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BAKOEVA NILUFAR MATYOKUB KIZI

THE ROLE OF KISSPEPTIN AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN PREDICTING THE DEVELOPMENT OF PREMATURE OVARIAN INSUFFICIENCY AFTER COVID-19

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List of abbreviations

AMH – Anti-Müllerian hormone

ACE2 – Angiotensin-converting enzyme 2

WHO – World Health Organization

HPG – Hypothalamic–pituitary–gonadal axis

HPA – Hypothalamic–pituitary–adrenal axis

GnRH – Gonadotropin-releasing hormone

BMI – Body mass index

LH – Luteinizing hormone

mRNA – Messenger ribonucleic acid

POI – Premature ovarian insufficiency

TSH – Thyroid-stimulating hormone

FSH – Follicle-stimulating hormone

IVF – In vitro fertilization

BDNF – Brain-derived neurotrophic factor

COVID-19 – Coronavirus disease 2019

KISS1 – Kisspeptin

KISS1R – Kisspeptin receptor

NTRK2 – Neurotrophic receptor tyrosine kinase 2

POIQoLS – Premature Ovarian Insufficiency Quality of Life Scale

SARS-CoV-2 – Severe acute respiratory syndrome coronavirus 2

TMPRSS2 – Transmembrane serine protease type 2

INTRODUCTION

Premature ovarian insufficiency (POI) is one of the most complex problems of the female reproductive system worldwide. According to the World Health Organization (WHO), the worldwide incidence is from 1-3% to 10% of women, and in Uzbekistan the incidence is 2.5%. Epidemiological studies have shown that POI is closely related to the age of women: up to 20 years old - with a frequency of 1:10,000, and from 30 to 40 years old - with a frequency of 1:1000.

Premature ovarian insufficiency is a disease characterized by the cessation of ovarian function in women under the age of 40. POI is associated with hypoestrogenism and loss of antral follicles, which leads to menstrual irregularities, infertility, and a reduced health-related quality of life.

The causes of POI development are multifactorial, including genetic, environmental, and intrinsic factors. The studied causes of POI etiology are: genetic factors, autoimmune diseases, environmental influences, infections and inflammatory processes, surgical interventions, chemotherapy and radiation therapy, and genetic syndromes. But in many cases, the cause of this disease remains unclear.

Currently, a number of scientific studies are being conducted around the world to study the causes of premature ovarian insufficiency, elucidate its etiology, predict and develop new treatment methods. Once the diagnosis of Premature ovarian insufficiency is confirmed, it is important to identify risk factors for its development at an early stage, since there is currently no way to restore ovarian function. It is known that coronavirus infection has led to disruptions in the reproductive function of women, but the exact mechanisms of the development of POI have not been identified. In this regard, studies are being conducted to determine the role of markers such as kisspeptin and brain-derived neurotrophic factor in cases of menstrual cycle disorders after coronavirus infection, especially in the context of premature ovarian insufficiency.

According to numerous studies in the world scientific literature, the number of diseases associated with reproductive health disorders in women due to the coronavirus pandemic is increasing. A number of authors have noted that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes premature ovarian insufficiency, which in turn indicates new pathogenetic mechanisms for the development of this pathology (James Wilkins, 2021; Entela Puca, 2022). It is known that COVID-19 can affect women's reproductive function through its effect on angiotensin-converting enzyme 2 receptors, which are widely distributed in the ovaries and uterus, and changes in their function can affect the menstrual cycle and reproductive health. There is a link between the hypothalamic-pituitary-adrenal axis, which controls the stress response, and the hypothalamic-pituitary-ovarian axis, in which activation of one axis can lead to suppression of the other. Chronic stress, caused by infection or other factors, can suppress estrogen production, which can lead to menstrual cycle disorders and anovulatory cycles (Guan W. et.al., 2019). Stress-induced menstrual cycle disorders encompass a variety of changes, so it is important to study the potential impact of COVID-19 (Devaux C. et al., 2020). It is important to note that the impact of COVID-19 on reproductive health is still being studied, and more detailed studies will help to better understand the relationship between infection and menstrual function. Maintaining women's health and reproductive function during times of stress and illness remains an important public health priority. It is also important to note that prior to the coronavirus outbreak, there were few controlled studies of ovarian insufficiency and the literature on it was limited.

