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Assessment of morphological changes and steroid receptors in the uteri of postmenopausal women

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Key words: uterus, morphology, steroid receptors, menopause

Short title: The uteri of postmenopausal women
Summary

Introduction. The morphology of the endometrium constantly changes in the reproductive period, depending on the levels of ovarian steroid hormones, and undergoes atrophic changes during menopause as a result of their insufficiency. The purpose of this study was to analyze morphological and morphometric changes in the mucous and muscle layers of the uterine wall in postmenopausal women, and to assess localization and number of cells showing the expression of steroid hormone receptors, namely estrogen receptor α (ER-α), progesterone receptor (PR), and androgen receptor (AR) in glandular epithelial cells and smooth muscle cells in particular groups of women.

Material and methods. The study material consisted of uterine specimens sectioned across the full thickness of the uterine wall, and embedded in 164 paraffin blocks. The specimens came from women without menopausal hormone therapy (MHT) operated due to reproductive organ prolapse or uterine myomas. The material was divided into four groups depending on the time interval from menopause to surgery: group I — from 1 to 5 years after menopause, group II — from 6 to 10 years after menopause, group III — more than 11 years after menopause, and group IV — women over 70 years of age. The sections were stained by standard HE, Masson’s trichrome, and immunohistochemical methods (ERα, PR, AR). Quantitative assessment of the results was based on computer image analysis.

Results. Analysis of morphological changes in the endometrium and myometrium revealed the presence of increasing regressive changes, such as various types of atrophy, fibrosis, and calcification, augmented over time from the last menstruation. Furthermore, endometrial polyps, foci of endometriosis, and leiomyomas were observed. Based on the results of morphometric measurements, a constant decrease in the endometrial and myometrial thickness was noticed in the studied groups (I-IV). Significant differences between the groups were observed in the number of ER-α positive cells in the myometrium, but not in the endometrial glandular epithelium. Statistically significant differences in the number of AR positive cells were detected in the endometrial epithelium and in the uterine muscle. The analysis the number of PR positive cells demonstrated differences between the groups in the endometrial stroma and the myometrium.

Conclusion. The uterus of postmenopausal woman undergo major morphological changes (mainly atrophic lesions in the endometrium and myometrium), leading to a decline in their morphometric parameters over time from the last menstruation. Localization and number of cells showing the expression of steroid receptors: ER-α, PR, and AR in the uterus of postmenopausal women, depending on the time interval from the last menstruation.
Introduction

The uterus is responsible for the stimulation of steroid hormones synthesized by ovaries. It consists of various types of cells that make up the endometrium, myometrium, and perimetrium. Hormonal stimulation of the uterus in the reproductive period involves cyclic changes in this organ during the menstrual cycle, which are no longer observed in the postmenopausal period (Jabbour et al., 2006; Otify et al., 2015).

Although there are many scientific publications describing the human uterus, most of them only concern the reproductive period (Mertens et al., 2001; Gregory et al., 2003; Shiozawa et al., 2003; Jakimiuk et al., 2003; Mehasseb et al., 2011). There are also comparative studies of changes occurring in the endometrium and myometrium in the pre- and postmenopausal periods (Sakaguchi et al., 2003; Arnett-Mansfield et al., 2004; Mendez et al., 2013; Dutta and Talukdar, 2015). An example is the morphological analysis performed by Dutta and Talukdar (2015), who demonstrated substantial differences in the endometrial thickness between the uteri of pre- and postmenopausal women. Changes in the uteri of postmenopausal women, depending on the time interval from the last menstruation, have not been thoroughly explored so far.

Moawia et al. (2013) informed about atrophic changes in the uterine body, and Simon et al. (2013) revealed that atrophic changes occur in the endometrium in the postmenopausal period. As stated by Dutta and Talukdar (2015), glandular epithelial cells become cuboidal or low columnar in shape. These authors also noticed that the uterine endometrium of postmenopausal women only consists of the basal layer. The postmenopausal endometrium does not show features of active proliferation or secretion. Immunoreactivity for estrogen and progesterone receptors also decreases. There is fibrosis of the endometrial stroma, which is caused by increased amount of collagen fibers. The number of endometrial glands declines (Otify et al., 2015).

