

Histological Changes of the Ampullary Epithelium of Cyclic Albino Rat Oviduct: Light and Ultrastructure Examination

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Abstract: The mammalian oviduct especially the ampulla part plays an essential role in female reproduction as fertilization, oocyte maturation and early embryonic development. The present study was focused on the microscopic changes of the rat ampulla epithelium during the oestrous cycle and correlates the findings with their possible functions. Twenty five rats of regular 5 day oestrous cycles were used in our study. They were divided into 5 groups; five rats in each phase of estrous cycle (early proestrus, late proestrus, estrus, metestrus and diestrus). They were sacrificed and the ampullae were excised, fixed and processed to be examined by light and electron microscopy. Marked changes in both relative number and the structure of ciliated and secretory cells throughout the estrous cycle were seen. Ciliated cells were increased in the relative number at the early proestrus till became the predominant cell type at the late proestrus then decreased at the estrus phase accompanied by deciliation. The secretory cells increased in the relative number and prevailed at the metestrus with increasing in the secretory activity. They became rare at the diestrus, a day on which the first sign of neociliogenesis was recorded. Intra epithelial glands were firstly appeared at the estrus phase which increased in number and size at the metestrus. The secretory material is showed PAS positive reaction in the estrus and the metestrus.

Key words: Oviduct ampulla epithelium • Electron microscopy • Oestrous cycle • Rat

INTRODUCTION

The oviduct is the key organ in the female reproductive system. It is not only a simple tubular conducting organ for the passage of gametes (ova & spermatozoa), but it is also considered as an active and vital secretory organ [1]. The mammalian oviduct can be divided into three regions: infundibulum, ampulla and isthmus. The site of fertilization is the ampulla part [2]. It creates an optimal environment for critical reproductive events leading to successful pregnancy as fertilization, oocyte maturation and early embryonic development. Its lining epithelium is adapted to perform these functions [3, 4]. It is lined by two types of cells ciliated columnar and non-ciliated secretory columnar cells. The ciliated cells have motile cilia which extend in the lumen. The secretory cells characteristically contain secretory granules [5]. The interactions of both cells are regulated by the ovarian steroid hormones associated with different phases of estrous cycle [6].

This study aimed to give a complete picture about the histological changes of the rat ampulla epithelium during oestrous cycle with the aid of light and TEM microscope and correlate the findings with their possible ampulla functions.

MATERIALS AND METHODS

In the present study 35 sexually mature female albino rats (*Rattus norvegicus albinos*) aged from (3 - 4 months) were obtained from Helwan Laboratory Animal Breeding Farm. The rats were confined in standard cages and maintained on food and water ad libitum, temperature of 22°C and a lightening schedule which was established as photoperiod of 12 hour light and 12 hour dark. All rats were observed for several days to ascertain their health before the samples were collected. Daily vaginal smears were taken at least two weeks for the determination of the different stages of the oestrus cycle. Only rats displaying at least three consecutive oestrous cycles of the

same length were selected for the experimental use. Twenty five of selected rats were used and divided into five groups. Five rats in each phase (early proestrus, late proestrus, estrus, metestrus and diestrus).

For light microscopy, at each cyclic phase 5 rats were sacrificed by using an over dose of ether inhalation, the ampullae of the oviducts were immediately excised and quickly fixed in Bouin's fluid, 10% neutral buffered formalin and stained with Harris Hematoxylin and Eosin for general studies, PAS – AB combination for identification and differentiation of neutral and acidic mucopolysaccharides. All the aforementioned fixatives and stains as outlined by Bancroft & Gamble [7].

For electron microscopy, 10 ampullae samples were collected; two samples of each phase of oestrus cycle. The collected specimen were cut into very small pieces and fixed in 4% gluteraldehyde in phosphate buffer solution, washed three times in the same buffer [8], post-fixed in 1% osmium tetroxide in phosphate buffer then embedded in epoxy medium. Semi-thin sections were obtained and stained by toluidine blue then examined by the light microscope [9]. Ultra-thin sections were obtained, mounted on copper grids and contrasted with 5% uranyl acetate followed by lead citrate stain [10], examined by transmission electron microscope JEOL (JEM-1400 TEM 80 kv) in the electron microscope unit of faculty of Agriculture, Cairo University.

RESULTS

Light microscopical examination of ampulla of rat oviduct at the early proestrus showed arranged four tunics from the inward to the outward i.e. the tunica mucosa, the tunica submucosa, the tunica muscosa and the tunica serosa. Ampulla could be distinguished from other oviductal segments by its highly folded mucosa and its thin-walled muscular coat. The mucosal folds were numerous, elongated and branched forming primary, secondary and sometimes tertiary folds which occupied the majority of oviductal lumen (Fig. 1.1). The submucosa formed of loose connective tissue and extending forming the core of mucosal folds. The thin-walled tunica muscosa was formed of very thin inner circular and outer longitudinal smooth muscle fibers housing many blood vessels. The outermost last layer was, the tunica serosa, lined with mesothelial layer rested on a thin layer of connective tissue sub serosa.

