

Morphological and ultrastructural aspects of the cyclical changes of human mammary gland during the menstrual cycle

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Analyzing the morphological and ultrastructural features of the mammary gland during the menstrual cycle, the authors observed that in the proliferative phase, the mammary gland consisted of few developed lobules and presented a relative absence of mitotic figures. In this phase, the great majority of the epithelial cells contained small, dark, irregular, rich heterochromatinic nuclei. The mammary stroma showed similar findings, presenting dense connective tissue with few enhanced fibroblasts. During the secretory phase, the lobules were well developed and mitotic figures were usually observed. In this phase, the great majority of the epithelial cells presented clear voluminous, rich euchromatinic nuclei; the stroma consisted of fibroblasts with voluminous nuclei full of euchromatin. There are evident modifications of the mammary tissue according to the phase of the menstrual cycle. The morphological and ultrastructural modifications indicate higher metabolic and mitotic activities during the secretory phase.

UNITERMS: mammary gland; menstrual cycle; morphology; ultrastructure.

INTRODUCTION

The cyclical function of the female genital apparatus has been known since the beginning of this century (6,14). The cyclical morphological modifications of the mammary glands were first described by Rosenberg in 1922, who observed gland hypertrophy in the premenstrual period and regressive signs during the catamenial flow (12). However, these findings could not be reproduced by other authors (2,16). Therefore, the concept of a "resting" mammary gland, suffering no significant modifications during the cycle, has been accepted for many years (5,18).

Recent investigations on ultrastructure and cellular kinetics of mammary tissue have demonstrated, however, conflicting results concerning the concept of a "resting" mammary gland in non-pregnant women (3,4). The purpose of the present study is to observe the morphological and ultrastructural features of the human mammary gland during the proliferative and secretory phases of menstrual cycle.

MATERIAL AND METHODS

Patient selection and study design.

Thirty patients who were submitted to excision of fibroadenomas were selected in order to perform this research. A detailed history was obtained from each patient; in this study we only included young women in the same age group, eumenorrheic in the last six months, presenting normal colposcopic and gynecologic examinations and with exact knowledge of the date of the last menstruation.

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Pregnant women, patients receiving hormonal treatment, those suffering from endocrine diseases, women who were lactating in the last 12 months and those who used any medications in the period of the biopsy were excluded.

The patients were divided at random into two groups of 15 women, according to the phase of the menstrual cycle (proliferative and secretory phase), which was defined not only by the date of the last menstruation but also by the determination of serum progesterone levels and the date of subsequent menstruation. The evaluation of good luteinic activity considered progesterone levels equal to or above 3.0 ng/ml (7). The specimens of normal mammary tissue were surgically obtained, excising a parenchymal fragment located at least about 1 cm from the edges of the lesion and which showed no alterations on gross examination. Table 1 shows the date of the menstrual cycle when the surgical procedure was performed as well as serum progesterone levels.

TABLE 1
CHARACTERIZATION OF MENSTRUAL CYCLE PHASE

CHASE Nº	DAY OF THE CYCLE	PROGESTERONE* (ng/ml)	PHASE OF THE CYCLE
1	8 ^o	1,1	PROLIFERATIVE
2	3 ^o	0,2	PROLIFERATIVE
3	3 ^o	0,2	PROLIFERATIVE
4	7 ^o	0,2	PROLIFERATIVE
5	10 ^o	0,2	PROLIFERATIVE
6	12 ^o	0,3	PROLIFERATIVE
7	12 ^o	1,1	PROLIFERATIVE
8	5 ^o	0,3	PROLIFERATIVE
9	11 ^o	0,2	PROLIFERATIVE
10	12 ^o	1,1	PROLIFERATIVE
11	12 ^o	0,2	PROLIFERATIVE
12	11 ^o	0,3	PROLIFERATIVE
13	2 ^o	0,2	PROLIFERATIVE
14	12 ^o	0,9	PROLIFERATIVE
15	5 ^o	<0,1	PROLIFERATIVE
16	20 ^o	25,0	SECRETORY
17	20 ^o	3,2	SECRETORY
18	27 ^o	3,1	SECRETORY
19	22 ^o	3,4	SECRETORY
20	30 ^o	6,3	SECRETORY
21	24 ^o	7,4	SECRETORY
22	29 ^o	3,1	SECRETORY
23	30 ^o	6,6	SECRETORY
24	26 ^o	4,1	SECRETORY
25	24 ^o	3,9	SECRETORY
26	22 ^o	3,8	SECRETORY
27	20 ^o	7,7	SECRETORY
28	22 ^o	5,5	SECRETORY
29	28 ^o	3,2	SECRETORY
30	24 ^o	18,0	SECRETORY

Normal values: Follicular phase = up to 1,5 ng/ml
Secretory phase >3.0ng/ml

The histopathological normality of the mammary parenchyma was previously verified and the morphological analysis was accomplished without previous knowledge of the phase of the menstrual cycle to avoid possible bias.

