# *Review*

# Human postmenopausal ovary – hormonally inactive fibrous connective tissue or more?

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**Summary.** The ovary undergoes several changes after the menopause. In this period, the main structural changes in both the cortex and medulla were observed. In the cortex, they included: 1) reduction of its thickness; 2) epithelial inclusions forming cysts; 3) blurring the line between medulla and cortex; 4) reduction of follicles number; 5) tendency to fragmentation of corpora albicantia; 6) surface epithelium invaginations. Whereas the changes in the medulla included: 1) fibrosis and scars in stroma; 2) architectonical changes in blood vessels with hyalinization of walls and constriction of lumen. The loss of follicles and several changes in the ovary are due to apoptotic processes. Despite age related atrophic changes, the postmenopausal ovary is not devoid of hormonal activity. Our results are coherent with the reports of other researchers, and reveal that postmenopausal ovary produces trace quantities of steroid hormones, mainly androgens, and confirm the presence of steroid receptors and activity of main enzymes involved in steroidogenesis process.

**Key words:** Postmenopausal ovary, Hormonal function, Immunohistochemistry, Pathology, Steroidogenesis, Epithelial inclusion cyst

## Introduction

In an everyday practice gynecologists are asked by their patients about the role of ovaries after menopause. Whether it is an irrelevant, hormonally inactive tissue that can be excised during an operation or something more affecting women's health. If postmenopausal ovary produces a significant amount of steroid hormones that

can affect women's health. We find it difficult to answer these questions mostly due to the scarce literature concerning structure and function of the postmenopausal ovary.

## The changes in the human ovary with women's age

Numerous published data reveal structural and functional changes of an ovary with women's age. Aging of the ovary means a transition from the organ rich in follicles, actively and cyclically secreting hormones like estrogen and progesterone to the organ that is follicle-depleted, rich in stroma and producing low levels of progesterone (Nicosia, 1987; Longcope, 2001; Jabara et al., 2003). The aging ovary shrinks and its volume is smaller in postmenopausal women which can be clearly seen in transvaginal ultrasound (Giacobbe et al., 2004a,b) as well as in the histological examination (Clement, 1987). The postmenopausal ovary is shrunken (sometimes it is called "ovarium gyratum") (Clement, 1987), presents various changes in vasculature (Gonzales et al., 1992; Kozik, 2000), may contain granulomas, hyaline scars and epithelial inclusion cysts (Clement, 1987). It also presents changes at the electron microscopy level, such as areas of loss of epithelium with an exposure of underlying stroma (Makabe et al., 1998) and the remodeling process of the corpus albicans (Focchi et al., 1996).

The most important histological aspect of ovarian aging is loss of oocytes leading to impaired fertility. Oocyte ages causing errors in chromosome segregation, meiotic divisions errors, mutations in mitochondrial DNA and disregulation of nuclear and cytoplasmic maturation (Dorland et al., 1998; Eichenlaub-Ritter, 1998; Kirkwood, 1998). Interestingly, the depletion of oocytes may influence the oncodenic behaviour of ovarian surface epithelium (Vanderhyden, 2005). The typical morphological change in the postmenopausal

ovary is a significant follicular depletion accelerating in years preceding menopause (Richardson and Nelson, 1990; Giacobbe et al., 2004b). Gougeon et al. (1994) tested regressions models of age-related decrease in human ovarian follicles. They showed acceleration of depletion rate of ovarian follicles in women older than 38-39 years with a total exhaustion of this pool at the age of 74.

Another interesting aspect of the aging ovary is vascular changes. Gonzales et al. (1992) analyzed vasculature of 35 postmenopausal ovaries and showed deposition of collagen and glycogen within the walls of veins, arteries and arterioles that leads to a significant reduction of vascular lumen. Also, numeral and structural changes in arteries anastomising from the arterial ovarian arch can be observed (Kozik, 2000).

