FUNCTIONAL STATE OF THE PINEAL GLAND IN WOMEN WITH THREATENED ABORTION IN THE FIRST TRIMESTER – FURTHER PROSPECTS OF PREGNANCY

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Summary
Melatonin is an important hormone for normal pregnancy development, but the influence of serotonin remains poorly understood. Low melatonin production can lead to spontaneous abortion by stimulating progesterone secretion and inhibiting prostaglandin synthesis. Melatonin has an effect on the induction and blocking of apoptosis of the villous cytotrophoblast and promotes the development of the syncytiotrophoblast. Analysis of the frequency of positive pregnancy showed a significant impact on the course of pregnancy and childbirth, compliance with sleep and rest also contribute to a positive effect, but there is no general concept, which makes the subject relevant.

The aim of the study. To evaluate the functional state of the pineal gland, in particular, the amount of melatonin and serotonin in the blood of pregnant women at 10-12 weeks of gestation with a threat of abortion, which was manifested by bloody discharge or formation of retrochorionic hematoma, and the consequences of pregnancy.

Materials and methods. Two groups were formed: I main group – 40 pregnant women with a threat of abortion and formation of retrochorionic hematoma, II control group – 44 healthy pregnant women. Obstetric examination, enzyme-linked immunosorbent assay for determination of melatonin, serotonin, chorionic gonadotropin, and ultrasound were performed.

Informed consent of pregnant women was obtained with adequate explanation of the purpose, objectives, methods and scope of laboratory and instrumental research methods.

The study protocol was approved by the Commission on Biomedical Ethics of the Bukovinian State Medical University, 2015 (minutes of the Commission meeting No 2 from the 19.10.2023 year). The study was conducted in accordance with the basic provisions of GCP (1996), the Convention of the Council of Europe on Human Rights and Biomedicine (April 4, 1997), the Declaration of Helsinki of the World Medical Association for the Ethical Principles of Scientific Medical Research Involving Human Subjects (1964-2008), the Order of the Ministry of Health of Ukraine 690 of September 23, 2009 (as amended by the Order of the Ministry of Health of Ukraine 523 of July 12, 2012).

Statistical analysis was performed using generally accepted methods of variance statistics. Reliability was assessed by Student’s t-test. Differences were considered significant if the significance level was p<0.05. We also performed correlation analysis in Microsoft Excel, using the method of squares (Pearson’s method) to determine the correlation coefficient.

The work is a fragment of the research work of the Department of Obstetrics and Gynaecology, UDC 618.1-053.34 + 618.1-0536 /8/. –07-084-08 «Prevention, diagnosis and treatment of disorders of the perinatal period and reproductive system of women and adolescent girls» (No. 201110H, state registration number 0111U006499. The term of implementation is 02.2011-12.2015).

Results of the study.
According to the obtained results, retrochorionic hematoma up to 1 cm is detected three times more often than over 1 cm, while the level of melatonin is not significantly lower, but with retrochorionic hematoma over 1 cm a significant decrease is observed in cases of further normal pregnancy and childbirth. With further complications of pregnancy in retrochoroidal hematoma up to 1 cm, there is a significant significant decrease in melatonin (p < 0.001), in contrast to retrochoroidal hematoma over 1 cm, where no significant difference was found in the complicated course of pregnancy (p > 0.05).

Serotonin levels were elevated in all subgroups regardless of hematoma size and subsequent pregnancy outcome.

Conclusions. In case of threatened abortion in the first trimester with formation of retrochorionic hematoma, the risk of complicated pregnancy remains in 60.87 % of cases. Determination of pineal hormones revealed a significant increase in serotonin with a simultaneous significant decrease in melatonin, while maintaining a positive relationship between the hormones, indicating a disruption of melatonin synthesis in the central nervous system. This is confirmed by the normal level of chorionic gonadotropin and the preservation of embryonic viability. Since the disruption of hormone formation in the pineal gland has been established, adherence to the recommendations for adequate rest may positively affect the further outcome of pregnancy and childbirth.

