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Ultrastructural analysis of the endometrial mucosa of female patients at menopause with hormonal substitutive therapy (TSH)

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Abstract

The objective of the paper was to observe the ultrastructural aspects of the endometrial biopsies taken from female patients at post-menopause with substitutive hormonal therapy (TSH). *Material and methods*. A number of three endometrial biopsies were taken from female patients at post-menopause with TSH. The ultrastructural analysis was carried out with the help of the electronic microscope Philips ME 301 using classical electronic microscopy methods. *Results and discussions*. The ultrastructural analysis has highlighted the presence of cuboidal and columnar epithelial cells, with basally situated nuclei, well represented cellular organelles, some cells having at the apical pole microvilli. At the electronic microscope, three types of epithelial cells are described, at the level of the endometrial mucosa of the woman who is in a fertile period: secretory cells (cells with an average electronic density with microvilli on the luminal surface), ciliated cells and clear cells (cells with a low electronic density). These cells have certain ultrastructural characteristics and of receptivity towards the steroid hormones. The stroma is axial with elongated cells with oval nuclei, with nucleoli and with smooth or undulated membrane. *Conclusions*. The ultrastructural aspects suggest the presence at endometrial level of epithelial active glandular cells, secretory cells and stromal active cells at female patients at post-menopause with TSH.

***Keywords*:** endometrium, post-menopause, ultrastructure, TSH.

Introduction

The menopause is defined as an endocrine deficiency characterized by the stopping of menstruum due to the ovarian atresia and ovarian follicle [1]. The average age when the menopause installs is 51 years old and is followed by changes that can be grouped in neuroendocrine modifications, urogenital atrophy, modifications of the tegument and hairiness, osteoporosis and cardio-vascular diseases [2, 3].

The hormonal substitutive therapy (TSH) recommended for oust these modifications has curative and prophylactic effects. The therapy can be done with unique or combined scheme, continuous or sequential, with local or oral administration [4, 5].

The aspects of the endometrial mucosa of the women under TSH are very varied, depending on [6]:

- endometrial hormonal receptors and local paracrine systems that mediate the hormonal effects and are responsible for the intra-endometrial interaction with different cellular types (glandular, stromal, vascular endothelium);
- the beginning of the menopause;
- the lasting ovarian function;
- the duration and doses of the estrogenic and progestogenic therapy;
- the nature of the used hormones.

The estrogens, the progesterone or the estro-progestatives have an increased influence on the endometrium. Their therapeutically administration or with a contraceptive aim determine endometrial structural modifications that sometimes are difficult to assess morphologically. The endometrium concentrates the estradiol and progesterone of the plasma against a concentration gradient and unites the hormones through receptors of small capacity [7, 8].

Material and methods

The electronic microscopy (EM) that was carried on three cases (endometrial biopsies) taken from female patients at post-menopause with TSH followed the ultrastructural changes of the endometrial mucosa in comparison with the ultrastructural aspects of the endometrium of women who are in a fertile period and at menopause (data from scientific literature).

The selected endometrial biopsies on which we have done studies of electronic microscopy showed from the mophopathological point of view certain combined aspects of inactive endometrium, with a "mix" of proliferative and secretory endometrium (seen by many authors as a stage in the evolving process of the atrophic

endometrium of female women at menopause who receive the hormonal substitutive therapy).

We used the classical method of electronic microscopy that works with the same principles of the optical microscopy, of the fixation and inclusion of the samples.

The following phases were carried out:

▪ *Fixation*:

– biopsies of 1 mm^3 are fixed between 90 minutes, 24 hours at 4ºC, in 4% glutaraldehyde, in cacodylate buffer 0.1 M, pH 7.2–7.4;

– washing in cacodylate buffer;

– post-fixation in 41% osmium tetraoxide, during 60 minutes;

– washing in cacodylate buffer 0.1 M, pH 7.2–7.4.

▪ *Desiccation and inclusion*:

– the desiccation is done in graduated series of ethylic alcohol (the concentration starting from 30% up to 100%);

– desiccation in propylene oxide;

– inclusion in Epon 812.