In this regard, one of the urgent tasks of endocrinology is to study markers of the development of Premature ovarian insufficiency after COVID-19 and develop new, more specific recommendations.

CHAPTER I. MECHANISMS OF POI IN THE CONTEXT OF COVID-19

§1.1. The impact of coronavirus infection on the female reproductive system

The coronavirus pandemic has affected the mental health of people around the world. Stressful situations are a common cause of ovarian dysfunction. Chronic stress is one of the main damaging factors affecting the endocrine glands. Increased physical and mental stress alters the regulatory function of the immune system and affects the hypothalamic-pituitary-ovarian axis by increasing the level of corticosteroid hormones [1, 20].

A study by Niamh Phelan et al. found links between menstruation, mental health and stress during the COVID-19 pandemic: 46% of women with regular menstrual cycles reported changes in their cycles during the pandemic (these changes could include longer, shorter, heavier or lighter periods); 17% of women did not menstruate during the pandemic, a 4% increase compared to before the pandemic.

In a study of the impact of COVID-19 infection on female fertility (1031 women), 46% of participants reported changes in their menstrual cycle and 53% reported worsening premenstrual symptoms since the start of the pandemic [6]. Thus, the detrimental effects of the pandemic on women's mental health may have additional unintended health consequences. It has also been suggested that COVID-19 may have potential adverse effects on ovarian reserve and endocrine function. The SARS-CoV-2 spike protein binds ACE2, a key cell surface receptor; the transmembrane serine protease TMPRSS2 primes the spike protein, facilitating cellular entry [4]. In order for the virus to enter the cell and interact with the AAC2 receptor on the cell membrane, the viral S protein must be cleaved or modified to enable it to bind to AAC2. Transmembrane serine protease type 2 (TMSP2) plays an important role in this process. TMSP2 helps to cleave the SARS-CoV-2 protein, which allows it to interact with AAC2 and enter the cell.

This process facilitates the entry of the virus into the cell and subsequent replication. SARS-CoV-2 affects not only the lungs, but also other organs with high expression of AAC2 [41], including the heart, kidneys, intestines and endothelial cells, testes, ovaries, vagina, uterus and placenta [5, 8, 14, 22]. In addition, some studies have shown that ACE2 is also expressed in human cumulus cells (cells that surround and nourish the oocyte) [35]. ACE2-modulated angiotensin II and angiotensin (1-7) can stimulate steroid secretion, promote follicular development and atresia, and regulate oocyte maturation and ovulation. Given these functions, SARSCoV-2 may disrupt female reproductive function through ACE2 [23].However, recent data suggest that the female reproductive system may be protected from viral infection [32, 12]. ACE2 plays a key role in the ovaries. It enhances steroid secretion [33], promotes follicular development [15] and oocyte growth, influences ovulation [16], and supports luteal function [36]. ACE2 receptors have previously been found in the ovaries of reproductive-aged and postmenopausal women [31]. Therefore, the ovaries and oocytes may be targets for SARSCoV-2 [23], ACE2 mRNA has been found in the human uterus and vagina [39]. We see the importance of studying the impact of coronavirus infection and the post-Covid state on reproductive health.

Takmaz et al. conducted a survey of 952 women aged 18–40 years who had regular menstruation in the year before the pandemic in Turkey in 2021. According to the questionnaires, 28.7% of women experienced menstrual irregularities, 10.7% had shorter or longer periods, and 12.9% had a change in period length of more than 9 days. 5.8% had prolonged cycles, and 6.5% had abnormal uterine bleeding. Women with irregular menstruation had significantly higher rates of depression, anxiety, and stress [37].

