ENDOMETRIAL CYTOLOGY DURING MENSTRUAL CYCLE, MENOPAUSE AND PROGESTINIC TREATMENT

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SUMMARY

The AA. examined 72 cytological smears of the human normal endometrium. Twentythree were taken during the proliferative phase, 17 during the secretory phase, 21 during menopause and 11 during estro-progestinic treatment.

The study pointed out that the endometrial smear, which has been employed up to now almost exclusively in the cyto-oncological field, is also quite suitable for a dynamic cyto-functional evaluation of ovarian function, because of the series of interpretative elements, easily and readily identifiable at the microscope, which it furnishes.

Functional cytology of the endometrium consists in the microscopic study of the nuclear-cytoplasmic modifications in cells taken directly from the uterine cavity in order to evaluate the nature and entity of the hormonal stimulus on the endometrial receptor.

Cyto-hormonal examination of the endometrium was first employed [1, 2, 3, 4] mostly for the study of ovarian dysfunctions and the diagnosis of ovulation in alternative to bioptic examination. In fact, endometrial cytological examination is an easily performed, non traumatic and almost painless procedure that may be repeated several times even during the course of a single menstrual cycle, with the advantage of a dynamic evaluation of the functional state of the endometrium. These characteristics render endometrial cytology a highly useful technique, even if for diagnostic purposes biopsy furnishes a greater variety of interpretative information at both the glandular and connective-vascular levels. Interpretation of the cytologic specimen instead, avails itself of a smaller amount of information that specifically regards the nuclear-cytoplasmic morphological aspect of the glandular and stromal cells.

The chromatinic intensity of the nucleus is a true index of the degree of trophism and estrogenic stimulation of the endometrial mucosa [5, 6], while the cytoplasmic aspects help identify the glandular or stromal nature of the cell and define its functional role.

Cytostructural data, constituted by intercellular relationships, have instead a secondary diagnostic importance compared to the cytomorphological characteristics.

Due to the small number of interpretative elements, the endometrial material must be carefully collected, stained and examined, keeping in mind, moreover, several important clinical facts such as the general character of the menses, the date the specimen was taken in relationship to the day of the patient's cycle, and the

nature of any possible hormonal therapies in course or recently undertaken.

An endometrial specimen for cytology may be collected by aspiration with different types of cannula, by abrasion of the endometrial surface with brushing-type equipment, or by washing the uterine cavity under negative pressure.

Naturally, specimen collection for cytology, as in every endocavity manoeuvre, may not be carried out during pregnancy or in the presence of uterine inflammation.

MATERIAL AND METHODS

Seventytwo endometrial smears were studied and consisted of:

23 smears taken during the first half of the menstrual cycle,

17 smears taken during the second half,

21 smears taken during menopause, and

11 smears taken during estro-progestinic contraceptive treatment.

The study was conducted in women presenting a completely negative gynecological history with normal menstrual cycles, or in menopause for at least two years. Regarding contraceptive therapy, only combined estro-progestinic treatment of at least 3 months duration was considered (ethinil-estradiol 0.05 mg + levonorgestrel 0.25 mg).

Specimens were obtained by abrasion, using an instrument called " endometrial brush ". This instrument consists of thin plastic cannula with many filaments in a ring formation at the distal end. It is introduced into the uterine cavity contained within its guide in order to avoid cervical contamination of the specimen. When the isthmus is passed, the distal end of the cannula is gently pushed out of the guide, and with a rotating motion, the endometrial specimen is obtained by abrasion of the endometrial surface by the filaments. When the specimen has been collected, the distal end of the cannula is re-inserted in its guide before removing it from the uterine cavity. The material thus obtained is rich in cells from the entire cavity, and is not contaminated by cervical cells. In the few cases where it was not possible to pass the isthmus, another probe was employed that is very similar to the endometrial brush except that it is thinner, more rigid, and its distal end is helical (endocyte) ([17]).

The endometrial smears were fixed in alcohol-ether (1:1) and then stained according to Papanicolaou.

RESULTS

A) *Cytologic aspect of the endometrium during menstrual cycle* (table 1).

