

Cytohystological Changes of the Apoptosis Ovarian Follicles in the Puberty Mouse Ovary

Liliana Petculescu-Ciochină, Nicolae Păcală, Gabi Dumitrescu

Banat's University of Agricultural Sciences and Veterinary Medicine - Faculty of Animal Sciences and Biotechnologies, 300645, Timisoara, Calea Aradului 119, Romania

Abstract

Apoptosis, as a normal physiological process is active in the ovary of all species of mammals, representing the essential factor in maintaining the homeostasis of this organ. Both steroid hormones and various other factors actively involve in this process and regulates it. This paper represents a cytohistologic study of the ovary of puber mouse, comprising the morphological changes accompanying cells through apoptosis, in different types of ovarian follicles and which lead ultimately to the establishment of bodies apoptotic and follicle atresia. The results obtained by histological analysis of ovarian tissue sections, in puber hood (28 days) shows that the apoptotic process occurs in all types of ovarian follicles, but with different intensity from one follicular stage to another and from one type of cell to another, the most critical stage being the transition to the stage of preantral follicle. From primordial follicle stage until the secundar follicle stage, a frequent cause of follicle atresia is the entry into apoptosis of the ovocyte, a process morphologically marked by the appearance of specific nucleo-cytoplasmic changes. Since preantral follicle stage until the stage, cause of atresia is the appearance of morphological changes, specific to apoptotic process first at granulosa cells level and its subsequent expansion in oocytes and then in internal sheath cells.

Key words: apoptosis, follicles, histology, mouse, ovary

1. Introduction

Of ovarian follicular reserve, only a small part of them reach adulthood, most suffering an involution process of follicular atresia suggestively called apoptosis. This is manifested in all stages of follicular development and lead to the elimination of over 90% of follicles entered in the growth phase. Evolution of ovarian follicles is stimulated by both growth factors and hormones through the FSH and estrogen hormone [1].

Before each wave of follicular development increased plasma levels of FSH caused the appearance in the ovary of the epidermal growth factor (EGF), fibroblast growth factor (FGF) and growth factors acting like insulin 1 (IGF1), which will act directly in cell proliferation leading to multiplying specific granulosa cell surface

receptors and in synergy with estrogen, cause the appearance of LH receptors on membrane of these cells [2].

Simultaneous morphophysiological differentiation of granulosa cells and internal sheath cells is adjusted by both by the presence of gonadotropins (FSH and LH) as well as by local acting factors, such as steroids, proteins, autocrine and paracrine growth factors, each specific for the stage of development. Thus, proliferating cells are sensitive to apoptosis and are dependent on the presence of the above mentioned factors, whereas cells that leave the cell cycle through differentiation become more resistant.

In rodents, leaving the cell cycle by granulosa cells after the LH preovulator peak causes an increased resistance to apoptosis of these cells, a very important property for future survival, longer term, once the corpus luteum is formed [3].

In addition, Fortune, et. al. [4], Beg, et. al. [5] and Ginther, et. al. [6] noted an association between

* Corresponding author: Petculescu Ciochină Liliana, Email: lilianapetculescuciochina@yahoo.com

increased bioavailability of IGF in follicular fluid and dominant follicle selection in the sense that free IGF concentration in subordinate follicles is very low at the time of follicular selection compared to the dominant follicle.

Similar results were obtained by Mihm, [7] and Austin, [8], who found that both the IGF and estradiol are important intrafollicular factors that support increased sensitivity to gonadotropins of dominant follicle when the follicular selection takes place.

At the beginning of each oestrous cycle, with increasing the concentration of serum FSH, granulosa cells produce large amounts of estrogen and inhibin. As feedback mechanism of inhibin, FSH secretion decreases, resulting in atresia of small size follicles.

Apoptosis as a fundamental process in the life cycle of ovarian cells may be triggered in several circumstances, but with the same final result, the elimination of damaged cells. Cell sensitivity to many stimuli, and the severity of apoptotic stimulus and / or cell cycle stage, determine at the cellular level a number of morphological changes, which aims to recognize this process [9-11].

2. Materials and methods

Our research was performed on ovarian tissue sections, from puber female mice (aged 28 days), the line NMRI, from Cantacuzino Institute, Station Baneasa, Bucharest.

