# The Effect of Melatonin on Endometrial Histology in Patients with Endometrial Proliferative Disorders: A Double-Blind Randomized Clinical Trial

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**Abstract**

**Introduction:** Endometrial hyperplasia represents a spectrum of morphological and biological changes in the endometrial glands and stroma, ranging from an exaggerated physiological state to carcinoma. In recent years, numerous laboratory studies have evaluated the role of melatonin in regulating endometrial cell proliferation. Emerging evidence indicates an inhibitory role of melatonin administration in various gynecological cancers. Therefore, the present study aims to investigate melatonin's effect on the endometrium's histology in patients with endometrial proliferative disorders.

**Method:** This double-blind, randomized clinical trial was conducted at the Al-Zahra Hospital in Tabriz university of medicail scinsses. The patients with complaints of abnormal vaginal bleeding and with diagnosis of endometrial proliferative disorders or non-atypical hyperplasia were included .The patients were randomly assigned to control and intervention group. The control group received 80 mg Megestrol acetate daily, the intervention group received 80 mg Megestrol acetate daily plus 5 mg of melatonin before bedtime for three months. Both groups were homogenized regarding demographic information, including age, gravidity, parity, and body mass index. The patients were followed up for three months, and after completing of treatment, an endometrial biopsy was performed. The endometrial histology was compared between two groups after three months.

**Results:** Out of the 40 enrolled patients in the study, 37 were included in the final analysis. 18 out of 19 patients (94.7%) in the intervention group and 12 out of 18 patients (66.6%) in the control group had normal biopsy results after treatment . This difference was statistically significant ($P<0.05$).

**Conclusion:** Adding melatonin to progestin for treatment of endometrial hyperplasia can enhance the treatment response in women with this condition and lead to a higher rate of treatment.

**Keywords:** disordered proliferative endometrium, endometrial cancer, melatonin, non-atypical endometrium hyperplasia, megestrol acetate

## Introduction

The endometrium undergoes morphological and functional changes during the menstrual cycle under the influence of steroid hormones, including estrogen and progesterone, to prepare for the implantation of an embryo (1). The endometrium returns to a new cycle through cellular apoptosis if embryo implantation does not occur. In contrast, if embryo implantation does occur, the endometrium undergoes further decidualization (2). Endometrial stromal cells (ESCs) are key cellular structures that contain estrogen and progesterone receptors, and they undergo morphological and structural changes under the influence of steroid hormones (3). Additionally, ESCs are involved in processes such as embryo recognition and selection, undergoing proliferation, migration, and cellular differentiation (4). Therefore, the normal functioning of ESCs is essential for proper implantation and successful pregnancy. The function of the endometrium is regulated by various factors, including hormones, circadian rhythms, and other factors (5).

Endometrial malignancies are among the most common gynecological cancers in the United States. According to recent epidemiological studies, they are the fourth most common cancer in women in the United States, with nearly 63,000 confirmed cases and 11,000 suspected cases reported in 2018 (6). The incidence of endometrial hyperplasia is approximately three times that of endometrial cancer. Studies have also indicated that endometrial hyperplasia is a precursor to endometrial cancer, highlighting the importance of prevention to avoid progression to cancer (6). Therefore, to reduce the incidence of endometrial malignancies, it is crucial to diagnose and treat endometrial hyperplasia in asymptomatic women accurately. Risk factors for endometrial cancer and hyperplasia include genetic factors, diabetes, obesity, hypertension, oligomenorrhea, tamoxifen therapy, physical inactivity, specific dietary habits, hormone therapy, and nulliparity (7-10). However, endometrial cancer may also occur in the absence of these underlying factors. In postmenopausal women, factors such as parity, high BMI, fibroids, tamoxifen use, uterine volume, ovarian volume, and estradiol levels influence endometrial thickness (11). Unlike cervical cancer, endometrial cancer is not associated with a history of sexual activity. Due to the earlier detection of endometrial cancer (often prompted by abnormal bleeding), its overall prognosis is generally better than that of other major gynecological cancers (12).