Proliferative phase: the glandular cells show a florid, round nucleus with a large nucleolus and a marked chromatin pattern. Many mitotic figures are observed. The nuclei are crowded closely together with a homogeneous, irregular, thin cytoplasmic halo (fig. 1: a, b).

The highly chromatinic cells are densely grouped together in a ribbon formation with straight, well outlined borders.

From a study of the 23 smears representing the entire arc of the proliferative

Figure 1. — a: Proliferative phase (10th day of cycle). Gland cell clusters with hyperchromatic nuclei. Nucleoli and mitosis are observed (×400). b: Proliferative phase (6th day of cycle). Nuclear and cytoplasmic characteristics are readily observed: chromatinic hyperactivity, large nucleolus and mitotic figures; the cytoplasm is seen as a thin perinuclear halo without secretory vacuoles (×1000). c: Proliferative phase (10th day of cycle). Reticular and fibroblastoid-type stromal cell cluster. Note irregular cell arrangement and fringe-like cytoplasm (×400). d: Secretory phase (20th day of cycle). Gland cell cluster showing finely reticulated nucleus with small nucleolus and no mitosis. The ample, vacuolated cytoplasm has distinct borders (×400). e: Secretory phase (28th day of cycle). Cell cluster showing finely granular nucleus in some zones and retracted, dark nucleus in others. The foamy appearance of the cytoplasm and several vacuoles indicate its secretory function. Cellular membrane is interrupted occasionally due to secretion into the lumen (×400). f: Secretory phase (28th day of cycle). Decidual-like stromal cells. The cytoplasm is ample, blue and finely vacuolated with fringe-like borders. The nucleus is retracted, uniformly dark and round (×400). g: Menopause. Strip of atrophic gland cells showing dark retracted nucleus. The cytoplasm has shrunk to a barely visible, thin perinuclear halo (×160). h: Estro-progestinic treatment. Cluster of gland cells with retracted, dense or pale nucleus. Ample, vacuolated cytoplasm has distinct borders (×400).

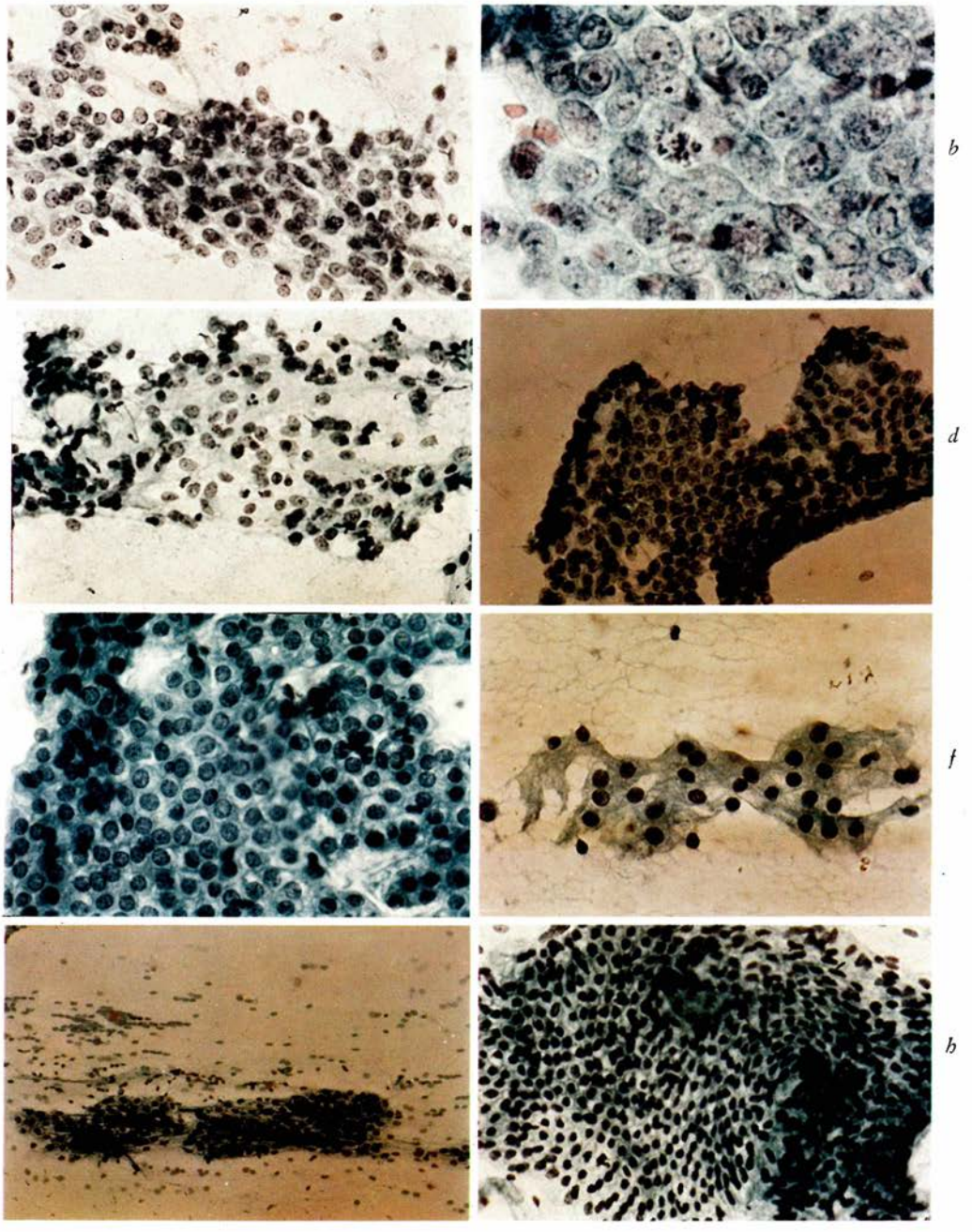
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Table 1. — *Cytology of the endometrium during menstrual cycle.*