Morphological and ultrastructural study

Fragments of normal mammary tissue were fixed in Bouin's solution, dehydrated, included in paraffin and the blocks were cut in a 5 µm settled microtome. These sections were stained with hematoxylin-eosin. For the electron microscope study, small fragments of about 1 mm were immersed in 2% glutaraldehyde in 0.2 M phosphate buffer, postfixation with 1% osmium tetroxide, dehydrated in crescent concentrations of ethanols and embedded in araldite. Thin sections of silver to light gold were double stained with 1% uranyl acetate and lead citrate, then examined with a Zeiss EM 9 s 2 60 kV electron microscope.

RESULTS

Homogeneity of the samples

The samples, in both phases of the menstrual cycle, were statistically homogeneous regarding the following features: age, time of menarche, number of gestations and deliveries, age of the first and last deliveries and presence or absence of lactation.

Proliferative phase

The analysis of the results of light microscopy revealed that the mammary gland consisted of poorly developed lobules with small or absent lumen. The cells presented basophilic nuclei and few evident nucleoli. The cell limits were not sharp and figures of mitosis were virtually absent. The connective tissue inside the lobules showed fibroblasts with basophilic and lengthened nucleus (fig. 1).

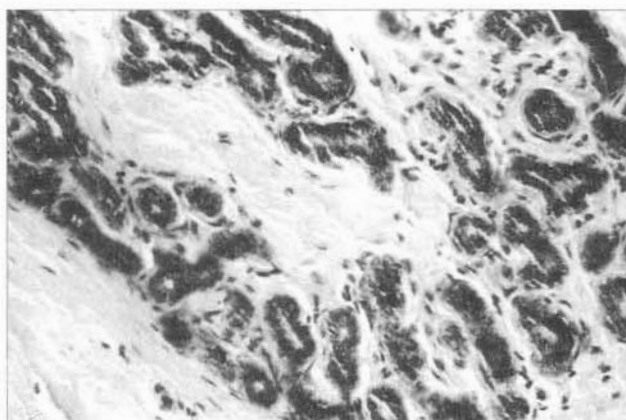


Figure 1 – Photomicrograph showing portion of a mammary lobule of a woman in the proliferative phase of the menstrual cycle. Note that the parenchyma and stroma contain cells with small condensed nuclei (H.E. 700 X).

The ultrastructural analysis showed alveolar cells containing small dark, rich heterochromatinic irregular nuclei in the proliferative phase. The cytoplasm showed a developed granular endoplasmic reticulum with enlarged cisternae; polysomies and mitochondrions were also observed. The upper portion was irregular and contained rare microvilli (fig. 2).

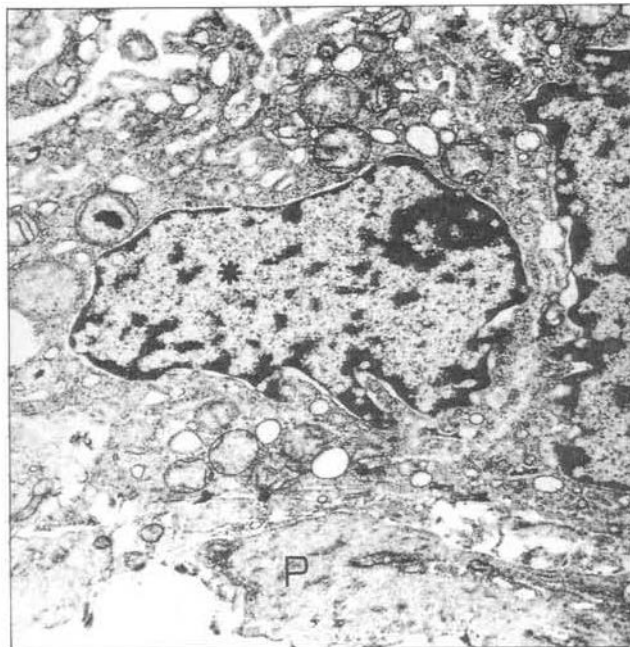


Figure 2 – Electronmicrograph of portion of mammary tissue in the proliferative phase. Observe that the epithelial cell contains an irregular, dark, rich, heterochromatinic nucleus (asterisk). The lumen is small and microvilli are scarce. In the basal portion, a prolongation of an epithelial cell (P) can be verified (17 000 X).

