SÃO PAULO MEDICAL JOURNAL

ORIGINAL ARTICLE

Mauro Abi Haidar, Edmund Chada Baracat, Manuel de Jesus Simões, Gustavo Rubino de Azevedo Focchi, Joaquim Evêncio Neto, Geraldo Rodrigues de Lima.

Premature Ovarian Failure: Morphological and ultrastructural aspects

Department of Obstetrics and Gynecology and Department of Morphology, Escola Paulista de Medicina, São Paulo, SP, Brazil.

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 woof, constituting a cellular syncicius.

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 diminuted size, wrinkled external surface and cerebroid aspect, resembling the postmenopausal ovary (23). Histologically, two modalities are described: follicular and afollicular. In the afollicular 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.

Adress for correspondence: *Prof. Dr. Mauro Abi Haidar Rua Botucatu, 740 - 7. andar - CEP 04023-900 - SP São Paulo - SP - Brasil*

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 mUI/ml. Furthermore, none of the patients presented background of radio or chemotheraphy 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 hemosedimentation 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 plasmatical 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 way. 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 correspondents 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 lenghtened 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 atipical ovarian follicles. These follicles showed cells with dismorphical 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.

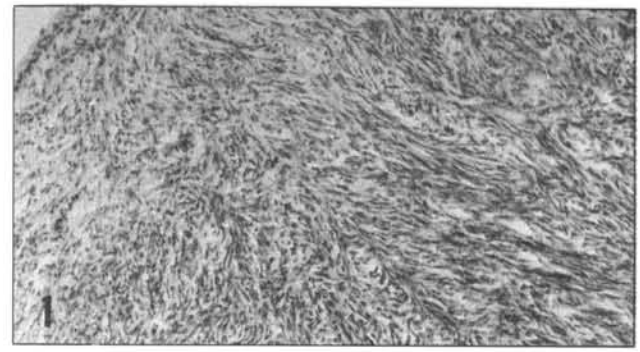

Figure 1 – Cortical region with depletion of oocytes - 300 ×.

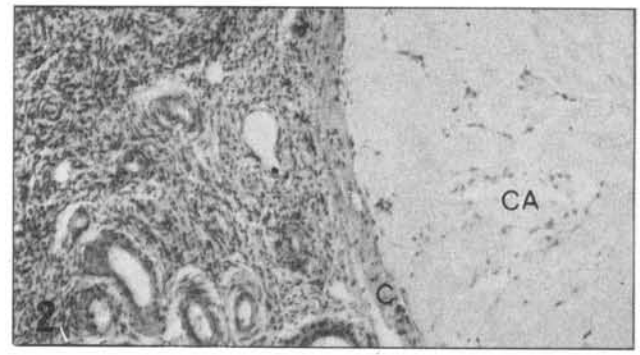

Figure 2 – Presence of a corpus albicans (CA) surrounded by a capsule of connective tissue (C) - 300 ×.

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-euchromatinic elipsoid 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 densitiy. 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

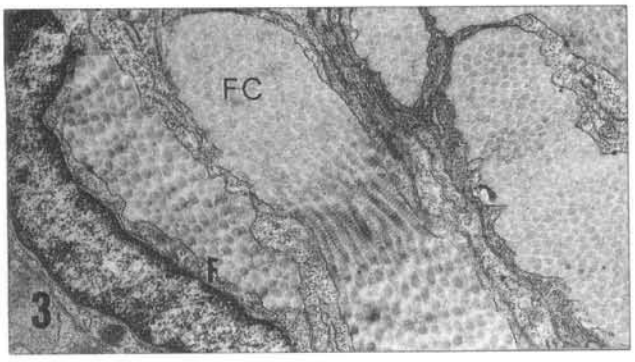

Figure 3 – Fibroblast (F) and collagen fibrils (FC) forming compact bundles - 16100 ×.