In the postmenopausal period, the myometrium becomes thin and atrophic. It consists of long, slender myocytes arranged into non-defined layers. External and the most internal layers are thin, and mainly composed of longitudinally and diagonally scattered myocytes, which was confirmed by Dutta and Talukdar (2015).

Imaging and histological examinations demonstrate features of involution. Fibrosis and atrophy are observed in both structures — the endometrium and myometrium. Lesions are observed in epithelial cells and stromal cells of the mucous layer, as well as in smooth myocytes (Bechcińska et. al., 2000; Sivridis et al., 2004).

Typical representatives of nuclear steroid receptors, estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR) are involved in hormonal stimulation of the uterus (Jabbour et al., 2006; Patel et al., 2015). They serve as transcription factors activated by ligands (Sever and Glass, 2013).

The available literature in the field offers numerous publications about immunolocalization and immunoexpression of these receptors (ER, PR and AR) in the uteri of premenopausal women (Mertens et al., 2001; Jakimiuk et al., 2003, Mylonas et al., 2005) and
also their comparison in the uteri of reproductive and postmenopausal age women (Sakaguchi et al., 2003).

At present, three types of estrogen receptors are known: alpha (ER-α), beta (ER-β) and gamma (ER-γ). The expression of ER depends on the phase of the menstrual cycle (Rich et al., 2002), age (Nephew et al., 2000), and the amount of estradiol and progesterone (Nephew et al., 2000). Strong immunosuppression of ER-α has been found (Skrzypczak, 2008) to occur in the uterine body — both in the endometrial and myometrial cell nuclei — and to increase after menopause.

Progesterone activity in target tissues during the menstrual cycle and pregnancy involves progesterone receptor. It has two isoforms: PR-A and PR-B that are not clinically differentiated (Patel et al., 2015). Both PR-A and PR-B are localized in epithelial and stromal cells in the endometrium, but also in smooth muscle cells in the myometrium (Patel et al., 2015).

Androgen receptor (AR) is a nuclear receptor that has two isoforms: A and B (Jabbour et al., 2006). This receptor is localized in the mucous membrane of the uterus, stromal cells, and smooth myocytes of the myometrium (Jabbour et al., 2006; Li et al., 2014).

Current knowledge of the localization and expression of steroid receptors (especially ER-α, PR, and AR) in the uteri of healthy women at different moments after menopause has not yet been fully explained and requires further investigation. These publications, however, only provide ambiguous results concerning changes in the expression of these receptors in the uterus with regard to the time interval from the last menstruation.

It is important to analyze localization of these receptors at various moments after menopause since they have effects on the pathogenesis of endometrial cancer (Goel and Kumar, 2012). This cancer is the most common neoplasm found in women in developing countries, and constitutes 91% of cancer cases among women aged over 50 (Goel and Kumar, 2012). A factor predisposing the majority of women to uterine cancer is exposure to estrogens that is not counterbalanced by progesterone.

The studies conducted so far have not provided details concerning changes in the morphology and expression of steroid receptors, and have not compared changes in the endometrium and myometrium in the postmenopausal period. Therefore, the purpose of our research was to analyze morphological changes in the body of the uterus (corpus uterus), especially the endometrium and myometrium, as well as to determine localization and number of cells showing the expression of steroid receptors: ER-α, PR, and AR in the uterus of postmenopausal women, depending on the time interval from the last menstruation.
Material and methods

Material

The material analyzed in our study consisted of uterine tissue sections embedded in paraffin blocks. These sections were taken from the uteri of postmenopausal women hospitalized in the gynecology wards of the Provincial Specialist Hospital in Slupsk and the Independent Public Specialist Healthcare Center “Zdroje” in Szczecin, and subjected to radical surgical treatment due to reproductive organ prolapse and uterine myomas.

The uterine sections included all layers of the uterine wall, namely the mucous, muscle, and serous membranes. They came from non-users of menopausal hormone therapy (MHT), which was confirmed by the results of serum follicle-stimulating hormone (FSH) levels (Table 1).

The material was divided into four groups with regard to the time interval from the last menstruation: group I — up to 5 years after menopause, group II — 6-10 years after menopause, group III — over 11 years after menopause, and group IV — women over 70 years of age. The total number of 164 paraffin blocks with uterine sections was collected: group I (48), group II (52), group III (50), group IV (14). Data were obtained from request forms for histopathological examination routinely issued by gynecologists.