▪ *Sectioning and coloring the grids*:

– out of the three blocks obtained for each case, semi-thin sections were taken for identifying the interest areas, by selecting only the representative blocks on which the semi-thin sections were carried out;

▪ *Obtaining the ultra-thin sections*:

– the ultra-thin sections are collected on grids and are colored with lead citrate and uranyl acetate.

The examination has been made at the electronic microscope Philips ME 301.

Results

The ultrastructural analysis highlighted:

▪ the presence of cuboidal and columnar epithelial cells, with basally situated nuclei with prominent nucleolus and regular contour (Figure 1);

▪ the mitoses are scarce;

▪ some epithelial cells show microvilli on the apical surface (Figure 1);

▪ the epithelial cells show a well developed endoplasmic reticulum, Golgi apparatus situated in the apical part of the cell (Figure 2);

▪ the epithelial cells show mitochondria dispersed in the cytoplasm;

▪ some epithelial cells have nuclei with a higher density of nuclear chromatin (Figure 3);

▪ some epithelial cells show basal vacuole;

▪ the stroma is axial with elongated cells with oval nuclei (some are dense, pyknotic, others are slightly increased), with nucleoli and smooth and slightly undulated membrane;

▪ in the cytoplasm of the stromal sells we can see the mitochondria (some of them with long cristae), rough endoplasmic reticulum (well developed in some cells), Golgi apparatus, lysosomes, lipids and vesicular bodies.

Discussions

The ultrastructural studying of the endometrial mucosa of female patients at post-menopause with hormonal substitutive therapy allows the appreciation of the epithelial and stromal modifications in comparison with endometrial ultrastructure of women during the fertile period.

At the electronic microscope, three types of epithelial cells are described, at the level of the endometrial mucosa of the woman who is in a fertile period: secretory cells (cells with an average electronic density with microvilli on the luminal surface), ciliated cells and clear cells (cells with a low electronic density) [9, 10].

These cells have certain ultrastructural characteristics and of receptivity towards the steroid hormones. The secretory cell in early proliferative stage is of columnar type with oval nucleus, basal, with prominent nucleolus and regulated contour; microvilli exist on the apical surface.

The cytoplasm of these cells contains mitochondria, well-developed endoplasmic reticulum (that has on the external surface numerous small granules of ribonucleoproteins), Golgi apparatus (apical, above the nucleus), big vesiculae, electrono-dense lipid granules (unregulated, basally and apically disposed), round or oval bodies with moderated electronic density (apically located).

At the end of the proliferative stage, at the basis of the cytoplasm between the nucleus and the basal membrane small deposits of glycogen appear. On the same surface, we can notice several big mitochondria surrounded by the endoplasmic reticulum. This first showing of the glycogen precedes the basal vacuole (observed at the optic microscope).

In the secretory phase, there is a tetrad characteristic:

1 – clear areas at the basis of the cells, rich in glycogen;

2 – voluminous mitochondria and rich in cristae around those areas;

3 – characteristic corpuscle in the nucleus;

4 – nuclear invaginations with undetermined function (probably with the role of getting close the membrane to the nucleolar basket.

The voluminous mitochondria exists for a limited period of time and are 3–5 times bigger than the normal mitochondria, have numerous ridges and contain two types of granules (it has been suggested that there is a correlation between the appearance of the gigantesque mitochondria, the hormonal variations and the metabolic modifications at glandular level).

In progesteronic regimen and after ovulation a complex nuclear structure appears which is made of tortuous channels called nucleolar basket, nucleolar canalicular system or nucleolar canalicular structure. The arrangement of these channels is similar to a honeycomb; they develop into nucleoli and migrate towards their membrane. The ciliated cell is scarcer and can be found especially in the surface epithelium and at the collum of the glands, more rarely on the basal part.

At the surface of the cells we can notice cilia and microvilli; the reticulum is less developed than the Golgi area and the mitochondria are similar to those in the secretory cell (lipid drops are also present). The clear cell is considered a pause cell or a cell with undetermined function.