The accommodation of animals was conducted according to European norms in force. For good reproductive performance, the room was illuminated for 12 hours of 24 and feeding of animals was performed ad libitum with mixed fodder grain, prepared in accordance with product technical standards of Cantacuzino Institute, Station Baneasa, Bucharest.

For biological sampling, females' killing was done by cervical dislocation. Ovarian tissue fragments were fixed in neutral formalin (10%) (Merck, Germany) and were then included in histological paraffin (Merck, Germany) and sectioned at 5µ size, using a manual rotary microtome by Leica RM2125RT.

Sections were stretched on microscopic slides, previously smeared with albumin Mayer, then were dewaxed, colored by Mallory and Masson

trichromic methods and analyzed using Olympus CX41 microscope with camera and image analysis software, with objectives 20x, 40x and 100x.

3. Results and discussion

Microscopic analysis of the ovary in puberty reveals its organization in two areas, fully separated, that is cortical and medullary area.

Cortical area occupies almost all the ovary, being composed of a large number of ovarian follicles, primordial, primary, secondary and preantral, but most of them exhibit the phenomenon of atresia through apoptosis [Fig1].

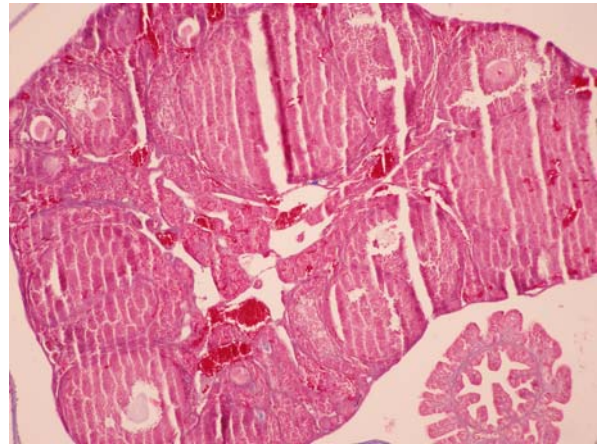


Figure 1. Ovary after puberty [Trichromic Mallory staining, 200x] - overall picture [original]

Primordial follicles, frequently organized in nests, just below ovarian albuginea, are composed of primary oocytes surrounded by a layer of flattened mesoderm cells. In these follicles one can frequently notice nucleo-cytoplasmic degenerations, translated in intense apoptotic processes that begin with the oocyte and are later extended to periovocitary cells.

In the early stage of apoptosis, the nucleus of oocytes exhibit the phenomenon of pyknosis due to the condensation process of genetic material and the cytoplasm shows small areas of vacuolation [Fig. 2].

In an intermediate stage of the same process, oocyte cytoplasm retracts and causes the appearance of some vacuolation areas, involving the detachment of the germinal cell in relation to the mesoderm cells.

Later, in a more advanced stage, oocytes appear very much retracted as a result of the enhancement of the dehydration process and as a result of degradation of fodrine, a protein associated with plasmalemma and membrane cytoskeleton [Figure 3].

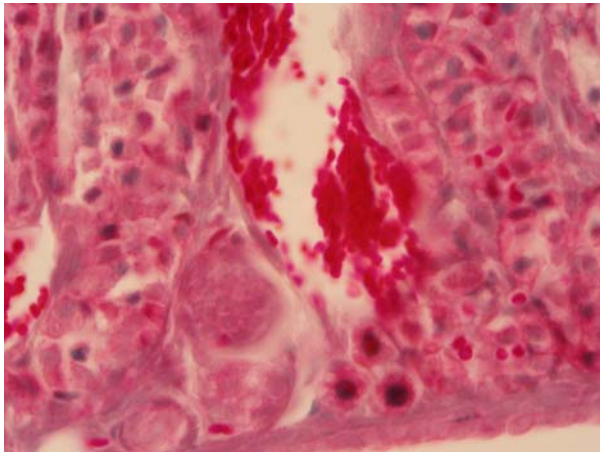


Figure 2. Primordial follicles in apoptosis [Trichromic Masson staining, 1000x]
1. Oocytes with pyknotic nucleus, 2. Vacuolation of ovary cytoplasm