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone that regulates the circadian, synthesized and secreted by the pineal gland, playing a key role in maintaining the body's circadian rhythm (16). Evidence also suggests that melatonin possesses antioxidant, anti-aging, and immune-regulation properties (17, 18). Recent research has increasingly focused on the role of melatonin in fertility and cancer control. New findings indicate a connection between melatonin and various physiological and pathological processes in the human body. Researchers today consider melatonin a hormone and compound with multiple functions, including cellular protection (19), immunomodulation, oxidative processes, and hematopoiesis (20). Furthermore, studies have demonstrated that melatonin has significant oncostatic properties exerted through receptor-dependent and receptor-independent mechanisms (21). Melatonin receptors, including MT1 and MT2, belong to the G protein-coupled receptor (GPCR) family (22). For instance, these receptors inhibit adenylate cyclase and cAMP, reducing linoleic acid uptake. The inhibition of linoleic acid uptake by melatonin is a mechanism underlying melatonin's antiproliferative effects (23). On the other hand, receptor-independent mechanisms include antioxidant activity, regulation of apoptosis, tumor metabolism, cancer immunity, inhibition of angiogenesis and cell migration, and suppression of circadian disruption (21, 24, 25). Recent studies have also reported that melatonin can be used as an adjuvant in cancer treatment, enhancing the effectiveness of drugs and reducing the side effects of chemotherapy or radiotherapy (26).

Based on a literature review, numerous laboratory studies have evaluated the role of melatonin in regulating endometrial cell proliferation, with results indicating a positive therapeutic effect. Additionally, evidence suggests that melatonin is inhibitory in various gynecological cancers and can reduce treatment-related side effects when administered alongside chemotherapy drugs. However, studies involving human samples to assess the use of melatonin for endometrial proliferative disorders are poorly understood. Therefore, the present study aims to investigate melatonin's effect on endometrial histology in patients with endometrial proliferative disorders.

## Method

### *Study Design and Setting*

This study is a double-blind, randomized clinical trial. The target population includes all women aged 38 to 55 with abnormal uterine bleeding with diagnosis of proliferative endometrial

disorders, or non-atypical hyperplasia who were visited in oncological clinic at Al-Zahra Educational and Medical Center in 2023.

*Participants and Study Groups*

Participants were randomly divided into a control group and an intervention group. The control group received routine treatment with Megestrol acetate, while the intervention group received Megestrol acetate plus melatonin. Megestrol acetate was administered at a daily dose of 80 mg for three months. Patients were excluded from the study if they experienced bleeding, required a change in medication dosage, or needed a repeat biopsy. Melatonin was prescribed at a daily dose of 5 mg (manufactured by Jalinoos Pharmaceuticals, Iran) to be taken before bedtime. Patients were followed up for three months, and approximately two weeks after completing the three-month treatment, the endometrial biopsy was repeated. Demographic information was recorded, including age, gravidity, parity, cancer history, history of oral contraceptive pills (OCP) use, and hormone therapy. Patients who had received OCPs or hormone therapy (estrogen and progesterone compounds) in the past three months were not included in the study. The two groups were homogenized based on age, gravidity, parity, and BMI, and their reports of the treatment results were used for analysis.

**Inclusion Criteria**

1. Age between 38 and 55 years with a history of abnormal uterine bleeding
2. Diagnosis of proliferative endometrial disorders or non atypical hyperplasia in endometrial biopsy samples
3. No history of hormonal treatment (estrogen and progesterone) or OCPs use in the past three months
4. Consent to participate in the study
5. BMI between 18.5 and 29.99

**Exclusion Criteria**

1. Diagnosis of endometrial cancer
2. History of hormone therapy in the past three months
3. Refusal to participate in the study
4. Diabetes

5. History of depression and use of antidepressant medications
6. Coagulation disorders
7. Hypertension
8. Seizures and use of antiepileptic drugs
9. Autoimmune diseases
10. Diagnosis of atypical hyperplasia, EIN, or endometrial cancer

The sample size was determined based on the study by Schwertner et al. (2013) and considering the results of our pilot study. For the variable "worst pain during the last 24 hours (daily)," the mean ± standard deviation was 2.78 ± 1.35 for the intervention group and 4.58 ± 1.46 for the control group. Using G Power software, with a maximum alpha error of 5% and a power of 80%, the sample size calculated for each group was nine, totaling 18 patients. To enhance the precision of the study, a final total of 40 patients was selected (20 patients per group).

