INFLUENCE OF ESTROGEN AND PROGESTERONE ON ULTRASTRUCTURAL INDICES OF OVIDUCTAL EPITHELIUM IN SEXUALLY IMMATURE MICE

I. LAUSCHOVÁ

Department of Histology and Embryology, Medicine Faculty, Masaryk University, Brno, Czech Republic

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Abstract


The influence of estrogen and progesterone on the oviductal epithelium of sexually immature mice aged 14, 21, and 28 days was studied using electron microscopy. These ages were used because the postnatal differentiation of the oviductal epithelium is completed in all substantial features. Each group was divided into three subgroups of 8-10 mice: controls, mice treated with estradiol, and mice treated with estradiol and progesterone.

The administration of steroid ovarian hormones generally induced acceleration of the development of oviductal epithelial cells. The ultrastructure of these cells was similar to that of adult mice with normal estrous cycle. While estradiol influenced both ciliated and secretory cells and stimulated their proliferation and differentiation, the ultrastructural marks of increased secretory activity and degeneration of ciliated cells were observed after the progesterone administration. Ultrastructural changes of the oviductal epithelium exposed to exogenous estrogen and progesterone showed distinct regional differences.

The height of the epithelium was evaluated as well. The measurement was made, both in controls and animals treated with hormones (mean values of differences of the epithelium height between estrogenized and progesteronized animals were 3.23 µm in the preampulla, 4.16 µm in the ampulla, and 2.02 µm in the isthmus), and also in the all segments of the oviduct: the epithelium is the lowest in the ampulla (14.25-18.34 µm), while, conspicuously, the higher epithelium lined the preampullary and isthmic segment (18.04-19.17 µm and 19.97-20.13 µm).

The results of present study serve to verify the sensitivity of the tubal epithelium of sexually immature mice to exogenous steroid hormones of the ovary and to give preliminary morphological concept for immunohistochemical study of respective hormone receptors.

Postnatal development, oviduct, ciliation, secretion, ovarian hormones, mouse
epithelial cells, fibroblasts of mucosal connective tissue and smooth muscle cells of the external muscle coat of the oviduct during estrous cycle were studied by Puri and Roy (1981), Batra and Iosif (1989), Yamashita et al. (1989). According to Ogunrati (1992) steroidogenic cells producing steroid hormones of tubal fluid were found in the oviductal epithelium.

The aim of present paper was to verify the response of the oviductal epithelium of the immature mice after the administration of exogenous estrogen and progesterone on the ultrastructural level.

Materials and Methods

Animals

The oviducts of sexually immature mice (C 57 BL/10 × CBA (F1)) aged 14, 21, and 28 days were used in the present study. The animals of the same age were divided into three groups of 8 – 10 animals. Mice of the 1st group were treated with estradiol, those of the 2nd group with estradiol and progesterone. The 3rd group of the examined animals served as intact controls.

Hormones

Microcrystalline water suspensions of steroid hormones were used for stimulation: Agofollin-Depot /Biotika/ (estradioli benzoas 10 mg/2 ml) and Agolutin-Depot /Biotika/ (progesteronom 50 mg /2ml). Aqua pro injectione /Biotika/ served as a vehiculum for the dilution of both hormones. 1 ml of vehiculum contained the total dose of hormones per day. Hormones as well as vehiculum were administered subcutaneously in the suprascapular region of the experimental animals.

Protocol of hormone administration (see Table 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
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<tr>
<td>I.</td>
<td>50 µg/kg.d E₂</td>
<td>50 µg/kg.d E₂</td>
<td>50 µg/kg.d E₂</td>
<td>50 µg/kg.d E₂</td>
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<tr>
<td>II.</td>
<td>50 µg/kg.d E₂</td>
<td>50 µg/kg.d E₂</td>
<td>25 µg/kg.d P</td>
<td>25 µg/kg.d P</td>
</tr>
<tr>
<td>III. (control)</td>
<td>Aqua pro inj. (1 ml)</td>
<td>Aqua pro inj. (1 ml)</td>
<td>Aqua pro inj. (1 ml)</td>
<td>Aqua pro inj. (1 ml)</td>
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Explanation: E₂ – estradiol, P – progesterone

