SCANNING ELECTRON MICROSCOPY OF THE HUMAN NORMAL ENDOMETRIUM

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SUMMARY

The AA. examined by scanning and transmission electron microscopy (SEM) the normal human endometrium of 21 females, 18 in fertile age and 3 in menopause.

The study pointed out the presence of cells with cilia and microvilli only in fertile women. This observation indicates the role these structures may have in the kinetic of spermatozoa, in the endovement and nutrition of oocyte and, at the same time, their dependence on the ovarian hormonal activity.

The medical interest in SEM is documented by the large number of publications on the subject which were issued during the last decade. As a matter of fact, this type of research permits a tridimensional study of tissue surfaces and of single cells under different functional conditions.

The first description of the human endometrium at the SEM dates back to D'Aquino ([1]), while the most numerous and interesting researches about the subject were made by Ferenczy et al. ([5, 6, 7, 8, 9]) and White ([15, 16]) who examined endometrium during menstruation, in menopause and in different pictures of organic and disfunctional pathology.

On the basis of data already collected by these Authors, we have started to study the human normal endometrium under the SEM, during menstrual cycle and in menopause in order to give our contribution to a field of research which is not much known yet.

MATERIAL AND METHODS

The endometrium specimens, taken by a Novak cannula, refer to 21 females. 18 of them, between 20 and 40 years of age, were regularly menstruated, with cycles of 28-30 days, while 3 had been in menopause for 2,3 and 15 years. The two-phase aspect of the menstrual cycle was always confirmed by the parallel histofunctional control of the endometrium and by the basal body temperature.

In 9 cases endometrium biopsy was performed during the fertile phase and in the remaining 9 cases during the secretory phase of the menstrual cycle.

As to the analysis of the biological sample to examine at the SEM, it's necessary to limit at the utmost the artifices that can condition the reading and interpretation of the preparation. For this reason, two techniques are described: freezing and drying. Air-drying, a simpler and quicker method was used, although — in some Authors' opinion — it involves a remarkable influence of structural artifices.

However, if we compare our documentation with similar publications, achieved results don't seem to show special interpretation difficulties.

After air-drying, the endometrium fragments (2-4 mm), were set on a small metal support by a thin stripe of bi-adhesive tape and then

Technical times were as follows

1) *Fixation:*	
— glutarhaldeide 4 %	10′
— buffer solut. pH 7.2	5′
2) *Dehydration*:	
— distilled water	20′
— ethyl alcohol 25 %	20′
— ethyl alcohol 35 %	20′
— ethyl alcohol 50 %	20′
— ethyl alcohol 75 %	20′
— ethyl alcohol 85 %	20′
— ethyl alcohol 95 %	20′
— absolute ethil alcohol	20′
— amile acetate	20′

subjected to vacuum nebulization with coal and a palladium-gold alloy.

We used the Philips PSEM, Model 50, which can give enlargements from 20 to 80.000 ×. Usually enlargements between 160 and 10.000 × were produced.

RESULTS

A) *The Endometrium in proliferative phase.*

The study of the endometrium at the SEM during the first half of the menstrual cycle includes 4 samples in intermediate fertile phase (8^{th} - 10^{th} day) and 5 in terminal fertile phase (11^{th} - 14^{th} day). We decided not to examine the endometrium in an earlier phase of the cycle to avoid possible interpretation problems due to a non-completely recreated mucosa.

Ferenczy has been able to recognize at the SEM the double origin of the post-menstrual endometrium from the free branches of the base layer and, secondarily, from the integral peritubaric and juxtaisthmic coating epithelium ([5, 6]). The stromal cells and the spongiosa glands don't seem to be involved in this process.

The recreation of the endometrium, which began on the 2^{nd} - 3^{rd} day of the cycle, as a rule is completed on the 5^{th} day ([5, 6, 15, 16]); it seems to be independent of the ovarian hormonal stimuli. As a matter of fact we note a progressive receptivity of the epithelium and periblastic cells to estrogens of the pre-ovulatory period ([2, 6, 8, 15, 16]) only after this date of the cycle.

