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PROSPECTS FOR INTRODUCING MELATONIN INTO PRACTICE IN REPRODUCTIVE MEDICINE

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Key words: melatonin, external genital endometriosis, reactive oxygen species.
Summary: Melatonin reduces the severity of pain syndrome, helps to reduce the dose of analgesics used, and also improves the quality of sleep.

Introductions. All this time, in science and medicine, there has been a persistent interest of researchers in the study of the biological properties and therapeutic possibilities of melatonin. This shows the participation of melatonin as a regulator of various biochemical processes and reactions. The results of numerous studies show that the action of melatonin is much wider than it was commonly believed a few decades ago. However, the mechanisms that control the production of melatonin, as well as its direct effect on various organs and tissues, are still not fully understood [8, 14]. Melatonin (K-acetyl-5-methoxytryptamine) is an indolamine hormone synthesized by pinealocytes of the pineal gland directly from serotonin. In turn, serotonin, which is also a neurotransmitter and found in significant concentrations in the pineal gland, is synthesized from the essential amino acid tryptophan by its 5-hydroxylation and then decarboxylation of the resulting hydroxytryptophan. In the pineal gland, serotonin is acetylated by acetyltransferase and then methylated by oxindole-O-methyltransferase to form melatonin. It should be noted that it is oxindole-O-methyltransferase that is the main factor determining the production of melatonin and, in fact, limiting it [8, 9, 26]. It is known that the synthesis of melatonin depends on the illumination. Thus, low plasma melatonin concentrations are observed during daylight hours, which increase to peak values in the dark. This is due to the presence of numerous neural pathways connecting retinal ganglion cells and the pineal gland. Information from the retina is primarily transmitted to neurons in the suprachiasmatic nucleus of the hypothalamus, a region of the brain well known as the coordinating region of biological circoral and circadian responses. Further, the nerve fibers from the hypothalamus descend to the spinal cord, and then switch to the neurons of the upper cervical sympathetic ganglion, from which, as part of the postganglionic neurons, they rise back to the epiphysis. Thus, the pineal gland is similar to the adrenal medulla in that it converts signals from the sympathetic nervous system into a hormonal response [26]. In addition to the pineal gland, melatonin is also produced and deposited by tissues of the retina, lens, ovaries, bone marrow, enterochromaffin cells of the gastrointestinal tract, however, it is the pineal gland that creates the plasma concentration of melatonin, and the hormone contained in the cells of other various tissues and organs realizes its action exclusively locally

[26].Melatonin is known to perform many different functions. This hormone is a trigger for the sleep process and one of the key regulators of the natural sleep cycle. It regulates the circadian rhythm, maintained by various mechanisms of the biological clock in the suprachiasmatic nucleus of the brain. The neuroprotective and neuroregenerative effects of this hormone have been noted [26]. Melatonin both directly and indirectly exhibits antioxidant activity. The direct antioxidant effect is manifested due to the direct inactivation of free radicals, in particular hydroxyl radicals, formed during the life of the cell. The indirect effect is realized by stimulating the synthesis of antioxidant cell enzymes such as superoxide dismutase, glutathione peroxidase and glutathione reductase, as well as by increasing the total level of glutathione. Thus, as an antioxidant, melatonin protects nuclear DNA, membrane lipids, and cytosolic proteins from free radical oxidative damage [19, 21]. Literature data show that melatonin has antitumor activity [1, 10, 19]. It is now generally accepted that experimental interventions that activate the function of the pineal gland, or exogenous administration of melatonin, reduce the number of occurrence and development of tumors, while pinealectomy stimulates tumor growth [40]. In addition, the pineal gland hormone can have an oncostatic effect through its immunomodulatory effect [12], antioxidant effect, the ability to block the mitogenic effects of prolactin [2], as well as the ability to influence the synthesis and secretion of hormones that regulate reproductive function, in particular, by affecting on the hypothalamic-pituitary system [14, 16]. Thus, given the many diverse effects of melatonin, the possibility of therapeutic use of this hormone is of great interest to medicine. In particular, an urgent problem is to study the effect of melatonin on the pathogenesis of external genital endometriosis (EGE) and the possibility of its use in clinical practice in this disease. Genital endometriosis is an estrogen-dependent pathological process characterized by the implantation, growth, and development of tissue similar in structure to the endometrium, outside the boundaries of the normal localization of the uterine mucosa [3]. This disease occurs, according to various sources, in 10-15% of women of reproductive age and is one of the leading causes of pelvic pain, infertility and miscarriage [3]. The etiology and pathogenesis of EGE are still insufficiently studied, however, factors such as retrograde menstruation, hereditary predisposition, immune system dysfunction, exogenous and endogenous toxins are involved in the emergence and development of this pathological condition [18, 19]. It is known that endometriosis is a hormone-dependent disease characterized by dysregulation of the hypothalamic-pituitary-ovarian system, with the development of absolute and relative hyperestrogenemia [3, 5, 9]. In various mammalian species, melatonin is able to regulate reproductive function through the activation of receptors in the hypothalamic-pituitary region, which, as is known, controls the activity of the gonads through the production of gonadotropic hormones, as well as through the regulation of estrogen secretion by the ovaries [22, 23]. In addition, the presence of melatonin receptors in the cells of antral follicles and corpus luteum in rat ovaries [24] also suggests a direct regulatory effect of this hormone on ovarian function. The presence of melatonin receptors in the CNS and gonads, as well as

