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Clinical and Morphological Examination of Fetal Growth Restriction: the Study of Melatonin Receptor Expression in Placenta

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ABSTRACT

BACKGROUND: Fetal growth restriction is one of the most significant problems in modern obstetrics, associated with a high risk of perinatal morbidity and mortality. Despite advances in diagnosis and treatment, fetal growth restriction remains a common cause of adverse pregnancy outcomes. One of the promising areas of research is the study of the role of melatonin and its receptors in the regulation of placental function.

AIM: The work aimed to conduct a comprehensive analysis of clinical and laboratory parameters in women with or without fetal growth restriction, including evaluating the expression of melatonin receptors (MT1A and MT1B) in the placenta.

METHODS: The study included women with fetal growth restriction and women in the control group. Immunohistochemical examination of placental tissue was performed using antibodies against MT1A and MT1B receptors, with clinical data analyzed. Confocal microscopy was used to quantify receptor expression.

RESULTS: We found a decrease in the expression of MT1A and MT1B receptors in the placenta of women with fetal growth restriction compared to the control group. The optical density of fluorescent signals was also lower in the fetal growth restriction group.

CONCLUSION: The data obtained suggest that decreased expression of melatonin receptors may play an important role in the development of fetal growth restriction. This opens up prospects for the development of new therapeutic strategies aimed at correcting placental function and improving pregnancy outcomes.

Keywords: fetal growth restriction; melatonin; melatonin receptors; placenta; immunohistochemistry; confocal microscopy.

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Клинико-морфологическое исследование при задержке роста плода: изучение экспрессии рецепторов мелатонина в плаценте

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АННОТАЦИЯ

Обоснование. Задержка роста плода является одной из наиболее значимых проблем современного акушерства, связанной с повышением перинатальных заболеваемости и смертности. Несмотря на достижения в области диагностики и лечения, задержка роста плода остается частой причиной неблагоприятных исходов беременности. Одним из перспективных направлений исследований является изучение роли мелатонина и его рецепторов в регуляции плацентарной функции.

Цель — провести комплексный анализ клинико-лабораторных показателей у женщин с задержкой роста плода и без патологии, в том числе оценить экспрессию рецепторов мелатонина (MT1A и MT1B) в плаценте.

Материалы и методы. В исследование включены женщины с задержкой роста плода и без нее (контрольной группы). Проведено иммуногистохимическое исследование плацентарной ткани с использованием антител к рецепторам MT1A и MT1B и анализ клинических данных. Для количественной оценки экспрессии рецепторов использована конфокальная микроскопия.

Результаты. Установлено значительное снижение экспрессии рецепторов MT1A и MT1B в плаценте у женщин с задержкой роста плода по сравнению с контрольным значением. Оптическая плотность флуоресцентных сигналов также была ниже в группе с задержкой роста плода.

Заключение. Полученные данные свидетельствуют о том, что снижение экспрессии рецепторов мелатонина может играть важную роль в развитии задержки роста плода. Это открывает перспективы для разработки новых терапевтических стратегий, направленных на коррекцию плацентарной функции и улучшение исходов беременности.

Ключевые слова: задержка роста плода; мелатонин; рецепторы мелатонина; плацента; иммуногистохимия; конфокальная микроскопия.

Как цитировать

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BACKGROUND

Infant mortality remains a key indicator of the socio-economic development and reproductive-demographic situation in the country [1]. One of the main factors affecting this indicator is fetal growth restriction (FGR), which occurs in 5%–10% of pregnancies, and in cases of complicated obstetric and gynecological history, its incidence increases to 10%–25% [2, 3]. FGR is characterized by pathologically small fetal size, not reaching its genetic growth potential [4]. It can result from maternal, fetal, or placental factors, and significantly increase the risks of intrauterine fetal death, neonatal morbidity and mortality [5]. The incidence of FGR is inversely proportional to the gestational age, reaching 60% among preterm newborns, which markedly increases mortality in this group [6]. FGR is associated with an increased risk of neurological disorders in newborns, including motor, behavioral, and cognitive impairment. The timing of the development of placental insufficiency plays a crucial role in the risk of neurological disorders in children born with growth retardation [7]. Placental histopathology in FGR includes inadequate trophoblast invasion, incomplete transformation of spiral arteries, and impaired uteroplacental blood flow [4]. FGR is often associated with preeclampsia, especially in the early development of the pathology, which warrants careful monitoring after the onset of preeclampsia [4]. Preeclampsia itself remains one of the leading causes of maternal mortality, and FGR substantially contributes to stillbirth statistics: according to the World Health Organization, one stillbirth is registered every 16 seconds worldwide [8].

In recent years, the role of melatonin in reducing the incidence of FGR and preeclampsia has been actively studied. Melatonin is a lipophilic and hydrophilic indolamine capable of quickly penetrating the placental and blood-brain barriers [9]. Known as a powerful antioxidant and regulator of circadian rhythms, it demonstrates a positive effect on placental neovascularization and oxygenation [10–13]. Moreover, melatonin also has anti-apoptotic and anti-inflammatory effects [14]. Studies have shown that melatonin is expressed in the placenta throughout pregnancy and contributes to the formation of the syncytium, highlighting its major role in maintaining normal placental function and favorable pregnancy outcome [15]. It acts as a mitochondria-targeted antioxidant, protecting cells from damage caused by reactive oxygen species [16]. Because of this property, melatonin is considered a promising agent for optimizing placental function and minimizing the risk of pregnancy complications [17, 18]. In addition, melatonin also exhibits protective effect on the embryos, securing them from the influence of exogenous stress factors, including oxidative stress and hypoxia [19]. Its ability to improve mitochondrial function and reduce the level of reactive oxygen species makes it an important constituent of prevention and treatment of FGR [20].