Cycle phase	Gland cells				Stromal cells		
Proliferative	Nucleus Chromatine	Mitosis	Cytoplasm	Arrangement	Nucleus	Cytoplasm	Arrangement
Initial	+	+	Thin perinuclear halo.				Non uniform clusters with irregular borders.
Middle	++	++		"Ribbons".	Large, dense, elongated	Thin, pale.	No cohesion.
Advanced	+++	+++	Secretive vacuoles absent.				
Secretory							
Initial	+	—	Small refracting perinuclear vacuole.			Ample, foamy with distinct borders.	Non uniform clusters with irregular borders.
Middle	—	—	Uniform vacuolization, clear borders.	"Clusters".	Large, clear, occasionally incised.	Oval, dark purple finely vacuolated.	Uniform clusters.
Advanced	—	—	Non-uniform vacuolization, indistinct borders.				

Table 2. — *Cytology of the endometrium during menopause.*

Gland cells			Stromal cells		
Nucleus	Cytoplasm	Arrangement	Nucleus	Cytoplasm	Arrangement
Large and pale or small, retracted and dark.	Thin, pale perinuclear halo.	Clusters. (Number and size dependent on degree of atrophy).	Oval and chromophilic or elongated, retracted and dark.	Thin, pale perinuclear halo. Thin ramifications from one or both nuclear poles.	Non uniform clusters.

Table 3. — *Cytology of the endometrium during combined estro-progestinic treatment.*

Cytologic type	Gland cells			Stromal cells		
	Nucleus	Cytoplasm	Arrangement	Nucleus	Cytoplasm	Arrangement
Secretory Hypoplastic Endometrium	Medium size finely reticulate and inactive. No mitosis.	Thin perinuclear halo. Small secretory vacuole.	Uniform clusters.	Round or elongated.	Ample, foamy with distinct borders. Perinuclear ramifications.	Dense uniform clusters.
Atrophic Endometrium	Small, pale. No mitosis.	Thin, indistinct perinuclear halo.	Medium and small clusters.	Finely reticulated.	Fine vacuolization.	Isolated cells or loosely grouped together.

phase, it was seen that as the date of ovulation approached, there was a progressive accentuation of the chromatin pattern and mitotic activity, along with a parallel increase in the diameter and number of the ribbon clusters.