estrogen receptors in the pineal gland, reflect a complex two-way relationship between melatonin and estrogen secretion. In humans, the role of melatonin on ovarian function has not yet been sufficiently studied, and the most clear evidence of a direct relationship between melatonin levels and ovarian function follows from observations reflecting a violation of the normal secretion of this hormone in dysfunctions of the reproductive system, or, conversely, disorders of the reproductive system with changes in the secretion of the hormone of the pineal gland. Two forms of melatonin receptors are expressed in human luteocytes. In these cells, melatonin modulates the expression of LH and GnRH receptors. In an experiment with cultured human granulosa cells, melatonin reduces LH- and FSH-stimulated estradiol secretion, which indicates in favor of a possible suppression of ovarian function by this hormone [24]. However, according to some authors, melatonin has no effect on the synthesis and secretion of estradiol by granulosa cells [17, 18], and high concentrations of melatonin in the follicular fluid are not the result of an increase in the level of its local synthesis, but the result of active absorption of the hormone by follicular cells [22]. The fact of the antiestrogenic effect of melatonin, by blockade of cytosolic a-estrogen receptors, is proven. The mechanism of blockade of these receptors is associated with the inactivation of the intracellular messenger complex Ca2+/calmodulin, which normally contributes to the phosphorylation of the estrogen receptor, which facilitates the binding of the estrogen-receptor complex to the AP-1 promoter site of the transcriptional apparatus, as well as with a decrease in the concentration of cAMP (through receptors for melatonin MT1) and a simultaneous increase in the level of cGMP, which prevents the implementation of estrogen signaling on the transcriptional apparatus of the nucleus [22]. An important fact is the absence of blockade of other forms of estrogen receptors [22], which makes it possible to use melatonin for a long period without the danger of ovarian stimulation, in contrast, for example, to drugs from the aromatase inhibitor group, which therefore cannot be prescribed as monotherapy. In addition, some researchers noted a change in the activity of the aromatase enzyme responsible for the conversion of androgens to estrogens under the influence of melatonin, which requires additional studies [22, 25]. It has been noted that melatonin reduces the activity and expression of aromatase, sulfatase, and 17β-hydroxysteroid dehydrogenase and increases the activity and expression of estrogen sulfotransferase, and also directly interacts with estrogen receptors, being a selective estrogen receptor modulator [16, 39]. These facts allow us to speak about the antiestrogenic effect of melatonin, which may be a topic for further research in the field of melatonin use in the treatment of estrogen-dependent diseases, including endometriosis.Endometriosis is a multifactorial disease associated with the development of an estrogen-dependent inflammatory response [20]. Oxidative stress has been proposed as one of the important factors in the pathogenesis of the disease [22]. Oxidative stress inducers can be erythrocytes, endometrial cells undergoing apoptosis, and rejected menstrual blood endometrial cells. Activated macrophages trigger the mechanism of oxidative stress, as a result of which a chain of redox and free radical reactions is activated.