Recent studies have shown that melatonin affects the expression of MT1A and MT1B receptors in the placenta, which plays a key role in regulating placental function. These receptors are involved in signal transmission related to antioxidant protection, angiogenesis, and immunomodulation [21]. It has been established that in cases of FGR and preeclampsia, the expression of melatonin receptors in the placenta decreases, which may be one of the reasons for impaired placental function [22]. Research on the role of melatonin in the pathogenesis of FGR and preeclampsia is of interest due to the high incidence of these complications and their significant contribution to infant and maternal mortality. Melatonin is currently considered one of the key signaling molecules between the mother and the fetus and a new potential candidate for the prevention of pregnancy complications such as preeclampsia and FGR [23]. Clinical trials have shown that the use of melatonin in pregnant women with FGR reduces oxidative stress levels and improves pregnancy outcomes [24, 13]. Further research should aim to clarify the mechanisms of action of melatonin, including its impact on MT1A and MT1B receptor expression in the placenta, and to develop guidelines for its use. This may lead to the introduction of new therapeutic approaches aimed at reducing perinatal complications and improving pregnancy outcomes.

The work aimed to conduct a comprehensive analysis of clinical and history data in women with or without FGR, including the expression of melatonin receptors (MT1A and MT1B) in the placental tissues.

METHODS

It was a two-stage study, including a clinical part—a prospective non-continuous study, and a non-clinical (laboratory) part—a prospective continuous study. The clinical part was conducted at the antenatal and maternity departments of Maternity Hospital No. 9 (St. Petersburg), and the morphological study was carried out at the St. Petersburg State Pediatric Medical University.

Inclusion criteria for the main group were as follows: age from 18 to 45 years, established diagnosis of FGR at up to 34 weeks, singleton pregnancy, comparable treatment within the group. The control group consisted of patients without FGR.

Exclusion criteria: multiple pregnancy; pregnancy achieved through assisted reproductive technologies; fetal chromosomal anomalies or developmental defects; acute infectious diseases during pregnancy; obesity diagnosed before pregnancy; chronic alcohol and/or nicotine intoxication; severe somatic pathology (bronchial asthma, chronic obstructive pulmonary disease, antiphospholipid syndrome,

diabetes mellitus, rheumatoid arthritis, neurodegenerative, oncological diseases, etc.).

Clinical examinations were conducted, including clinical and anamnestic study, general clinical examination, ultrasound examination (fetometry, amniotic fluid assessment, Doppler ultrasound), cardiotocographic monitoring, laboratory tests.

For *the immunohistochemical study*, samples obtained from the central part of the placenta (near the site of umbilical cord attachment) were used. They were fixed in 4% paraformaldehyde for at least 24 h at 4 °C. After fixation, the samples were washed in phosphate-buffered saline (PBS) to remove excess fixative. For further processing, the samples were embedded in paraffin using the standard method. We used 3-μm paraffin tissue sections, obtained using a Leica microtome (Leica Biosystems, Germany). Polylysine-coated slides were placed in a thermostat at 37 °C for 24 h to improve adhesion.

The following primary antibodies were used: Anti-Melatonin Receptor [1B] (rabbit polyclonal antibodies, ab203346, Abcam, USA) at a 1:50 dilution and Anti-Melatonin Related Receptor [1A3] (mouse monoclonal antibodies, ab167108, Abcam, USA) at a 1:50 dilution. Incubation with primary antibodies was carried out for 1 h at 37 °C.

The immunohistochemical reaction was performed with a secondary label to determine common receptors on one section. For this, incubation with secondary antibodies Anti-Mouse IgG H&L (Alexa Fluor 647, ab150115, Abcam, USA) at a 1:200 dilution and Goat Anti-Rabbit IgG H&L (Alexa Fluor 647, ab150079, Abcam, USA) at a 1:200 dilution was used for 30 min at room temperature in the dark.

Cell nuclei were stained with Hoechst 33258 (Sigma, USA) at a 1:100 dilution for 1 min in the dark. After staining, the sections were rinsed in distilled water. Prepared slides were covered with cover slips in the Dako Fluorescent Mounting Medium (Dako, Denmark).

For quantitative assessment of melatonin receptors, all sections were analyzed using a Zeiss LSM 710 confocal microscope (Carl Zeiss AG, Germany).

For visualization of cell nuclei, the DAPI stain (Thermo Fisher Scientific, USA) was used (excitation at 405 nm, signal intensity 1.053 arbitrary units). For visualization of fluorescent labels, the TRITC dye (Thermo Fisher Scientific, USA) was used (excitation at 543 nm, signal intensity 59.58 arbitrary units). The beam splitter (dichroic mirror) was set to position 1/2 for optimal signal separation. The background signal was 926 arbitrary units.

Visualization control parameters: signal level 2.4, noise level 0.4.

Sanitation was performed on 10 sections of the slide for each sample under study, after which the data were processed by calculating the mean.

The images were analyzed using ZEN (Carl Zeiss AG, Germany) and ImageJ (National Institutes of Health, USA) software.

Indicators, including tissue area, relative expression area, and average optical density were analyzed. Measurements were performed three times, then the mean was analyzed.