Starting from the 6^{th} day of the cycle the surface of the endometrial mucosa gets increasingly wavy, due to the alternation of plicae and folds (fig. 1) and it is covered with two varieties of cells which are irregularly distributed: the ciliated and the non-ciliated cells. The ciliated cells are more numerous near the gland orifices, where they are arranged as a crown ([6, 8]), at the tubal corners and close to the endocervical mucosa ([2]). Their role is to remove the secretion products of the bordering cells and take an active part in the kinetics of the spermatozoa and in the captation of the ovocyte ([8]).

As a matter of fact, the relation between ciliated and non-ciliated cells, equivalent to 1/30 during the 5^{th} - 6^{th} day of the cycle, gradually increases in the pre-ovulatory period ([8]).

Under the SEM the ciliated cells (fig. 2) appear with some straight or variously folded branches. The number of cilia varies from cell to cell and includes an average of 60 branches: their length is 8 micron and width is 0.2 micron ([14]). The cilia growth is in relation with the estrogenic stimulus; consequently their number and size are greater during the peri-ovulatory phase. It was not possible to define under the SEM the kinetics of the cilia, since their arrangement is extremely various and has no unidirectional character.

The non-ciliated cells are predominant elements of the endometrium and have a finely irregular surface due to the presence of numerous hairy branches. In some areas they are arranged to form a more or less visible cobbled paving which is intersected by deep grooves.

The number and size of the microvilli depend on the importance of the estroge-

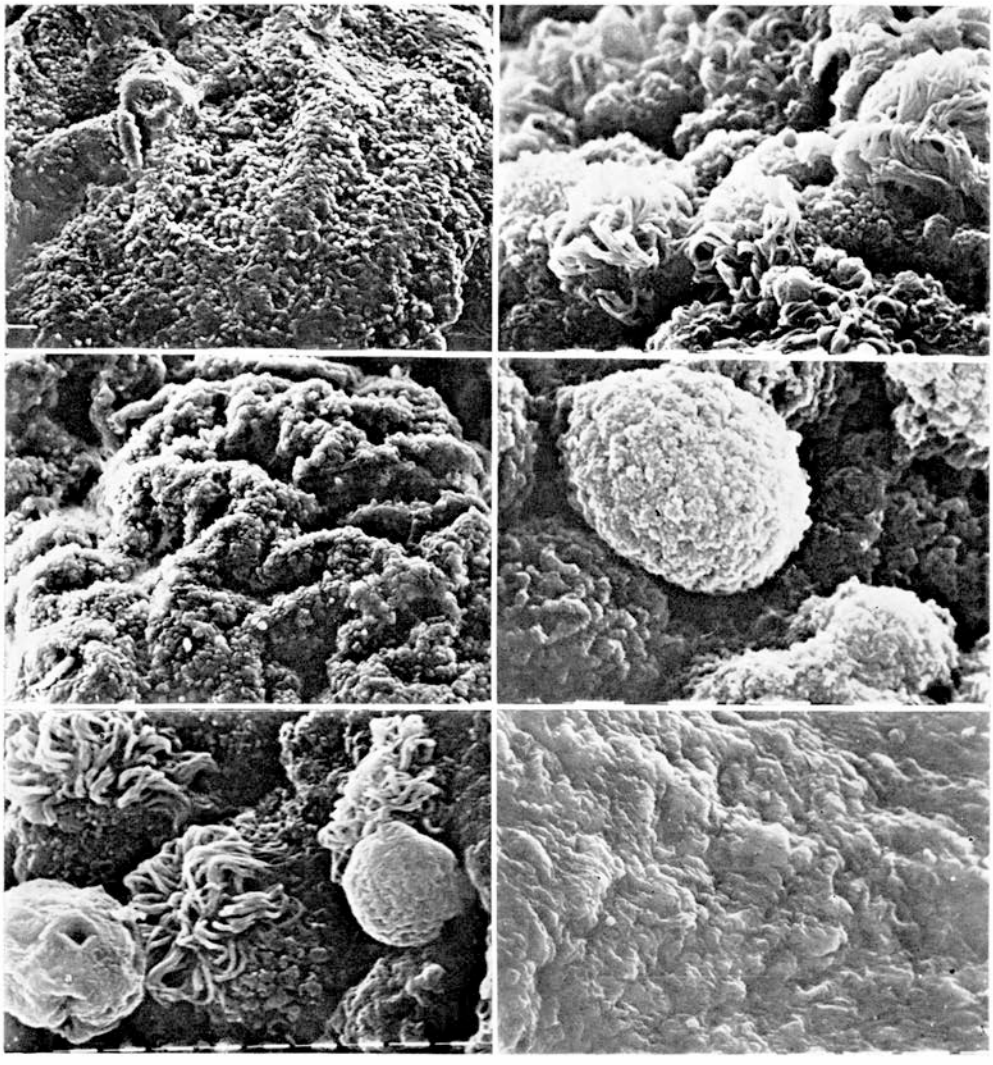

Fig. 1. — Proliferative phase (8th day of the cycle); (225 ×).