The mucosal folds were lined with simple columnar epithelium which consisted of two types of cells i.e., columnar ciliated cells and columnar non ciliated cells

(secretory cells). They appeared with acidophilic cytoplasm containing oval vesicular nuclei. Both cells intermingled irregularly with each other and the ciliated cells could be distinguished from the secretory cells by their apical numerous hair-like processes (Fig. 1.2).

At EM level, the ciliated cells were more numerous than the secretory cells and could be easily demonstrated by their cilia on the free surface. (Fig. 1.3). They were columnar cells containing oval euchromatic basally situated nuclei. The cytoplasm contained numerous mitochondria which distributed at the apices and bases of the cells. Also, sparse RER cisternae and supranuclear Golgi apparatus were seen. Numerous rounded basal bodies immediately close or beneath the apical ciliated cell membrane were present. In some ciliated cells, sparse short and thick microvillus processes were scattered between the cilia. (Fig. 1.4 & 1.5).

The secretory cells were few in number having convex slightly smooth apical surface which carried sparse, short and thick microvilli. They showed darkly stained cytoplasm containing heterochromatic, elongated and indented nuclei. Few cytoplasmic organelles were seen as short tubular cisternae of RER, swollen mitochondria, poorly developed GA and remnant of degenerated bodies (Fig. 1.6). The ampullary epithelium showed negative staining reaction after PAS-AB application (Fig. 1.7).

During the late proestrus, the lining epithelium of mucosal folds showed few focal areas of pseudo stratification and the ciliated cells were more numerous than the early proestrus which became clearly the most dominant cell type. They carried elongated tightly-packed cilia on their luminal surfaces forming a brush border (Fig. 2.1). Ultrastructurally, well developed columnar ciliated cells were arranged closely to each other while the observed secretory cells became shrunk and located in lower level than the ciliated cells which didn't reach the oviductal lumen (Fig. 2.2). The apical basal bodies of the ciliated cells were fewer than that of the early proestrus. Most of them changed to be elongated structures to form ciliary rootlets which implanted into the cell membrane and their apical free tips extended from the luminal surfaces. Moreover, the cilia were dense, long and slender-like. Numerous of different shaped mitochondria, RER cisternae, Golgi apparatus and polysomes were distributed throughout the cytoplasm (Fig. 2.3). No pronounced ultrastructural changes in the secretory cells as well as in PAS - AB staining reaction from the early proestrus.