Statistical analysis was conducted on a personal computer using the Python 3.8 programming language, the Scipy 1.6.3 library, and the SPSS Statistics 26.0 software (SPSS Inc., USA). The mean and standard deviation ($M \pm \sigma/\sqrt{n}$) were used to characterize position and dispersion for quantitative data. Discrete parameters were described by absolute values and fractions. Non-parametric data were analyzed using the χ^2 test (distribution of the sum of squares of independent standard normal random variables), the Mann–Whitney test, and the Kolmogorov–Smirnov test. The differences were considered statistically significant at $p < 0.05$ for the possibility to reject the null hypothesis. Graphical data processing was performed using Microsoft Excel 2013 (USA).

RESULTS

The main group consisted of 34 women with FGR, and the control group consisted of 32 women without FGR.

No statistically significant differences in age and height were found when comparing the groups of patients. The weight of pregnant women with FGR was statistically significantly lower, as confirmed by statistical data processing ($p = 0.003$). The distribution by blood type and Rh factor did not differ from the average in the population. Primiparous women with FGR accounted for 38.23%, while women without FGR accounted for 46.87% ($p = 0.24$; $\chi^2 = 0.3$). The proportion of primiparous women was 58.82% and 59.375% in the main and control group, respectively ($p = 0.48$; $\chi^2 = 0.73$). The differences were statistically insignificant.

No statistically significant differences were found between the groups in terms of obstetric and gynecological history parameters, including endometriosis, uterine fibroid or previous myomectomy, cervical scarring, polycystic ovary syndrome, as well as cases of pregnancy loss, infertility, and anatomically narrow pelvis.

The hypothesis of intergroup differences cannot be rejected for such somatic diseases as hypertension, urolithiasis, pyelectasis, hydronephrosis, varicose veins, biliary dyskinesia, subclinical hypothyroidism and euthyroidism.

Interestingly, pregnant women without FGR had a significantly greater overall weight gain during pregnancy (14.2 ± 0.95 kg) compared with pregnant women with FGR (10.7 ± 0.79 kg; $p = 0.007$). It is partly related to the term of delivery, but when calculating the value for women with comparable terms in different groups, the statistical difference remained.

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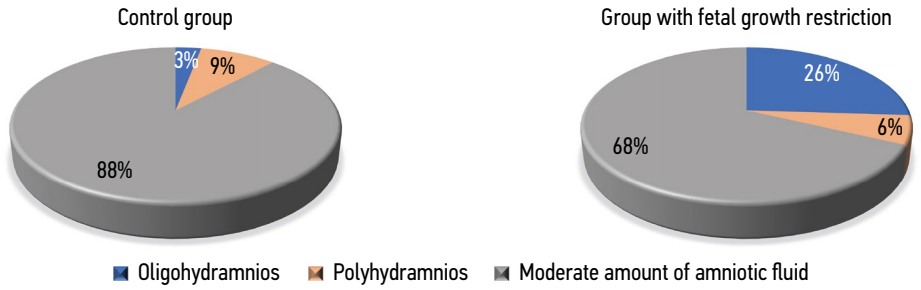

Fig. 1. Amount of amniotic fluid.

Рис. 1. Количество околоплодных вод.

In the control group, 50% of women had no edema, 30% of women had mild edema of the limbs, and 20% had moderate to severe edema of the limbs. In the group with FGR, edema was absent in only 10% of women, 32% of patients had mild edema of the hands and shins, and 58% had severe edema of the limbs. The difference between the groups was statistically confirmed ($p = 0.0002$).

Increased blood pressure was recorded in 41% of pregnant women with FGR, whereas there were no cases of hypertension in the control group ($p < 0.00001$; $\chi^2 = 0.00001$).

Preeclampsia occurred in 44.1% of cases in the FGR group, while in the control group it was reported in only 6.25% of cases ($p = 0.00025$).

When assessing the amount of amniotic fluid, statistically significant differences were identified (Fig. 1). In the control group, oligohydramnios was diagnosed in only one woman (3.125%), while polyhydramnios accounted for 9.37% of cases, and a moderate amount of amniotic fluid was reported in 87.5% of cases. In the main group, 26.5% of patients had oligohydramnios, 5.8% had polyhydramnios, and 67.7% had a moderate amount of amniotic fluid. The null hypothesis was rejected ($p = 0.02$).

The average amniotic index in the FGR group was significantly lower (10.5 ± 0.9) than that of the control group (13.9 ± 0.7; $p = 0.0013$), indicating a significant reduction in the volume of amniotic fluid in FGR. This may be due to the impaired placental function and deterioration of uteroplacental blood flow, which highlights the importance of monitoring the amniotic index for early diagnosis and prediction of FGR.

During the study, anemia of pregnancy and thrombocytopenia were quantitatively assessed, however, no statistically significant differences between the groups were found. Leukocytosis was noted exclusively among patients in the control group. Elevated fibrinogen and proteinuria were more frequently observed in the group with FGR; however, these differences did not reach statistical significance.

The study showed that the localization of the placenta does not affect the risk of FGR. The placenta was located on the posterior uterine wall in 58.6% of pregnant women without FGR, and on the anterior wall in 41.4%. In women with FGR, the placenta was located on the posterior wall in 46.7% of cases, and on the anterior wall in 53.3% ($p = 0.18$; $\chi^2 = 0.35$).

During the ultrasound examination, the fetometric parameters of the control group were within the 10th percentile for the corresponding gestational age, whereas in the main group, 58.8% of patients showed signs of FGR.