The alveolar cells exhibited cellular junctions and interdigitations connecting one to another. The cellular apical portion showed junctional complexes and the basal portion presented junctions of desmosome type which were connected to prolongations of myoepithelial cells. These cells were attached to the basal lamina by hemidesmosomes.

The alveolar lumen was irregular, diminutive and contained some material inside. The myoepithelial cells contained irregular processes, full of filaments, surrounding the alveolar cells and directly attached to the basal lamina. The basal lamina was irregular and showed numerous folds. Fibroblasts with irregular dark nuclei and poorly developed cytoplasm containing few organelles were observed in the stroma.

Secretory phase

The light microscopy revealed well developed lobules with enlarged ducts. The alveolar were sharply limited and presented enlarged, rich euchromatinic elliptical nuclei at the basal direction of the cell and with an evident nucleolus. The cytoplasm of the apical region was acidophilic and showed a granulated aspect, sometimes presenting prolongations directed to the lumen. Some mitotic figures were also observed in the lobules (fig. 3).

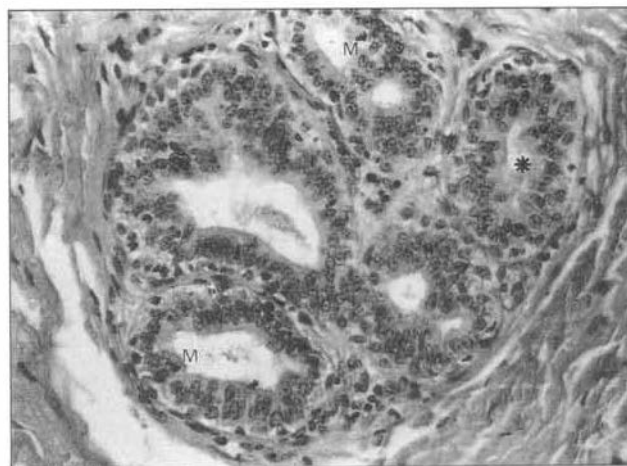


Figure 3 – Photomicrograph of a portion of a mammary lobule in the secretory phase of the cycle. The parenchyma consists of cylindrical cells with ellipsoid rich euchromatinic voluminous nuclei and evident nucleolus (asterisk). Mitotic figures can be observed (M). (H.E. 700 X).

Fibroblasts with enlarged fusiform rich-euchromatinic nuclei were observed in the lobular stroma. On the electron microscopy, the great majority of the alveolar cells presented elliptical, clear, regular, voluminous nuclei full of euchromatin with evident nucleolus. (fig. 4).

The basal portion of the cytoplasm showed cisternae of the granular endoplasmic reticulum, mitochondrions and polysomies. The apical region shows cisternae of the granular endoplasmic reticulum, Golgi apparatus and numerous mitochondrion. The alveolar cells presented interdigitations and typical cellular junctions as well as desmosomes and junctional complexes at its apical portion. At the luminal side we observed numerous microvilli. Prolongations of myoepithelial cells containing filaments established contact with the basal lamina and the basal aspect of alveolar cells. The myoepithelial cells contacted the alveolar cells and lamina basal by means of desmosomes and hemidesmosomes, respectively. The mammary stroma consisted of enhanced fibroblasts intermixed with amorphous and fibrillar intercellular substance.

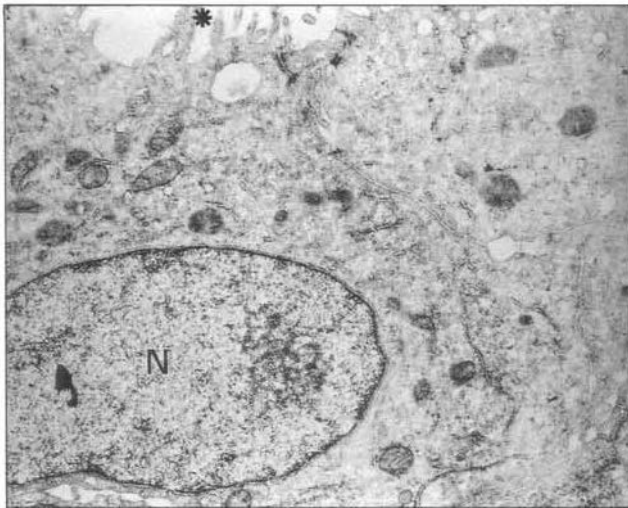


Figure 4 – Electronmicrograph of portion of a mammary gland in the secretory phase of the cycle. Observe numerous microvilli (asterisk) at the lumen. The luminal epithelial cells present regular, clear voluminous rich euchromatinic nucleus (N). (17 000 X).