The study protocol was approved by the Bioethical Commission of the Pomeranian Medical University in Szczecin (permission number: KB-0012/56/11/11).

Morphological studies

The uterine sections were routinely fixed in 4% buffered paraformaldehyde, and embedded in paraffin. The paraffin blocks were cut into 4 µm sections, which were next stained using a standard hematoxylin and eosin method (HE; Sigma Aldrich, Germany). The samples were examined under an Olympus BX46 light microscope (Olympus Optical Co., Japan).

Masson’s trichrome method

Masson’s trichrome staining was employed to assess fibrosis. Four dyes were applied: Weigert’s iron hematoxylin for nuclear staining, picric acid for staining erythrocytes, a mixture of acid dyes (acid fuchsin — Ponceau S) for staining cytoplasm, and aniline blue for staining collagen connective tissue fibers. The following tissue colors were obtained: collagen fibers — blue; stromal cells, epithelial cells, and smooth myocytes — red (Armed Forces Institute of Pathology, 1994). Reaction was performed on 30 slides in each group from I to III, and on 14 slides in group IV.

Immunohistochemical analysis

The following antibodies were used for immunohistochemical reactions: monoclonal mouse antibody IgG against ER-α (1:50 ratio, Ventana Medical System, Inc., Confirm anti-Estrogen Receptor α, Clone SP1, USA); monoclonal mouse antibody IgG against PR (ready-to-use; Ventana Medical System, Inc., Confirm anti-Progesteron Receptor Clone 1E2, USA); and monoclonal mouse antibody IgG against AR (1:50 ratio, Ventana Medical System, Inc., Anti-Androgen Receptor Clone SP107, USA).
The uterine slides were deparaffinized, hydrated, and boiled in target retrieval solution (Dako Denmark A/S) for 30 minutes at pH 9.0 to retrieve the antigens. The activity of endogenous peroxidase was blocked by using peroxidase blocking solution (Dako Denmark A/S) for 10 minutes. The sections were incubated at room temperature with mouse monoclonal primary antibodies in a humid chamber for 30 minutes. Primary antibodies were diluted in antibody diluter (Dako Denmark A/S). Next, the sections were incubated with a complex containing secondary antibody conjugated with horseradish peroxidase (Dako REAL EnVision Detection System Peroxidase/diaminobenzidine +, rabbit/mouse; Dako Denmark A/S). After washing out the secondary antibody, diaminobenzidine was applied for 5-10 minutes (1:50 ratio, Dako Denmark A/S). As the final step, the slides were counterstained with Mayer’s hematoxylin, dehydrated, and coverslipped. Washing the slides with phosphate-buffered saline preceded each step of the procedure. All reactions were performed simultaneously under the same conditions. The slides were examined under a light microscope (BX 41). Each reaction was performed on 30 slides in each group from I to III, and on 14 slides in group IV.

Quantitative computer image analysis

The slides were scanned with the Aperio AT Turbo scanner (LeicaBiotechnologies). The endometrial and myometrial thickness of the uterine body was measured using the Image Scope Viewer software (Aperio Technologies). Six measurements of each of the tested structures (endometrium, myometrium) were made for each microscope slide, and extreme results were disregarded.

Computer image analysis of immunohistochemical reactions was performed using the Image Scope Viewer software v. 11.2.0.780 (Aperio Technologies). Histological structures of the uterine wall (epithelium of endometrial glandular ducts, endometrial and myometrial stroma) were marked on virtual microscope slides obtained from the Aperio AT2 Turbo scanner (Leica Biotechnologies). In the fields of similar size (0.1mm² for epithelial cells of glandular ducts; 0.25mm² for endometrial and myometrial stromal cells), cell nuclei showing positive reaction (brown color) were counted, and we estimated what percent of all nuclei counted using the nuclear algorithm v.9 (Aperio Technologies, Inc.) they constituted.

30 randomly chosen fields were counted for each slide to obtain reliable and repeatable results.

Statistical analysis

Statistical analysis was performed using Statistica 5.0 for Windows (StatSoft). Basic parameters such as a mean value ($\bar{x}$) ± standard deviation (SD) were determined. Normality of the distribution was assessed by the Shapiro-Wilk test, the Kruskal-Wallis ANOVA test and the Mann-Whitney U test were applied to determine differences between the groups. The level of significance was set at $p < 0.05$. The results are shown in tables and figures.
Results

The study presented here focuses on the morphology of the uterine wall depending on the time interval from the last menstruation. We observed changes associated with the organ involution and pathological processes, such as foci of benign proliferative tumors of the uterine smooth muscle (leiomyomas), and ectopic endometrial tissue in the muscle membrane of the uterus (adenomyosis).