According to the Doppler ultrasound data, hemodynamic parameters of the mother-placenta-fetus system in the main group were outside the normal range, while there were no abnormalities in the control group. The data are presented in Table 1.

In fetuses of women with FGR, the basal fetal heart rate was higher than in the control group, while oscillations and myocardial reflex were significantly lower. The data are presented in Table 2.

All women without FGR delivered on time, while in the FGR group, three patients had a preterm delivery, which accounted for 8.25%.

Table 1. Doppler ultrasound parameters of the mother–placenta–fetus system in the presence or absence of fetal growth restriction

Таблица 1. Показатели допплерометрии системы мать–плацента–плод при наличии и отсутствии задержки роста плода

Artery	Systolic-diastolic ratio	Resistance index	Pulsation index
Umbilical	3.56 ± 0.8 / 2.41 ± 0.07 ($p = 0.046$)	0.59 ± 0.02 / 0.55 ± 0.01 ($p = 0.048$)	1.19 ± 0.06 / 0.8 ± 0.16 ($p = 0.049$)
Middle cerebral	4.1 ± 0.24 / 4.43 ± 0.4 ($p = 0.19$)	0.73 ± 0.02 / 0.74 ± 0.02 ($p = 0.33$)	1.5 ± 0.08 / 1.6 ± 0.29 ($p = 0.34$)
Right uterine	2.66 ± 0.16 / 1.87 ± 0.05 ($p = 0.0002$)	0.57 ± 0.02 / 0.45 ± 0.02 ($p = 0.0005$)	1.08 ± 0.09 / 0.6 ± 0.08 ($p = 0.01$)
Left uterine	2.29 ± 0.1 / 1.98 ± 0.06 ($p = 0.027$)	0.54 ± 0.02 / 0.48 ± 0.01 ($p = 0.033$)	0.98 ± 0.07 / 0.71 ± 0.1 ($p = 0.08$)

Note: The data are presented as the mean and standard deviation.

Table 2. Cardiotocography parameters in the study groups
Таблица 2. Показатели кардиотокограммы в исследуемых группах

Parameter	Group with fetal growth restriction	Control group	*p*-value
Baseline fetal heart rate, per minute	137.88 ± 1.39	132.2 ± 1.04	<0.05
Myocardial reflex, per minute	19.92 ± 0.58	23.43 ± 0.53	<0.01
Oscillations, per minute	6.96 ± 0.13	8.06 ± 0.19	<0.01

Note: The data are presented as the mean and standard deviation.

Also, 31.75% of pregnant women in the control group and 58.82% of patients with disorders related to the mother-placenta-fetus system delivered by cesarean section (p = 0.02). Indications for cesarean section in 14 patients with FGR were related to the studied pathology, two of them underwent surgery based on ophthalmologist's conclusion, one—due to uterine scar failure, and another one due to scar deformation of the cervix (Fig. 2).

Among women with FGR, the following delivery complications were noted: two patients experienced precipitous labor, two underwent transfusion of fresh frozen plasma, one had primary uterine inertia, one had neonatal asphyxia, and one underwent drainage of the peritoneal cavity. During natural delivery, an episiotomy was performed in five women, and one underwent manual examination of the uterus due to retained placental lobe.

In the control group, the complications of delivery were as follows: three experienced discoordination of labor activity, two had a drainage placed in the peritoneal cavity during a cesarean section, and one had neonatal asphyxia. In addition, 12 women had an episiotomy, and two underwent a manual examination of the uterine cavity and removal of the retained placental lobe.

The mean term of delivery in women with FGR was 38.0 weeks, while in the control group it was 39.75 weeks. The null hypothesis was rejected (p < 0.0000001).

In the control group, 64.5% of the women gave birth to boys and 35.5% to girls, while in the main group, 52.9% of patients gave birth to girls and 47.1% to boys (p > 0.05).

The average body weight of newborns from women with FGR was (2654 ± 85 g), which is significantly lower than that of conditionally healthy women (3488 ± 68 g; p < 0.00000001). Undoubtedly, this reflects the presence of fetal mass deficiency as the leading component of FGR. The mean fetal height in the main group (48 ± 0.54 cm) was

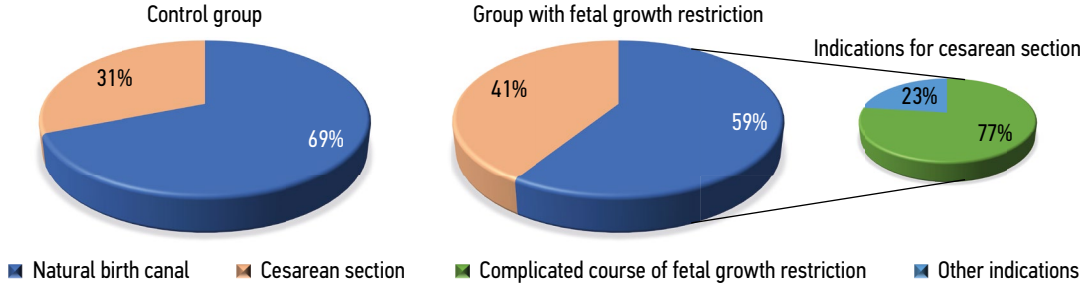

Fig. 2. Method and indications for delivery.
Рис. 2. Способ родоразрешения и показания для него.