Electron microscopy

The animals were killed by decapitation, and oviducts were fixed in 300 mmol/l glutaraldehyde and 80 mmol/l OsO₄ in 100 mmol/l cacodylate buffer, after dehydration, the samples were embedded into Durcupan ACM (Fluka), cut on Ultratome III ultramicrotome and stained with uranyl acetate and lead citrate according to Reynolds (1963). The ultrathin sections were viewed and photographed by transmission electron microscope Tesla BS 500 (90 kW).

Height of the epithelium

The height of the epithelium was measured in electron micrographs at magnification 6 000×. In each electron micrograph 6 – 12 measurements of height were made. The obtained data were used for statistical evaluation of significance of differences by means of simple variance analysis (Student’s t test).

Results

Control animals

Day 14

Four types of ciliated cells were identified in the epithelium of the preampulla and ampulla. Type I cells with short microvilli on the luminal surface contained generative complexes in the supranuclear region of the cytoplasm. The generative complex was made up of 2 or more procentrioles arranged radially around deuterosome (Plate VI, Fig.1). Besides generative complexes, microtubules and solitary centrioles were found in some of these cells. Type II cells with microvilli contained numerous centrioles corresponding to
basal bodies with regard to localization and orientation (Plate VI, Fig. 2). Type III cells were characterized by luminal surface covered partly with cilia, partly with short microvilli. Below the microvillous region of plasmalemma, solitary centrioles were usually present. Type IV cells showed the signs of well differentiated ciliated cells with abundant cilia, covering the whole cell apices. They were classified into two subtypes: the cells with numerous organelles (elongated mitochondria, long tubules of rough endoplasmic reticulum (RER) and 2-3 well developed Golgi apparatuses), and the cells with poor organelle equipment (containing a small amount of spherical mitochondria, a small amount of short tubules of RER and 1 small Golgi apparatus beneath the nucleus).

Secretory cells with secretory granules of different size and maturity level (homogenous and heterogenous immature and homogenous mature granules) were observed in the ampulla and isthmus. The granules were mostly dispersed among cisternae of the RER and Golgi apparatus within the cytoplasm. Tubules and cisternae of both organelles were dilated regularly.

Uniform indifferent cells with microvilli were found in the epithelium of all the segments of the oviduct. These cells occurred in a lesser amount than ciliated and secretory ones. Their cytoplasm contained the abundance of polysomes and elongated mitochondria, while the RER and Golgi apparatus were poorly developed.

The concentric bodies, composed of densely and spirally arranged narrow tubules with glycogen granules among them were observed in secretory and indifferent cells (Plate VII, Fig. 3). The bodies were often localized in the subnuclear zone of the cytoplasm.

Mitoses were sporadically found in the epithelium of all oviductal segments.

Day 21
Type IV ciliated cells were predominant in the preampullary and ampullary epithelium. The remaining types seen in the oviductal epithelium of the 14-day animals occurred sporadically.

The secretory cells with apically localized mature secretory granules were found among predominated ciliated cells in the preampulla and indifferent cells in the isthmus. The apices of these cells were flat or dome-shaped and protruded into the oviductal lumen. Except well matured cells with secretory granules in their apical protrusions, the secretory cells with dilated profiles of the RER and Golgi apparatus, and scattered immature and mature secretory granules were present in the ampulla. Regardless of localization and level of secretory granules maturation, the concentric bodies were found in many secretory cells.

The density of indifferent cells was higher in the isthmus; in the ampulla and preampulla these cells were observed sporadically. Solitary mitotic figures were also found in the preampulla and ampulla.

Day 28
Ciliated cells were always well differentiated in all the segments of the oviduct. Secretory cells with mature secretory granules in protruding apices and with the concentric bodies were identified in the whole oviductal epithelium. The number of mitotic figures was the same as that in the oviductal epithelium of the 21-day-old animals.