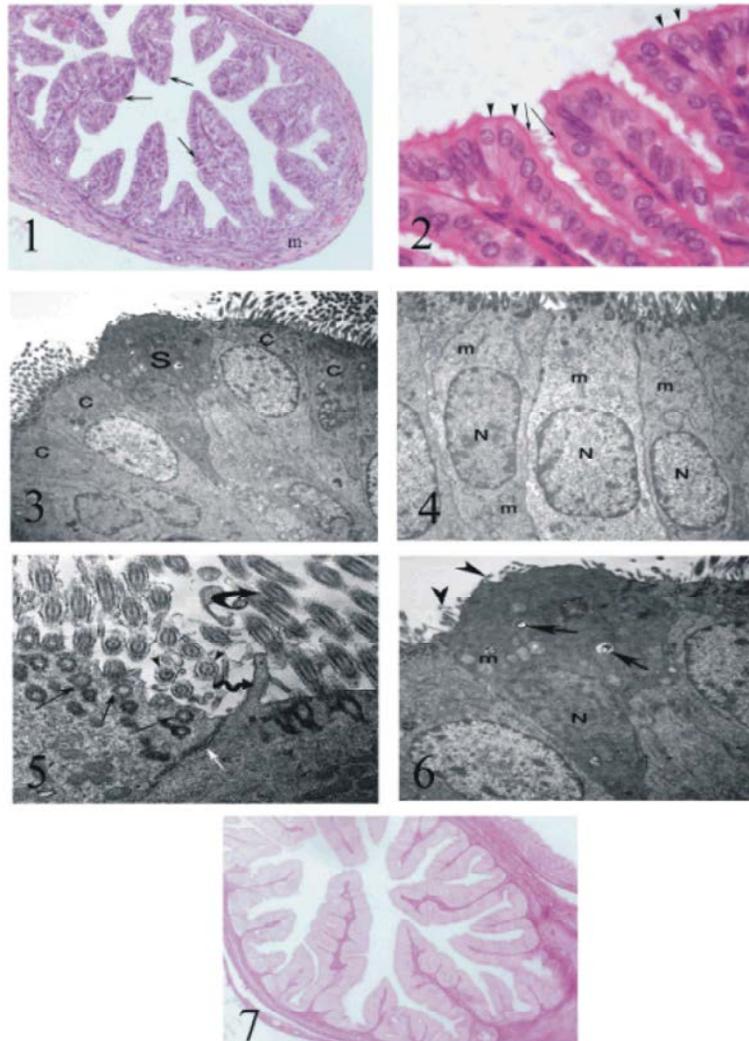


Fig. 1: 1. A photomicrograph of ampullated part of rat oviduct at the early proestrus showing numerous, elongated, branched and highly folded mucosa occupied the majority of oviductal lumen (arrows) and thin-walled tunica muscosa (m). H&E, X200. 2. A higher magnification of an ampullary mucosal fold of rat oviduct at the early proestrus showing its lining epithelium was simple columnar cells; ciliated carrying apical hair-like processes (arrows) and non-ciliated cells (arrowheads) containing acidophilic cytoplasm, oval, vesicular and basally situated nuclei. H&E, X1000. 3. An electron micrograph of the lining epithelium of ampulla of rat oviduct at the early proestrus showing numerous ciliated cells with apical cilia (C) and few electron dense secretory cells (S). Uranyl acetate and lead citrate stain, X3600. 4. An electron micrograph of ciliated cells of ampulla of rat during the early proestrus showing oval euchromatic nuclei (N) surrounded by numerous mitochondria (m). Uranyl acetate and lead citrate stain, X5200. 5. A higher magnification of the apical portion of a ciliated cell of rat ampulla at the early proestrus showing apical rounded basal bodies (arrows), typical structure of cross-sectioned cilia (arrowheads), longitudinally cut cilia (a curved arrow) and intercellular junction (a white arrow). Note, apical thick microvillus processes (a wavy arrow). Uranyl acetate and lead citrate stain, X25000. 6. An electron micrograph of a secretory cell of ampullary epithelium of rat during the early proestrus showing convex apical surface with few and short microvilli (arrowheads), electron-dense cytoplasm containing heterochromatic and elongated nucleus with marked indentations (N), swollen mitochondria (m) and small sized degenerated bodies (arrows). Uranyl acetate and lead citrate stain, X5800. 7. A photomicrograph of rat ampulla during the early proestrus stained with PAS/AB showing -ve reaction. X 200