Key words: Melatonin; Serotonin; Retrochorionic Hematoma; Threatened Abortion

Introduction
The pineal gland (epiphysis) belongs to the midbrain and functions as an endocrine gland whose main functions are to regulate circadian (daily) biological rhythms, normalize immune responses, endocrine connections, metabolism, neutralize the effects of stress hormones, and adapt the body to changing light conditions that determine its activity. The main hormones of the pineal gland (PG) are melatonin (MT) and serotonin (ST).

It is believed that the cells of the pineal gland secrete ST during the day and begin to synthesize MT in the dark, while information about the level of external illumination is transmitted from the retina to the pineal gland via sympathetic fibers, which is important for the normal functioning of the gland.

MT, also known as 5-methoxy-N-acetyltryptamine, is a neuroendocrine hormone produced mainly in the pineal gland that is regulated by light levels and follows a circadian
an N-acetyl side chain, which determine its specificity and amphiphilicity. MT biosynthesis starts with its precursor tryptophan and consists of four enzymatic steps. In the biosynthesis of MT, serotonin N-acetyltransferase is the rate-limiting enzyme and ST is an important intermediate [3, 4, 5]. The half-life of MT in the circulation is usually short, ranging from 30 to 60 minutes [6]. It has amphiphilic properties that allow MT to easily diffuse and cross all membranes. Approximately 70 % of MT binds to albumin, and the remaining 30 % diffuses into surrounding tissues after release into the bloodstream [7]. Due to its multiple actions, MT acts as a circadian regulator, natural antioxidant, anti-inflammatory and anti-neoplastic agent, among many other functions [8, 9]. Regarding the anti-inflammatory and circadian regulatory functions, a study by Hobson et al. 2018 shows a correlation between the circadian clock and the immune system. It is emphasized that MT is powerful in terms of circadian regulation of lymphocyte proliferation, enhancing phagocytosis and stimulating cytokine production, which is essential for the normal development of pregnancy. Thus, in the context of obstetrics and fetal growth, MT is important for the prevention of preterm labor, gestational diabetes, and pre-eclampsia [10, 11, 12].

The majority of endogenous MT production occurs in the pineal gland, which is regulated by light levels. However, studies by McCarthy R, 2019 using human placenta and ovarian tissue report melatonin-synthesizing enzymes capable of converting ST to MT. Such non-pineal sources are not regulated by light and therefore do not show circadian fluctuations. However, no systematic evaluation of the metabolism of endogenous circulating MT during the course of pregnancy and identification of the placenta as a source of MT during pregnancy has been reported [26].

Low production of MT in the pineal gland may lead to spontaneous abortion in cases where uterine abnormalities and/or chromosomal abnormalities have been excluded. Recurrent pregnancy loss is thought to be associated with oxidative damage and immunologic imbalance; in this context, MT acts as a direct potent free radical scavenger with immunomodulatory effects. In addition, MT stimulates progesterone secretion and inhibits the synthesis of prostaglandins, which are potent inducers of miscarriage and preterm labor [13, 14, 15].

MT has an effect on inducing and blocking apoptosis of the villous cytotrophoblast, promotes the development of syncytiotrophoblast through paracrine, autocrine and/or intracrine action of MT1 and MT2 receptors in the placenta [16, 17, 18], which are used by the placenta to maintain the balance between villous cytotrophoblast and syncytiotrophoblast. This is how the placenta develops normally. The fusion of villous cytotrophoblasts forms a syncytiotrophoblast through a regulated MT process [19].

In addition, placental MT interacts with MT1 and MT2 receptors and reactive oxygen species to reduce oxidative damage to the placenta. Because MT protects the placenta from antioxidant effects, it has been recognized as a regulator of placental homeostasis [20]. In the first trimester of pregnancy, the MT1 receptor is more important in promoting synchronization of the villous cytotrophoblast by protecting trophoblastic cells from oxidative damage and promoting apoptosis in altered cells [21].

The involvement of ST in pregnancy is not well understood. ST is a phylogenetically ancient neurotransmitter that is widely distributed in key brain regions and influences emotional state, impulsivity, learning and memory, attention, sleep, aggression, and neurovegetative control [22]. It is also found in enterochromaffin cells of the intestine — apodocytes, a set of which is a special endocrine organ belonging to the APUD (Amine Precursor Uptake and Decarboxylation) system, blood platelets, mast cells of connective tissue, bronchial epithelium, ovaries, thyroid cells, which also belong to the APUD system [23]. It is believed that the threat of miscarriage increases the number of ST, which may lead to the induction of habitual abortions [24].