Results. The main active form of oxygen, the presence of which must be taken into account, is superoxide anion, mainly produced by mitochondria, hydrogen peroxide, formed from superoxide anion under the action of superoxide dismutase and destroyed by another enzyme, catalase, and peroxynitrite, formed by the interaction of two oxygen molecules with a nitric oxide molecule [30, 36]. It has been determined that reactive oxygen species directly damage cellular macromolecules such as proteins, lipids, and nucleic acids [45]. These processes are key factors in maintaining the inflammatory response, which is one of the main pathogenetic mechanisms for the development of endometriosis. Many studies have shown that the volume of peritoneal fluid in patients with EGE is increased, and its composition is changed [30]. An increase in the concentration of reactive oxygen species produced by macrophages [38], an increase in the oxidation of low-density lipoproteins [42], an increase in the content of the end product of lipid peroxidation - malondialdehyde, a decrease in the level of antioxidant enzymes, as well as lower levels of vitamin E in the peritoneal fluid in endometriosis compared with healthy women [30]. In addition to the direct damaging effect of reactive oxygen species on cells, they are also able to indirectly support the inflammation process by stimulating the release of various cytokines and inflammatory mediators by macrophages, such as interleukin-1, interleukin-6, tumor necrosis factor-a (TOT-a) [36]. An increase in the peritoneal fluid of patients with EGE was noted in the levels of interleukin-f, interleukin-6, interleukin-8, TNF-a, a factor that inhibits the migration of macrophages and monocyte chemoattractant protein-1 (MCP-1), RANTES (a chemokine that regulates activation, expression and secretion of normal T-cells), MU (monokine induced by interferon-y), as well as some other cytokines [4, 50]. Interleukin-1 and TNF-a are the main pro-inflammatory cytokines that increase the expression of many other chemokines, such as interleukins-6, -8, -18, and stimulate angiogenesis processes in endometrioid heterotopias [50]. A relationship has been established between an increase in the level of cytokines, in particular interleukin-1, and the severity of chronic pelvic pain in endometriosis [7]. However, the direct effect of interleukin-1 on nerve endings and its impact on the occurrence and nature of pelvic pain associated with endometriosis remains to be clarified [50]. Interleukin-6, together with interferon-y, is involved in stimulating the production of various growth factors, as well as in the process of adhesion of ectopic endometrial cells [50]. Interleukin-8 is a powerful leukocyte chemoattractant, angiogenesis stimulator, and a potential autocrine growth factor that promotes the proliferation of endometrioid stromal cells [50]. Thus, endometriosis can be considered an autoimmune disease due to a local increase in the level of some pro-inflammatory cytokines, as well as an increased level of production of autoantibodies (anti-endometrial, etc.) and disorders of local and systemic cell-mediated immunity [50]. However, it is not known whether the processes of inflammation in the pelvis and dysfunction of the immune system can be the cause or trigger mechanism that contributes to the growth of the endometrium in ectopic foci outside the uterus [50]. Melatonin, having a pronounced antioxidant effect, is able to inactivate reactive oxygen

species, increase and stimulate the activity of antioxidant enzymes such as superoxide dismutase and catalase, thus preventing the formation of free radicals and the development of oxidative stress [9]. This is evidenced by a significant decrease in the production of reactive oxygen species, products of lipid peroxidation, in particular malondialdehyde, an increase in the antioxidant potential of cells against the background of melatonin [34]. Inhibition of the oxidative stress process leads to the extinction of the inflammatory response and, therefore, prevents the development of endometriosis. This is confirmed by the regression of endometriotic foci in rats with experimentally modeled endometriosis in the presence of melatonin [36]. In a 2009 study, there was a greater regression of surgically induced endometrial lesions in a rat model in the melatonin group compared to the third-generation aromatase inhibitor letrozole group. In addition, after drug discontinuation, the relapse rate in the melatonin group was statistically lower than in the letrozole group [43]. Some researchers [13] note a significantly higher level of cyclooxygenase-2 (COX-2) activity found in ectopic endometriotic lesions compared to normal endometrium [13]. A decrease in the activity of this enzyme is one of the therapeutic targets in the treatment of endometriosis [37] Melatonin is a selective inhibitor of COX-2 (in contrast to the non-selective COX inhibitors that are actively used in most cases, such as most NSAIDs), inhibits the production of prostaglandins, and thus has an anti-inflammatory effect [37]. In addition, there is indirect evidence that melatonin inhibits the production of cell adhesion molecules that promote adherence of leukocytes to the endothelial cell, thereby preventing the development of a local inflammatory response and the progression of endometriosis [35].

