Comparative microscopic study of the ovarian blood vessels

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Abstract
The present study puts forth the morphometric analysis of modifications that occur in the ovarian structures (stroma, parenchyma, blood vessels) before and after menopause onset. Our study was performed on 30 human ovary specimens from patients with no history of genital pathology that were split in two groups (15 ovaries harvested from fertile women and 15 ovaries harvested from women at menopause). The specimens were processed following the classic histological technique of paraffin embedding and stained using Hematoxylin–Eosin technique and trichromic Goldner–Szeckelly technique. The samples were examined and assessed using Nikon E-600 light microscope. To evaluate the histological modifications, we chose the following parameters: Area Fraction of blood vessels and Circularity of blood vessels. The study shows that modifications of the ovary components are synchronized. The vascular changes that occur after menopause onset lead to a poor vascular supply of all ovarian components: germinal epithelium, ovarian follicles, and ovarian stroma. The comparative morphometric analysis on premenopause and postmenopause ovarian specimens sustains the idea that the ovarian involution starts from the ovarian vessels, which are the first structures affected by hyaline degeneration.

Keywords: ovary, blood vessels, morphometric analysis.

Introduction
The study broadens the microscopic knowledge about some ovarian structures, and intends to explain an important problem such as the ovarian involution. At the present level of medical knowledge on ovarian morpho-physiology, we considered that our observation may shed some light on this issue.

In this research we used routine histological methods, but also special methods, adequate to the studied tissue (trichromic staining methods, and specific staining methods for elastic and reticular fibers), in order to observe the characteristic structures of the organ. Our observations and the conclusions that arose from it represent a contribution to the theoretical knowledge on which the medical practice is based.

Material and methods
Our study was performed on human ovary specimens, necroptic pieces (ovaries harvested during autopsy), from women with ages between 20 and 65 years.

We studied a total of 30 ovaries from women with no history of genital pathology, as resulted from examining their medical files. Also from the data found in the medical files we were able to split our pieces into two groups:
• group I – 15 fertile women;
• group II – 15 women at menopause.

The studied material belongs to the laboratory of Histology Department, Faculty of Medicine, “Ovidius” University, Constanta.

The ovary fragments were fixed using 10% formaldehyde and then processed following the classic histological technique of paraffin embedding.

The pieces were sectioned at 5 micrometers and stained using Hematoxylin–Eosin technique in order to observe the general structure of the ovaries, and trichromic Goldner–Szeckelly technique.

After staining, we examined and assessed the histological specimens using Nikon E-600 light microscope.

The images captured by computer were processed using LUCIA G 4.10 software.

In order to evaluate the histological modifications, we chose the following parameters:
• Area Fraction of blood vessels;
• Circularity of blood vessels.

Results
General ovarian morphology demonstrated significant changes as the subjects approached menopause.

Until the menopause onset, the germinal epithelium shows invaginations in the depth of ovarian cortex, forming at this level certain cellular nests, which are similar to the ovarian surface epithelium (Figure 1).
In the women at menopause of even shortly before menopause, these small irregularities of surface epithelium tend to disappear, the ovarian epithelium becoming smooth along its entire length. Decreased ovarian weight is accompanied by progressive and generalized atrophy, there is present a proliferation of fibrous connective tissue with vascular sclerosis in variable degrees and an increased tendency to produce follicular cysts. The abundant connective tissue invades parenchyma and the ovarian medulla becomes hypertrophic. The Berger’s cells within hilum increase in volume [1] (Figure 2).

The ovarian artery decreases in diameter faster that the uterine artery. Helicine arteries undergo atrophic processes and degenerate [2].

Blood vessels in the ovarian cortex are in lesser number than in the rest of the organ. With ageing, the ovarian cortex possesses fewer ovarian follicles and blood vessels; it becomes less vascularised [3], while the stroma suffers an important condensation (Figure 3).

In women at post-menopause age, the ovaries contain no follicles in the cortex, therefore the ovarian cortex is comprised only by the dense stroma and few blood vessels; some of these vessels underwent hyaline degeneration. Inside the ovarian parenchyma are located corpora albicantia – hyaline structures that result from the involution corpora lutea. Contrary to corpora lutea – that disappear as women approach menopause – corpora albicantia are still present after menopause onset (Figure 4).

Corpora albicantia is embedded in a denser stroma along with hyalinated vascular structures designated as hyaline vascular bodies. The vascular hyalinization is generally present in the medulla, but it may occur as well in the ovarian cortex (Figure 5).