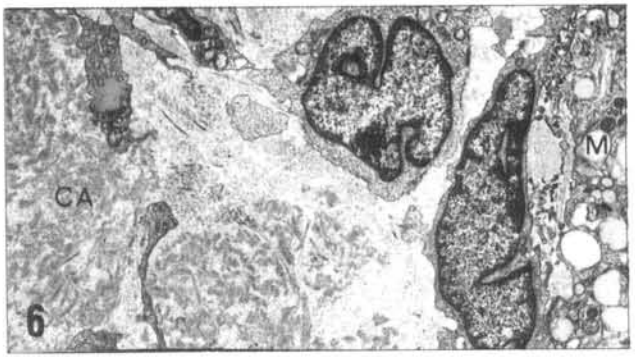

Figure 6 – Macrophage (M) located at the peripheral region of corpus albicans - 4250 ×.

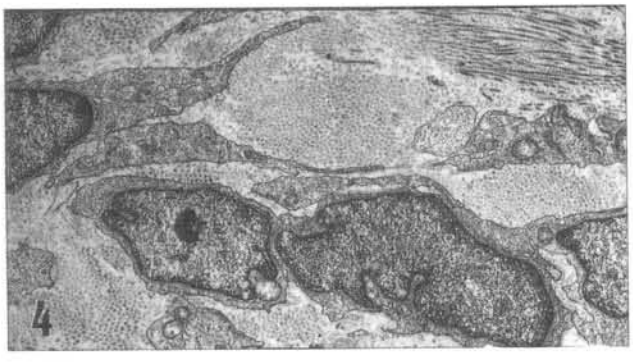

Figure 4 – Great concentration of fibroblasts and collagen fibrils in the stroma - 4600 ×.

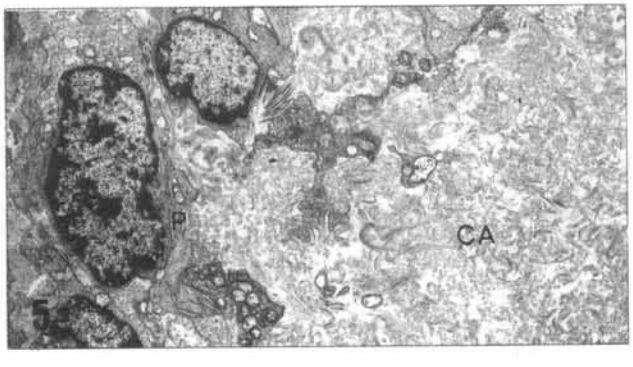

Figure 5 – Peripheral region (P) of the corpus albicans (CA) - 3450 ×.

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 cytoplasmatical myofilaments (Fig.6). We also identified macrophages with several eletrondense and electrun 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,

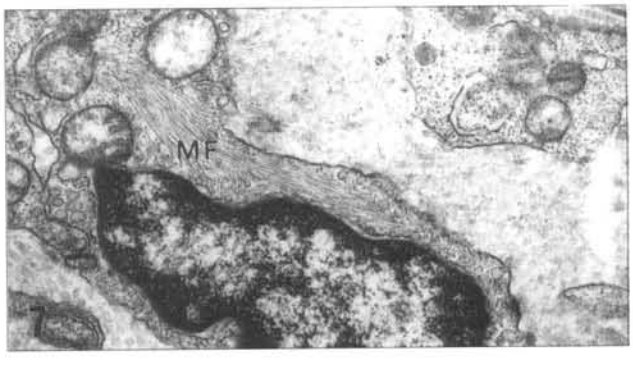

Figure 7 – Fibroblast showing great concentration of cytoplasmatical myofilaments (MF) - 14900 ×.

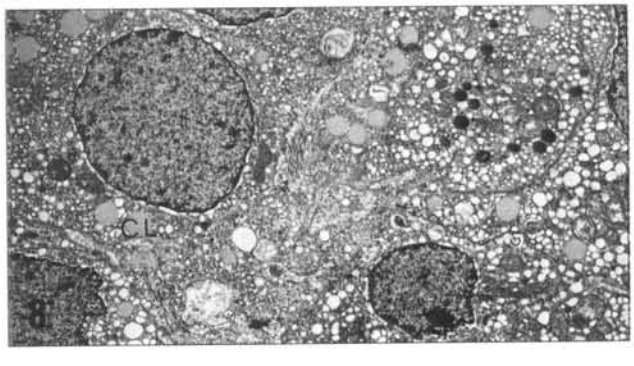

Figure 8 – Lutheinic cells (CL) from a corpus luteum (case 10) - 3260 ×.

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 electrun 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 aetiology (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 chromosomical 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.