The evidence base for the effectiveness of MT and ST is currently being formed, however, an analysis of the frequency of positive pregnancy in patients showed a significant impact on the course of pregnancy and childbirth, the presence of complications in the case of synthesis disorders [25, 26]. Compliance with sleep and rest regimens also contributes to a positive effect.

The aim of this study was to assess the functional state of the gonads, in particular the amount of MT and ST in the blood of pregnant women at 10-12 weeks’ gestation with threatened abortion, manifested by bloody discharge or formation of retrochorionic hematoma (RCH), and the outcome of pregnancy.

Materials and methods. To achieve this goal, we formed two groups: I Main Group (MG) – 40 pregnant women with signs of threatened abortion, especially the presence of bloody discharge or RHG, II Control Group (CG) – 44 pregnant women without complications in the first trimester of pregnancy. As known from the studies of De Almeida Chuffa et al. 2019, the main functions of MT, such as decidualization and implantation, are important, which makes this period crucial for the normal development of pregnancy, so the results can be used to predict the further course of pregnancy and birth outcomes [7]. The two groups are representative in terms of age, lifestyle, and obstetric and gynecologic history.

In the course of the study, we used a general clinical examination (clarification of complaints, anamnesis, general somatic medical examination), obstetric examination, special research methods to determine the number of MT, ST, ultrasound to determine the condition of the fetus, the size of the RCH. Chorionic gonadotropin (hCG) measurement to determine the functional state of the chorion.

Preparation of patients to determine the amount of MT, ST, hCG according to the general rules. Time of sampling: 8.00-9.00 a.m., material: blood serum.

Melatonin and serotonin ELISA kits are designed for determination of MT and ST levels in blood of pregnant women. The principle of analysis is based on enzyme-linked immunoassorbent assay.
Determination of free beta-hCG is part of prenatal screening in the first trimester at 10-12 weeks of gestation, calculated by PRISCA or ASTRAIA programs. It is used for early confirmation of pregnancy and dynamic monitoring of its course, assessment of the risk of fetal anomalies and complicated pregnancy.

Considering that the determination of hCG, MT and ST in CG was performed at 10-12 weeks of pregnancy during the first prenatal screening, the results of the study were compared for data reliability with similar indicators of 23 pregnant women in MG who presented with complaints at 10-12 weeks of pregnancy.

Statistical analysis was performed using generally accepted methods of variance statistics. Reliability was assessed using Student’s t-test. Differences were considered significant at a significance level of p≤0.05. Correlation analysis was also performed in Microsoft Excel, using the method of squares (Pearson’s method) to determine the correlation coefficient.

Informed consent of pregnant women was obtained with adequate explanation of the purpose, objectives, methods and scope of laboratory and instrumental research methods.

The study protocol was approved by the Commission on Biomedical Ethics of the Bukovinian State Medical University, 2015 (minutes of the Commission meeting No 2 from the 19.10.2023 year). The study was conducted in accordance with the basic provisions of GCP (1996), the Convention of the Council of Europe on Human Rights and Biomedicine (April 4, 1997), the Declaration of Helsinki of the World Medical Association for the Ethical Principles of Scientific Medical Research Involving Human Subjects (1964-2008), the Order of the Ministry of Health of Ukraine 690 of September 23, 2009 (as amended by the Order of the Ministry of Health of Ukraine 523 of July 12, 2012). The work is a fragment of the research work «Prevention, diagnostics and treatment of perinatal and reproductive disorders of women and adolescent girls» (No. 2011I10H, state registration number 0111U006499).

### Results of the study and their discussion.

Determination of MT, ST was performed in pregnant from MG and CG. To determine the functional state of chorion, hCG was evaluated.

According to the studies of Ejaz, 2020 [9], placental synthesis of MT was established, so it is obvious that in case of impaired blood supply to the chorion, the level of hormones formed in it should decrease. However, the results obtained were somewhat different, indicating the possibility of compensatory extraplacental synthesis of MT.