Assessment of morphological and morphometric changes in the uteri of postmenopausal women

Morphological analysis of the uterine wall in group I (women up to five years after menopause) did not reveal significant differences between the endometrium of postmenopausal women and commonly known the normal uterine structure observed in reproductive-age women (Gartner and Hiatt, 2007).

The endometrium was covered with simple columnar epithelium, invaginating into stroma and forming glandular ducts of a similar size and shape. Epithelial cells were characterized by a normal ratio of a nucleus to cytoplasm. Oval mesenchymal stromal cells filled tightly the stroma of the endometrium. The myometrium was visible beneath the basal layer of the endometrium. The muscle membrane consisted of preserved myocytes with a distinct ‘cigar-like’ nucleus and quite abundant cytoplasm. Bunched smooth muscle cells intertwined, thus forming the uterine muscle. In the deeper layers of the myometrium, blood vessels with normal morphology were found. In none of the tissues described above, mitotic figures were observed. In two cases, foci of the endometrial tissue were visible in the myometrium (Fig. 1 A, B). Leiomyomas were found in four microscope slides (Fig. 1 C).

In group II (women 6 to 10 years after menopause), the microscopic image of the uterine wall revealed discrete features of atrophy. At the side of the uterine lumen, the endometrium was covered with columnar epithelium, showing no significant changes. The epithelium penetrated the stroma, and formed glandular ducts. In ten cases, the ducts were dilated and lined with columnar epithelium, which locally evolved into cuboidal epithelium (Fig. 2 A). Some of the endometrial stromal cells were spindle-shaped. The uterine muscle consisted of myocytes with spindle-shaped, blunt-ended cell nuclei. The size of smooth muscle cells slightly decreased. No features of mitotic activity were found in the tested sections. Foci of myometrial adenomyosis were observed in two microscope slides, and uterine leiomyomas in six slides.

In group III (women more than 11 years after menopause), histological analysis of the endometrium revealed cystic dilation of glandular ducts in more than a half of cases. In three slides, atrophic lesions took the form of mucous membrane invaginations into the lumen of the uterus, and atrophic endometrial polyps (Fig. 2C). Histological features of atrophy were accompanied by cytological indicators, such as conversion of columnar epithelial cells into cuboidal ones, and consequently the visible flattening of the glandular duct epithelium (Fig. 2B). Many of endometrial stromal cells were spindle-shaped. They were scattered within polyps compared with the endometrium outside them. The myometrium consisted of myocytes with a small nucleus and scanty cytoplasm. Three of the studied women had
myometrial leiomyomas. In single cases, we observed calcification in the walls of blood vessels.

In all microscope slides showing the uterine wall of postmenopausal women over 70 (group IV), atrophic features of the uterine wall tissue were noticeable (Fig. 3A, B). In the endometrium, they were manifested by a decline in the number of glandular ducts. Cystic dilation of the lumen accompanied by the flattening of the lining epithelium was visible in some ducts. Both epithelial and stromal cells in the endometrium were smaller than cells in other groups. The majority of stromal cells of the mucous membrane were comma-shaped. The uterine muscle consisted of substantially thinned smooth muscle fibers with scanty cytoplasm. In one third of cases, calcification was present in the myometrial blood vessel walls (Fig. 3B). There were no pathological lesions.

The process of fibrosis in the uterine muscle membrane was observed in all groups. It was especially visible in the myometrial area under serous membrane, and was more severe in the deeper layers of the uterus. In the myometrial part adjacent to the endometrium, fibrosis was insignificant. All these slightly changed over time from the last menstruation but the fibrosis-free area was preserved (Fig. 4).

Results of morphometric measurements

Morphometric measurements of the endometrial and myometrial thickness were performed in the material divided into groups with regard to the time interval from the last menstruation. Based on the data obtained, the mean value and standard deviation were established. A decrease in the mean thickness of both uterine wall structures was observed (Table 2).