Fig. 2. — Proliferative phase: ciliated cells (13th day of the cycle); (7500 ×).

Fig. 3. — Secretory phase (19th day of the cycle); (450 ×).

Fig. 4. — Secretory phase: cells with microvilli (23rd day of the cycle); (15000 ×).

Fig. 5. — Secretory phase: in the left-lower side of the figure one may note a rounded-umbilicated formation corresponding to a product of cellular secretion (20th day of the cycle); (7500 ×).

Fig. 6. — Atrophic menopausal endometrium; (450 ×).

nic stimulus and on the time of the menstrual cycle. As a matter of fact, during the proliferative phase a greater number of microvilli was noticed than in the secretory phase.

Furthermore, these are thinner and protruding, but in any case shorter than cilia (0.5 micron according to Ferenczy).

B) *The Endometrium during the Secretory Phase.*

The endometrium specimens are taken as follows: 3 during the initial secretory phase (17^{th} - 20^{th} day), 3 during the intermediate secretory phase (21^{st} - 24^{th} day and 3 during the final secretory phase (25^{th} - 28^{th} day).

The endometrium surface, widely examined at the SEM, looks much convoluted, due to the irregular alternation of plicae and deep grooves (fig. 3). This aspect can already be observed during the initial secretory phase and seems to be in relation with a stromal rearrangement of cellular type (deciduous reactio) and of extracellular type (oedema and congestion).

Ferenczy ([7, 8]) speaks about a slight decrease in ciliated cells on the 20^{th} - 21^{st} day of the cycle, in relation with higher levels of progesterone. This quantity would remain unchanged until the next menstrual cycle.

In our study no considerable numerical change in ciliated cells was noticed during the two phases of the menstrual cycle. On the contrary, during the secretory phase a lower number of non-ciliated cells was observed, with short and thick microvilli (fig. 4).

In this connection Nilsson ([14]) describes the presence of microvilli with irregular crests of various size to which he attributes an important role in the captation and nidation of the blastocyst.

The apocrine secretory activity of non-ciliated cells is proved by the presence of round apex prominences with a wrinkled and umbilicated surface, with 2-4 micron diameter (fig. 5), which are irregularly distributed and more numerous about the 20^{th} - 24^{th} day of the cycle ([8]).

C) *Atrophic Endometrium (Menopause).*

We examined under the SEM 3 specimens of endometrium of females in physiological menopause lasting from 2,3 and 15 years. The cytologic vaginal and the histofunctional pictures of the endometrium were proved to be atrophic, thus excluding any estrogenic activity.

In all 3 cases, in spite of the different lenght of menopause, the endometrium mucosa showed the same aspect, characterized by a comparatively smooth surface without the plicae and the grooves noted during the menstrual cycle (fig. 6).

Non-ciliated cells with microvilli were not found, although Ferenczy indicates the presence of cells with 1-15 cilia ([7]) as a rare finding.

The surface of the cells looked smooth and flat so that it was impossible to identify the limits among the single cell elements.

CONCLUSIONS

The study of the endometrium under the SEM points out the various aspects of the uterine surface during the proliferative and the secretory phase of the menstrual cycle, presumably on account of spermatozoa kinetics, and nidiation and nutrition of the fecundated ovocyte.

In this connection and during the first phase of the cycle, Ferenczy could document a greater number of ciliated cells, while in the post-ovulatory phase the secretory activity of non-ciliated cells is prevailing.

In the course of menopause the endometrial epithelium does not show any microvilli and cilia, thus proving the dependence of these structures on the ovarian hormonal activity.

The results of the study of the normal endometrium under the SEM integrate

knowledge already acquired by the optic and electronic microscopy and represent a valid contribution to further research in the field of disfunctional and organic uterine pathology.

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