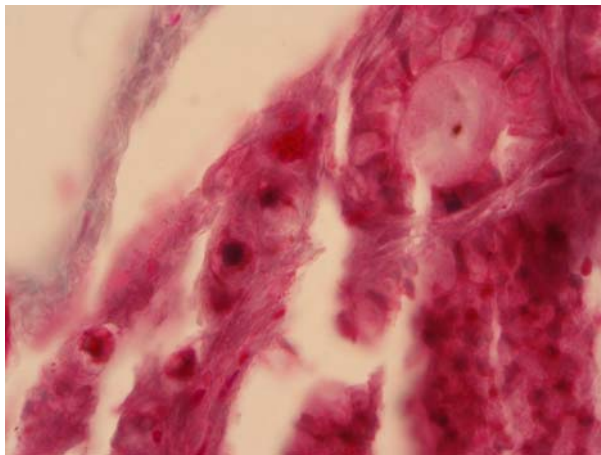


Figure 3. Primordial follicles in apoptosis [Trichromic Mallory staining, 1000x]
1. Oocytes with pyknotic nucleus, 2. Vacuolation of ovary cytoplasm

Similar results were found by Hussein, et al. [12] who emphasized that the degradation of fodrine causes not only reduction of cell volume, but also wrinkling of the membrane, with the appearance of vesicles.

Towards the end of the apoptotic process, the space between germinal cells and mesoderm cells expands, cytoplasmic vacuolations become multiple and oocyte nucleus acquires the

characteristic appearance of the crescent, with fragmented chromatin, arranged on the internal side of envelope nuclear [Fig. 3].

Yakovlev, et al. [13] found that the process of fragmentation of nuclear chromatin and of arrangement of these fragments on the internal side of envelope nuclear is correlated with the proteolysis of vimentines and laminines, nuclear non-histone proteins, responsible for the attachment of chromosomes to the nuclear membrane. Consequently, the two strands of DNA are no longer protected by these proteins and they become accessible to endonucleases dependent on Ca^{2+} and Mg^{2+} ions, enzymes involved in uniform chromatin filaments fragmentation in oligonucleosome.

Primary follicles are disposed both under the ovarian albuginea and in the peripheral layers of the cortical area. They are composed of the primary oocytes with spherical nucleus, disposed slightly eccentric, in which 1-2 nucleoli are present. The oocytes is surrounded by a thin pellucida area, overlapped by a cubic monolayer follicular epithelium [Fig.4].

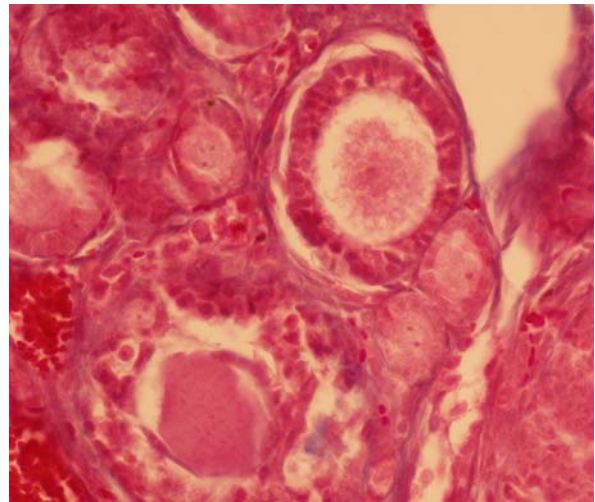


Figure 4. Primary follicles [Trichromic Mallory staining, 400x][original]
1. Nucleus with 1-2 nucleoli

Similar to primordial follicle stage, in primary follicles apoptosis comprises first the oocytes and is caused by the break of the communication junctions (gap) between it and follicular cells due to the degradation of connexine 37, the key protein of these links, and further extension of the process in follicular cells [Fig. 5].

Secondary follicles occupy large areas of the cortical area and the majority of them are in various stages of apoptosis. In this case, too, apoptosis starts with the oocytes, where nuclear events are represented by fragmentation and marginalization of chromatin and the cytoplasmic ones by deep vacuolation, strong dehydration, followed by whole cell retraction, which causes enlargement of the space between it and the pellucida area [Fig. 6].