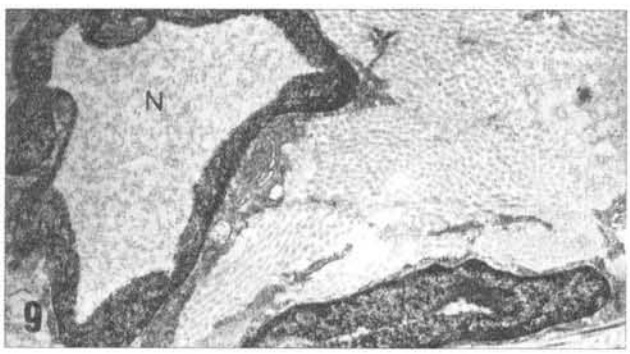

Figure 9 – Nucleus (N) presenting an electrontranslucent area inside it (case 9) - 15100 ×.

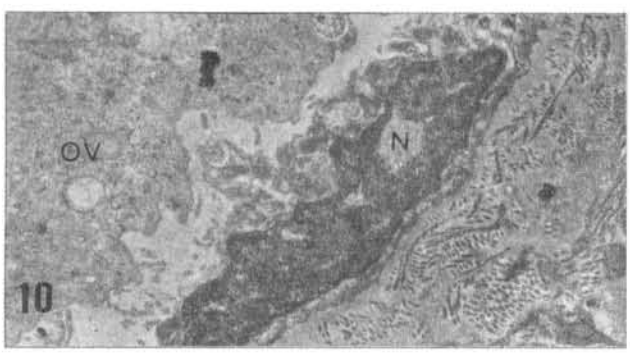

Figure 10 – Nucleus of a follicular cell (N) from case 9 (oocyte: OV) - 12000 ×.

Our light microscopical findings were similar to the literature data: the gonads consisted of connective tissue and rare corpora albicantia were observed. Description of atipical 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 plasmatical levels. We believe that the patients who presented atipical ovarian follicles developed the POF due to structural changes on the hypophyseal 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 gonadotrophical 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 syncicius.

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 myofilaments 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 aetiology of POF, early morphological, endocrinal and immunological studies should be performed.