Ding et al. in 2021 studied 78 women under the age of 50 with COVID-19 (61 of whom had mild and 17 had severe cases). In severe cases, women had increased dysmenorrhea, irregular menstruation, and amenorrhea. The authors attributed these findings to lower anti-Mullerian hormone (AMH) levels,

increased FSH levels, and higher testosterone and prolactin levels in women in the COVID-19 group compared to the same control group. age. AMH is one of the most accurate markers of ovarian reserve. The results of this study suggest that SARS-CoV-2 infection may affect ovarian reserve. Overall, 48% of patients in this study had mental disorders (anxiety, depression, sleep disorders) during this period [12, 38].

The results of a study by Herrero et al. showed a potential decrease in ovarian reserve and a decrease in reproductive capacity in a short period of time. Direct viral attack, excessive immune or inflammatory response, stress, and HPA axis dysfunction may contribute to ovarian dysfunction in COVID-19 and ultimately lead to ovarian damage. [21].

Thus, one of the mechanisms of POI development is stress and its exact mechanism of reproductive system dysfunction as a result of the pandemic remains unknown. In recent years, attention has been paid to the role of kisspeptin and brain-derived neurotrophic factor (BDNF).

§1.2. The effect of kisspeptin and brain-derived neurotrophic factor on the development of premature ovarian insufficiency

In the context of the development of modern medicine, the discovery of hypothalamic kisspeptin neurons has had a major impact on the world of reproductive neuroendocrinology. In women, hypothalamic kisspeptin acts on GnRH neurons and mediates the release of luteinizing hormone and follicle-stimulating hormone, which stimulate the synthesis of sex hormones. Kisspeptin has both direct and indirect effects on the hypothalamic-pituitary-ovarian axis. The direct effect of kisspeptin is to act on pituitary gonadotrophs, promoting the release of LG and FSG. The indirect effect is the main physiological pathway, which affects the GnRH system of the hypothalamus, resulting in the production of LG and FSG. At the end of the follicular phase of the menstrual cycle, high levels of estrogen activate KISS1 neurons, which increase the frequency and

amplitude of GnRH pulses. This leads to an increase in LG and ovulation [34, 19].

Kisspeptin not only affects the hypothalamus and pituitary gland, but also the ovaries. The rise in LG at the end of the follicular phase leads to an increase in brain-derived neurotrophic factor (BDNF) in the granulosa cells of the ovary.Transduction of BDNF signals to the oocyte plays a crucial role in disrupting oocyte development[44].

In a study by Fumihiko K. et al., serum sex hormone levels and plasma kisspeptin levels were determined in postmenopausal women. Serum gonadotropin levels were high (LG, FSG) and estradiol were very low, indicating decreased ovarian function.Plasma kisspeptin levels were significantly higher in women in the luteal phase than in women in the follicular phase and in postmenopausal women. However, there were no significant differences in values in postmenopausal women [17].

In response to stress, the body reacts by activating the hypothalamic-pituitary-adrenal axis with increased secretion of corticoliberin and glucocorticoids. Studies on kisspeptin have allowed us to understand and look at the mechanisms of menstrual disorders under the influence of stress. Under stress, the actiPOI of KISS1 neurons decreases and kisspeptin synthesis decreases. This, in turn, can lead to a violation of the pulsatile secretion of gonadotropins and disorders in the reproductive system, such as anovulation and amenorrhea [25].

According to the results of a study by Melka et al., a potential mechanism for the development of POI suggests a synergy between the signaling by kisspeptin and BDNF, which are essential for the survival of oocytes in antral follicles [27].

Another important discovery was the importance and role of BDNF in the development of POI. In the last few years, BDNF has received special attention as a marker of post-Covid depression and cognitive impairment. According to a study by Lorkiewicz et al., a decrease in AAF2 levels was shown to be associated

with BDNF [26].This theory is supported by a study conducted to determine BDNF levels in the serum of patients with COVID-19, which concluded that BDNF may be a good biomarker of post-COVID depression [26]. Zaman Emon et al. (2020) argue that decreased serum BDNF levels can be used as a marker for early detection of the risk of developing depression, since, as mentioned above, BDNF plays an important role in the pathophysiology of depressive disorders [43].

A study by Begliuomini et al. [7] revealed lower levels of BDNF in plasma in a group of patients with natural menopause and amenorrhea compared to women with regular menstrual cycles.Furthermore, the authors observed that circulating BDNF concentrations were negatively correlated with menopausal age in healthy women.