A constant decline in the mean endometrial thickness observed in all groups was a permanent process. A decrease in the mean myometrial thickness, on the other hand, proceeded dynamically in groups I and II, and was visibly less dynamic in groups III and IV, which is illustrated in Figure 4.

Statistical analysis of the endometrial and myometrial thickness

Analysis of the endometrial and myometrial thickness demonstrated statistically significant differences in the myometrial thickness, and no statistically significant differences in the endometrial thickness between the groups (Table 2).

Expression of ER-α in uterine tissues

A statistically significant difference in the expression of ER-α (Table 3, Fig. 5) within the endometrial stroma was only observed between groups II and III (p = 0.00003). In the myometrium, statistically significant differences were found between groups I and II (p = 0.0005); groups I and III (p = 0.000001); groups I and IV (p = 0.0005); groups II and III (p = 0.005); and groups II and IV (p = 0.02). There were no statistically significant differences in the expression of ER-α in the endometrial glandular epithelium between the groups.

Expression of AR in uterine tissues

There were statistically significant differences in the percent of cells showing AR expression (Table 3, Fig. 6) in the endometrial epithelium between groups I and II (p =
Expression of PR in uterine tissues

In the endometrial stroma, a statistically significant difference in the expression of PR (Table 3, Fig. 7) was found between groups I and II (p = 0.002). Statistically significant differences were also observed in the myometrium between groups I and IV (p = 0.04); groups II and III (p = 0.039); and groups II and IV (p = 0.03). There were no statistically significant differences in the expression of PR in the endometrial epithelial cells.

Discussion

As confirmed by Moawia et al. (2013), morphological and morphometric changes in the uteri of postmenopausal women are mostly observed in the body of the uterus (corpus uterus). Ultrasound examination of healthy postmenopausal women reveals a decrease in the thickness of the uterine wall layers, especially the endometrium and myometrium. In the study of Singh et al. (2016), endometrial thickness of 4-5 mm was regarded as requiring histopathological verification due to possible hyperplasia.

Microscopic images of the uterine layers in group I showed the endometrium with tubular glands lined with simple columnar epithelium. The ducts were surrounded by numerous oval stromal cells not adjacent to each other. This may have been caused by the fact that some women from group I were in the early phase of the postmenopausal period. At this stage, the thickness of the endometrium slightly decreases, and morphological features of the secretory and proliferative phases gradually disappear as was shown by Dutta and Talukdar (2015).

In group II (6-10 years after menopause), we noticed increasing atrophic changes in the endometrium, whose average thickness did not exceed 1 mm. The endometrium was covered with simple columnar epithelium with various size glandular ducts found underneath. In 18% of the endometrium, enlarged cystic ducts partially lined with cuboidal epithelium were observed. Similar results were obtained by Heyn et al. (2005) on the basis of intraoperative biopsy in 45-72-year-old postmenopausal women. Atrophic endometrial changes were also described by Bechcińska et al. (2000), who reported on cystic atrophy of the endometrium, in which stromal cells adjoined to each other and were focally spindle-shaped in the upper endometrium layers.

In group III, we noticed advanced atrophic features in a morphological picture of the endometrium. The average thickness of the mucous membrane did not exceed 0.7 mm, and simple columnar epithelium covering the endometrium was locally replaced with cuboidal epithelium. Numerous fairly dilated cystic glandular ducts, lined with cuboidal epithelium, were present in the stroma. This was seen in 29% of the microscope slides. Non-dilated ducts were constricted by dense stroma with spindle-shaped cells adjacent to each other. In 6% of cases, atrophic endometrial polyps with enlarged cystic glandular ducts were found in the
lumen of the uterus. In most cases, endometrial lesions in group III corresponded with the endometrial picture in group IV (women over 70).

Our results for the endometrium in groups III and IV correspond with those obtained by Bechcińska et al. (2000), who analyzed microscopic images of the normal and pathological endometrium from the fetal period to 20 and more years after menopause.

In our study, a constant decrease in the myometrial thickness was observed with time from menopause. This process was especially dynamic during the first decade following menopause (groups I and II). In our study, a decrease in the myometrial thickness in groups I — IV was probably associated with processes occurring in myocytes, and leading to changes in their size and morphology. Publications presenting in-depth analysis of the myometrium are very rare in the literature.

In our study, nuclear immunolocalization of steroid receptors ER-α, PR and AR was found in all groups. A positive immunohistochemical reaction was present in epithelial and endometrial stromal cells, as well as in myometrial myocytes.