*Randomization and Blinding*

In this study, simple randomization was performed using a computer. A list of numbers from 1 to 40 was generated and then randomly divided. Each patient was assigned one of these numbers based on the time of patient visits. According to the created list and the even-odd sequence, patients were allocated to either the intervention or control groups. The intervention group was assigned even numbers, while the control group received odd numbers. A sampling of participants was done convenient sampling from patients who visited in Al-Zahra Medical Center with complaints of abnormal uterine bleeding and had undergone either diagnostic curettage or pipeline biopsy. patients with diagnosis of proliferative endometrial disorders or atypical hyperplasia were included.

Due to the intervention's open-label design, blinding the clinicians who administered the treatments was not feasible. However, pathologist and the statistical analyst were blinded to the group assignments (double-blinding).

*Measurements*

In this double-blind, randomized clinical trial, patients with menorrhagia who were visited at the outpatient clinics of Al-Zahra Hospital in Tabriz university of medical siencess were enrolled

based on the inclusion and exclusion criteria. After clinical suspicion of proliferative endometrial disorders, endometrial biopsies were performed on the patients. A pathologist at Al-Zahra Medical Center examined all samples. With the diagnosis of proliferative endometrial disorders or atypical hyperplasia confirmed, patients were referred to the researcher at the treating physician's discretion. After explaining the study, including its objectives, potential side effects, and benefits of participation, patients were reminded that participation was voluntary and were asked to complete an informed consent form to join the study.

Demographic information of the patients, including age, gravidity, parity, history of cancer, history of OCP use, and hormone therapy, was recorded. Patients who had received OCPs or hormonal treatments (estrogen and progesterone compounds) in the past three months were excluded from the study. The two groups were homogenized in terms of age, gravidity, parity, and BMI.

The control group received routine treatment with 80 mg of megestrol acetate daily. The intervention group received 80 mg of megestrol acetate daily and 5 mg of melatonin before bedtime for three months. During this period, patients were monitored, and after completing the treatment, endometrial biopsies were performed. The endometrial tissues of both groups were compared after three months.

*Outcomes*

After treatment, patients were assessed for the extent of proliferative endometrial disorders and endometrial hyperplasia regression. The study compared the degree of disease regression between the intervention and control groups and the incidence of medication-related side effects in both groups.

**Statistical Analysis**

The data were analyzed using SPSS version 22. The Shapiro-Wilk test was used to assess data normality. If the data were normally distributed, the independent T-test was used to compare quantitative variables between the two groups; otherwise, the Mann-Whitney U test was applied. The chi-square test was utilized to compare categorical variables. Fisher's exact test was used if the expected frequency in more than 80% of cells was less than 5. A significance level of less than 5% was considered for all tests.

## Results

In this study, 40 women aged 38 to 55 years with a history of abnormal uterine bleeding and a diagnosis of proliferative endometrial disorders or atypical hyperplasia, as confirmed by endometrial biopsy, were recruited from the outpatient clinic of Al-Zahra Educational and Medical Center in Tabriz university of medical scinsess. These participants were randomly assigned to either the intervention or control groups.

Of the 40 enrolled patients, 37 were included in the final analysis. Two patients, one from the intervention group and one from the control group, discontinued treatment due to continued severe bleeding and sought alternative therapies. Additionally, one patient from the control group stopped treatment due to intolerance to the medication. Consequently, the final analysis included 19 patients in the intervention group and 18 in the control group. The patient flow throughout the study is illustrated in the flow diagram shown in Figure 1.

