hypothesize this destination to be the testis. Alternatively, the amoeboid cells have a scavenging role and remove the secretory granules from the epithelium of the base particularly when the animal enters the regression phase. There is at least one instance of leucocytic cells performing a comparable function. The epididymal epithelium of mammals has cells known as basal cells. The basal cells were earlier considered to be a resident cell population and even to be stem cells producing the tall columnar cells (Sun and Flickinger, 1979; Robaire and Hermo, 1988). But, recent studies have shown that the basal cells arrive at the epididymal epithelium from the peritubular tissue and are derivatives of tissue monocytes (Roitt, 1984). The basal cells are, thus, a migratory cell population (Yeung et al., 1994; Seiler at al., 2000, Holschbach and Cooper, 2002). Studies have shown that the basal cells acquire the disintegration products from the overlying principal cells in the form of lipofucsin material (Akbarsha et al., 2000). The lipofucsin material accumulates in the cytoplasm of the basal cell to a large size and brings about a change in shape of the nucleus. Subsequently, the basal cells leave the epithelium carrying the lipofucsin material (Yeung et al., 1994; Seiler et al, 2001). The situation obtaining in the amoeboid cell of the epithelium of the basal portion of the tubular glands of caecilian Mullerian gland, in a way, matches that in the basal cells of the epididymal epithelium.
The mechanism of release of secretory granules from the secretory cells through the apical membrane as structured granules makes another interesting observation. In general, the glandular epithelial cells fabricate the secretory material as discrete granules, but at the time of release of the material into the lumen the condensed material undergoes some kind of solublization; the membrane of the vesicle and the apical plasma membrane fuse and the content is released in a solublized form (Luke and Coffey, 1994; Hermo and Robaire, 2002; Burgoyne and Morgan, 2003). In another situation, the granules are released in a structured form through an exocytotic mechanism when the granules maintain the identity even after their release into the lumen. This mechanism has been reported in the epididymal epithelium of several reptiles (Depeiges and Dufaure, 1977; Manimekalai and Akbarsha, 1992; Akbarsha and Manimekalai, 1999) and the mammalian prostate gland (Gesase and Satoh, 2003; Cohen et al., 2001). Thus, the mechanism of release of granules from the secretory cells of the tubular glands of the caecilian matches that in the epididymal epithelium of several reptiles and the mammalian prostate gland.

The few published reports have suggested the retention of Mullerian duct as a functional glandular structure in male caecilians, in the absence of other male accessory reproductive glands, as of significance in the secretion of a medium in which sperm could be suspended during ejaculation (Wake, 1981; Exbrayat, 1986, 1992). This primitive form of internal fertilization, making use of an eversible phallodeum as the intromittent organ, has been interpreted as an attempt towards terrestrialization in the amphibian stage of evolution itself. The present study, while confirming the role of caecilian Mullerian gland in secretion of a material, also expounds major differences between the secretory cells and secretory processes of the column and the base of the tubular glands. The scope for multiplicity of the secretory processes at the base of the tubular glands suggests not only origin of the secretory material from elsewhere, but destination from the tubular glands to elsewhere. Identification and characterization of the proteins of the secretory material of Mullerian gland and finding their antigenic homogeneity with proteins of caecilian testis, blood and liver, the secretory material of lizard epididymis and the secretory material of mammalian prostate and seminal vesicles are under investigation.
REFERENCES


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FIGURE LEGENDS

Fig. 1. Low power TEM picture of the secretory cells of the column of the tubular gland of *Uraeotyphlus narayani* showing the basal nucleus (NU), basal and perinuclear cytoplasm being free from secretory granules (SG) but abounding with mitochondria (MI) and endoplasmic reticulum (ER), and abundant secretion granules in the cytoplasm apical to the nucleus but mostly confined to the medullary portion (MD) and few in the cortical portion (CX). Scale bar = 10μm.

Fig. 2. A portion of figure 1 magnified showing the cortico (CX)- medullary (MD) differentiation of the cytoplasm. The secretory granules (SG) are confined to the medullary portion. The SGs are in different sizes and electron densities. Scale bar = 1.5μm.

Fig. 3. TEM picture of the basal portion of secretory cell of the column showing the nucleus (NU), and mitochondria (MI) and endoplasmic reticulum (ER) in the perinuclear and basal cytoplasm. Secretion granules (SG) are found in the supranuclear cytoplasm. Scale bar = 6μm.

Fig. 4. TEM picture of the supranuclear cytoplasm of the secretory cell of the column showing stacks of Golgi apparatus (GA) and newly formed secretory vesicles (SV). Scale bar = 2.8μm.

Fig. 5. High power TEM picture of the Golgi apparatus of the secretory cell of the column. Cisternae of rough endoplasmic reticulum (RE) associate with the *cis*- phase of the Golgi apparatus (GA). Fully formed secretory vesicles (SV) appear at the *trans*-phase. Scale bar = 1.5μm.