The expression of ER-α was observed in numerous epithelial and endometrial stromal cells, however there were statistically significant differences in the number of stromal cells showing ER-α expression between the groups. The expression of ER-α was also evaluated during the menstrual cycle, and was found to be stable in endometrial cells (Sivridis et al., 2004; Singh et al., 2016). According to Jakimiuk et al. (2003), who described the expression of ER-α in premenopausal women, the expression of the receptor is stronger in endometrial mucosa cells than in myometrial muscle cells.

Analysis of the number of PR positive cells in the endometrial epithelium in the first (groups I and II) and the second (groups III and IV) decades after menopause did not demonstrate statistically significant differences between the groups. Differences in the number of cells showing the PR expression in the endometrial stroma between the groups (I, II, III, IV) were statistically significant. There were also statistically significant differences in the number of myometrial PR positive cells between the groups (Table 3).

A similar study was conducted by Mehasseb et al. (2011), who established that the PR expression in endometrial epithelial cells was highest during the beginning of the secretory phase, while in the stromal cell nuclei of the mucous membrane, and myometrial myocytes it remained unchanged. In the postmenopausal period, the increased PR expression in endometrial polyps, proliferation, and endometrial tumors was accompanied by a decline in the expression of ER-α. The expression of PR in healthy women, both in the endometrium and myometrium, remained on the same level (de Carvalho et al., 2011). According to Arnett-Mansfield (2004, 2001), the disturbed expression of progesterone receptor isoforms (PRA and PRB) can result in endometrial pathologies in the postmenopausal period. The reports on the lack of changes in the PR expression within the endometrium of healthy postmenopausal women correspond with our findings.

In the reproductive age, the expression of AR is observed in all cells of the uterine wall. Its strength varies depending on the phase of the menstrual cycle — it is strongest in the proliferative phase, and undetectable in the late secretory phase (Mertens et al., 2001). In the postmenopausal period, the expression of AR is visibly stronger in epithelial cells of the endometrial gland than in cell nuclei of other uterine wall structures (Maia et al., 2001).
Our study provided evidence for the presence of AR in endometrial and myometrial cells. The number of cells showing the expression of AR in endometrial stromal cells was stable, and no statistically significant differences were observed between the groups (Table 3). In the myometrium and the epithelium of the uterine mucous membrane, the number of AR positive cells increased with the time from the last menstruation. The differences between the groups were statistically significant. AR plays a stimulatory role in the proliferation of smooth muscle cells, and fulfills an antiapoptotic function, which was confirmed by Li et al. (2014) and Lan et al. (2016). Morphological analysis of the uterine wall in the groups of women suggests gradual atrophy of the organ, dependent on the length of time from the last menstruation. Morphological and immunohistochemical analyses concerning the expression of steroid receptors let us conclude that changes in the uterus of postmenopausal women are complex and specific to this organ. Our findings indicate a constant decrease in morphometric parameters of the uterus, progressive fibrosis, and changes in the cell morphology of particular tissues. In our study, morphological changes were accompanied by an increase in the number of ER-α, PR and AR positive cells in the myometrium, as well as an increase in the number of AR positive cells in the endometrial epithelium (Table 3). A comparison of the above mentioned processes revealed that the peak of their intensity is during the first ten years after menopause (groups I and II). After the first decade from menopause (groups III and IV), the intensity of changes becomes visibly lower. The studies of Brodowska et al. (2005, 2010) seem to support this hypothesis. These authors analyzed sex hormone levels in ovarian tissue homogenate, and found that during ten years from the last menstruation, the levels of hormones in ovarian tissue were higher than in serum. Furthermore, female gonads retained their capacity for steroidogenesis, which was the highest during the first five years following menopause (Brodowska et al., 2005, 2010). This phenomenon is favorable to nearby tissues, including the uterine tissue, however it does not prevent atrophy in the skeletal system, the cardiovascular system, and the skin (Lang et al., 2003; Archer, 2007; Zegarska et al., 2011; Zhao, 2012). In the uteri of postmenopausal women, major morphological changes occur. These mainly include atrophic lesions in the endometrium and myometrium, leading to a decrease in their morphometric parameters. Our study demonstrated that atrophic changes in the uteri of postmenopausal women slightly differed, which could be associated with the length of time from the last menstruation. Further investigation is needed to understand aging-related processes occurring in female reproductive organs.
References


androgen receptor expression in surface epithelium and inclusion cyst in the ovary of premenopausal and postmenopausal women. J. Ovarian. Res. 6, 85.


