Premature Ovarian Failure: Morphological and ultrastructural aspects

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The authors documented by means of light and transmission electron microscopy that the ovaries of women with premature ovarian failure (POF) displayed dense connective tissue and rare corpora albicantia. Eight of the ten studied cases did not present ovarian follicles; in two cases, it was verified the presence of ovarian follicles, atypical primordial follicles and in one case, a corpus luteum was identified (after stimulation with exogenous gonadotrophin). Regarding the ultrastructural analysis, it was noted that the fibroblasts were united one to each other by cellular prolongations that formed a web, constituting a cellular synciкус.

UNITERMS: premature ovarian failure, corpora albicantia, morphology.

INTRODUCTION

Premature ovarian failure (POF) is an infrequent entity, representing about 5.0 to 18.0% of all secondary amenorrhea (1,10,25). In the POF the gonads usually present dimitted size, wrinkled external surface and cerebriform aspect, resembling the postmenopausal ovary (23). Histologically, two modalities are described: follicular and afolicular. In the afolicular type, the ovary consists of stroma, corpora albicantia and do not show atretic follicles. Active primordial follicles are rarely observed (2,3,8,9,12,20,23,28). The follicular type, on the contrary, shows numerous primordial follicles and total or almost complete absence of growing follicles; in some cases, an linfoplasmocitical infiltration can be observed surrounding the primordial follicles (3,9,14,15,24,27).

Regarding the ultrastructure, there are few studies in the literature. Multiple blood vessels intermixed with collagen fibrils, as well as active fibroblasts and rare normal primordial follicles were registered in ovarian fragments of women with premature ovarian failure (4,16). On account of these data, we intend, in this paper, to analyse ovarian fragments of patients who presented premature ovarian failure using the light and transmission electron microscopy.

PATIENTS AND METHODS

We utilized ovaries from 10 women with ages ranging from 28 to 39 years who presented premature ovarian failure and were submitted to ovarian biopsy in the period from 1989 to 1990. Our study only included patients with secondary amenorrhea of one year or more time of duration, and with serum FSH levels above 40 mU/mL. Furthermore, none of the patients presented background of radio or chemotherapy or were submitted to ovarian surgery. In order to improve the characterization of our study group, we attained a propaedeutic method which included a complete blood test with hemoskmentation velocity, fasting glucose, proofs of renal and hepatic function, overall rheumatological evaluation,
investigation of viral infection, evaluation of the thyroid gland function, cytogenetical study and determination of anti-ovarian antibodies in the peripheral blood and in the ovary. These examinations were normal and the karyotype was 46 XX in all patients. We accomplished an oestradiol plasmatic evaluation before and after stimulation with postmenopausal gonadotrophin. For this test, the patients received 75 UI of follicle stimulating hormone for 5 consecutive days.

Ovarian fragments of these women were fixed in 10% formaldehyde, processed and included in wax. Sections of 7 um were prepared for the hematoxylin-eosin staining. The other fragments were fixed in 2% glutaraldehyde at 0.1 M phosphate buffer of pH 7.2 for 4 hours and then refixed in 1% osmium tetroxyde for one hour. After immersion in 0.5% uranyl for 12 hours, this material was dehydrated at progressive concentrations of ethanol and finally included in araldyte. Semithin sections were prepared and analysed in a Carl Zeiss EM 9s2 - 60- kV electron microscope (18,26).

RESULTS

Light Microscopy

Two regions are identified in the ovary: an inner portion, presenting several blood vessels, which corresponds to the medulla, and another outer portion, containing a lot of collagen fibers, which constitutes the cortical region.

These two regions are united with no good delimitation. The cortical connective tissue consists of great quantity of fusiform cells with lengthened nuclei intermixed with high concentration of collagen fibers. The distribution of cellular and fibrilar elements shows lack of homogeneity: next to the lining epithelium these components are parallel to the ovarian surface, forming the albuginea; the deeper cortex presents irregular spirals of cells and collagen fibers, characterizing a net. Between these bundles, at the woofs of the net, we can observe clearer areas containing less quantity of collagen fibers. Ovarian follicles were not found in eight cases (Fig.1). However, in all fragments, we registered the presence of corpora albicantia consisting of an eosinophilic mass with sharp boundaries, surrounded by a rich- collagen connective tissue capsule (Fig.2). The medullar connective tissue is similar to the cortical one, excluding the presence of numerous blood vessels.

We verified, in two patients (cases 9 and 10), rare atypical ovarian follicles. These follicles showed cells with dissimorphical eosinophilic cytoplasm and central spherical nuclei. In one of those cases (9), we also noted the presence of a corpus luteum; in case 9 the majority of stromal cells contained nuclei with clear areas.