According to a study by Great et al., in women with infertility undergoing IVF treatment, low levels of nerve growth factor and low (nonsignificant) levels of BDNF were found in the follicular fluid of women with diminished ovarian reserve compared to the control group [10]. Palumbo et al. found low (nonsignificant) levels of BDNF in plasma after 3 months compared to baseline in women who had undergone chemotherapy, despite a previous decrease in follicular reserve [29].

The above data indicate the importance of research aimed at understanding the relationship between coronavirus infection, stress, and the development of POI in women of reproductive age. In this context, studies that reveal the role of kisspeptin and brain-derived neurotrophic factor (BDNF) may be important for understanding the molecular mechanisms of POI development after coronavirus infection and stress. This may have practical implications for the early diagnosis, treatment, and prevention of POI in women infected with COVID-19 and experiencing stress. Understanding hypothalamic markers such as KISS1 and BDNF may help develop effective strategies to maintain women's reproductive health and reduce the risk of developing POI. Further research in this area may

lead to new ways to treat and prevent POI, as well as a better understanding of the impact of coronavirus infection on women's health and reproductive function.

Recent studies have demonstrated the important role of kisspeptin and its receptors and BDNF in regulating the hypothalamic-pituitary-ovarian reproductive axis. Since the neuropeptide kisspeptin is a stimulator of GnRH secretion, responsible for both positive and negative feedback, it can be considered as a potentially promising marker for the correction of reproductive disorders.

Disturbances in the psychological state of women, especially those infected with COVID-19, can significantly affect reproductive health, including amenorrhea. Studying the pathogenetic mechanisms in this direction and finding effective methods for timely correction of these conditions remains highly relevant.

CHAPTER II. GENERAL CHARACTERISTICS OF THE STUDY COHORT AND METHODS

§2.1. Clinical characteristics of the examined women and research methods

Women with complaints of menstrual cycle disorders, in particular, after coronavirus infection, were examined at the "Consultation Clinic" department of the Republican Specialized Scientific and Practical Medical Center of Endocrinology named after Academician Y.Kh. Turakulov. The study is based on an analysis of studies conducted on 112 women aged 18 to 40 years (52 women who developed POI after COVID-19; 28 women with confirmed POI who were not infected with COVID-19; 32 healthy women of childbearing age).

The inclusion of patients in the main study group was carried out according to the following criteria:

I. Inclusion criteria: age 18 to 40 years; presence of menstrual cycle disorders (absence of menstruation for more than 4 months) after coronavirus infection; regular menstrual cycle before COVID-19 infection; absence of hormone replacement therapy (for the last three months).

II. Exclusion criteria: age under 18 and over 40 years; iatrogenic hypergonadotropic ovarian insufficiency (chemotherapy, ovarian surgery, radiation therapy); presence of POI disease in close relatives of the 1st and 2nd degree; organic diseases of the reproductive system (uterine fibroids, adenomyosis, endometrial pathologies) or chronic extragenital diseases associated with menstrual disorders; endocrinopathies (polycystic ovary syndrome, hyperprolactinemia, adenoma and other diseases of the pituitary gland); congenital chromosomal, genetic diseases (Turner syndrome) or malformations of the genitals associated with menstrual disorders; taking psychotropic drugs.

The comparison group was formed according to the following criteria:

I. Inclusion criteria: age 18 to 40 years; confirmed Premature ovarian insufficiency (disease duration 2 to 5 years); no hormone replacement therapy (for the last three months).

II. Exclusion criteria: age under 18 and over 40 years; history of COVID-19; iatrogenic hypergonadotropic ovarian insufficiency (chemotherapy, ovarian surgery, radiation therapy); organic diseases of the reproductive system (uterine fibroids, adenomyosis, endometrial pathologies) or chronic extragenital diseases associated with menstrual disorders; endocrinopathies (polycystic ovary syndrome, hyperprolactinemia, adenoma and other diseases of the pituitary gland); congenital chromosomal, genetic diseases (Turner syndrome) or malformations of the genital organs associated with menstrual disorders; taking psychotropic drugs.