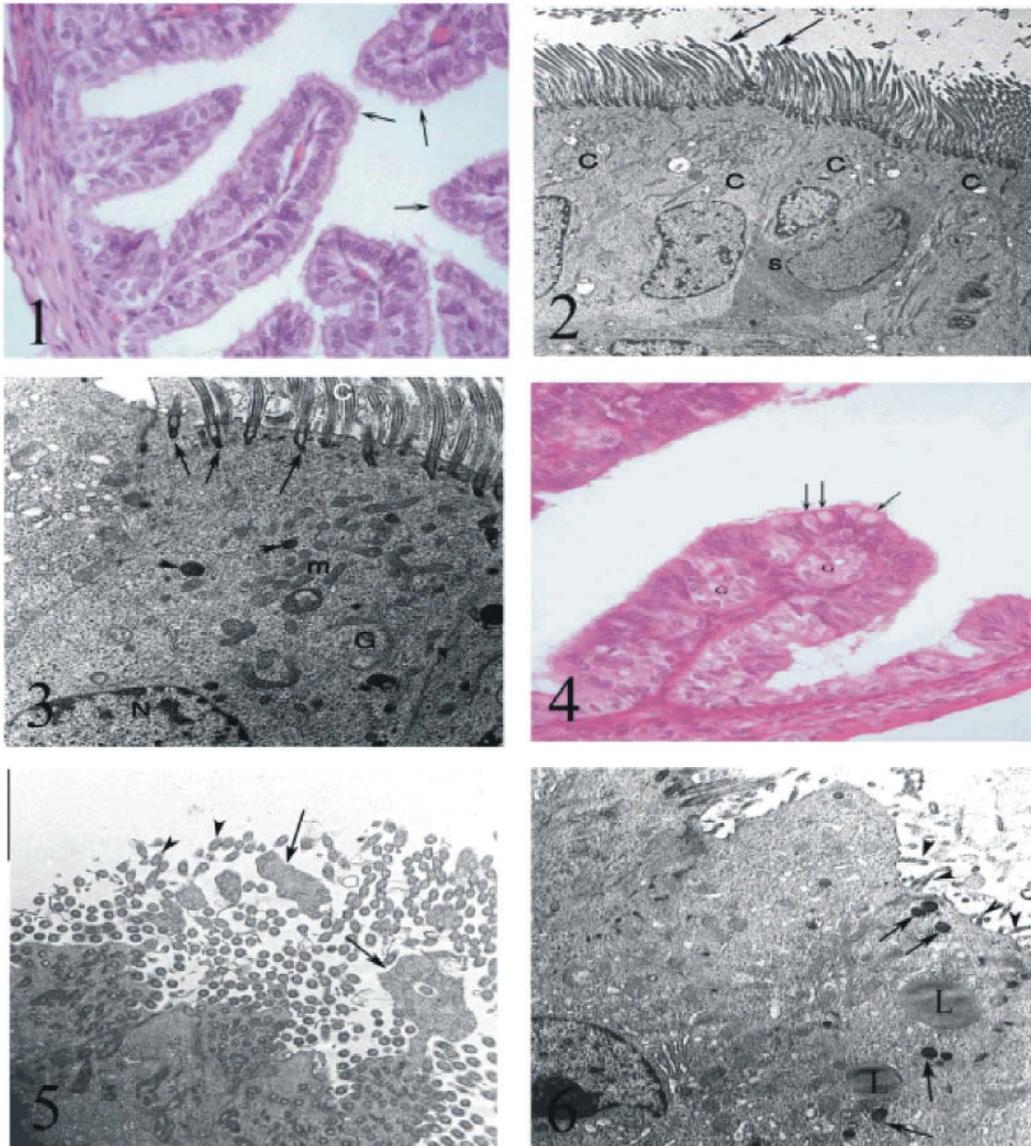


Fig. 2: 1. A photomicrograph of rat ampulla during the late proestrus showing heavily ciliated epithelium (arrows) with focal areas of pseudostratification. H&E, X400. 2. An electron micrograph of rat ampulla at the late proestrus showing predominant of ciliated cells (C) with dense and well- developed elongated cilia (arrows), sparse, electron dense secretory cell located in low level (S).Uranyl acetate and lead citrate stain, X4000. 3. An electron micrograph of a ciliated cell of rat ampullary epithelium at the late proestrus showing numerous ciliary rootlets were implanted in the apical surface (arrows), elongated cilia (C), numerous mitochondria (m), well developed Golgi apparatus (G) and euchromatic nucleus (N). Notice, remnants of secretory granules (arrowheads).Uranyl acetate and lead citrate stain, X12000. 4. A Photomicrograph of rat ampullary mucosal fold at the estrus showing marked pseudostratified epithelium with decreasing of ciliation and increasing of lightly - stained secretory cells (arrows). Note, invagination of groups of secretory cells into the mucosal folds forming intraepithelial glands with narrow lumina (G).H&E, X400. 5. An electron micrograph of ciliated cells of rat ampullary epithelium at the estrus showing marked deciliation. Notice, solitary sloughed cilia (arrowheads) and ciliary packets (arrows) float in the oviductal lumen. Uranyl acetate and lead citrate stain, X10000. 6. An electron micrograph of the secretory cells of rat ampullary epithelium at the estrus showing well-developed microvilli (arrowheads), electron dense secretory granules (arrows) and lipid granules (L).Uranyl acetate and lead citrate stain, X10000.