**Figure 1: CONSORT Flow Diagram of Patient Recruitment in the Study**

| | | |
|---|---|---|
| **Patient recruitment** | Checking inclusion and exclusion criteria<br>Randomization: n=40 | |
| **Allocation to groups** | Megestrol acetate (control group): n=20 | Megestrol acetate combined with melatonin (intervention group): n=20 |
| **Follow up** | Continued bleeding n=1<br>Drug intolerance n=1 | Continued bleeding n=1 |
| **Statistical analysis** | Statistical analysis n=18 | Statistical analysis n=19 |

Table 1 shows the demographic and obstetric characteristics of patients with proliferative endometrial disorders, categorized by the control and intervention groups before the intervention. The mean age and body mass index (BMI) were reported as 43 years and 27.8, respectively, for both the intervention and control groups ($p>0.05$). The groups had no significant differences in obstetric characteristics, such as gravidity and parity ($p>0.05$).

**Table 1: Demographic and Obstetric Characteristics of Patients by Group (Pre-Intervention)**

| **Variables** | **Groups** | | **Significance Level** |
|---|---|---|---|
| | **Intervention n=20** | **Control n=20** | |

| | SD | Mean | SD | Mean | |
|---|---|---|---|---|---|
| **Age (years)** | 4.6 | 43.15 | 4 | 43.45 | 0.827 |
| **Body Mass Index (kg/m²)** | 2.3 | 27.93 | 2.9 | 27.8 | 0.850 |
| **Gavida** | 1.5 | 2.45 | 1.6 | 2.95 | 0.310 |
| **Parity** | 1.3 | 2 | 1.25 | 2.25 | 0.539 |

Table 2 shows the biopsy results of patients in both groups before the therapeutic interventions. The biopsy results showed no significant difference between the two groups regarding the type of biopsy findings, and the patients were evenly distributed between the two groups ($p>0.05$). Additionally, none of the patients in either group had a history of OCP use, hormone therapy, cancer, or underlying conditions such as hypertension and diabetes.

**Table 2: Biopsy Results and Clinical History of Patients Before the Intervention**

| **Variable** | | **Groups** | | **Significance level** |
|---|---|---|---|---|
| | | **Intervention n=20** | **Control n=20** | |
| **Biopsy Results Before Intervention** | Disordered proliferative endometrium | 18 | 18 | 0.988 |
| | Endometrial hyperplasia without atypia | 2 | 2 | |

Table 3 shows the biopsy results three months after treatment, broken down by the intervention and control groups. Endometrial regression to a normal or atrophic state was considered a response to treatment, while disease progression or no change in the pathological result of the endometrium was considered a lack of response to treatment. Overall, 18 out of 19 patients (94.7%) in the intervention group had a normal biopsy result, whereas in the control group, 12 out of 18 patients (66.6%) had a normal biopsy result. This difference was statistically significant ($p=0.037$).

However, no significant difference was observed between the two groups regarding the type of biopsy results. Table 2 details the frequency of each type of biopsy result by study groups.

| Table 3: Biopsy Results Three Months After the Intervention by Study Groups | | | | |
|---|---|---|---|---|
| **Variable** | | **Groups** | | **Significance level** |
| | | **Intervention n=19** | **Control n=18** | |
| **Biopsy Result after Intervention** | **Normal** | 18 | 12 | 0.037* |
| | **Abnormal** | 1 | 6 | |
| **Biopsy Result After Intervention (by Type)** | Pill effect | 12 | 10 | 0.248 |
| | Weakly proliferative endometrium | 1 | 0 | |
| | Disordered proliferative endometrium | 1 | 3 | |
| | Anovulatory endometrium | 1 | 0 | |
| | Inactive endometrium | 3 | 1 | |
| | Late secretory endometrium | 1 | 1 | |
| | Hyperplastic-type endometrial polyp | 0 | 2 | |
| | Fragments of endometrial polyp | 0 | 1 | |

Table 4 shows the frequency of complications observed in patients after therapeutic interventions, broken down by study groups. Regarding complications, a higher frequency of spotting, weight gain, and increased appetite was observed in the intervention group, and these differences were statistically significant ($p<0.05$). However, for other complications, details provided in Table 4, no significant differences were found between the two groups. Additionally, none of the study groups reported complications such as sexual dysfunction, diarrhea, anemia, thrombophlebitis, pulmonary embolism, deep vein thrombosis (DVT), decreased consciousness, daytime fatigue, dizziness, or headache.