The size of postmenopausal ovary is mostly determined by the number and the size of corpora albicantia, structures derived from involuting corpora lutea. The corpus albicans is an elongated structure composed of centrally located collagen-producing fibroblasts, macrophages located centrally and peripherally and myofibroblasts located peripherally. Corpora albicantia may remain in the ovary for many years, but the longer from the menopause the more fragmented they are. The number and the size of corpora albicantia of a woman depends on the number of ovulations in the reproductive period and on phagocytic cell activity. The number and the size of corpora albicantia diminish with age after the menopause and they become more fragmented, but no studies were performed to estimate the longest period of their presence in postmenopausal ovaries (Focchi et al., 1995, 1996; Brown et al., 2004).

The peripheral zone of an ovary, known as the cortex, and the internal part - the medulla, can be distinguished within the structure of an ovary during the reproductive period of women's life. In the cortex the follicles at different stages of development (primordial, primary, secondary and Graafian follicles) can be seen. The medulla however, is made of loose connective tissue with elastic fibers, scarce myocytes, blood and lymphatic vessels and nerves (Clement, 1987). In postmenopausal ovary the division between the cortex and the medulla is not clear as the cortex reduces with time. The ovary in this period of women's life is devoid of follicles, and its structure is mainly composed of fibrous tissue, corpora albicantia, vessels and nerves (Fig. 1). Follicles follow developmental stages from the primary follicles through secondary, Graafian follicles to corpora lutea and albicantia that yields some hormonal changes during the reproductive and post-reproductive period (Focchi et al., 1995; Dorland et al., 1998; Burger et al., 2002). The loss of follicles occurs at each stage of their development. The primary and secondary follicles vanish without a trace. Only the corpus luteum forms the corpus albicans of menstrual or pregnancy which vanishes at the end of pregnancy. The loss of follicles and several changes in the ovary are due to apoptotic

processes (Vinatier et al., 1996; Amsterdam et al., 2003; Vaskivuo and Tapanainen, 2003; Rolaki et al., 2005).

## Morphologic changes of the aging ovary and their pathologic consequences

Postmenopausal ovaries frequently present stromal hyperplasia. Some authors found a significant correlation between the degree of stromal hyperplasia and the presence of endometrial carcinoma (Snowden et al., 1989; Jongen et al., 2003).

From the point of view of general practice in pathology, the most important and life-threatening pathology of postmenopausal ovary is epithelial ovarian cancer. Epithelial ovarian tumors consist of a spectrum of pathologies of different aggressiveness from benign ones found in younger women, through borderline malignancy found most frequently between the ages of 30 and 60, to frankly malignant cancers found in women at the age of 40 to 70 (Rosai, 2004; Scott and McCluggage, 2006). Pathogenesis of the ovarian cancer is far beyond the scope of this paper but some morphological changes in postmenopausal ovary can be linked with the risk of ovarian cancer. As we mentioned before, one of the morphological changes in an aging ovary is the presence of epithelial inclusion cysts (Clement, 1987). Most pathologists consider them as clinically irrelevant but whether they may be precursors of cancers is still under debate (Scott and McCluggage, 2006). Heller at al. (2003) analyzed proliferative activity of surface epithelial inclusion cysts from benign ovaries using Ki-67 immunostaining and found it very low. However, "atypia" of epithelial cells in these structures in benign ovary of women who underwent oophorectomy for unilateral ovarian cancer is much more common than in the control group (Sherman et al., 1999). Moreover, the epithelium in inclusion cyst seems to be more prone to undergo malignant transformation than the epithelium on the surface of the ovary (Scully, 1995). Also, a case of serious carcinoma in inclusion cysts was described (Aoki et al., 2000; Auersperg et al., 2001). Thus, the epithelial inclusion cyst, a morphological change occurring more commonly in ovaries of postmenopausal women, may be considered a
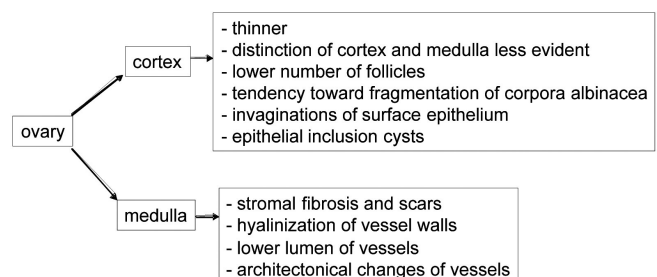


**Fig. 1.** Structural changes of ageing ovary

precursor lesion for epithelial ovarian carcinoma (Auersperg et al., 2001).