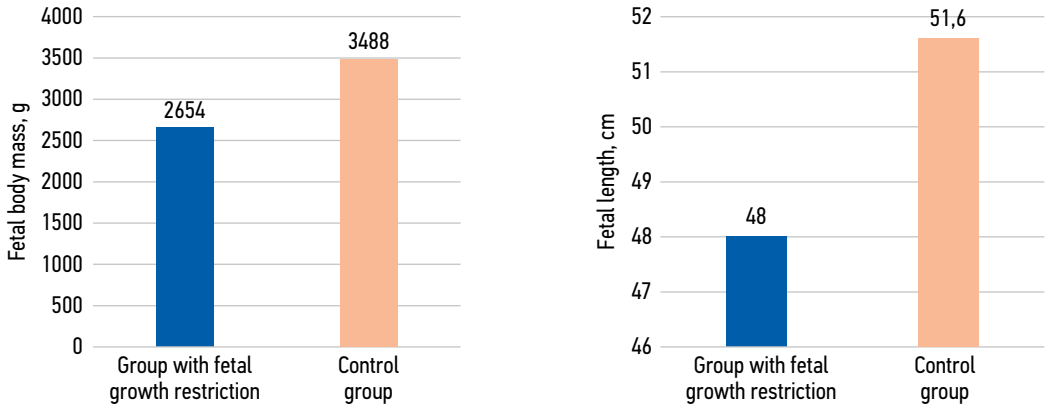

Fig. 3. Fetal parameters.
Рис. 3. Антропометрические параметры плодов.

Table 3. Fetal and placental parameters
Таблица 3. Параметры плодов и плацент

Parameter	Group with fetal growth restriction (n = 34)	Control group (n = 32)	p-value
Fetal head circumference, cm	33.8 ± 0.28	35.2 ± 0.24	<0.001
Fetal chest circumference, cm	31.4 ± 0.45	33.9 ± 0.25	<0.0001
Umbilical cord length, cm	62.4 ± 1.7	63.8 ± 1.7	>0.05
Placental mass, g	489.3 ± 22.8	613.1 ± 21.6	<0.001
Placental area, cm^2	278.8 ± 21.8	337.1 ± 14.0	<0.05

Note: The data are presented as the mean and standard deviation.

statistically lower than in the control group (51.6 ± 0.37 cm; $p < 0.001$) (Fig. 3).

The measurements of head circumference, chest circumference, umbilical cord length, placental mass, and placental area are presented in Table 3.

Fetal hypoxia was noted in 27.3% of women with FGR, and in 18.75% of women without the studied pathology ($p = 0.21$; $\chi^2 = 1.17$).

Women without the studied pathology showed the following Apgar scores for newborns in 1 min after birth: 9 points (9.375%), 8 points (81.25%), 7 points (6.25%), and 5 points (3.125%). The scores for patients in the main group were as follows: 8 points (64.7%), 7 points (32.35%), 6 points (2.95%). According to the data, statistically significant differences between the groups were noted in 1 min after birth ($p < 0.05$), while in 5 min there were no differences identified ($p > 0.05$). Apgar score in 5 min after birth in the control group was 9 points (31.25%), 8 points (62.5%), 7 points (3.125%), and 6 points (3.125%). In the main group, it was 9 points (44.1%), 8 points (47.1%), and 7 points (8.8%).

The groups did not differ significantly in terms of blood loss during surgery: in the main group, the average volume of blood loss was 582 ± 20 mL, while in the control group it was 630 ± 16 mL ($p > 0.05$). No differences were found in natural delivery either: the average blood loss in mothers with FGR was 227 ± 12 mL, and in those without FGR it was 247 ± 20 mL ($p > 0.05$).

In 44% of cases, FGR was accompanied by preeclampsia. The severe course of the condition led to surgical delivery in 58% of cases, on average 2 weeks earlier than in the control group. All this confirms the need for new treatment and prevention regimens for FGR. As a result of the study using the quantitative test method, the relative area of expression and optical density of melatonin receptors MT1A and MT1B were determined, which allowed for a quantitative characterization of their expression in placental tissues. For the MT1A receptor, the mean relative expression area in the FGR group was 0.259 ± 0.183 arbitrary units, while in the control group, it was significantly higher, reaching 0.402 ± 0.226 arbitrary units. Statistical analysis using the Mann–Whitney test revealed significant differences between the groups ($p = 0.002$), confirming the reduction of MT1A expression in FGR. The graphical representation of the data (Fig. 4) clearly illustrates the identified differences and the significance of the obtained results.

Quantitative test revealed that the average relative area of MT1B expression in the FGR group was 0.472 ± 0.245 arbitrary units, whereas in the control group it was 0.544 ± 0.191 arbitrary units. Statistical analysis using

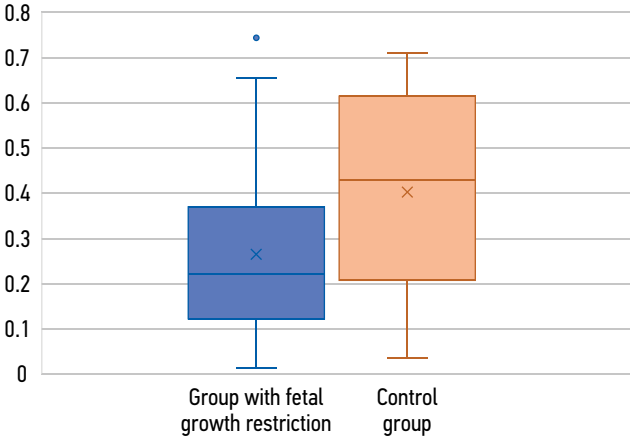

Fig. 4. Relative area of MT1A melatonin receptor expression.
Рис. 4. Относительная площадь экспрессии рецептора мелатонина MT1A.