Estrogen
The occurrence of well differentiated ciliated cells and tall secretory epithelial cells (Plate VII and VIII, Figs. 4, 5 – day 28) protruding into the lumen of the oviduct was recorded after the administration of estrogen.

Day 14
Scattered cells with ciliogenic activity (type II and III cells) were found among type IV cells abundant in the preampulla and ampulla. Well differentiated secretory cells containing
mature secretory granules lined both the ampullary and isthmic tubal segments. The granules were accumulated in apical regions of the cytoplasm, which constantly protruded into the lumen. The concentric bodies located subnuclearly were typical of secretory cells (Fig. 4).

Days 21 and 28

An ultrastructural appearance of the epithelium corresponded well with the pictures of the oviductal epithelium seen in adults during proestrus and early estrus (Figs. 4, 5). Ciliated cells with marks of ciliogenic activity (type I-III cells) were not observed in any oviductal segment.

Mitotic figures occurred sporadically in the epithelium of the ampullary segment in animals of all age groups. However, the number of such cells was lower than that in controls.

Progesterone
Day 14, 21 and 28

The progesterone administration after pretreatment of the animals with estradiol caused a reduction in the number of ciliated cells. The height of the epithelium was always decreased and the apices of most secretory cells were flat and only with a few secretory granules (Plate VIII, Fig. 6). Cytoplasmic processes containing secretory granules or smooth electronlucent vesicles were pinched and protruded from the apices of many secretory cells (Plate IX and X, Figs. 7, 8, 9); narrow and long stalks connecting the cell apices and cytoplasmic processes were often observed (Plate X, Fig. 10). Cytoplasmic fragments of secretory cells were seen within oviductal lumina (Plate XI, Fig. 11). Moreover, the degenerating ciliated cells, characterized by condensation of nuclear chromatin, mitochondrial defects and vacuolization of the cytoplasm, were found in the epithelium of the preampulla and ampulla (Plate XI, Fig. 12). Mitotic figures were never observed in any oviductal segment.

Height of the epithelium

The results of measurement of the epithelial height are given in Table 2.

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<thead>
<tr>
<th></th>
<th>C</th>
<th>E</th>
<th>P</th>
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<tbody>
<tr>
<td>14</td>
<td>19.17</td>
<td>20.89</td>
<td>18.37</td>
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<tr>
<td>21</td>
<td>18.34</td>
<td>19.69</td>
<td>15.23</td>
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<tr>
<td>28</td>
<td>20.13</td>
<td>22.20</td>
<td>21.17</td>
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Table 2

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>A</th>
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<tbody>
<tr>
<td>14</td>
<td>19.47</td>
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<td>14.25</td>
<td>15.89</td>
<td>13.84</td>
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<tr>
<td>28</td>
<td>19.97</td>
<td>22.37</td>
<td>15.38</td>
</tr>
</tbody>
</table>

Explanation to the table: P – preampulla, A – ampulla, I – isthmus, 14,21,28 – age of animals in days, C – control, E – estradiol, P – progesterone, n = 8, statistical significance: p < 0.05 (*) and p < 0.01 (**), mean value ± SEM

The oviductal epithelium of control animals aged 14 and 21 days was higher (15.75-20.13 µm) than that of control animals aged 28 days (14.25-19.97 µm). In view of topography of the oviduct, the epithelium is the lowest in the ampulla (14.25-18.34 µm), while, conspicuously, the higher epithelium lined the preampullary and isthmic segment (18.04-19.17 µm and 19.97-20.13 µm). Statistically significant differences of epithelial height in the animals treated with estradiol or estradiol + progesterone were observed: mean values of differences of the epithelium height between estrogenized and
progesteronized animals are 3.23 µm in the preampulla, 4.16 µm in the ampulla, and 2.02 µm in the isthmus (see graph).