Morphological changes in aging ovary, considered as pathological, may also induce some hormonal changes. Ovarian hyperthecosis, a diffuse stromal hyperplasia with nests of luteinized cells, as well as stromal hyperplasia, may be associated with estrogenic or progetagenic effects. Patients with stromal hyperplasia or hyperthecosis presented endometrial abnormalities or endometrioid endometrial cancer (Sasano et al., 1989; Nagamani et al. 1992; Jongen et al., 2003) and this relation is thought to be dependent on the conversion of androgens to estrogens by cytochrome P 450 aromatase (Jongen et al., 2002). Some patients present direct androgenic effects such as virilization, hirsutism and hair loss (Manieri et al., 1998; Krug and Berga, 2002). Other non-neoplastic pathologies found in postmenopausal ovaries are described as case reports and include massive ovarian edema (Shirk et al., 1996), hilus cell hyperplasia (Hayes et al., 1997; Braithwaite et al., 2001) and cases of atypical hyperthecosis (Madiedo et al., 1985).

It can be seen that postmenopausal ovary is a site of very characteristic, histological changes related to the normal aging process as well as some pathological changes that occur more commonly in this period of women's life.

## Apoptosis in the human ovary

Apoptosis is one of the most crucial processes that causes most of the atrophic changes in the human ovary, and its activity accelerates after the menopause. Apoptosis is a multi-step process leading to the condensation of nucleus and cytoplasmatic structures and to the formation of apoptotic bodies and elimination of the cell (Vinatier et al., 1996; Vaskivuo and Tapanainen, 2003; Quintana et al., 2004; Brodowska et al., 2005; Parborell et al., 2005).

The ovulating follicle apoptosis causes shrinkage of the oocyte (up to 30-50% of its initial volume) and loss of contact with neighboring granulosa cells. The follicar antrum collapses and zona pellucida shrinks. Basal membrane becomes thicker and forms hyaline membrane and the whole follicle becomes corpus albicans (Tilly, 1998; Morita and Tilly, 2000; Markstrom et al., 2002; Denkova et al., 2004, Brodowska et al., 2005; Rolaki et al., 2005).

The apoptosis of cells in Graafian follicle also leads to numerous functional changes: lower expression of gonadothropins receptors, lower activity of steroidogenic enzymes such as 17- α-hydroxylase and 17,18-liase, which leads to a decrease in estradiol production and diminishes the concentrations of IGF-1, the factor stimulating proliferation of granulosa cells (Gospen and Spears, 1997; Pushkala and Gupta, 2001; Vital Reyes et al., 2001; Brodowska et al., 2005; Hussein, 2005).

Apoptosis causes the atrophy of follicles before reaching the ovulation capability, degenerative changes in formed follicles, and atrophy of primordial cells (Gospen and Spears, 1997; Tilly, 2003; Kamo et al., 2004; Denkova et al., 2004, Hussein, 2005).

During the perimenopausal period, the activity of apoptosis within an ovary accelerates, which leads to lower feritility, a higher number of spontaneous abortions and a higher number of chromosomal abnormalities. The number of granulosa cells per one follicle is lower and those cells are more sensitive to anoxia, the production of steroid hormones, mostly progesterone, and inhibine diminishes (Chun and Hsueh, 1998; Tilly, 2003; Denkova et al., 2004; Dharmarajan et al., 2004).

Based on our previous studies applying the TUNEL method and expression of caspase-3, the highest activity of apoptosis in ovary was found in the wall of blood vessels, in corpus albicans and connective tissue. The highest number of apoptotic cells was found in the ovaries of women of up to 5 years after the menopause but some were also present in the ovaries of women of more than 5 years after the menopause (Brodowska et al., 2007). The literature data and our studies show that apoptotic activity can be still observed in the postmenopausal ovary, however, it is not as prominent as it is in the follicles at the reproductive period (Marti et al., 1999; Pru and Tilly, 2001; Xavier 2002; Manabe et al., 2004; Nicholas et al., 2004).