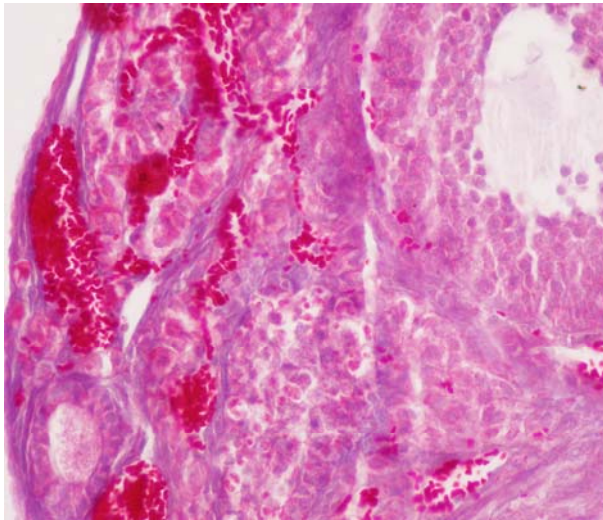


Figure 5. Primary follicle apoptosis [Trichromic Mallory staining, 400x] [original]

1. Ovocită with fragmented nucleus; 2. Vacuolation of ovary cytoplasm

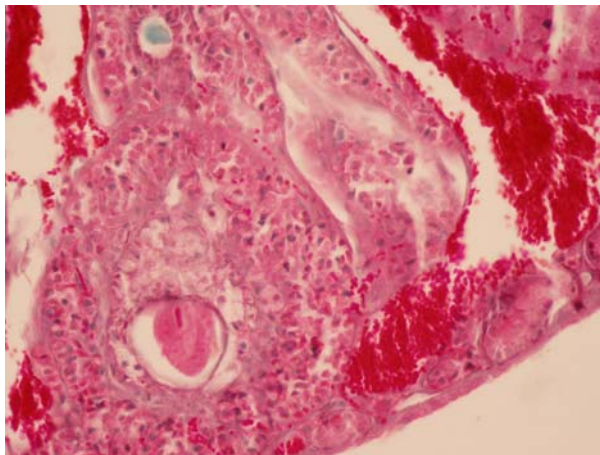


Figure 6. Secondary follicle in apoptosis [Trichromic Masson staining, 400x] [original]

1. Retraction of the oocytes and its distancing from ZP distance
2. Granulosa cells disorganization
3. Hypertrophy of vascular network
4. Abundant leukocyte infiltration

In a later stage of apoptosis, after removal of the oocyte, the process expands to the granulosa cells, too. Thus, they are detached from each other, through disassembling of communication junctions (gap) between them, as a result of the damage of connexine 43 out of their structure. Destabilization of communication links lead to the widening of the intercellular spaces and, hence, to the entry into apoptosis of follicular epithelial cells by depriving them of growth factors, hormones support and trophic substances [Fig. 6]. The results are consistent with data in the specific literature, both in mice and humans [14, 15].

In the perifollicular stroma we observe the presence of leukocyte cells, in large numbers, in migration, a process linked to their "attraction" towards the apoptotic follicle. Macrophages have the ability to recognize apoptotic signal, represented by phosphatidylserine, membrane lipid molecule, translocated from the endoplasmic side of the cellular membrane to the exoplasmic one [Fig. 6].

The same conclusion was reached by Svanberg, [16] through his studies on the human ovary.

Preantral follicles are located exclusively in the peripheral area of the ovarian cortex, presenting different degrees of development, but most of them show the process of atresia. In this stage of development, the follicle is composed of the primary oocytes surrounded by the developed pellucida area, overlapped by the follicular epithelium, which indicates the presence of multiple cavities, where a small amount of follicular fluid is accumulated. At the periphery of the follicle, out of the mesenchymal cells of the ovarian stroma, internal sheath cells are distinguished through metaplasia, over which a condensation of the perifollicular conjunctive is superimposed and forms the external sheath.

Starting from this stage a frequent cause of follicular atresia is represented by the entry of granulosa cells into apoptosis, followed by the expansion of the process into the oocytes and eventually into the internal sheath cells. In the granulosa cells, respectively in the internal sheath cells, apoptosis starts with the breaking of the communication ties between them (process caused, as previously shown, in other follicular stages, by the destruction of connexine 43 and, consequently, the appearance of large intercellular spaces. In addition, another aspect noted is the detachment of granulosa cell from the basal

membrane, followed by its fragmentation and the emergence of large spaces between granulosa cells and sheath cells [Fig. 7].