Discussion

The oviducts of newborn mice are lined with simple columnar epithelium, in which uniform indifferent cells with microvilli and isolated cells showing ciliogenic activity occur. Postnatal changes of the mouse oviductal epithelium from birth to day 14 are characterized by proliferation manifested by the presence of mitotic figures, and by differentiation of ciliated cells (Lauschová 1996). The occurrence of generative complexes with procentrioles and microtubules is one of typical ultrastructural marks of ciliated cells differentiation. The first secretory cells containing immature and mature secretory granules, dispersed in the infra- and perinuclear cytoplasm, were observed in animals aged 14 days. The well-developed rough endoplasmic reticulum and the Golgi apparatus were typical of most developing secretory cells. The concentric bodies located subnuclearly were described in secretory and indifferent cells by Lauschová (1996) and Čech and Lauschová (1996). We first observed them in the oviductal epithelium on day 14. Their number increased to day 28 and decreased from days 28 to 35 after birth. After day 35 and in adult mice the concentric bodies persisted only in the cells lining the isthmus.

The occurrence of the ultrastructural signs of ciliated and secretory cells was lower after hormonal treatment. The number of mitotic figures decreased in the epithelium of estrogenized animals. Mitoses were not observed in the oviducts of progesteronized animals.

After the estrogen administration we observed well differentiated ciliated and secretory cells. These findings agree to the results of other authors (Brenner et al. 1974; Verhage and Brenner 1975; Pathak et al. 1979; Eroschenko 1982; Odor et al. 1980 and 1983; Brenner and Slayden 1994). Malette et al. (1995) have demonstrated the synthesis of specific glycoproteins in secretory cells.
The influence of progesterone on the oviductal epithelium was manifested by a decrease of the total number of ciliated cells, the epithelium height decrease (Brener and Slayden 1994) and by ultrastructural signs of increased secretory activity (Uhrín 1992). The decrease of ciliated cells number is usually explained as a result of deciliation. Gaddum-Rose et al. (1975) described tufts of cilia in oviducts of postmenopausal women as a clear and doubtless sign of deciliation. Brenner (1969) has described a process of separating the apical part of cilium and presented it as "pinching off". This term was used later by Abe and Oikawa (1990) in the description of the formation of apical cytoplasmic processes in secretory cells. Hollis et al. (1984) have observed ciliated cells showing an apocrine release of apical vesicles on the surface of isthmic ciliated cells, and they claimed that this process was probably not connected with deciliation. We have not observed any ultrastructural evidences, which could be referred to as deciliation marks. On the other hand, some ciliated cells in the degenerative process were seen. By that time, there has not been answered the question, if the decrease of ciliated cells number results only from the deciliation or only degeneration or from both processes. An antiproliferative effect of progesterone was mentioned by Kim et al. (1996b). Sawyer et al. (1984) described the transformation of the columnar ciliated and secretory cells into the cuboidal cells with wrinkled nuclei without nucleoli, and with split apical ends of the cells in the oviductal epithelium. They have found cell fragments and macrophages in the lumen of the oviduct, too.

We have observed the pinched apical parts of the cytoplasm, containing secretory granules, electronlucent vesicles, tubules of the rough endoplasmic reticulum, ribosomes and isolated mitochondria in the epithelium. Some cytoplasmic segregations, usually without any organelles, were connected with the secretory cell apices by long and narrow stalks. The similar structures on the apical surfaces of secretory cells in the oviducts of adult mice during later estrus and metestrus were described by Čech and Lauschová (1990). Abe and Oikawa (1990) found them in the oviducts of a pig during the luteal phase, and used the term “pinching off” for this process. Recently Novotný et al. (1998a, b) described protrusions of the apices of the epithelial cells of the endometrium in women during luteal phase and after hormonal treatment. They have referred them, according to the size, as pinopodia and micropinopodia.