## Immunohistochemical study in human postmenopausal ovary

The immunohistochemical studies of reproductive and postmenopausal ovaries showed significant differences in the expression and localization of numerous antigens, cell markers and subcellular structures of the ovary.

It is known that in women estrogen levels decline at menopause as a result of the loss of ovarian follicles. The actions of estrogens in an ovary are mediated by binding to one of the two specific estrogen receptors (ERs), ERα or ERß (Pelletier, 2000; Pelletier and EL-Alfy, 2000; Taylor and Al-Azzawi, 2000; Slomczyńska et al., 2001).

Pelletier and EL-Alfy (2000) and Taylor and Al-Azzawi (2000) showed the expression of both estrogen receptors (ERs), ERα and ERß in the ovary of women at the reproductive age. In the ovary, ERα was weakly expressed in the nuclei of granulosa cells, but strongly in the theca cells and in the corpora lutea cells, whereas ERß was present in granulosa cells, in small, medium and large follicles, theca and corpora lutea cells. Immunohistochemical distribution of ERα and ERß were also observed in the stromal cells and ovarian epithelial cells (Pelletier et al., 2000; Taylor and Al-Azzawi, 2000).

Our studies on the expression and localization of ERα in postmenopausal ovaries (unpublised results) showed the nuclear expression of ERα in the stromal cells and epithelial cells. We also noticed cytoplasmic
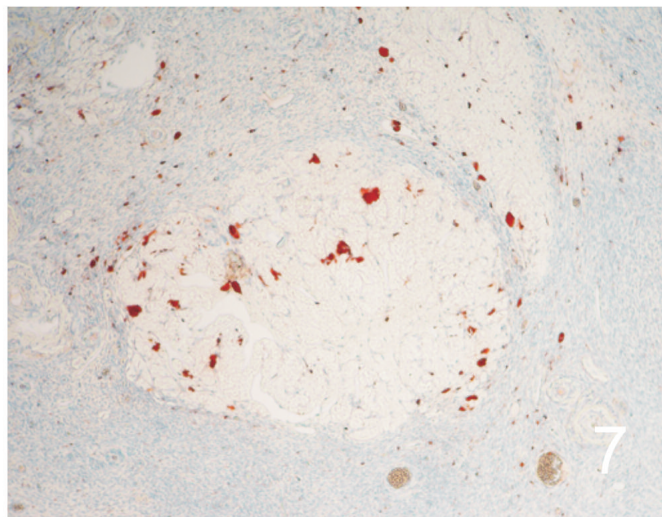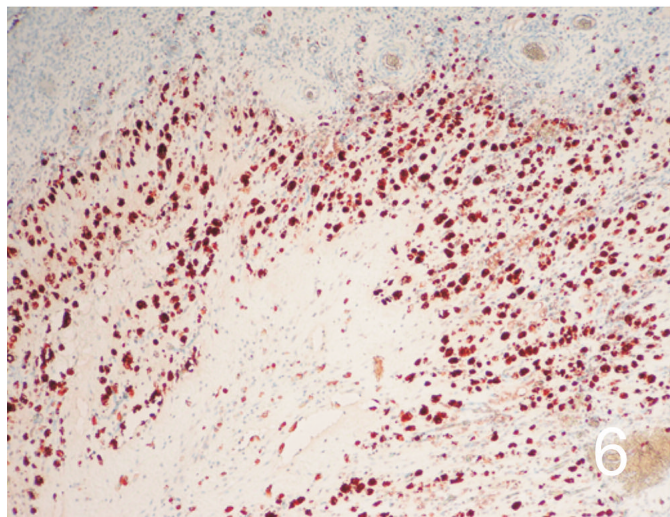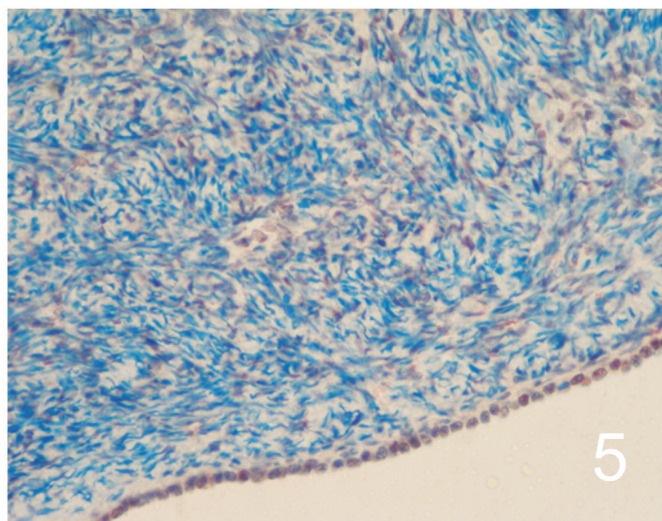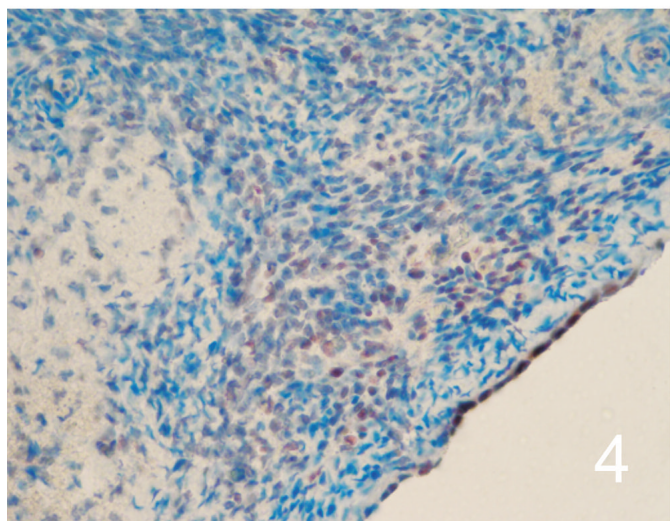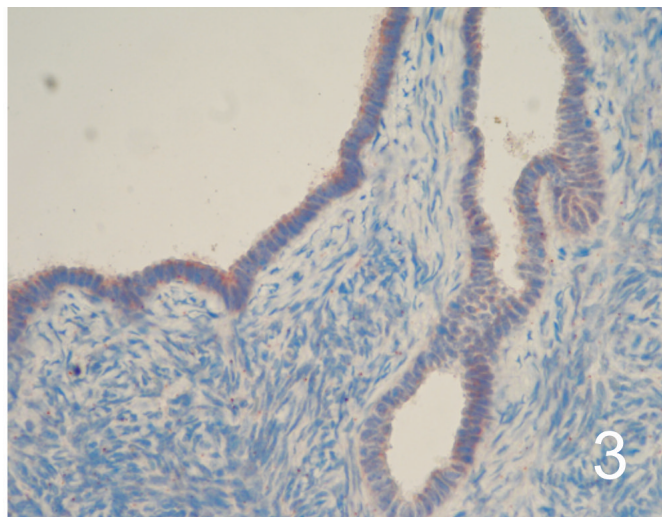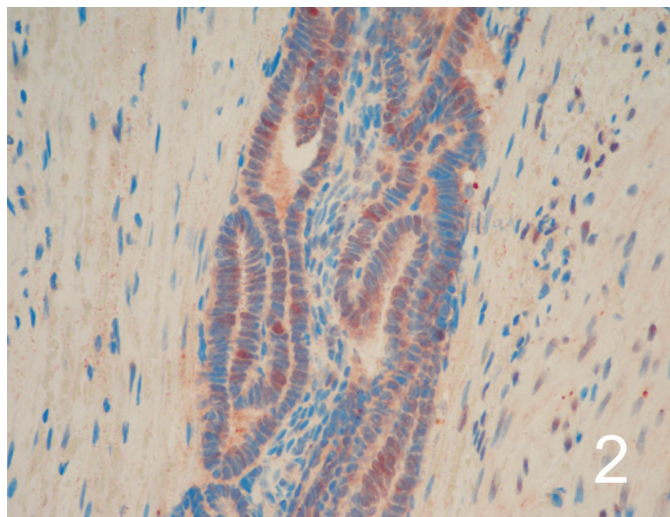
expression of ERα in luteal and paraluteal cells of disappearing corpus luteum. The expression of ERα seems to lower in ovaries of women after 5 and 10 years from menopause (Figs. 2, 3).