Fig. 1. Hematoxylin and eosin (HE) staining: A (x 40), B (x 200) — internal endometriosis. A centrally located focus of internal endometriosis (red arrow) with a distinct glandular duct (black arrow), surrounded by rare stromal cells (white arrow); myometrium (M); C (x 100) leiomyoma of the uterine body. Chaotically intertwined bundles of myocytes, and fibrosis of stroma visible in the tumor. Myoma (*); myometrium (M)

Fig. 2. HE staining: A (x 400) — epithelium in the enlarged cystic glandular duct of the uterine mucous membrane. The dilated lumen of the glandular duct (asterisk) lined with cuboidal epithelium (black arrow), columnar epithelium (red arrow) in unchanged ducts (black quarter); B (x 400) — changing epithelium in the enlarged cystic glandular ducts. Normal columnar epithelium (red arrow); cuboidal epithelium (black arrow); flattened squamous epithelium (thin arrow); the lumen of the glandular ducts (*); C (x 20) — an endometrial polyp showing cystic atrophy features. In the central part, the polyp invaginating into the lumen of the uterine cavity (triangle). Numerous enlarged cystic glandular ducts (white arrow) in the polyp and the endometrium; the lumen of the uterine cavity (*)

Fig. 3. HE staining: A (x 20) — the endometrium with the features of cystic atrophy (black arrow), thickened walls of blood vessels with calcification (red arrow), serous membrane (perimetrium) (white arrow), myometrium (M); B (x 40) — calcification in the walls of arterial vessels of the uterine wall. The lumen of the vessels (*): calcification (thin arrow)

Fig. 4. Histochemical staining by Masson’s trichrome method: fibrosis of the myometrium; cross-sections of the uterine wall. Collagen fibers — blue color; stromal cells, epithelial cells, myocytes — red color; A (group 1), B (group 2), C (group 3) (x20) — progressive fibrosis of the uterine myometrium (F) with a fibrosis-free area (SM) adjacent to the endometrium (E); serous membrane (perimetrium) (N)

Fig. 5. Location of estrogen receptor α (ER-α) in the uterus in postmenopausal women; In the endometrium: A (group 1, x200), B (group 2; x200), C (group 3; x200), D (group 4; x200); In the myometrium: A (group 1; x100), B (group 2; x100), C (group 3; x100), D (group 4; x100); ER-α (+) cells (red arrow), ER-α(–) cells (black arrow)

Fig. 6. Location of androgen receptor (AR) in the uterus in postmenopausal women; In the endometrium: A (group 1, x 200), B (group 2; x 200), C (group 3; x 200), D (group 4; x 200); In the myometrium: A (group 1; x 100), B (group 2; x 100), C (group 3; x 100), D (group 4; x 100); AR-α (+) cells (red arrow), AR-α(–) cells (black arrow)

Fig. 7. Location of progesterone receptor (PR) in the uterus in postmenopausal women; In the endometrium: A (group 1, x 200), B (group 2; x 200), C (group 3; x 200), D (group 4; x 200); In the myometrium: A (group 1; x 200), B (group 2; x 200), C (group 3; x 200), D (group 4; x 200); PR-α (+) cells (red arrow), PR-α(–) cells (black arrow)
### Table 1. The mean serum FSH levels in the studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
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<tbody>
<tr>
<td>FSH level (mlU/mL)</td>
<td>62.26 ± 23.3</td>
<td>77.26 ± 32.52</td>
<td>82.24 ± 33.59</td>
<td>82.27 ± 29.27</td>
</tr>
</tbody>
</table>

Mann-Whitney U; Group I — women up to 5 years after menopause; Group II — women 6 to 10 years after menopause; Group III — women more than 11 years after menopause; Group IV — women over 70 years of age; \( \bar{x} \) — arithmetic mean; SD — standard deviation