The results of pregnancy and childbirth in both groups are not encouraging: we considered the CG as a group of healthy pregnant women whose first trimester of pregnancy was uneventful, but the results of further pregnancy and childbirth did not differ significantly between groups. In this regard, we formed subgroups according to the subsequent normal course of pregnancy and childbirth and the complicated course of pregnancy and childbirth. The complications are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Main Group (n=23)</th>
<th>Control Group (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal delivery</td>
<td>9 (39.13 %)</td>
<td>21 (47.72 %)</td>
</tr>
<tr>
<td>Premature rupture of the membranes</td>
<td>3 (13.04 %)</td>
<td>4 (9.09 %)</td>
</tr>
<tr>
<td>Abnormalities of labor activity</td>
<td>2 (8.7 %)</td>
<td>7 (15.91 %)</td>
</tr>
<tr>
<td>Polyhydramnios</td>
<td>1 (4.35 %)</td>
<td>1 (2.27 %)</td>
</tr>
<tr>
<td>Preterm labor</td>
<td>1 (4.35 %)</td>
<td>3 (6.82 %)</td>
</tr>
<tr>
<td>Premature detachment of the normal placenta</td>
<td>1 (4.35 %)</td>
<td>2 (4.55 %)</td>
</tr>
<tr>
<td>Placental dysfunction</td>
<td>3 (13.04 %)</td>
<td>-</td>
</tr>
<tr>
<td>Fetal growth retardation syndrome</td>
<td>1 (4.35 %)</td>
<td>-</td>
</tr>
<tr>
<td>Childbirth Distress</td>
<td>1 (4.35 %)</td>
<td>4 (9.09 %)</td>
</tr>
<tr>
<td>Gestosis</td>
<td>1 (4.35 %)</td>
<td>1 (2.27 %)</td>
</tr>
</tbody>
</table>

From the results obtained, it can be concluded that there is a slight difference in the number of complications between the two groups, except for placental dysfunction and fetal growth retardation syndrome, which were found only in MG, and gestosis, which was found twice as often in MG. However, obstructed labor and abnormal uterine activity were twice as common in CG. Thus, complications related to placental function were observed in MG, confirming the results of our study on the effect of MT on chorionic and placental development.

Pregnancy and delivery were completed by cesarean section in 12 cases of CG (27.3 %), half of which were emergency cesarean sections (50 %). The situation is similar in MG: 12 cases of cesarean section (30 %), but one case of emergency cesarean section (8.3 %). The main indications for cesarean section in both groups were uterine scarring, abnormal uterine contractions that could not be corrected by medication, and breech presentation. In both groups, cesarean section was performed with premature abruption of the normally positioned placenta. There were no significant differences in the number or outcome of operative deliveries.

As a result of the study, it was found that the level of MT and ST depends on the size of the RCH, so we calculated the PG hormones depending on the size of the RCH – up to 1 cm and more than 1 cm (Table 2).

To determine the reliability of the indicators, the results were compared with the CG, where pregnancy and delivery were normal.
According to the results obtained, the presence of RCH up to 1 cm was found to be three times more frequent, while the level of MT was not significantly lower compared to CG (p > 0.05), but in the case of RCH over 1 cm and dangerous for the embryo, a significant decrease was observed in the case of further normal pregnancy and delivery. With further pregnancy complications, a significant decrease in MT was observed for RCH up to 1 cm (p < 0.001), in contrast to RCH over 1 cm, where no significant difference was found for complicated pregnancies (p > 0.05).

### Table 2

**Comparative characteristics of melatonin (pg/ml) and serotonin (ng/ml) in the main and control groups with different sizes of retrochorionic hematoma in normal and complicated pregnancy and delivery**

<table>
<thead>
<tr>
<th></th>
<th>CG (n=44: –21 with normal course, –23 with complicated course)</th>
<th>MG RCH up to 1 cm (n=17: –6 with normal course, –11 with complicated course)</th>
<th>MG RCH over 1 cm (n=6: –3 with normal course, –3 with complicated course)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Melatonin</strong></td>
<td>-124.9 ±15.84, -123.7 ±14.29</td>
<td>-82.99±27.52, -68.7±7.1**</td>
<td>-86.35±7.55, -101.6 ±33.32</td>
</tr>
<tr>
<td><strong>Serotonin</strong></td>
<td>-354.4±61.57, -654±138.57*</td>
<td>-537.13±168.76, -690.03±105.2**</td>
<td>-783.89±160.93, -622.17±147.65*</td>
</tr>
</tbody>
</table>