Some authors, simultaneously with morphological studies of the oviductal lining in the adults during the estrous cycle, evaluated the height of the epithelial cells. In bovine oviducts Uhrín (1992) found the highest values of the epithelium height during proestrus and estrus (37.3 µm); in metestrus and diestrus the height was decreased (35.8 and 29.9 µm). Verhage et al. (1990) have recorded cells between 31-39 µm in the follicular phase and 19-32 µm in the luteal phase in a baboon. The differences in height of hormonally treated and non-treated oviductal epithelium exhibited the same trends in our experiments. In addition, we have observed some regional differences in sensitivity of the oviductal epithelium to the ovarian steroid hormones. The differences in the epithelium height after estrogen or progesterone treatment were the highest in the ampulla and the smallest in the isthmus. Similar findings were published by Gupta et al. (1969) and Fredricsson and Holm (1974).

Vliv estrogenu a progesteronu na ultrastrukturní ukazatele tubárního epitelu pohlavně nezralých myší

Aplication steroidních hormonů ovaria urychnila vývoj buněk tubální výstelky, jejíž ultrastrukturální obraz připomíná poměry u dospělých myší s normálním estrálním cyklem. Zatímco estrogeny proliferaci a diferenciaci buněk, a to jak řasinkových, tak sekrecních stimulovaly, progesteron působil zejména na sekrecní buňky a indukoval degenerativní změny v řasinkových buňkách. Ultrastrukturální obraz tubálního epitelu po aplikaci exogenního estrogenu a progesteronu vykazoval zřetelné regionální rozdíly.

Současně se studiem ultrastrukturních charakteristik autorka vyhodnotila věšku epitelu ve všech segmentech vejcovodu u kontrolních zvířat i u zvířat, kterým byly aplikovány steroidní hormony. V ampule byl epitel nejnižší (14,25-18,34 µm), v preampule a isthmu věška epitelu kolísala v rozmezí 18,04-19,17 µm a 19,97-20,13 µm. Střední hodnoty rozdílů věšky epitelu zvázaly „estrogenizovaných“ a „progesteronizovaných“ činily 3,23 µm v preampule, 4,16 µm v ampule a 2,02 µm v isthmu.

Výsledky studie potvrdily, že tubální epitel pohlavně nezralých myší je senzitivní vůči exogenním steroidním hormonům ovaria a poskytly předběžný morfoanatomický korelát pro cílené imunohistochemické studium příslušných receptorů.

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Fig. 1. Ampulla (day 14 - control): Type I cell with the signs of ciliogenesis. Generative complex (gc), deuterosomes (→). Bar = 0.5 µm

Fig. 2. Preampulla (day 14 - control): Type II cell with short microvilli on the surface and centrioles localized supranuclearly. Bar = 0.5 µm
Fig. 3. Preampulla (day 14 - control): The concentric body (CB) in secretory cell. Bar = 1µm

Fig. 4. Preampulla (day 28 - estrogen): Well developed ciliated cells. Bar = 2.5 µm
Fig. 5. Preampulla (day 28 - estrogen): Subnuclearly localized concentric bodies (→) in well developed secretory cells. Bar = 5 µm

Fig. 6. Preampulla (day 14 - progesterone): Flattened surfaces of secretory cells with only a few secretory granules in the cell apices. Bar = 2.5 µm
Fig. 7. Ampulla (day 28 - progesterone): Part of the cytoplasm pinched of secretory cell (SC). Poorly developed ciliary apparatus in a ciliated cell (CC). Bar = 1µm

Fig. 8. Preampulla (day 28 - progesterone): The cytoplasmic process of secretory cell containing electronlucent vesicles (*). Bar = 1µm
Fig. 9. Isthmus (day 21 - progesterone): The cytoplasmic process of secretory cell containing the RER (ER) and secretory granules (SG). Bar = 1 µm

Fig. 10. Isthmus (day 28 - progesterone): The apex of secretory cell – narrow stalk (→) connects the cytoplasmic segregation with cell surface. Bar = 0.5 µm
**Plate XI**

**Fig. 11.** Ampulla (day 28 - progesterone): Secretory cell fragment with secretory granules in the oviductal lumen. Bar = 1µm

**Fig. 12.** Preampulla (day 28 - progesterone): Ciliated cell showing chromatin condensation and defect mitochondria (→). Bar = 1µm