It is to be presumed that a hyalinated blood vessel with an almost obstructed lumen finally provides insufficient blood supply to the tissues. This process leads to the hyaline degeneration of corpora lutea into corpora albicantia (Figure 6).

The decreased vascular area fraction in postmenopause age is due to the degenerative processes that occur in the vascular walls (Figure 7).

The decreased value of vascular circularity in postmenopause age is not statistically significant. This means that the general shape of ovarian blood vessels remains unchanged. Therefore the process of hyaline degeneration is diffuse and affects the vascular wall without changing its shape.

Discussions

The examined ovaries appear, at all ages, covered by a simple cuboidal epithelium. The ovarian epithelium represents a specialized dependence of peritoneum.

At young ages, but also in adult women, this epithelium is comprised by one single row of cuboidal-columnar cells, whose dark-stained nuclei are very well seen using the staining techniques mentioned above. After menopause onset germinal epithelium becomes low cuboidal or even squamous, and it doesn’t show cellular nests as it happens in young ages.

The morphological modifications are the consequences of ovarian involution, the ovaries diminish in weight, the cortex reduces its dimensions, the follicular apparatus is progressively diminished [4], the primary follicles completely disappear [5], then the follicles in different stages of development and recent corpora lutea; the abundant connective tissue invades the parenchyma and the ovarian medulla becomes atrophic. With ageing, both atretic follicles and corpora lutea decrease in number [6–8].

A condensation of the cortical stroma is also noticed. Hyaline blood vessels surround corpora albicantia. This is a morphologic feature that may explain the transformation of corpora lutea into corpora albicantia. The relationship: hyaline blood vessels – corpora albicantia suggested that involution of corpora albicantia is determined by involution processes that take place in the neighboring blood vessels [1].

Patients at menopause showed narrowing and hyalinization of blood vessels and focal fibrosis of the ovarian cortex.

The size of the follicle’s stockpile is age-related and the number of remaining follicles in the ovaries declines with age in a logarithmic fashion. It was shown in recent studies that, after the age of 37, the slope representing the rate of diminishing ovarian reserve are sharper as more follicles are destroyed.

Blood flow to ovarian stroma is significantly reduced in older women due to marked reduction in number and caliber of blood vessels with thickening of the vascular walls and changes in endothelial cells in aged patients.

Conclusions

The study shows that modifications of the ovary components: stroma, parenchyma and blood vessels are synchronised. Samples from subjects approaching or undergoing the menopausal transition demonstrated evidence of ovarian senescence, having scattered and atretic follicles, low numbers of primordial follicles and reduced stromal tissue.

The ovarian stroma in fertile women is dense, but richly cellular. Among fibroblasts the stroma contains collagen and reticular fibers, forming a dense network. Elastic fibers are absent in the ovarian stroma. With ageing ovary undergoes follicular reservoir depletion.

With ageing, there are noticed certain hyaline structures in the ovary. Vascular hyaline degeneration is present both in the cortical and medullary stroma.

The hyaline involution of blood vessels surrounding corpus luteum can be identified before the transformation of this structure into corpus albicans.

The vascular changes lead to a poor vascular supply of all ovarian components: germinal epithelium, ovarian follicles and ovarian stroma. The involutive process of the ovarian stroma occurs after the local vascular hyaline degeneration. The comparative morphometric analysis on premenopause and postmenopause ovarian specimens sustains the idea that the ovarian involution starts from the ovarian vessels, which are the first structures affected by hyaline degeneration.
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Figure 1 – Ovarian cortex from a woman of 32 years of age. At the surface, the simple cuboidal epithelium slightly invaginates into the organ (H&E staining, ×100)

Figure 2 – Ovarian medulla: woman of 42 years of age. In the condensed stroma are present blood vessels with hyaline modifications in their walls (H&E staining, ×100)

Figure 3 – Ovarian medulla: woman of 41 years of age. There are present large blood vessels, surrounded by a loose connective tissue (H&E staining, ×100)

Figure 4 – Section through the ovary of a woman of 56 years of age. Deep inside the cortex is located a corpus albicans. Collagen fibers organized in bands cross its structure. The medullary blood vessels, especially veins, are also present in the specimen (Goldner–Szeckelly trichrome staining, ×100)

Figure 5 – Ovarian medulla: woman of 64 years of age. There are present numerous fibrocytes and large bundles of collagen fibers, surrounding the blood vessels (Goldner–Szeckelly trichrome staining, ×100)

Figure 6 – Comparative morphometric analysis of vascular area fraction in premenopause and postmenopause periods

Figure 7 – Comparative morphometric analysis of vascular circularity in premenopause and postmenopause periods
References


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