The fibroblasts were fusiform and showed numerous prolongations that maintained contact with prolongations of other fibroblasts, resembling a net. The nuclei were voluminous, elliptical, contained great amount of euchromatin and evident nucleolus. The cytoplasm presented mitochondria, Golgi apparatus and granular endoplasmic reticulum.

DISCUSSION

Previous observations of normal histology of the mammary gland were made in the beginning of this century and showed proliferation of the gland epithelium during the premenstrual phase and inhibition of growth after catamenium (12). Other authors, however, did not find any histological cyclical modifications in the mammary tissue and considered the non-pregnant mammary gland as being at "functional rest" (6,16). This concept endured until studies on ultrastructure and cellular kinetics demonstrated the presence of marked cyclical modifications in the mammary gland during the menstrual cycle (3,4).

In the present study, we intend to analyze the morphology of the mammary gland during the proliferative and secretory phases of the cycle.

Since our study material was obtained from glands that presented fibroadenoma, one may speculate that the pathogenetic factor responsible for the onset of this lesion might eventually have caused change in the mammary

gland as a whole. However, because the type of associated lesion was circumscribed and always the same (fibroadenoma), if eventual subtle physiological abnormalities had been presented, they should have been equally present in both phases of the cycle (9).

Our light microscopic results were corroborated by the ultrastructural analysis and were similar to the literature data (3,8). In fact, Fanger and Ree (3) postulated that in the proliferative phase the cell undergoes a relative inactive state, characterized by simple ultrastructural features and a metabolic activity that is limited to the synthesis of endogenous protein. The nuclei appear small, irregular and rich in heterochromatin; the nucleoli are rarely observed and microvilli are scarce. Paradoxically, the maximum degree of inactivity of the mammary gland epithelial cell was observed during the period of presumably highest levels of estrogen. During the secretory phase we can observe voluminous, regular, rich euchromatinic nuclei with evident nucleolus; the microvilli are prominent, all these data indicating an intense proteic synthesis. Mitotic figures appear frequently (9,11).

The predominance of epithelial cells with clear nuclei in the luteal phase and dark nuclei in the proliferative one corroborates the hypothesis that these elements constitute the same cellular type at different functional states according to their metabolic activity (13,15).

The description of an enlarged granular endoplasmic reticulum in the proliferative phase contrasts with decrease of nuclear volume that occurs in this phase. It could probably be explained by a contraction of the cellular volume which would cause an apparent enlargement in the concentration of organelles (3).

It is interesting to note that the interdigitations and desmosomes are well developed in the mammary epithelium and are necessary for uniting the cells against tension forces, such as secretion or decreasing of cellular volume, according to the phase of menstrual cycle; in this manner, cellular deformation would be avoided (1).

The stroma shows morphological and ultrastructural modifications that are similar to those found in the epithelial component. The observation of an evident intercellular substance is possibly explained by the stromal edema induced by sexual steroids, which can produce a histaminic effect on the mammary microvasculature or increase the synthesis of proteoglycans with high osmotic power, which consequently leads to the accumulation of fluids (10,17).

In conclusion, we verified that mammary glands undergo clear modifications during the menstrual cycle. The morphological and ultrastructural alterations indicate that the mitotic and metabolic activities of the mammary tissue are more intense in the secretory phase.

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RESUMO

Com a finalidade de avaliar se a glândula mamária humana sofre alterações morfológicas e ultra-estruturais durante o ciclo menstrual, selecionaram-se 30 pacientes atendidas no período de janeiro de 1991 a setembro de 1992. As pacientes foram divididas, ao acaso, em dois grupos, isto é, 15 mulheres na fase proliferativa e igual número na fase secretora. Os espécimes de tecido mamário normal foram obtidos por ocasião da exérese de fibroadenomas. Na fase proliferativa, a glândula mamária encontra-se constituída por lóbulos pouco desenvolvidos e as figuras de mitose são virtualmente ausentes. A maioria das células epiteliais apresenta núcleos pequenos, escuros, irregulares, ricos em heterocromatina. O estroma mamário apresenta comportamento semelhante; é compacto e denso e os fibroblastos são desenvolvidos. Já durante a fase secretora, os lóbulos são bem desenvolvidos e foi relativamente comum o encontro de mitoses. A grande maioria das células epiteliais, nesta fase, apresenta núcleo claro, regular, volumoso, rico em eucromatina. Da mesma maneira, o estroma é constituído por fibroblastos com núcleos volumosos e ricos em eucromatina. Concluindo, pudemos constatar alterações marcantes na glândula mamária humana consoante a fase do ciclo menstrual.