Note* **p < 0.05$ **p < 0.001

In his study, McCarthy, 2019, argues that just as placental MT can cross into maternal blood, maternal plasma MT can cross into the placenta unchanged. In the placenta, MT is thought to protect the mononuclear villous cytotrophoblasts from apoptosis, allowing them to continuously regenerate and fuse with the syncytiotrophoblast layer, keeping it healthy. This layer is in direct contact with the mother’s blood and ensures the exchange of gases, nutrients and wastes. One possible explanation for the initial decrease in serum melatonin levels in the first trimester is that placental mitochondria and polymorphonuclear leukocytes generate large amounts of superoxide free radicals, which may lead to a temporary decrease in maternal serum melatonin levels to protect developing tissues from oxidative stress [22]. The results of our study partially confirmed the data of the study – in MG with RCH up to 1 cm with subsequent complicated pregnancy and delivery, the level of MT is significantly reduced, so the next task of our study was to determine ST, which is an intermediate hormone for the synthesis of MT.

The amount of ST, in contrast to melatonin, is increased in all subgroups, despite the fact that ST is an intermediate hormone in the synthesis of MT, so we determined the correlation in the subgroups. An almost twofold increase in ST in MG compared to CG, where pregnancy and labor were uncomplicated, was observed both at RCH over 1 cm and at RCH less than 1 cm. An increase in ST was also observed in CG where pregnancy and labor were complicated. The results of CG and ST were presented in Table 3.

### Table 3

**Results of correlation analysis of melatonin (pg/ml) and serotonin (ng/ml) in the main and control groups with different sizes of retrochorionic hematoma in normal and complicated pregnancy and delivery**

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>MG RCH up to 1 cm</th>
<th>MG RCH over 1 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>With normal course of pregnancy</td>
<td>R = –0.14</td>
<td>R = –0.05</td>
<td>R = 0.97</td>
</tr>
<tr>
<td>With complicated course of pregnancy</td>
<td>R = 0.39</td>
<td>R = –0.3</td>
<td>R = 0.77</td>
</tr>
</tbody>
</table>

Positive correlations were found in CG with complicated course of second half of pregnancy, delivery and strong positive correlations in case of RCH over 1 cm, regardless of further course of pregnancy. Therefore, we can conclude that MT synthesis in the CNS is impaired. Our opinion is confirmed by the results of hCG, which in pregnant women from MG was 53.63 ± 2.56 ng/ml and in CG – 45.85 ± 3.71 ng/ml. Thus, no significant difference was found (p=0.05) despite the presence of RCH. Also, no significant difference was found in the subsequent normal and complicated course of pregnancy.

Normal hCG levels may be evidence of chorionic function and pregnancy maintenance despite a significant decrease in MT. A significant increase in the level of ST and an insufficient level of MT may indicate a disturbance in the synthesis of MT of central genesis, while an increase in MT at an RCH over 1 cm may be due to an increase in synthesis in the CNS as a stress response to compensate and normalize the fetal state in extreme conditions of blood supply disruption for further pregnancy development. The results obtained are important for the development of recommendations for the observance of sleep and rest regimens for the full formation of MT in the CNS.

### Conclusions.

1. In the case of threatened abortion in the first trimester with the formation of retrochorionic hematoma, the risk of complicated pregnancy remains in 60.87 % of cases.

2. Determination of pineal hormones revealed a significant increase in serotonin with a simultaneous significant decrease in melatonin with a positive relationship between the hormones, indicating a disorder
of melatonin synthesis in the central nervous system. This is confirmed by the normal level of chorionic gonadotropin and the preservation of embryonic viability.

3. Since the formation of hormones in the pineal gland has been found to be impaired, adherence to the recommendations for adequate rest can positively influence the subsequent outcome of pregnancy and childbirth.