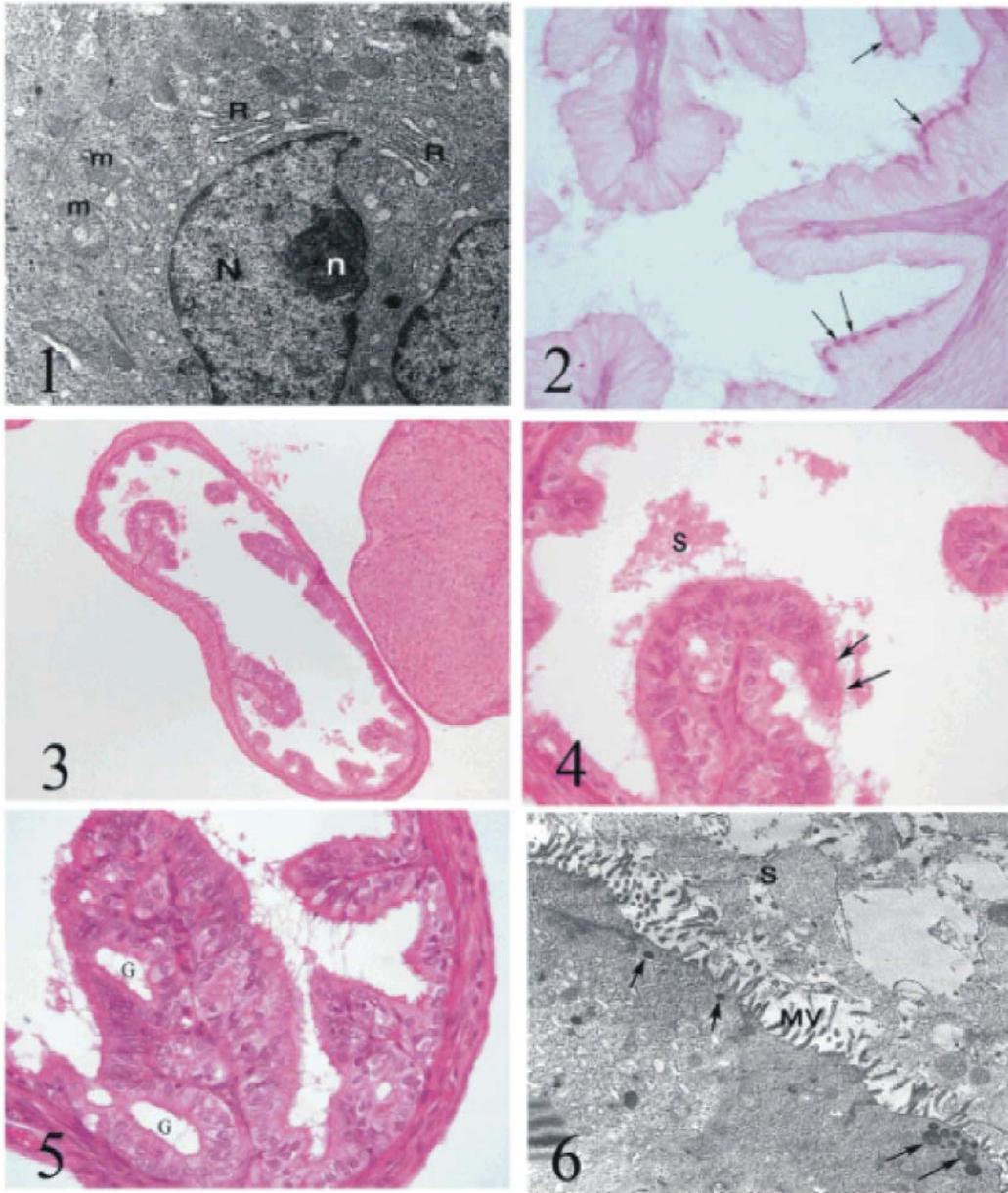


Fig. 3: 1. A higher magnification of (Fig.2.6) showing well developed cytoplasmic organelles as dilated cisternae of RER (R), numerous mitochondria (m) with lamellar crista. Notice, oval euchromatic nucleus (N) with prominent nucleoli (n).Uranyl acetate and lead citrate stain, X15000. 2. A section of ampulla of rat oviduct at the estrus stained with PAS/AB showing strong apical PAS +ve granules (arrows). X 400. 3. A section of ampulla of rat oviduct at the metestrus showing decreasing in the number and the height of most of mucosal folds with misshaping appearance. H&E, X40. 4. A higher magnification of (Fig. 3.3) showing massive pseudostratified epithelium, presence of detached cells with secretory material and nuclei (arrows). Note, acidophilic secretory material accumulated in the oviductal lumen (S). H&E, X400. 5. A photomicrograph of mucosal fold of rat ampulla at the metestrus showing numerous large -sized intra epithelial glands with wide lumina (G).H&E, X400. 6. An electron micrograph of secretory cells of rat ampulla at the metestrus showing numerous and elongated well developed microvilli (MV), electron dense secretory granules (arrows) and and secretory material accumulated in the oviductal lumen (S).Uranyl acetate and lead citrate stain, X10000.