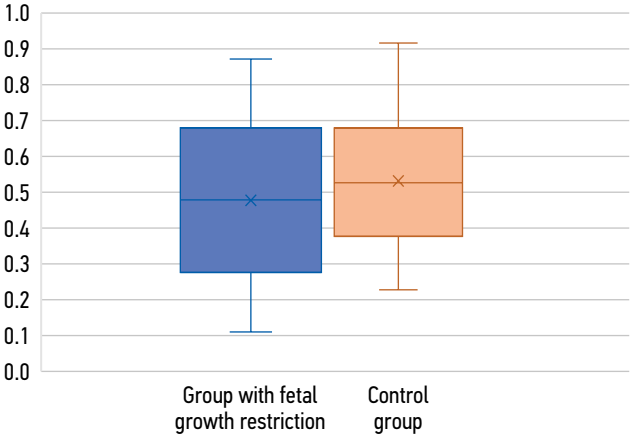

Fig. 5. Relative area of MT1B melatonin receptor expression.
Рис. 5. Относительная площадь экспрессии рецептора мелатонина MT1B.

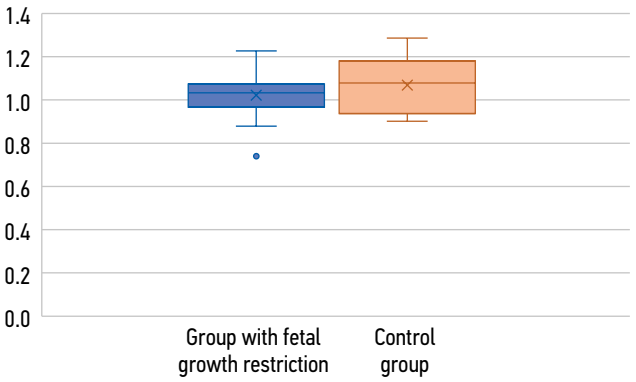

Fig. 6. Average optical density of MT1A melatonin receptor fluorescent signals.

Рис. 6. Средняя оптическая плотность флуоресцентных сигналов рецептора мелатонина MT1A.

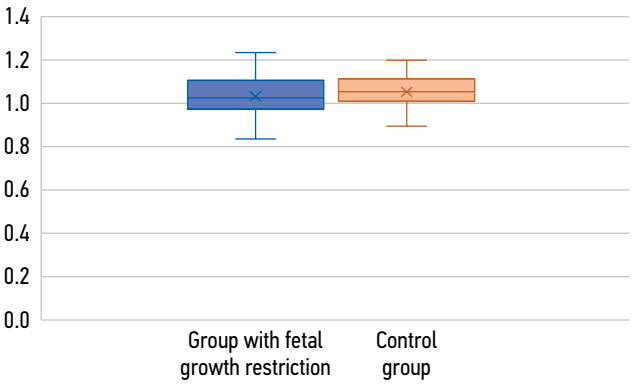

Fig. 7. Average optical density of MT1B melatonin receptor fluorescent signals.

Рис. 7. Средняя оптическая плотность флуоресцентных сигналов рецептора мелатонина MT1B.

the Mann–Whitney test showed that the differences between the groups were statistically significant (p = 0.039). This indicates a decrease in MT1B expression in FGR (Fig. 5).

The results confirm that the relative expression area of MT1A and MT1B in the FGR group is lower than in the control group, which may indicate a link between their reduced expression and the pathogenesis of FGR.

The optical density of fluorescent signals was measured to quantitatively assess the intensity of melatonin receptor expression. The mean optical density of the MT1A receptor in the FGR group was 1.021 ± 0.104 arbitrary units, while in the control group it was 1.073 ± 0.118 arbitrary units, with the differences between the groups being statistically significant (p = 0.039; Mann–Whitney test). Similarly, for the MT1B receptor, the mean optical density in the FGR group was 1.037 ± 0.088 arbitrary units, while in the control group it was 1.063 ± 0.081 arbitrary units, with the differences being statistically significant (p = 0.039; Mann–Whitney test). The distribution of optical density values for both receptors is presented in Fig. 6 and 7.

It was shown that the expression of MT1A is reduced in the FGR group compared with the control values, which may indicate a disruption in the regulation of melatonin receptors in the placenta in FGR. MT1B expression was also reduced in the FGR group, but the differences between the groups were less pronounced compared with MT1A. The results of the immunohistochemical study of the expression of melatonin receptors MT1A and MT1B in the main and control groups are presented in Figs. 8–11. The distribution of MT1A and MT1B receptors in the placenta

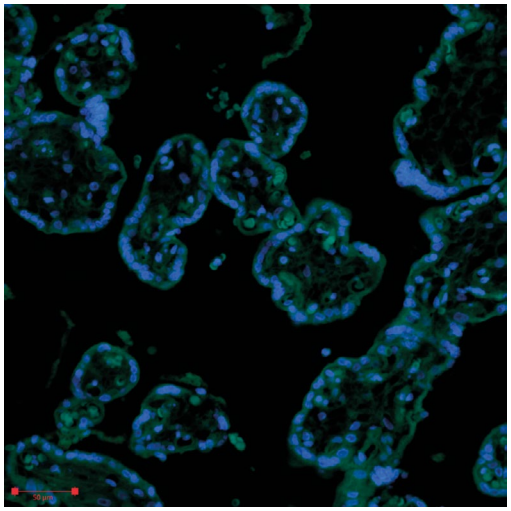

Fig. 8. Expression of MT1A melatonin receptors in terminal and intermediate chorionic villi in the group with FGR. Immunohistochemical examination using a confocal microscope, zoom ×200.