### Table 2. The endometrial and myometrial thickness in the studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Thickness of the endometrium (mm) (( \bar{x} \pm SD ))</th>
<th>Thickness of the myometrium (mm) (( \bar{x} \pm SD ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.22 ± 0.67</td>
<td>16.11 ± 2.86</td>
</tr>
<tr>
<td>Group II</td>
<td>0.94 ± 0.63</td>
<td>12.66 ± 2.86</td>
</tr>
<tr>
<td>Group III</td>
<td>0.72 ± 0.47</td>
<td>9.86 ± 2.12</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.58 ± 0.18</td>
<td>8.64 ± 2.23</td>
</tr>
</tbody>
</table>

Statistical significance

<table>
<thead>
<tr>
<th>Group vs. Group</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs. II</td>
<td>0.063</td>
<td>0.0001*</td>
</tr>
<tr>
<td>I vs. III</td>
<td>0.0007*</td>
<td>0.000001*</td>
</tr>
<tr>
<td>I vs. IV</td>
<td>0.014*</td>
<td>0.0005*</td>
</tr>
<tr>
<td>II vs. III</td>
<td>0.038*</td>
<td>0.0001*</td>
</tr>
<tr>
<td>II vs. IV</td>
<td>0.03*</td>
<td>0.013*</td>
</tr>
<tr>
<td>III vs. IV</td>
<td>0.424</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Mann-Whitney U; Group I — women up to 5 years after menopause; Group II — women 6 to 10 years after menopause; Group III — women more than 11 years after menopause; Group IV — women over 70 years of age; \( \bar{x} \) — arithmetic mean; SD — standard deviation, * - statistically significant parameter
Table 3. The number of cells showing expression of ER-α, PR, and AR in particular uterine wall structures

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-α</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epithelium</td>
<td>90.55 ± 6.71</td>
<td>83.62 ± 10.67</td>
<td>86.62 ± 9.76</td>
<td>83.92 ± 9.45</td>
<td>0.07</td>
</tr>
<tr>
<td>stroma</td>
<td>74.98 ± 16.48</td>
<td>69.29 ± 13.49</td>
<td>83.79 ± 9.24</td>
<td>80.60 ± 6.48</td>
<td><strong>0.0008</strong> *</td>
</tr>
<tr>
<td>myometrium</td>
<td>47.22 ± 18.09</td>
<td>66.41 ± 20.18</td>
<td>79.58 ± 14.71</td>
<td>86.3 ± 5.07</td>
<td><strong>0.0001</strong> *</td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epithelium</td>
<td>76.45 ± 16.76</td>
<td>83.49 ± 10.75</td>
<td>80.30 ± 9.85</td>
<td>78.42 ± 8.10</td>
<td>0.35</td>
</tr>
<tr>
<td>stroma</td>
<td>80.65 ± 13.86</td>
<td>69.31 ± 15.68</td>
<td>73.79 ± 14.88</td>
<td>69.32 ± 64.70</td>
<td><strong>0.023</strong> *</td>
</tr>
<tr>
<td>myometrium</td>
<td>73.76 ± 10.40</td>
<td>71.48 ± 14.08</td>
<td>77.81 ± 11.87</td>
<td>83.22 ± 64.70</td>
<td><strong>0.034</strong> *</td>
</tr>
<tr>
<td>AR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epithelium</td>
<td>7.99 ±10.78</td>
<td>26.05 ±17.49</td>
<td>29.35 ±16.14</td>
<td>33.06 ±13.30</td>
<td><strong>0.000001</strong> *</td>
</tr>
<tr>
<td>stroma</td>
<td>26.82 ±29.69</td>
<td>26.06 ±19.08</td>
<td>25.85 ±20.51</td>
<td>17.04 ±16.03</td>
<td>0.83</td>
</tr>
<tr>
<td>myometrium</td>
<td>12.10 ±14.17</td>
<td>23.20 ±15.06</td>
<td>37.53 ±13.55</td>
<td>36.14 ±13.65</td>
<td><strong>0.000001</strong> *</td>
</tr>
</tbody>
</table>

Kruskal-Wallis ANOVA test; ER-α - estrogen receptor α, PR - progesterone receptor, AR - androgen receptor, Group I — women up to 5 years after menopause; Group II — women 6 to 10 years after menopause; Group III — women more than 11 years after menopause; Group IV — women over 70 years of age; x — arithmetic mean; SD — standard deviation; * - statistically significant parameter; p — statistical significance, intergroup comparison in the analyzed part of the uterus (endometrium = epithelium + stroma), myometrium