The control group consisted of 32 healthy women aged 18 to 40 years with regular menstrual cycles confirmed by hormonal and ultrasound results.

Hormonal studies included determination of the levels of luteinizing hormone, follicle-stimulating hormone, estradiol, thyrotropin, anti-Müllerian hormone, kisspeptin (KISS1) and brain-derived neurotrophic factor (BDNF) in the blood. Ultrasound examination of the pelvic organs was performed using a 7.5 MHz transvaginal convex sensor and a 3.5 MHz abdominal sensor 2000 CHISON (Japan). The psychological state of women was assessed using the DASS-21 questionnaire. Statistical processing of all obtained data was carried out using the statistical software packages "Microsoft Excel", "Minitab 14" (USA) and "RStudio" (USA).

The level of anxiety, stress and depression was determined in all examined women (100%), the assessment was carried out using the Russian-adapted version of the DASS-21 questionnaire (V.V. Ruzhenkova, V.A. Ruzhenkov, I.S. Xamskaya, 2019). The questionnaire consists of 21 questions and consists of three scales designed to diagnose depression, anxiety and stress. Questions indicating the level of stress: 1,6,8,11,12,14,18; anxiety level: 2,4,7,9,15,19,20;

Depression level: 3,5,10,13,16,17,21. Answers to the questions are scored: 0 points – this statement does not apply to me; 1 point – this statement applies to me to some extent; 2 points – this statement applies to me to a large extent; 3 points – this statement applies to me completely.

"DASS-21" questionnaire

1 (stress)	It was difficult for me to overcome the tension.
2 (anxiety)	I felt a dry mouth.
3 (depression)	I didn't feel any positive emotions.
4 (anxiety)	I felt short of breath.
5 (depression)	It was hard for me to force myself to do anything.
6 (stress)	I tended to overreact to the situation.
7 (anxiety)	I felt a tremor (hands shaking)
8 (stress)	I felt like I was wasting a lot of nervous energy.
9 (anxiety)	I used to worry about situations where I might panic and act stupidly.
10 (depression)	I felt like I had nothing in the future.
11 (stress)	I felt a surge of excitement.
12 (stress)	It was hard for me to relax.
13 (depression)	I felt depressed.
14 (stress)	I looked forward to everything that bothered me.
15 (anxiety)	I felt panic approaching.
16 (depression)	I have no passion for anything.
17 (depression)	I felt a little less valuable as a person.
18 (stress)	I felt very nervous.
19 (anxiety)	I felt uncomfortable in the heart area without exercise.
20 (anxiety)	I felt an unreasonable fear.
21 (depression)	I felt that life was meaningless.

Interpretation of results: to calculate the final score, the points obtained must be multiplied by 2. The higher the scores, the higher the level of stress, anxiety, and depression.

CHAPTER III. RESEARCH RESULTS

§3.1. Clinical characteristics of the examined women

A total of 3612 women aged 18 to 40 years were consulted in an outpatient setting with complaints of menstrual disorders. After analyzing the data, it was found that 76.4% of the patients (2758 out of 3617) reported the absence of menstruation for several months (three to six months). This indicates that the majority of women seeking medical care had significant disturbances in their menstrual cycle, reflecting a high burden of reproductive health issues in this age group. Of these, the following types of menstrual cycles were identified: oligomenorrhea in 10% of women (361 out of 3617), amenorrhea in 76.4% (2758 out of 3617), and abnormal uterine bleeding in 13.6% (493 out of 3617). These indicators demonstrate the diverse nature of menstrual disorders and their varying severity, ranging from mild infrequent menstruation to complete absence of menstrual bleeding.