| Table 4: Comparison of Post-Treatment Complications by Study Groups | | | |
|---|---|---|---|
| **Variable** | **Groups** | | **Significance level** |
| | **Intervention n=20** | **Control n=20** | |

| | | | | |
|---|---|---|---|---|
| **Insomnia** | Yes | 1 | 0 | 0.999 |
| | No | 19 | 20 | |
| **Mood Swings** | Yes | 2 | 0 | 0.487 |
| | No | 18 | 20 | |
| **Rash** | Yes | 1 | 0 | 0.500 |
| | No | 19 | 20 | |
| **Sweating** | Yes | 2 | 2 | 0.999 |
| | No | 18 | 18 | |
| **Amenorrhea** | Yes | 2 | 0 | 0.487 |
| | No | 18 | 20 | |
| **Spotting** | Yes | 6 | 0 | 0.020 |
| | No | 14 | 20 | |
| **Nausea** | Yes | 1 | 0 | 0.500 |
| | No | 18 | 20 | |
| **Hot Flashes** | Yes | 1 | 1 | 0.999 |
| | No | 19 | 19 | |
| **Weight Gain** | Yes | 8 | 2 | 0.032 |
| | No | 12 | 18 | |
| **Increased Appetite** | Yes | 6 | 1 | 0.046 |
| | No | 14 | 19 | |
| **Depression** | Yes | 1 | 0 | 0.500 |
| | No | 19 | 20 | |
| **Drowsiness** | Yes | 2 | 0 | 0.487 |
| | No | 18 | 20 | |

## Discussion

Endometrial hyperplasia is a condition characterized by the excessive proliferation of cells in the endometrium, which is the inner lining of the uterus. Most cases of endometrial hyperplasia are caused by high levels of estrogen combined with insufficient levels of the hormone progesterone, which normally counteracts the proliferative effects of estrogen on this tissue. Endometrial hyperplasia can occur due to various factors, including obesity, polycystic ovary syndrome

(PCOS), estrogen-producing tumors like granulosa cell tumors, and certain forms of estrogen replacement therapy.

Oral progestins have been the mainstay of conservative management for endometrial hyperplasia. In cases where oral progestins are not tolerated, intrauterine progesterone treatment is a valid alternative. GnRH analogs, metformin, and hysteroscopic resection, when combined with progestins, appear to enhance the overall effectiveness of the treatment. Alternatively, maintenance therapy with careful monitoring has been suggested to reduce the risk of recurrence. Well-designed and homogeneous studies are essential for standardizing and identifying the best treatment protocols.

The present study showed that 40 women aged 38 to 55 with a history of abnormal bleeding and diagnosed with proliferative endometrial disorders or non atypical hyperplasia were treated at the Al-Zahra Educational and Therapeutic Center in Tabriz univrsty of medical scincess with two different treatment regimens. It was also demonstrated that melatonin administration with megestrol acetate was superior to megestrol acetate for treating proliferative endometrial disorders or nonatypical hyperplasia. Overall, 18 out of 19 patients (94.7%) in the intervention group had a normal biopsy result, whereas in the control group, 12 out of 18 patients (66.6%) had a normal biopsy result. This difference was statistically significant ($p=0.037$).

Based on the literature review, no study has yet been conducted on human samples to investigate the effects of melatonin on proliferative endometrial disorders.

In 2020, Mustafa Can Sivas et al. investigated the effect of melatonin on estradiol-induced uterine hypertrophy/hyperplasia in Wistar albino rats. The study demonstrated a significant reduction in the height of epithelial cells compared to the control group (31).

In a study conducted in 2019 by Mosher et al. on melatonin activity and receptor expression in endometrial tissue and endometriosis, 20 patients with confirmed endometriosis, 11 patients with peritoneal lesions, and 15 patients without surgical evidence of endometriosis (as a control group) were included. The expression of melatonin receptors was examined. The study investigated the status of MR1A and MR1B receptors in biopsies from epithelial gland cells of the endometrium in women with and without endometriosis. The results showed that both receptors were present in both eutopic and ectopic endometrial tissues. The mRNA expression

for MR1A and MR1B receptors was significantly higher in peritoneal lesions than in endometrial tissue. Additionally, melatonin administration at concentrations (0.1 nanomolar - 0.1 micromolar) inhibited estrogen-induced proliferation of endometrial epithelial cells within 48 hours of cell culture (14).