Other authors point out that ER genotype may determine the function of the sex-steroid system not only at the receptor level but also at the level of hormone synthesis (Zofkova et al., 2002).

The expression of androgen receptors (AR) and progesterone receptors (PR) was found immuno-histochemically in the ovaries of reproductive and postmenopausal women (Meza-Munoz et al., 2006). Immunohistochemical expression of AR in postmenopausal women is similar to that at the reproductive age (Figs. 4, 5). In contrast, immuno-histochemical expression of PR seems to be lower in the ovaries of women after 5 and 10 years from menopause (unpublised results).

Inkster and Brodie (1991) immunohistochemically detected the expression of aromatase cytochrome P-450 in the ovaries of pre- and postmenopausal women. In all cases, the aromatase immunostaining reaction was cytoplasmic. The results provide the direct evidence for the existence of thecal cell aromatase, and of stromal cell aromatase in postmenopausal women, which proves the activity of steroidogenesis in these ovaries.

The immunohistochemical studies on CD68 expression revealed the presence and localization of macrophages in the ovary. These cells were mainly found in the surrounding of corpora albicantia (Figs. 6, 7). Focchi et al. (1995, 1996) and Motta et al. (2002) analyzed changes in corpora albicantia in post-menopausal women. They proved that the remodeling process of these structures appears to depend on the activity of three essential cell types: the fibroblasts which provide collage synthesis, the macrophages which phagocytize the flaky material, and the myofibroblasts mainly located in the peripheral region of the corpora albicantia, which may have a retracting action on the remodeling site of the corpus albicans.

## Hormonal function of the postmenopausal ovaries, steroidogenesis

During the reproductive period the androgens are produced by thecal cells and aromatised to estrogens by the zona granulosa cells. The activity of thecal cells is regulated by LH, while the activity of aromatase P-450 of granulosa cells depends on FSH. The ovarian steroidogenesis depends mainly on activity of enzymes such as aromatase P-450 that transforms testosterone to 17-ß estradiol, 3-ß steroid dehydrogenase transforming dehydroepiandrosterone into androsterone, and 17-ß steroid dehydrogenase catalyzing the transformation of dehydroepiandrosterone into androstenediole and then into testosterone (Buffet and Bouchard, 2001; Brodowska et al., 2005).

The ovarian steroidogenesis activity depends also on steroid hormones receptors such as estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR). Those receptors are located in the nucleus but they are also detected in the cytoplasm and cell membrane (Plouffe, 1998; Lobo, 2001; Brodowska et al., 2005).

The menopause does not indicate the complete cessation of hormonal function of an ovary. The atrophy of follicles means the end of cyclic function of ovary as a reproductive organ but does not change into an inactive connective tissue. The function of granulosa and theca cells is still maintained, and some amounts of steroid hormones, mostly androgens, are still produced (Manieri et al., 1998; Plouffe, 1998; Burger, 2002; Basaria and Dobs, 2006).

The androgenesis after the menopause is especially important because of general action of androgens, the hormones that influence the quality of woman's life. They influence the mineral bone density, libido and sexual activity, memory and intellectual abilities, mood and physical activity (Focchi et al., 1995; Bancroft and Cawood, 1996; Manieri et al., 1998; Plouffe, 1998; Longcope, 2001; Basaria and Dobs, 2006; Wierman et al., 2006.). Thus the ovary as a source of androgens

**Fig. 2.** Ovary of postmenopausal woman examined no more than 5 years after menopause. Immunohistochemical localization of ERα. Strong nuclear staining is present in some columnar epithelial cells (invagination of ovarian surface epithelium, metaplastic changes) and in some stromal cells. x 400