Melatonin can lead to regression of endometrioid ectopic foci, exerting an inhibitory effect on the activity of matrix metalloproteases, as well as to triggering the process of apoptosis in endometrioid cells [44, 46]. Matrix metaloproteinases are zinc-dependent endopeptidases involved in the destruction of the extracellular matrix, ensuring the invasion of endometrial cells in the ectopic region, and the autocrine type of regulation of apoptosis [5, 44]. Several metalloproteinases, such as matrix metal prostheses -1, -2, -3, -7, -9, have attracted particular attention as key players in the pathogenesis of endometriosis. Matrix metalloprotease-9 (MMP-9) deserves special mention, whose role in the process of invasion of endometrioid cells has been confirmed by numerous studies [15, 32]. Another metalloproteinase, MMP-3, is the central link of the proteolytic system, since it has the ability to activate other metalloproteinases and is associated with various pathological processes [44]. The role of MMP-3 in the pathogenesis of endometriosis has not been sufficiently studied, however, there is already evidence of a high concentration of MMP-3 and the transcription factor AP-1 associated with it, as well as an increase in the expression of the proto-oncogenic c-Fos gene in the early stages of endometriosis development, in contrast to MMP -9, the concentration of which increases at later stages of the disease [44]. This fact suggests the importance of MMP-3 as one of the triggers for the process of invasion of endometrioid cells, remodeling of endometrioid foci, and activation of other matrix

metalloproteinases, such as, for example, proMMP-9. Against the background of the use of melatonin, a significant decrease in the expression of c-Fos, a decrease in the DNA-binding activity of AP-1, and a decrease in the activity of both MMP-9 and MMP-3 are observed [44]. At the same time, in the foci of endometrioid heterotopias, an increase in the expression of the tissue inhibitor MMP-3 (TIMP-3) is observed, which correlates with a decrease in the concentration of TNF-a. According to studies [6, 11], TIMMP-3 is one of the factors involved in the initiation of the apoptosis process through the classical pathway of caspase-3 activation, independent of the Fas-L-mediated pathway [44]. It is assumed that melatonin exerts its therapeutic effect, which manifests itself in the form of regression of endometriotic foci, by enhancing apoptosis through the classical mitochondrial pathway, which is associated with a decrease in the expression of anti-apoptotic proteins Bc1-2 and a simultaneous increase in the expression of pro-apoptotic proteins Bax and caspase-9 [44] . This mitochondrial pathway of apoptosis in the absence of melatonin is insufficient in endometriosis foci, which leads to delayed regression of endometrioid foci [44].

Discussion. A study conducted in 2013 [31] shows the effectiveness of melatonin as a drug that reduces the intensity of chronic pelvic pain in patients with endometriosis. Melatonin reduces the severity of pain syndrome, helps to reduce the dose of analgesics used, and also improves the quality of sleep [31]. There is an assumption that melatonin reduces the secretion of brain-derived neurotrophic factor (NMF) [31]. NMF is a neurotransmitter of hyperalgesia and a central sensitizer of the spinal cord to pain signals. It is known that the concentration of this factor increases under the influence of estrogens, which is observed in endometriosis. It is this process, an increase in the concentration of NMF against the background of hyperestrogenemia, that is one of the important links in the pathogenesis of chronic pelvic pain in patients with EGE. The mechanism of the effect of melatonin on the neurotrophic factor has not been studied, but it is obvious that it is not associated with the blockade of the formation of prostaglandins by melatonin and its anti-inflammatory effect. In addition, melatonin reduces the activity of local nerve growth factors, which prevents the growth of C-nociceptive nerve fibers in the focus of endometriosis and also has an effect on reducing the intensity of pelvic pain [12].Suppression of tumor neoangiogenesis was revealed against the background of the use of melatonin [27]. This effect is realized through direct suppression of angiogenesis under the action of melatonin, and by suppressing the production of tissue factors that initiate and implement the processes of cell proliferation [27]. The direct antiproliferative effect is due to an increase in the activity of intracellular p53 and Bax proteins, with a simultaneous decrease in the Bcl-2 protein [17]. Against the background of the use of melatonin, a decrease in the concentration of vascular endothelial growth factor - VEGF [28] is observed, both directly and due to a decrease in the production of a hypoxia-induced factor - HIV [27].

Conclusion. Thus, having antiestrogenic, anti-inflammatory, antioxidant and analgesic effects, melatonin can be considered as one of the drugs for the treatment

of external genital endometriosis. Further experimental and clinical studies will help to study in more detail the mechanism of the effect of this hormone on the development or regression of endometrioid foci, determine the indications, effective doses and duration of therapy, and also answer the question of the effectiveness of melatonin as a pathogenetically substantiated method of treating endometriosis.

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