A total of 89% of women (3217) were infected with coronavirus. This extremely high proportion of COVID-19 cases among the consulted patients suggests a potential link between the infection and subsequent menstrual or endocrine disturbances. Among them, 73.4% of women (2364) had menstrual dysfunction after coronavirus infection, indicating that cycle irregularities may be one of the common post-infectious manifestations. A more detailed analysis revealed the following pathologies: gynecological problems and inflammatory diseases – 27.1% (641), polycystic ovary syndrome – 24.3% (574), functional hypothalamic amenorrhea – 21% (496), abnormal uterine bleeding – 13.4% (?), hyperprolactinemia – 12.1% (286), and Premature ovarian insufficiency – 2.1% (52). These findings demonstrate the wide spectrum of endocrine and gynecological conditions associated with post-COVID changes, reflecting the multisystem influence of the virus on female reproductive health.

It is important to note that all 52 women who developed Premature ovarian insufficiency had regular ovulatory cycles and had not experienced menstrual irregularities prior to contracting the coronavirus infection. This observation is particularly significant, as it suggests a possible causal association between COVID-19 and the onset of POI in previously healthy women with normal reproductive function. Such data highlight the potential pathogenic impact of coronavirus infection on ovarian reserve and endocrine regulation, reinforcing the need for further comprehensive studies in this direction. Moreover, this group of women may represent a new clinical phenotype of post-COVID reproductive dysfunction, which requires careful monitoring, timely diagnosis, and the development of targeted preventive and therapeutic measures.

To address these issues, anamnestic data, clinical, instrumental and laboratory studies of 112 women were analyzed.

- Group I (main group) - 52 women (average age 31.05±1.78 years old) complained of menstrual dysfunction that occurred after stress due to coronavirus infection.
- Group II (comparison group) - 28 women with confirmed diagnosis of POI (mean age 34.28±2.56 years).
- Group III (control group) – 32 women (mean age 28.68±2.1 years), with regular menstrual cycles, no complaints and no chronic diseases.

There were no significant differences in mean age of menarche and body mass index between the three groups (Table 1). This indicates that the groups were comparable in terms of key baseline characteristics, and therefore the observed clinical differences are unlikely to be influenced by these factors. According to age at menarche, two (1.7%) patients were younger than 12 years, 99 (88.3%) were between 12 and 14 years, and 11 (9.8%) were older than 14 years. These data suggest that the majority of participants experienced menarche within the typical physiological range, while only a small proportion had early or delayed onset of menstruation. Such distribution further supports the

homogeneity of the study population and minimizes the potential confounding effect of pubertal development on the outcomes analyzed.

Table 1

Age, BMI, and menstrual cycle.

Parameters	Group I (n=52)	Group II (n=28)	Group III (n=32)
Age, years	31.05±1.78	34.28±2.56	28.68±2.1
BMI, kg/m2	24.93±1.56	24.97±1.64	24.53±1.21
Menarche, years	13.28±0.33	13.17±0.54	12.87±0.27

According to anamnestic data, the following common infectious diseases can be identified: chickenpox – in 32 (61.53%) women of the main group; in 20 (71.42%) women of the comparison group; in 18 (56.25%) women of the control group; mumps – in 15 (28.84%) women of the main group; in 5 (17.85%) women of the comparison group; in 4 (12.5%) women of the control group. These findings indicate that a considerable proportion of women in all groups had a history of childhood viral infections, which are known to potentially influence long-term reproductive health. Although these conditions are common and typically self-limiting, their different prevalence across the groups may suggest variations in immune response or exposure that could be relevant when interpreting the study outcomes.

According to obstetric anamnesis, 17 (32.69%) pregnancies with delivery were recorded in the main group, 13 (46.42%) in the comparison group, and 20 (62.5%) in the control group. This distribution shows that the reproductive history differed among the groups, with the highest proportion of full-term pregnancies observed in the control group, suggesting more favorable reproductive outcomes in women without the primary condition under investigation. The lower rates of successful deliveries in the main and comparison groups may reflect underlying

endocrine, metabolic, or gynecological disturbances that could have contributed to reduced reproductive capacity. These differences are important for understanding the potential impact of the studied condition on fertility and pregnancy outcomes.

The co-occurring extragenital diseases of women in all three groups are presented in Table 2.