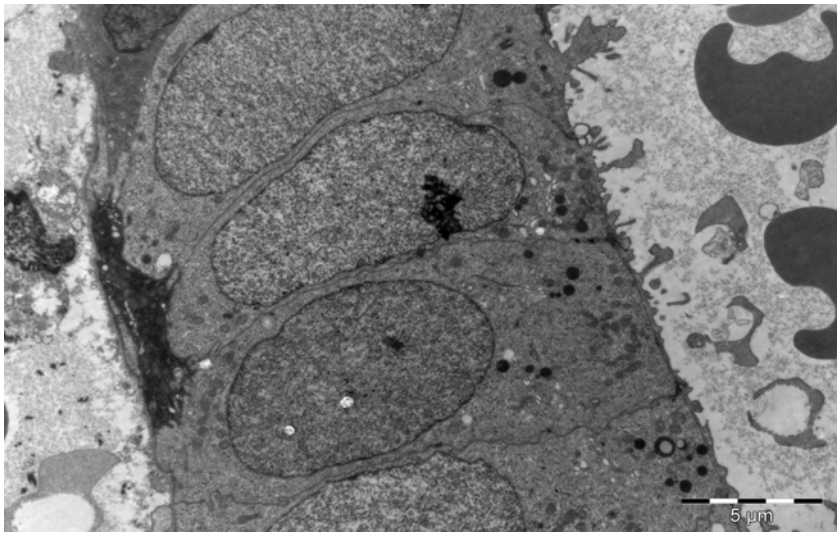

Figure 1 – ***Endometrium: active columnar epithelial cells with microvilli on the apical surface and intact basal membrane (EM)***

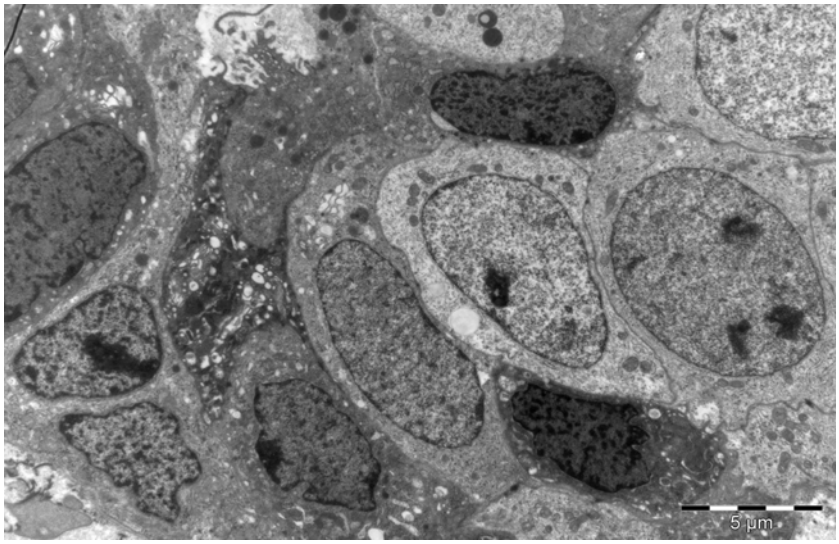

Figure 2 – ***Endometrium: columnar glandular epithelial cells, arranged in stratified way, secretory aspects, clear and dark cells (EM)***