Lin et al. (2020) investigated the inhibition of cellular proliferation in uterine leiomyomas through melatonin-induced activation of cell death pathways. They found that melatonin treatment increased the sub-G1 phase and DNA condensation in ELT3 cells (uterine leiomyoma cells). The study also observed melatonin-induced apoptosis and autophagy in ELT3 cells. Additionally, melatonin reduced cellular proliferation in ELT3 cells by activating MT1 and MT2 receptors, which resulted in the down-regulation of the Akt-ERK1/2-NFkB signaling pathway (13).

Dastaranj Tabrizi (2014) compared the anti-estrogenic effect of metformin on endometrial histology with that of progesterone. Their findings showed that metformin could induce endometrial atrophy in 21 out of 22 patients (95.5%), whereas this positive response was achieved in only 13 out of 21 patients (61.9%) in the megestrol group (32).

In an observational study by Emrah (2012), the effectiveness, safety, and acceptability of cyclic versus continuous administration of medroxyprogesterone acetate (MPA) for treating atypical endometrial hyperplasia (EH) were evaluated. Forty patients received MPA cyclically for 12 days in each menstrual cycle, while another 40 patients received MPA daily for six months. Regression of endometrial hyperplasia was 90% in the cyclic MPA group compared to 82.5% in the continuous MPA group, with no significant difference between the two groups in this regard (33).

In a 2022 systematic review, Al-Sal et al. compared the effectiveness of the levonorgestrel-releasing intrauterine device (LNG-IUD) with systemic progestins. The rate of endometrial hyperplasia regression was 91.3% with LNG-IUD versus 68.6% with systemic progestins (34). However, LNG-IUD is considered an invasive method and may not be tolerated by some patients.

Although some studies have reported that systemic progestin administration is effective in treating endometrial hyperplasia with efficacy rates above 80%, most previous studies have

reported this rate to be between 60% and 70% (32, 33, 34). This discrepancy highlights the need for new medications and more effective treatments for this condition.

Melatonin, with its antioxidant activity, regulation of apoptosis, tumor metabolism, cancer immunity, inhibition of angiogenesis and cell migration, and suppression of circadian disorders and antiproliferative effects (21, 23, 24, 25), opens a new therapeutic window for various health conditions in women. Research on melatonin in this field is increasing. This study, by examining the impact of melatonin on proliferative endometrial disorders and demonstrating its efficacy, introduces a new treatment option to gynecologists and draws attention to the potential of melatonin.

**Limitations**

Future studies should use larger sample sizes and longer follow-up periods. Few studies have been conducted in this area for comparison, so similar studies with more patients and comparisons with other methods are advised to reach a comprehensive conclusion and suitable protocol. In addition, it is advisable for researchers to further analyze the potential effects of melatonin in alternative anti-cancer therapies, its application in the treatment of endometrial hyperplasia, and the consequences of administering higher doses.

**Conclusion**

This study indicated that adding melatonin to the standard treatment for endometrial hyperplasia with progestins can enhance the treatment response in women, leading to a higher rate of endometrial tissue returning to a normal state in more individuals.

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**Ethical Considerations**

All ethical considerations for observational studies, including the ethical use of documents and scientific evidence in executing this thesis, were adhered to. No disclosures that could jeopardize the intellectual property rights of the participants were present in this study. All patients were fully informed about the study by reviewing and approving the informed consent form, and the

patients incurred no additional costs. All aspects of the Helsinki Declaration regarding the findings' conduct, collection, interpretation, and publication were considered. Additionally, the proposal for this study was approved by the Regional Ethics Committee with the code IR.TBZMED.REC.1402.060 registered with the Clinical Trials Center of Iran under the code IRCT20120922010901N10.

**Trial registration:** The study protocol was registered and confirmed in the Iranian Registry of Clinical Trials under the number (IRCT20120922010901N10).

**Registration date:**1402/02/18 , 2023-05-08

**URL to registration on the trial registry website:**
https://irct.behdasht.gov.ir/user/trial/68200/view

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