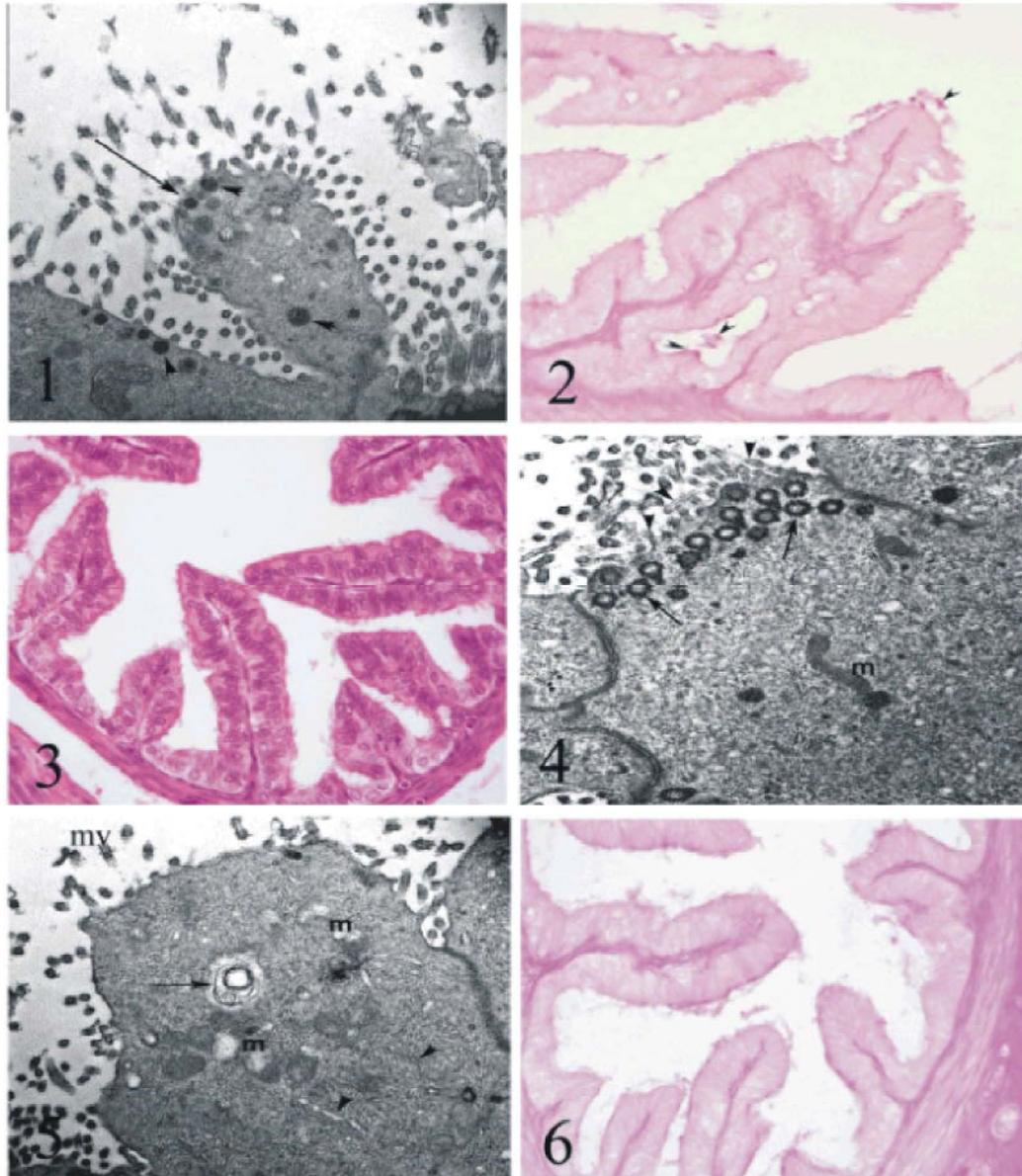


Fig. 4: 1. An electron micrograph of the apical part of the secretory cell of the ampullary epithelium at the metestrus showing a constricted apical cytoplasmic protrusion (an arrow) containing secretory granules (arrowheads). Uranyl acetate and lead citrate stain, X20000. 2. A photomicrograph of rat ampulla at the metestrus stained with PAS\AB showing strong intra and extracellular PAS +ve secretory material as well as in the lumen of intra epithelial glands (arrowheads).X400. 3. A photomicrograph of rat ampulla at the diestrus showing the mucosal folds returned to be elongated and lined with simple columnar epithelium with basophilic cytoplasm containing oval and vesicular nuclei. H&E, X200. 4. An electron micrograph of a ciliated cell of rat ampullary epithelium at the diestrus showing the first sign of neociliogenesis with few apical newly formed basal bodies (arrows) and few elongated mitochondria (m).Notice, apical short microvilli in the same cell (arrowheads).Uranyl acetate and lead citrate stain, X20000. 5. An electron micrograph of a secretory cell at the diestrus showing bulged apical surface with detached microvilli in the oviductal lumen (MV), few cisternae of RER (arrowheads) and mitochondria (m) as well as presence of a degenerated lamellated body (an arrow) .Uranyl acetate and lead citrate stain, X20000. 6. A photomicrograph of ampulla at the diestrus stained with PAS\AB showing moderate PAS +ve materials in the apical surface of the lining epithelium. X400.