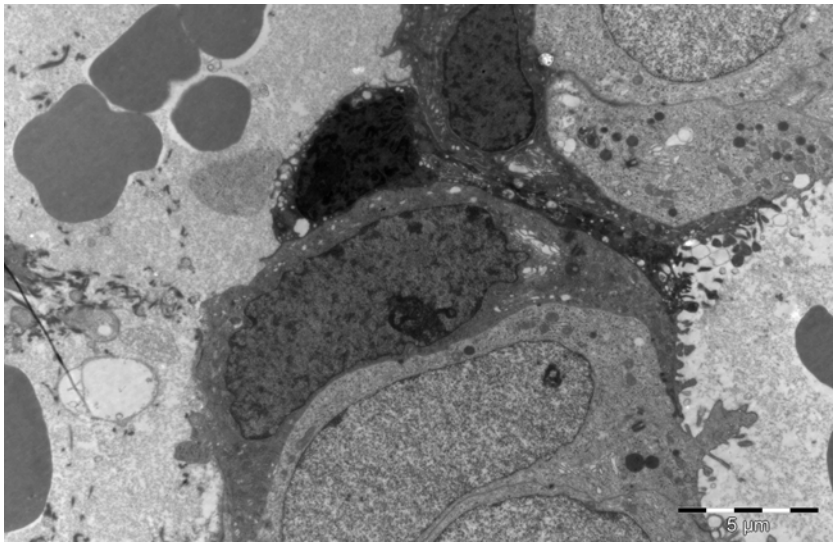

Figure 3 – ***Endometrium: active glandular epithelial cells, with granules in the cytoplasm, nuclei with chromatin condensation (ME)***

It has a reduced quantity of endoplasmic reticulum, rare mitochondria and a prominent Golgi apparatus; it is devoid of basal membrane and does not touch the luminal surface of the gland. From a metabolic point of view, they are inactive cells (they do not contain ARN, mucins, lipids, alkaline phosphatases).

The stromal cells can be divided into superficial cells that are near the surface of the epithelium, of the glands and vessels, and deep cells that are not exposed to these structures. Initially, the stromal cells are immature, similar to the stem cells, acquiring afterwards a fibroblastic morphology.

The maturation of the stromal cells consist in the appearance of the predecidual cells (characterized by the surface and cytoplasmic complexity of the cell); in the first place the superficial cells are maturated and afterwards the deep cells. Initially, the superficial cells are elongated and the deep ones become round. The nucleus is ovoidal, with 1–2 nucleoli, surrounded by smooth nuclear membrane. The mitochondria are few with a small number of cristae, the Golgi complex and the smooth endoplasmic reticulum is weakly developed; the lipid bodies and the lysosomes are the cellular membrane has certain irregularities called "condensation boundaries".

The stromal cells have the capacity to produce collagens or a precursor of collagen that polymerize afterwards in the extracellular space. Experimentally, it has been established that the synthesis of collagen from the stromal cells is dependent on the estrogens; some authors consider that the existence of the intracellular collagen in the proliferative phase would represent an abnormal answer of the fibroblasts at the estrogenic stimulation.

The future maturation of the stromal cells leads to an improvement of their hormonal receptivity, the collagen fibers included in the lysosomes suggesting an autolysis process of the intracellular collagen. In the late secretory phase, the stromal cells are elongated similarly to fibroblasts: the nuclei are ovalar, undulated with peripherally disposed chromatin; the mitochondria have long thin cristae; the rough endoplasmic reticulum is well developed; the vacuole of the Golgi apparatus are distributed in the whole cytoplasm and lysosomes; lipid and multivesicular bodies are present; the glycogen appear under the form of granules either as floccular material, arranged in clusters or diffuse in the cytoplasm. The condensation margins are rare and the collagen fibers are in the lysosomes.

Researchers from Italy and Japan (Makabe S *et al.*, 1998) considered that the micro-anatomic modifications at the level of the genital organs, including at the level of the endometrium of women aged between 45 and 65 years at menopause and post-menopause, by using the Scanning Electron Microscopy (SEM) [11].

The endometrium taken by hysteroscopy fixed immediately in 2.5% glutaraldehyde in 0.1 mol/L saline phosphate buffer solution for minimum 48 hours, the fragments being then post-fixed in 1% osmium tetraoxide in 15 mol/L buffer solution, and then desiccated in ethanol.

Each of obtained fragments was divided into two parts: one for scanning electron microscopy examination and another one for transmission electron microscopy (TEM).

The surface of the examined endometrium appeared thin and smooth, devoid of the plies specific to the reproduction period, observed especially in the secretory phase.

The glandular orifices are more reduced in numbers and have a round irregular shape.