Рис. 8. Экспрессия рецепторов мелатонина MT1A в терминальных и промежуточных ворсинах хориона в группе с задержкой развития плода. Иммуногистохимическое исследование на конфокальном микроскопе, увеличение ×200.

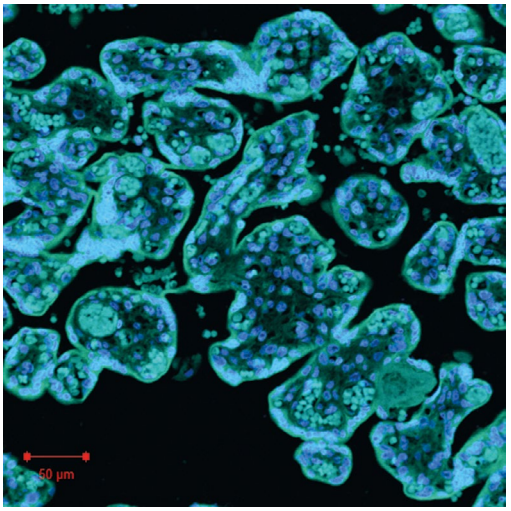

Fig. 9. Expression of MT1A melatonin receptors in terminal and intermediate chorionic villi in the control group. Immunohistochemical examination using a confocal microscope, zoom ×200.

Рис. 9. Экспрессия рецепторов мелатонина MT1A в терминальных и промежуточных ворсинах хориона в контрольной группе. Иммуногистохимическое исследование на конфокальном микроскопе, увеличение ×200.

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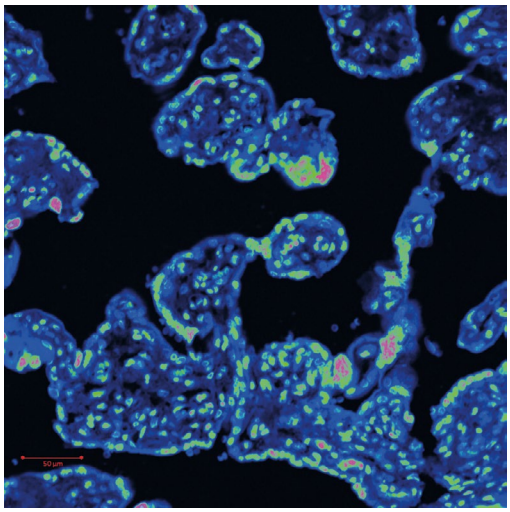

Fig. 10. MT1B melatonin receptor expression in terminal and intermediate chorionic villi in the fetal growth restriction group. Immunohistochemical examination using a confocal microscope, zoom ×200.

Рис. 10. Экспрессия рецепторов мелатонина MT1B в терминальных и промежуточных ворсинах хориона в группе с задержкой развития плода. Иммуногистохимическое исследование на конфокальном микроскопе, увеличение ×200.

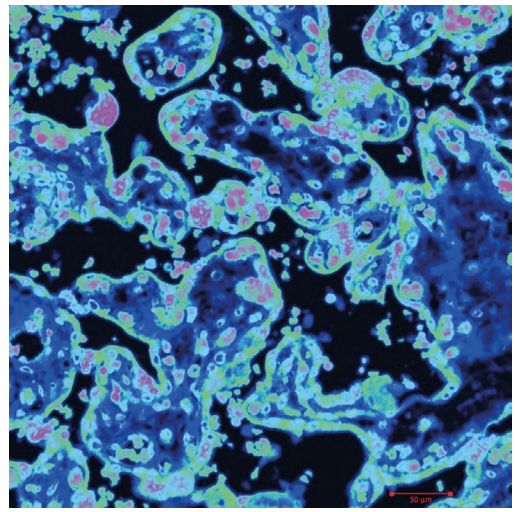

Fig. 11. MT1B melatonin receptor expression in terminal and intermediate chorionic villi in the control group. Immunohistochemical examination using a confocal microscope, zoom ×200.

Рис. 11. Экспрессия рецепторов мелатонина MT1B в терминальных и промежуточных ворсинах хориона в контрольной группе. Иммуногистохимическое исследование на конфокальном микроскопе, увеличение ×200.

is likely to vary depending on the functional characteristics of the area (e.g., villi), which requires further in-depth study.

The evaluated area of placental tissue for MT1A receptors in the FGR group was 871.432 ± 194.732 arbitrary units, while in the control group it was 934.732 ± 537.892 arbitrary units (Mann–Whitney test; $p = 0.512$), the differences were statistically insignificant. Similarly, for MT1B receptors, the average area in the FGR group was 869.732 ± 459.812 arbitrary units, while in the control group it was 891.270 ± 537.892 arbitrary units (Mann–Whitney test; $p = 0.512$), the differences were statistically insignificant. Thus, the study results established that the groups did not significantly differ in placental tissue area when analyzing the melatonin receptors A and B.

DISCUSSION

To date, FGR remains one of the most pressing issues in obstetrics, as evidenced by the high rates of perinatal mortality and morbidity associated with this condition.