During the estrus, epithelial pseudo stratification increased and prevailed than the simple columnar cells. The epithelium of the mucosal folds appeared with crowded nuclei which located in different levels. The most characteristic feature was the increase of secretory cells which became outnumbers of ciliated cells. They appeared as swollen cells with pale or foamy cytoplasm containing oval or rounded vesicular nuclei. Groups of arranged secretory cells showed to be invaginated within the thickness of mucosal folds forming intra - epithelial glands with narrow central lumina (Fig. 2.4).

Ultrastructurally, the ciliated cells became fewer and carried shorter cilia than that of the late proestrus and most of them showed pronounced signs of deciliation. The sloughed cilia were shedded in the oviductual lumen either solitary or in packets and apical cytoplasmic protrusions of ciliated cells containing many ciliaryaxonemes were seen (Fig. 2.5). Numerous secretory cells were easily distinguished ultrastructurally. Their apices were bulged into the oviductual lumen and carried microvilli. Their nuclei were oval euchromatic with obvious nucleoli. The cytoplasm contained electron dense secretory granules, supranuclear GA, mitochondria and extensive network of RER (Fig. 2.6&3.1). After application of PAS-AB, the secretory cells showed positive staining reaction with PAS only. PAS positive magenta secretory granules were accumulated in the apical part of the cytoplasm (Fig. 3.2).

During the metestrus, the most obvious feature was decreasing in number and the height of most mucosal folds with misshaping appearance (Fig. 3.3). They showed marked pseudo stratification with more crowded cells and nuclei. Some of detached cells containing secretory material were clearly seen in the oviductual lumen while others were still attached with the mucosal folds (Fig. 3.4). Also, other examined sections showed increasing in the number and size of the intra - epithelial glands housing wide lumina (Fig. 3.5). Sparse small sized ciliated cells were noticed and their ultrastructural features were similar to that seen at estrus. Whereas the secretory cells became the most dominant cell type. They characterized by numerous well- developed microvilli, apical electron dense secretory granules and numerous well - developed cytoplasmic organelles. The oviductual lumen was filled with secretion (Fig. 3.6). Also, apical cytoplasmic protrusions containing secretory granules were noticed (Fig. 4.1). Strong PAS positive materials accumulated in the cell apices of both ampullary epithelium and the intra epithelial glands as well as in their lumina (Fig. 4.2).

During the diestrus, the ampulla regained its elongated highly folded mucosa with simple columnar epithelium. They characterized by basophilic cytoplasm

containing oval and vesicular nuclei (Fig. 4.3). Some of examined cells ultrastructurally showed first signs of neociliogenesis which characterized by appearance of apical newly formed basal bodies beside the presence of short microvilli (Fig. 4.4). Few secretory cells were recorded. The cytoplasm became darkly stained containing few organelles and degenerated lamellated bodies (Fig. 4.5). Moderate PAS positive materials were seen in the apical surface of the lining epithelium (Fig. 4.6).

DISCUSSION

The structure of rat ampullary mucosa in our study, similar to the most of mammalian species, is highly folded and occupied the majority of the oviductual lumen. It is lined by simple columnar epithelium consisting of two types of cells; ciliated and non-ciliated (secretory cells). Similar results were recorded by previous studies [11- 17]. The interactions of ciliated and secretory cells in the oviduct with gametes both in vitro and in vivo obviously support the normal growth of early embryos and maintainance of sperm functions [6].

Our findings showed marked histological changes of the ampullary epithelium during different phases of the oestrous cycle. These changes are regulated by the circulating ovarian steroid hormones [6]. The lining epithelium of ampulla of rat oviduct showed alteration from simple to pseudostratified throughout the estrous cycle. Gradual increase in the epithelial pseudo stratification was seen from the late proestrus to the estrus till became obviously extensive with more crowded cells and nuclei at the metestrus. Then it returned to be simple columnar cells at the diestrus and the following proestrus. Similar observations were recorded in ampulla of mare [3]. While the epithelial pseudo stratifications were high at the proestrus and the estrus in ampulla of sow oviduct [18].

Moreover, the relative numbers and the structure of ciliated and secretory cells were greatly altered throughout the oestrus cycle. Similar findings also recorded by other studies [11, 19]. Steroid hormones have an effect on cyto differentiation of both ciliated and secretory cells [20]. Ovariectomized rabbits showed atrophy of the oviductual epithelium and pronounced decrease in the number of cilia which were renewed again after administration of estrogen [21]. The ampullary epithelium in our study showed extensive ciliated cells at the late proestrus followed by decreasing at the estrus with further decreasing at the metestrus. Similar findings were noticed in different animal species [11, 14, 16, 22].