REFERENCES

1. AIMAN, J. & SMENTEK, C. - Premature ovarian failure. **Obstet Gynecol, 66:** 9-14, 1985.
2. ASCH, R.H.; BALMACEDA, J.P.;ORD, T.; BORRERO, C.; CEFALU, E.; GASTALDI, C. & ROJAS, F. - Oocyte donation and gamete intrafallopian transfer in premature ovarian failure. **Fertil Steril, 49:** 263-267, 1988.
3. CLEMENT, P.B. - Anatomy and histology of the ovary. In: Blaustein's, A. 3ª ed. Pathology of the female genital tract. **New York, Springer-Verlag,:** 438-470, 1987.
4. COSTOFF, A. & MAHESH, V.B. - Primordial follicles with normal oocytes in the ovaries of postmenopausal women. J **Amer Geriat Soc, 23:** 193-6, 1975.
5. COULAM, C.B.; ADAMSON, S.C.; ANNEGERS, J.F. - Incidence of premature ovarian failure. **Obstet Gynecol, 76:** 604-606, 1986.
6. DAMEWOOD, M.D.; ZACUR, H.A.; HOFFMAN, G.J. & ROCH, J.A. - Circulating antiovarian antibodies in premature ovarian failure. **Obstet Gynecol, 68:** 850-854, 1986.
7. GABBIANI, G.; RYAN, G.B.`& MAJNO, G. - Presence of modified fibroblast in granulation tissue and their possible role in wound contraction. **Experientia, 27:** 549-550, 1971.
8. GADIR, A.A. & RAMADAN, A.A. - Anormal ovarian cell line: a cause for ovarian failure: Case report. **Br J Obstet Gynecol, 97:** 446-448, 1990.
9. HUNG, W.; MACLAREN, N.K.; KAPUR, S.; ANDERSON, K.D. & AUGUST, G.P. - Premature ovarian failure in a 15-years-old. **Clin Pediatr, 25:** 40-42, 1986.
10. JEWELEWICZ, R. & SCHWARTZ, M. - Premature ovarian failure. Bull NY **Acad Med, 62:** 219-236, 1986.
11. JOEL, R.V. & FORAKER, A.G. - Fate of the corpus albicans: a morphologic approach. **Am J Obstet Gynecol 80:** 314-316, 1960.
12. KINCH, R.A.H.; PLUNKETT, E.R.; SMOUT, M.S. & CARR, D.H. - Primary ovarian failure. **Am J Obstet Gynecol, 91:** 630-644, 1965.
13. MAJNO, G. - The story of the myofibroblast. **Am J Surg Pathol, 3:** 535-542, 1979.
14. MORAES-RUEHSEN, M. & JONES, G.S. - premature ovarian failure. **Fertil Steril, 18:** 440-461, 1967.
15. PLATIA, M.P.; BLOOMQUIST, G.; WILLIAMS, R.F. & HODGEN, G.D. - Refractoriness to gonadotropin therapy: how to distinguish ovarian failure versus pseudoovarian resistance cause by neutralizing antibodies. **Fertil Steril, 42:** 779-784, 1984.
16. REBAR, R.W.; ERICKSON, G.F. & YEN, S.S.C. - Idiopathic premature ovarian failure: clinical and endocrine characteristics. **Fertil Steril, 37:** 35-41, 1982.
17. REBAR, R.W. & SILVA DE SÁ, M.F. - The reproductive age: Premature ovarian failure. In: Serra, G.B. The ovary. **New York, Raven Press,:** 241-256, 1983.
18. REYNOLDS, E.S. - The use of lead citrates of high pH as an electron opaque stain in electron microscopy. **J Cell Biol, 17:** 208-213, 1963.
19. RYAN, G.B.; CLIFF, W.G.; IRLÇ,C.; MONTANDON, D.; STATKOV, P.R. & MAJNO, G. - Myofibroblasts in human granulation tissue. **Hum Pathol, 5:** 55-67, 1974.
20. SILVA DE SÁ, M.F.; BREGIEIRO, L.O.R.; FERRIANI, R.A.; MOURA, M.D. & COSTA, H.L.F.F. - Pode a biópsia dos ovários diferenciar entre a falência ovariana prematura e a síndrome dos ovários resistentes?. **Femina, 19:** 120-122, 1991.
21. SILVA DE SÁ, M.F.; MATTHEWS, M.J. & REBAR, R.W. - Altered forms of immunoreactive urinary FSH and LH in premature ovarian failure. **Infertility, 11:** 1-11, 1988.
22. SILVA, P. & GILULA, N.B. - Gap junctions in normal and transformed fibroblasts in culture. **Exp Cell Res, 71:** 397-401, 1972.
23. STARUP, J.; SELE, V. - Premature ovarian failure. **Acta Obstet Gynecol Scand, 52:** 259-268, 1973.
24. TALBERT, L.M.; RAJ, M.H.G.; HAMMOND, M.G.& GREER, T. - Endocrine and immunologic studies in a patient with resistant ovary syndrome. Fertil Steril, 42: 741-744, 1984.
25. TAN, S.L.; HAGUE, W.M.; BECKER, F.; JACOBS, H.S. - Autoimmune premature ovarian failure with polyendocrinopathy and spontaneous recovery of ovarian follicular activity. **Fertil Steril, 45:** 421-4, 1986.
26. WATSON, M.L. - Staining of tissue section for electron microscopy with heavy metals. **J Biophys Biochem Cytol, 4:** 475-478, 1958.
27. WEHBA, S. - Falência ovariana prematura ou precoce. **Femina, 16:** 101-111, 1988.
28. WOLFFENBUTTEL, B.H.R.; WEBER, R.F.A.; PRINS, M.E.F.; VERSCHORR, L. - Premature ovarian failure and autoimmune hypothyroidism in the absence of Addison"s disease. **Neerth J Med, 30:** 128-134, 1987.

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 são constituídos por tecido conjuntivo denso e raros corpos albicantes. Oito dos 10 casos não apresentavam folículos primordiais; em um caso verificou-se a presença de folículos ovarianos atípicos e, em outro, identificou-se corpo lúteo (após estimulação com gonadotrofina exógena). À ultra-estrutura, verificou-se que os fibroblastos achavam-se unidos uns aos outros através de prolongamentos celulares, formando um sincício celular.