In a big proportion at the women in the menopause, we could notice the reduction of the number of ciliated cells and the vaulted aspect of those not ciliated that have numerous microvilli with small signs of secretion. Other cells with microvilli showed an apical narrow poll. The microvilli were very numerous at some cells and concentrated at the apical pole.

By the agglutination of the close microvilli it lead to the establishment of certain micro-bridges or micro-plies similar to those highlighted in the squamous cells of the exocervix. This last aspect has become progressively more frequently with the passing of time since the installing of the menopause.

The examinations with SEM have highlighted the above-mentioned modifications a lot less than the conventional ones of optic microscopy and than those with TEM.

The observations with SEM proved that the progressive decrease of estrogens in post-menopause induces important and complex modifications in the three-dimensional system of microanatomy – tissular and cellular.

The authors consider that these modifications need to be taken into consideration when using the hormonal substitution therapy.

Conclusions

The selected endometrial ultrastructural biopsies have at the level of the epithelial cells oval nuclei with basal distribution and in some basal cells, the presence of basal vacuole can be noticed; on the surface of some cells, microvillus can be noticed.

At epithelial level, the nuclei have a prominent nucleolus, regular contour and in some nuclei, we can notice a higher density of the chromatin.

The epithelial cells have a well-developed endoplasmic reticulum, Golgi apparatus situated in the apical side of the cell and mitochondria spread in the cytoplasm.

The mitoses are rare. The ultrastructural aspects are in accordance with the histopathological aspects and with the scientific data, in the cases we have studied.

References

[1] BERG G., HAMMAR M. (eds), *The modern management of the menopause. A perspective for the 21st century. Proceeding of the VIIth International Congress of the Menopause*, Stockholm, Sweden, 1993, Parthenon, London, 1994.

[2] LOMAX P., SCHÖNBAUM E., *Postmenopausal hot flushes and their management*, Pharmacol Ther, 1993, 57(2–3):347–358.

[3] BRENNER D. E., KUKULL W. A., STERGACHIS A., VAN BELLE G., BOWEN J. D., MCCORMICK W. C., TERI L., LARSON E. B., *Postmenopausal estrogen replacement therapy and the risk of Alzheimer's diseases: a population-based case-control study*, Am J Epidemiol, 1994, 140(3):262–267.
[4] BARRET-CONNOR E., *Epidemiology of the menopause: a global overview*, Int J Fertil Menopausal Stud, 1993, 38(Suppl 1):6–14.
[5] LUISETTO G., ZANGARI M., TIZIAN L., NARDI A., RAMAZZINA E., ADAMI S., GALUPPO P., *Influence of aging and menopause in determining vertebral and distal forearm bone loss in adult healthy women*, Bone Miner, 1993, 22(1):9–25.
[6] SMITH R. N., STUDD J. W., *Recent advances in hormone replacement therapy*, Br J Hosp Med, 1993, 49(11):799–808.
[7] BERGERON C., FERENCZY A., SHYAMALA G., *Distribution of estrogen receptors in various cell types of normal hyperplastic, and neoplastic human endometrial tissues*, Lab Invest, 1988, 58(3):338–345.
[8] BELCHETZ P. E., *Hormonal treatment of postmenopausal women*, N Engl J Med, 1994, 330(15):1062–1071.
[9] CORNILLIE F. J., LAUWERYNS J. M., BROSENS I. A., *Normal human endometrium. An ultrastructural survey*, Gynecol Obstet Invest, 1985, 20(3):113–129.
[10] DOCKERY P., LI T. C., ROGERS A. W., COOKE I. D., LENTON E. A., *The ultrastructure of the glandular epithelium in the timed endometrial biopsy*, Hum Reprod, 1988, 3(7):826–834.
[11] MAKABE S., MOTTA P. M., NAGURO T., VIZZA E., PERRONE G., ZICHELLA L., *Microanatomy of the female reproductive organs in postmenopause by scanning electron microscopy*, Climacteric, 1998, 1(1):63–71.

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