Analysis of clinical and history data showed that women with FGR had a significantly lower body weight and less weight gain during pregnancy compared with the control group. This may be related to both genetic characteristics and placental dysfunction, and requires further investigation. Moreover, edema, increased blood pressure, and preeclampsia were more frequently observed with FGR, confirming their role as predictors of FGR. These data are consistent with the data from other researchers who also noted the association between FGR and preeclampsia [19, 20].

In 8.25% of women with FGR, preterm delivery occurred, and the average delivery term was almost 2 weeks earlier than in the control group. This highlights the need for a more thorough monitoring and timely intervention in patients with FGR. In addition, newborns from mothers with FGR showed significantly lower anthropometric parameters (body weight, height, head and chest circumference), which confirms the presence of fetal weight deficiency as a key component of FGR. These data are consistent with the results of other studies, which also demonstrated the association between FGR and low anthropometric parameters in newborns [4, 6].

The study allowed for the identification of key aspects related to the role of MT1A and MT1B receptor expression in the pathogenesis of FGR. The results demonstrate that their expression in the placenta is significantly reduced in women with FGR compared with the control values. This supports the hypothesis that dysregulation of melatonin receptors may contribute to FGR.

It is known that melatonin, synthesized predominantly by the pineal gland as well as the placenta itself, plays a major role in the regulation of oxidative stress, angiogenesis, and immune tolerance during pregnancy [16]. Placental melatonin receptors modulate vasodilation, trophoblast invasion, and apoptosis, which is crucial for adequate fetoplacental blood flow [25]. Disruption of their expression can lead to dysfunction of the placental barrier and, consequently, to hypoxia and FGR.

The obtained data demonstrate a decrease in the expression of melatonin receptors in placentas with FGR, which correlates with the results of recent studies. Other researchers demonstrated that suppression of melatonin signaling impairs trophoblastic invasion and reduces the production

of placental lactogen, confirming the key role of melatonin in placental development [26].

Melatonin has powerful antioxidant properties, and its receptors activate enzymes (superoxide dismutase, catalase) that protect the placenta from injury [27]. Disruption of this system may contribute to placental ischemia, and reduced expression of MT1A and MT1B may be associated with impaired antioxidant protection, angiogenesis, and immunomodulation, ultimately leading to placental dysfunction and FGR. The data are consistent with the results of previous studies, which also showed that melatonin plays a key role in maintaining normal placental function [10–13].

CONCLUSION

The obtained data support the hypothesis about the possible influence of melatonin on the development of FGR. Further studies, including molecular and functional tests, are needed to confirm the causality between the reduction in melatonin receptor expression and FGR. Study of the MT1A and MT1B receptor distribution in various morphological structures of the placenta (e.g., villi, basement membrane) is of particular interest, as it may help to better understand their role in the pathogenesis of FGR.

The study confirms that a decrease in the expression of melatonin receptors MT1A and MT1B in the placenta may be associated with FGR. This reveals new prospects for the development of therapeutic strategies aimed at correcting this pathology. In particular, the use of melatonin may become a promising approach for improving placental function and reducing the risk of FGR. However, further research, including clinical trials, is necessary to confirm these assumptions.

ADDITIONAL INFORMATION

Author contributions: E.V. Novitskaya: investigation, formal analysis, funding acquisition, writing—original draft; V.O. Polyakova, V.M. Bolotskikh: conceptualization; writing—review & editing; T.S. Kleimenova, S.S. Pyurveev: investigation; M.A. Mikhailova: investigation, writing—original draft. All authors approved the version of the manuscript to be published, and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of it are appropriately reviewed and resolved.

Ethics approval: The study received no ethics committee approval, as the institution has no ethics committee. However, all fundamental ethical research practices were maintained The participants were informed on the study aim and objectives, its methods and possible risks, and gave their consent to participate in the study. The study did not impose any potential physical, mental, or social harm to the participants. Their data were kept confidential. The study and its protocol were not registered.

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Data availability statement: All data generated during this study are included in this article.

Generative AI: No generative AI was used in preparing this article.

Provenance and peer-review: This work was submitted unsolicited and reviewed following the standard procedure. The review involved two internal reviewers.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Е.В. Новицкая — проведение исследования, анализ данных, привлечение финансирования, написание черновика рукописи; В.О. Полякова, В.М. Болотских — определение концепции, пересмотр и редактирование рукописи; Т.С. Клейменова, С.С. Пюрвеев — проведение исследования; М.А. Михайлова — проведение исследования, написание черновика рукописи. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

Этическая экспертиза. Одобрение этического комитета на проведение исследования не получали в связи с отсутствием этического комитета в организации, однако были соблюдены базовые принципы проведения исследований. Участники были полностью информированы о целях, методах и возможных рисках исследования, дали добровольное согласие на участие в исследовании. Данное исследование не несло потенциальный вреда, в том числе физического, психологического, социального. Обеспечена конфиденциальность данных. Исследование и его протокол не регистрировали.

Источники финансирования. Отсутствуют.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. При создании настоящей работы были использованы фрагменты собственного текста, опубликованного ранее [DOI: 10.21638/spbu11.2024.20], распространяется с разрешения правообладателя (© Санкт-Петербургский государственный университет (СПбГУ), 2024. Источник: https://medicine-journal.spbu.ru/article/view/18665/12412).

Доступ к данным. Все данные, полученные в настоящем исследовании, доступны в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внутренних рецензента из состава редакционной коллегии.

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