Subsequently, in the nuclei of these cells, genetic material appears fragmented and attached to the internal side of envelope nucleare. In the cytoplasm the specific process of vacuolation occurs.

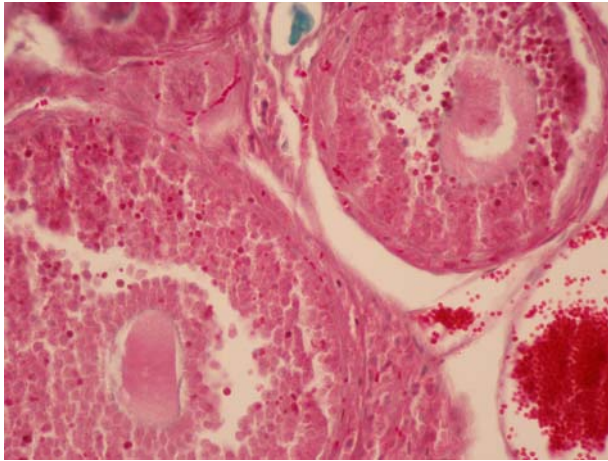


Figure 7. Preantrali follicles in apoptosis [Trichromic Masson staining, 400x] [original]

1. Vacuolation of ovary cytoplasm, 2. Retraction of the oocyte and its distancing from ZP;
3. Destabilisation and detachment of granulosa cells from the sheath cells 4. Lympho-leukocyte infiltration
5. Apoptotic bodies

At the end of the process, both the nucleus and the cytoplasm of apoptotic cells fragment, forming apoptotic bodies, a process accompanied by the invasion of intercellular spaces with lympho-leukocyte infiltrations [Fig. 7].

After the onset of apoptosis in granulosa cells, communication links between the oocytes and the follicular periovocitary cells are destroyed, too, as a consequence of the degradation of connexine 37, out of the structure of these junctions' conaxon. Destabilization of these links causes deprivation of the oocytes of nutrients and hormonal factors, a process that triggers its entry into apoptosis.

In this period of ontogenic development, in the mouse ovary we notice the presence of the interstitial gland, whose cells insert both among ovarian follicles of the cortical area and medullary area. In the gland parenchyma two types of cells can be distinguished: some with clear cytoplasm and large nucleus, centrally located, the others with acidophilous cytoplasm and small nucleus

positioned eccentrically. Moreover, in the periglandular capsules, we notice blood capillaries and fibroblasts, which are considered the source of glandular parenchyma generation [Fig. 6].

4. Conclusions

In the stage of puberty, the ovary of female mouse is populated predominantly with primordial and secondary follicles and, less frequently, with primary follicles and follicles undergoing cavitation.

Since the primordial follicle stage to the stage of secondary follicle the frequent cause of follicle atresia is represented by the entry into apoptosis of the oocyte, followed later by follicular cell apoptosis.

From the stage of preantral follicle, the frequent cause of atresia is the apoptosis of granulosa cells and then the expansion of the process to the oocytes, and, finally, to the internal sheath cells.

Although apoptosis occurs at every stage of follicular development, the most critical stage is the transition to preantral follicle.

At nuclear level the apoptotic process is initially manifested through the condensation of the genetic material in a single block (pyknosis), then it fragments due to the activation of endonucleases and the resulting chromatic bodies are placed on the internal side of envelope nucleare;

In the granulosa cells, respectively in the internal sheath cells, apoptosis starts with the breaking of the communication ties between them, process caused by the destruction of connexine 43 and, consequently, the appearance of large intercellular spaces, which are subsequently invaded by lympho-leukocyte infiltrates.

The disruption of intracellular water distribution, both in the oocytes, and in granulosa cells and internal sheath cells, causes the condensation of their cytoplasm, a process morphologically marked by the appearance of a large number of vacuoles and, finally, cell retraction,

At the end of the process, the nucleus and the cytoplasm of apoptotic cells fragments progressively, resulting apoptotic bodies which reach the interstitial space, where are rapidly recognized and phagocytosed without involving an inflammatory process.

Acknowledgements

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