Along the course of oestrus cycle, the ciliated cells in the present study showed regular cyclic process of ciliogenesis and deciliation. The first sign of ciliogenesis was noticed at the diestrus which indicated by the presence of few newly formed rounded ciliary basal bodies in the apical cytoplasm of cells then increased in number in the following proestrus. While, massive well-developed cilia with ciliary rootlets were clearly shown and predominant at the late proestrus, a day of ovulation. That's indicative of importance of cilia to transport the oocyte [11, 23, 24]. The decreasing of ciliated cells at the estrus and the metestrus is augmented by the deciliation phenomena [25, 14, 16]. The first sign of deciliation in the present study was noticed in the estrus. The cilia were detached singly or in packets which manifested by shedding of the apical cytoplasmic portion of ciliated cells with their ciliary axonemes. Our investigation was met with the results recorded by Reeder and Shirley [25] who suggested that according to the short estrus cycle of rat which spans only 4 or 5 days, rapid deciliation occurred by pinching off clusters of cilia from ampullary epithelium. While, in animals with long oestrous cycle as beagle, the deciliation occurred by resorption of cilia and complete regression of ciliated cells took place during a period of 6 to 9 days [26]. Many authors recorded that the regular ciliogenesis and deciliation of ampulla is depended on the female hormonal levels i.e., ciliogenesis in follicular phase occurs under the influence of estrogen [27]. Whereas, the appearance of progesterone lead to deciliation which has an anti-proliferative effect on the oviductal epithelium [25, 28, 29].

In the present study the secretory cells showed marked alteration in the relative number accompanied by changes in the ultrastructure and histochemical properties as well as the secretory activity throughout the oestrus cycle. More secretory cells were easily observed at the estrus than the proestrus and continued in increasing till became the predominant cells at the metestrus as recorded in different animal species [11, 30, 31, 16]. With increasing their number, the secretory activity was enhanced which manifested ultra-structurally by the presence of electron dense secretory granules, well-developed elongated apical microvilli and numerous of well-developed cytoplasmic organelles as dilated cisternae of RER, GA and mitochondria. This result is in harmony with that recorded by other researchers [14, 12, 11]. Shirley & Reeder [11] stated that the secretory activity was heightened in the early post-ovulatory period when the oocyte was present in the ampulla.

In our study the picture of progressive epithelial detachment explain the characteristic feature of rat ampulla during the metestrus. Most of mucosal folds were lost except few of them characterized by misshaping as a result of extensive crowding of detached cells with their secretory material and their nuclei. Regards the mode of secretion our findings lead us to agree with the hypothesis stated that the mode of secretion is apocrine type which augmented by the presence of apical cytoplasmic protrusions containing secretory granules [30, 32].

Our study revealed the presence of intra-epithelial glands in the mucosa of rat ampulla throughout the oestrous cycle which firstly appeared at the estrus as few and small in size with narrow lumina then increased in number and size with wide lumina at the metestrus followed by decreasing till disappeared at the diestrus and the next proestrus. Similar results were recorded in oviducts of mare and bitch [3, 33]. Such glands could be classified as glands of tubular type [5, 34]. Moreover, marked decreasing of the secretory activity was recorded in the diestrus which manifested by decreasing of apical microvilli and the cytoplasmic organelles became few and ill-developed accompanied by presence of degenerated lamellated bodies as recorded by Shirley & Reeder [11] and Morita *et al.* [14].

Many researches recorded that the rat oviductal glycoprotein is produced and released by the secretory cells of the oviductal epithelium [31, 35]. An immunohistochemical study of mouse showed the oviductal glycoprotein was localized particularly in the secretory granules of the secretory cells in infundibulum and ampullary epithelia but not in isthmus [36]. Regarding the histochemical observations in our study, the type of secretion is neutral mucopolysaccharides. That's augmented by results obtained after application PAS /AB, the secretory cells showed negative reaction with AB while was given positive reaction with PAS technique. Similar findings were detected by Özen *et al.* [16]. The intense staining of reaction varied from moderate in the estrus, strong in the metestrus, weak in the diestrus till became negative at the next proestrus. This result is in harmony with that recorded in the present study by the electron microscopical observation.

The presence of remnant secretory granules and scattered microvilli beside the cilia in the newly formed ciliated cells as well as the rapid ciliation and deciliation lead us to agree with the findings reported by Shirley & Reeder [11] and Kress & Morson [37]. They stated that the oviductal epithelial cells are multipotential,

as they may transform from one functional cell type to another, which supported by the fact that the ciliated cells and the secretory cells alternately increase and decrease in number without evidence of much mitotic activity in either cell type.

CONCLUSION

Pronounced variation in the ampullary epithelial cells of rat oviduct according to the phases of oestrous cycle. Proliferation of the ciliated cells occurred during the early and the late proestrus just before the ovulation. Increasing of the secretory cells with their activity appeared during the estrus and the metestrus after ovulation to maintain suitable environment for early embryonic development.

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