Unlike the glandular cells, the stromal cells appear isolated or loosely grouped together in irregular clusters (fig. 1: c). The nuclei also show estrogenic stimulation with hyperchromatism and mitotic figures, while the cytoplasm is thin and fringe-like, and shrinks to one or more perinuclear ramifications.

Secretive phase: the glandular cells lose their ribbon arrangement and group together in more or less wide cellular sheets with a polygonal outline. Study of the 17 specimens representing the 16th to the 29th day of the cycle disclosed three evolutive steps at the cytoplasmic level:

– initial secretory activity which manifests with the appearance of a perinuclear vacuole, not always readily observed, followed by other vacuoles irregularly distributed in the ample cytoplasm with distinct borders;

– in the middle of the secretory phase (19-25 day of cycle) the cytoplasm is uniformily vacuolated or foamy. Due to initial expulsion of the secretion into the glandular lumen, the cellular membrane at some points loses its individuality, a phenomenon that is readily observed along the edges of the cellular clusters (fig. 1: d);

– in the advanced secretory phase (after the 25th day) the glandular cells seem to constitute large syncytia. In fact, with the almost total disappearance of the cellular borders, the cyptoplasmic spaces become confluent. The foamy and vacuolated aspect of the cytoplasm is highly accentuated (fig. 1: e).

The gland cell nuclei which are hyperchromatic only in the first days following ovulation, become hypochromatic and finely reticulated or more or less retracted and dark. Mitotic figures are not observed.

Under progestinic stimulation, the stromal cells also undergo cytoplasmic metamorphosis which is readily observed cytologically. The cells which were loosely united, re-arrange and form clusters with a decidual appearance that are fairly homogeneous with ample, finely vacuolated cytoplasm (fig. 1: f).

B) *Cytological aspect of the endometrium during menopause* (table 2).

Cytological findings in 21 endometrial smears from women in menopause for at least two years were rather uniform.

Cellular material was usually scanty and contained mostly atrophic epithelial and stromal cells. Small islands of glandular cells dotted uniformly by hypochromatic or intensely retracted, dark nuclei were observed, and the cytoplasmic space was practically not distinguishable (fig. 1: g). The presence of rare clusters of cells mostly fibroblastic in type with an elongated and dense nucleus indicated stromal atrophy. The cytoplasm is pale and fringe-like.

C) *Cytological aspect of the endometrium during estro-progestinic treatment* (table 3).

The cytological picture in the 11 cases observed was not uniform. In fact, from an analysis of the degree of trophism and secretory activity of the gland cells, two cytofunctional types were distinguished: atrophic endometrium, and secretory but hypoplastic endometrium.

In the atrophic variety, the smear is very similar to that seen during menopause. Medium sized clusters of glandular cells, where the small, hypochromatic or dense nuclei are closely crowded together due to the thin cytoplasmic space, without mitotic figures, were seen.

In the secretory type, the cytoplasm of the glandular cells appears poorly vacuolated and foamy, with distinct borders. The nucleus is inactive with a fine chro-

matinic, or intensely dark reticulum, without mitotic figures (fig. 1: h). The stroma shows the progestinic action of the contraceptive, even if the glandular cells are clearly atrophic. The smear contains numerous stromal cells that are isolated, loosely grouped together or arranged in uniform clusters. In this last case, the cell assumes the features of decidual transformation. The cytoplasmic space is progressively more ample and vacuolated, while the nucleus is round, finely reticulated or dark.

CONCLUSIONS

From an examination of the data obtained, it seems reasonable to state that the endometrial smear, which has been employed up to now almost exclusively in the cyto-oncological field, is also quite suitable for cyto-functional evaluation. In fact, the smear furnishes a series of interpretative elements, some of which are easily and readily identified at the microscope. Our study, accurately defines the various cyto-functional aspects of the endometrium in different phases of life in the woman, and therefore demonstrates that endometrial cytology represents an investigative technique that is particularly useful in dynamic evaluation of ovarian function in view of its easy application and interpretation.

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