Figs. 6-8. High power TEM pictures showing gradual condensation of the content of the secretory vesicles (SV). Fully formed (mature) secretory granules (SG) and cisternae of rough endoplasmic reticulum (RE) are also indicated. Scale bar = 1.5μm.

Fig. 9. High power TEM picture showing nascent secretory vesicles (SV) and the maturing secretory granules (SG) in the process of condensation and even fusion (arrowheads). Scale bar = 1.5 μm.

Fig. 10. TEM picture showing apical portion of the secretory cells (SC) of the column. The secretory granules (SG) are in different phases of condensation. The lumen of the tubular gland is also shown (LU). Scale bar = 3μm.

Figs. 11, 12. High power TEM pictures showing the different stages in the apocrine mechanism of release of the secretory granules (SG) into the lumen (LU). The
membrane of the secretory granule becomes continuous with the apical plasma membrane of the cell (arrowheads). Scale bar = 1.5\mu m

Fig. 13. TEM picture showing the different structural variants / functional stages of the secretory cells of the base of the tubular glands. A freshly recruited cell (FC) lacks secretory granules. Mature cells are of two kinds, those with the electron-lucent cytoplasm (EL) and those with electron-dense cytoplasm (ED). Scale bar = 4\mu m

Fig. 14. A portion of figure 13 magnified, highlighting the differences between cells with electron-lucent cytoplasm (EL) and electron-dense cytoplasm (ED). Secretory granules (SG) of the two cells also differ in shape and electron density. Scale bar = 1\mu m

Figs. 15-19. High power TEM pictures of the Golgi area (GA) of the secretory cells of the base showing the different stages in the formation of the secretory granules. 15. Minute vesicles are pinched off (arrowheads) from the rough endoplasmic reticulum (RE) which associate with the cis-phase of the Golgi apparatus; on the opposite side in the trans-phase. 16. There are a few mitochondria (MI) in the Golgi area. 17, 18. The secretory vesicles (SV) are produced in the Golgi apparatus (GA). The Golgi apparatus (GA) abounds with secretory vesicles (SV) and mitochondria (MI). 19. Mitochondria (MI) associate with the nascent secretory vesicles (SV). Scale bar = 15-18, 1\mu m; 19, 0.5\mu m

Figs. 20-26. TEM pictures illustrating the origin of amoeboid cells (AC) present in the base of the tubular glands, from fibroblasts (FB) of the peritubular tissue. 20. A fibroblast (FB) in the peritubular tissue. 21. Migration of a fibroblast (FB) through the basement membrane (BM). 22. Early establishment of an amoeboid cell in the basal epithelium, among the secretory cells (SC). 23-26. The cells put forth pseudopodial protuberances (arrowheads) and reach deeper destinations. Scale bar = 20-24, 0.3\mu m; 25, 1.5\mu m; 26, 3.0\mu m

Figs. 27-30. TEM pictures showing the different phases in the acquisition / accumulation of secretory granules by the amoeboid cells of the base. 27. The amoeboid cell (AC) is rich in organelles but contains few secretory granules (SG). 28. The amoeboid cell (AC) contains immature secretory granules (SG). 29. The amoeboid cell (AC), lying below a secretory cell (SC), accumulates minute granules in large vesicles (VE). The content of the granules apparently arrives from outside (arrowheads) or, alternatively, the content is being released to outside. 30. Picture shows an amoeboid cell containing highly condensed minute granules in membrane-bound vesicles (arrowheads) in the vicinity of vesicles containing less condensed material (SV). Scale bar = 2.4\mu m

Figs. 31-38. TEM pictures showing arrival of amoeboid cells at the base of a regressing Mullerian gland. 31. Plenty of amoeboid cells (AC) arrive at the base
amongst the secretory cells (SC) from the peritubular tissue. Scale bar = 10μm. 32. Secretory cells (SC), with electron-lucent (EL) and electron-dense (ED) cytoplasm associate with the amoeboid cells (AC) through pseudopodial protuberances (arrowheads). Granules start appearing in the cytoplasm of amoeboid cells (arrows). Scale bar = 0.3μm. 33. Secretory cells with the electron-lucent (EL) cytoplasm, and the granules forming into dense aggregates (arrowheads). Scale bar = 1.5μm. 34. Same as figure 33, but the granules form into a large heterogeneous mass (HM). Scale bar = 5.0μm.

35. Same as figure 34, but the secretory material forms into dense heterogeneous aggregates (HA). Scale bar = 4.0μm. 36. Large dense heterogeneous aggregates (HA) are present in the cytoplasm of amoeboid cells (AC). Scale bar = 5.0μm. 37, 38. The amoeboid cells (AC), loaded with the dense heterogeneous aggregates of secretory material (HA), leave the epithelium, penetrating (arrowheads) through the basement membrane (BM). Scale bar = 4.0μm.