**Fig. 3.** Ovary of postmenopausal woman examined more than 10 years after menopause. Immunohistochemical localization of ERα. Weak nuclear staining is present in some columnar epithelial cells (invagination of ovarian surface epithelium, metaplastic changes). x 400

**Fig. 4.** Ovary of postmenopausal woman examined no more than 5 years after menopause. Immunohistochemical localization of AR. The high level of AR expression was observed in nuclei of ovarian surface epithelium and in some stromal cells. x 400

**Fig. 5.** Ovary of postmenopausal woman examined more than 10 years after menopause. Immunohistochemical localization of AR. The high level of AR expression was observed in nuclei of ovarian surface epithelium and in some stromal cells. x 400

**Fig. 6.** Ovary of postmenopausal woman examined no more than 5 years after menopause. Immunohistochemical localization of CD68. The highest level of CD68 expression was observed in macrophages of the corpus albicans and stroma. x 400

**Fig. 7.** Ovary of postmenopausal woman examined more than 10 years after menopause. Immunohistochemical localization of CD68. The CD68 expression was observed in macrophages of the corpus albicans and stroma. x 400

seems to be important even after cessation of its reproductive function.

Burger et al. (2002) reported that the amount of androgens, namely testosterone and androstendione, produced by ovaries, diminishes with age. This process is gradual: it starts about the age of 20 and accelerates after the menopause. Thus, the postmenopausal ovary produced testosterone of about 50% of the production at the reproductive age remains the important source of this hormone.

Plouffe (1998) observed rapid and significant decrease in the estadiol, testosterone and androstendione concentration in blood serum up to 50% after surgical ovariectomy at the perimenopause.

Numerous papers report lipid production in stromal cells of postmenopausal ovary that may indicate production of steroid hormones and more or less intense hormonal changes (Ala-Fossi et al., 1998). Some authors point to the accelerated ovarian steroidogenesis in case of stromal cell hyperplasia. Those changes are designated as neoplasma-like and are accompanied by different metabolic changes or hirsutism and virilisation (Ala-Fossi et al., 1998; Sluijmer at al., 1998). Interestingly, hirsutism may be induced by gonadotropin-dependent ovarian androgen production in women many years after the menopause (Ayuk et al., 1998; Lindgren et al., 2000; Lobo, 2001). In those cases surgical ovarectomy is a matter of choice (Manieri et al., 1998).

Longcope proved the expression of gonadotropin receptors (FSH, LH) and activity of steroidogenesis enzyme such as P-450 aromatase in postmenopausal ovaries, which suggested the ovarian production of androgens and estradiol (Longcope, 1998, 2001). However, some papers suggest adrenal glands to be the only source of androgens after the menopause. Couzinet et al. (2001) analyzed groups of women with normal and insufficient adrenal glands and showed significantly higher androgenes production in women with normal adrenal glands. The supplementation of gonadotropins did not influence the blood serum concentration of androgens, but blockage of adrenal gland function with dexametasone caused a significant decrease of androgen concentration in blood serum (Couzinet at al., 2001; Brodowska et al., 2005). Jabara et al. recenty reported that the predominant stromal cells of the postmenopausal ovary are not a significant site of androgen biosynthesis. They showed that stromal cells in the postmenopausal ovary could be distinguished from fibroblast and did not appear to have significant steroidogenic potential, but did metabolize cholesterol into hydroxysterols (Jabara et al., 2003).

Thus, it can be suggested that the ovary undergoes several structural, functional and immuno-histochemically detected changes after the menopause. The postmenopausal ovary partially sustains its hormonal function.

The postmenopausal ovaries, frequently considered as inactive connective fibrous tissue, are still important for women for good psychological condition as well as physical wellbeing. Thus, in some countries, including the USA, the substitution of androgens is proposed for women whose ovaries were surgically removed (Wierman et al., 2006).

We hope that more extensive studies will determine the role of the postmenopausal ovary and enable the optimization of therapy in many clinical situations balancing the risk of ovarian cancer and the benefits from its hormonal function.

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