References:
ФУНКЦІОНАЛЬНИЙ СТАН ШИШКОВИДНОГО ТІЛА У ЖІНОК ІЗ ЗАГРОЗЛИВИМ АБОРТОМ В ПЕРШому ТРИМСТРІ – ПОДАЛЬШІ ПЕРСПЕКТИВИ ВАГІТНОСТІ

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Резюме.
Мелатонін є важливим гормоном, який забезпечує нормальний розвиток вагітності, однак вплив серотоніну при цьому залишається недостатньо вивченим. Низька продукція мелатоніну може спричинити розвиток спонтанного абортів, так як він стимулює секрецію прогестерону та пригнічує синтез простагландинів. Мелатонін має вплив на індукуцію та блокування апоптозу ворсинчастого цитотрофобласта, сприяє розвитку синцитотрофобласта. НАПАД частоти позитивного перебігу вагітності показав суттєве значення впливу на перебіг вагітності та пологів, дотримання режиму сну та відпочинку також сприяють позитивному ефекту, проте загальній концепції, це не існує, що робить тему актуальною.

Мета дослідження. Оцінити функціональний стан шишковидного тіла, зокрема кількість мелатоніну та серотоніну у крові вагітних у терміні 10-12 тижнів вагітності із загрозою переривання вагітності, яка проявлялася кров'яністими виходами чи формуванням ретрохоріальної системи та насадження лігатури.

Матеріали та методи. Сформовано дві групи: I основна – 40 вагітних з загрозою переривання вагітності та формуванням ретрохоріальної гематоми, II контрольна – 44 здорових вагітних. Проведено акушерське дослідження, імуноферментне для визначення мелатоніну, серотоніну, нормального, шишковидного гонадотропіну, УЗД.

Передбачено інформаційну згущену вагітних при відповідному роз'ясненні мети, завдань, методів та обсягу лабораторних та інструментальних досліджень.

Протокол дослідження схвалено Комісією з питань біомедичної етики Буковинського державного медичного університету, 2015 (протокол засідання комісії № 2 від 19.10.2023 року).


Статистичний аналіз виконували за загальнодержавними методами кореляційної статистики. Достовірність оцінювали за t-критерієм Стьюдента. Відповідь значностей p < 0,05, а також проведені кореляційний аналіз системи Microsoft Excel, для визначення коефіцієнта кореляції застосовували метод квадратик (метод Персона).

Робота є фрагментом НДР кафедри акушерства та гінекології, УДК 618.1-053.34 + 618.1-053.6/.8 – 07-084-08 «Профілактика, діагностика та лікування розладів перинатального періоду та репродуктивної системи жінок і дівчат-підлітків» (№ 201110Н, номер державної реєстрації 0111У006499). Термін виконання 02.11.2012-2015.

Результати дослідження. Згідно отриманих результатів ретрохоріальна гематома до 1 см виявляється втрічі частіше, ніж більше 1 см, при цьому рівень мелатоніну не є достовірно нижчим, однак при ретрохоріальній гематомі, яка більше 1 см спостерігається достовірне зниження у випадках подальшого нормального перебігу вагітності та пологів. При подальшому ускладненні перебігу вагітності при ретрохоріальній гематомі до 1 см спостерігається значно виражене достовірне зниження мелатоніну (p < 0,001), на відміну від ретрохоріальної гематоми більше 1 см, де при ускладненному перебігу вагітності не виявлено достовірної різниці (p > 0,05). Кількість серотоніну в підвищенні у всіх різних групах, незалежно від розмірів гематоми та подальшого перебігу вагітності.

Висновки. У випадку загрози переривання вагітності у першому тримстру з утворенням ретрохоріальної гематоми у подальшому зберігаються ризики ускладненого перебігу вагітності у 60,87% випадків. При визначенні гормонів шишковидного тіла встановлено наявність достовірного збільшення серотоніну з одночасним достовірним зниженням мелатоніну зі збереженням позитивного взаємозв'язку між гормонами, що свідчить про порушення синтезу мелатоніну у ЦНС. Підтвердженням є нормальний рівень хоріногенного гонадотропу та збереження життєздатності ембріонів. Оскільки встановлено порушення утворення гормонів у шишковидному тілі, дотримання рекомендацій щодо повноцінного відпочинку може позитивно вплинути на подальші результати вагітності та пологів.

Ключові слова: мелатонін; серотонін; ретрохоріальна гематома; загроза переривання вагітності.
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