TRANSMISSION ELECTRON MICROSCOPY

The ovarian stroma presents great concentration of fibroblasts and collagen fibers, the latter one grouped forming compact bundles. We could note prolongations of fibroblasts among these bundles. The distribution of cells at the stromal tissue is not homogeneous; some regions are filled with collagen fibers (Fig.3) and other regions show greater cellular density. The fibroblasts usually present rich-euchromatic ellipsoid voluminous nuclei with evident nucleoli (Fig.4). The cytoplasm shows several prolongations which sometimes touch the prolongations of other cells; at the junction site, the membranes present higher electron density. At some regions we could note the presence of corpora albicantia consisting of flaky material and rare collagen fibrils (Fig.5). At its peripheral region, there was a major concentration
of cells and collagen fibrils. Inside the flaky material, we could verify spread cells, mainly fibroblasts, with numerous prolongations that touched each other frequently. Some of those fibroblasts inside the corpora albicantia and at its periphery showed great concentration of cytoplasmatic myofilaments (Fig.6). We also identified macrophages with several electron-dense and electron translucent granulations at the peripheral region of the corpora albicantia (Fig.7). In the ovarian fragment which presented a corpus luteum (case 10) we could note great concentration of lutheinic cells with central spherical nucleus and obvious nucleolus; the cytoplasm contained high concentration of agranular endoplasmatic reticulum, mitochondrions,
electrodense granulations and lipid droplets (Fig. 8).

Regarding to the atypical ovarian follicles observed in cases 9 and 10, we identified oocytes surrounded by squamous follicular cells with fusiform nucleus and several interdigitations at the apical portion (Fig. 9). An interesting finding is that, in case 9, all the nuclei presented an electron translucent area inside it (Fig. 10).

**DISCUSSION**

As seen before, the POF is an infrequent entity; 0.9% of all women can develop POF (5), which was classified according to the etiology (5,10,16,17,24,27). Genetic changes, autoimmune diseases, viral infections, iatrogenic factors, changes of gonadotrophic action and the idiopathic modality are quoted as the more important causes. Owing to these facts, our study group did not include women with chromosomal changes, general immunological disturbances, previous viral infections (specially mumps and rubella), hepatic or renal pathology, nor those with antithyroid or antiovarian antibodies, which could all be the causal factor of amenorrhea.

Our light microscopical findings were similar to the literature data: the gonads consisted of connective tissue and rare corpora albicantia were observed. Description of atypical primordial follicles is also reported in the literature (2,3,8,9,12,20,23,28). These findings were expected by the authors, since the stimulation with chorionic gonadotrophin in cases 9 and 10 led to an increase of oestradiol plasmatic levels. We believe that the patients who presented atypical ovarian follicles developed the POF due to structural changes on the hypophysal gonadotrophins or on its receptors (6,21). The finding of a corpus luteum could be a consequence of the stimulation with menopausal gonadotrophin; despite of considering those ovarian follicles as anormal ones, they possibly responded to the gonadotrophic stimulus.

The ultrastructural study corroborated the light microscopical observation. Great concentration of fibroblasts and collagen fibrils were registered in the ovarian stroma. These elements presented heterogeneous distribution, showing areas filled with collagen fibrils and areas with higher concentration of fibroblasts, which explained the whirl-like aspect. A curious fact was the description of junctions between the fibroblasts; this detail has already been observed in fibroblasts of tissue culture and human endometrium, but has not been registered in the ovary. These junctions are of the “gap” type and might be important for the transmission of any kind of stimulus from one cell to each other (22); in this manner, the cells (fibroblasts) would behave like a cellular synciosis.

In regards to the corpora albicantia, we believe that these structures could be undergoing a remodelling process, since there were typical macrophages at its periphery, with signs of intense phagocytical activity. Furthermore, the fibroblasts, mainly those located at the peripheral portion of the corpora albicantia, showed myofibrils in its cytoplasm, characterizing myofibroblasts. These myofibroblasts appear at sites of tissular retraction (7,13,19). By the way, under physiological conditions, it is observed that the majority of the corpora albicantia is reabsorbed, undergo fibrosis and is incorporated to the ovarian stroma (11).

Therefore, as could be verified in our study material, the majority of the gonads showed follicular depletion and the morphology was similar to that of physiological menopause. In this manner, the histological features of the ovary, after the development of POF, would have only diagnostic importance. We believe that, in order to clarify the etiology of POF, early morphological, endocrinical and immunological studies should be performed.
REFERENCES


RESUMO

Os autores analisaram, através de microscopia de luz e de microscopia eletrônica de transmissão, os ovários de mulheres com insuficiência ovariana prematura. Observaram que está constituídos por tecido conjuntivo denso e raros corpos albinantes. Oito dos 10 casos não apresentavam foliculos primordiais; em um caso verificou-se a presença de foliculos ovarianos atípicos e, em outro, identificou-se corpo lúteo (após estimulação com gonadotrofina exógena). À altura-estrutura, verificou-se que os fibroblastos achavam-se unidos uns aos outros através de prolongamentos celulares, formando um sincício celular.