Table 2

Types and frequency of extragenital diseases in the examined patients

Diseases	Main group (n=52)	Comparison group (n=28)	Control group (n=32)
ENT diseases, n (%)	3 (5.76%)	3 (10.71%)	2 (6.25%)
Respiratory diseases, n (%)	8 (15.3%)	2 (7.14%)	-
CVD diseases, n (%)	-	1 (3.57%)	-
Breast disorders, n (%)	4 (7.69)	2 (7.14%)	-
Gastrointestinal diseases, n (%)	3 (5.75)	5(17.85%)	5 (15.62%)
Urinary system diseases, n (%)	1 (2%)	-	3 (9.37%)
Other endocrinopathies (thyroid diseases, diabetes mellitus, pituitary diseases, etc.), n (%)	9 (17.3%)	4(14.28%)	6 (18.75%)

At the time of examination, all concomitant diseases were in remission and did not require therapy. This ensured that the clinical picture was not influenced by acute comorbid conditions, allowing for a more objective assessment of reproductive function. The functional state of the reproductive system of the examined women was determined by hormonal analysis and transvaginal ultrasound of the pelvic organs, which together provide the most reliable diagnostic information regarding ovarian function, follicular reserve, and endocrine regulation.

In the main group and in the comparison group (patients with POI), the level of FSH and LH in the blood was significantly higher than in women in the control group. The elevation of gonadotropins is a classic diagnostic hallmark of ovarian insufficiency, reflecting the loss of negative feedback due to estrogen deficiency. The range of FSH levels in the main group was from 25.7 mIU/ml to 196.6 mIU/ml, and in the comparison group these indicators were from 26.6 mIU/ml to 139.9 mIU/ml. Such a wide variation indicates that patients with POI may demonstrate both moderate and extremely high elevations of FSH, depending on the duration and severity of follicular depletion. The level of LH in the blood was from 10.1 mIU/ml to 127.7 mIU/ml in the main group, and in the comparison group its level was from 16.8 mIU/ml to 77.1 mIU/ml. These data confirm the presence of hypergonadotropic hypogonadism in both groups, which is characteristic of premature ovarian insufficiency and serves as one of the key diagnostic criteria.

The level of FSH and LH in the blood of women with POI was significantly higher ($p < 0.05$) compared with healthy women (Table 3). This statistically significant difference demonstrates the reproducibility of the diagnostic parameters and confirms the reliability of the selected patient groups. Serum estradiol levels were also lower in women with POI in the main and comparison groups: the average values were 39.8±7.7 pg/ml and 46.73±14.25 pg/ml, respectively. Reduced estradiol concentrations reflect the decrease in ovarian estrogen-producing capacity, which leads to hypoestrogenic symptoms and contributes to the clinical picture of POI.

Estradiol levels ranged from 5.0 pg/ml to 175.1 pg/ml in the main group. It was also found that 1 patient in the main group had an estradiol level of 175.1 pg/ml, since the amenorrhea period was 4 months. High levels of estradiol were detected in women who had recently developed POI. This phenomenon is well described in the literature: in early stages of POI, ovarian activity may fluctuate, leading to transient estrogen production despite

elevated gonadotropins. Such fluctuations can explain occasional ovulation and spontaneous pregnancies observed in some POI patients.

According to the comparative analysis (Table 3.3), the average level of estradiol in the blood in the group of patients with POI was significantly lower than in the group of healthy women ($p < 0.05$). This confirms the diagnostic consistency of hypoestrogenism in POI and highlights its clinical significance for menstrual dysfunction, bone health, and overall endocrine balance.

AMH is one of the main biomarkers that allows you to assess the ovarian reserve. In all patients with POI, the AMH level was below 1.2 ng/ml. Such uniformly low AMH values indicate a severe depletion of the follicular pool, supporting both the clinical diagnosis and the laboratory findings of elevated gonadotropins and low estradiol. AMH deficiency in this population is an important prognostic marker, demonstrating the irreversibility of ovarian decline and the necessity for timely counseling on fertility preservation and hormonal therapy.

Table 3

Levels of follicle-stimulating hormone, luteinizing hormone, estradiol, and anti-Mullerian hormone by study group

Study groups	FSH, mIU/ml	LH, mIU/ml	Estradiol, pg/ml	AMG, ng/ml
	(norm: 3.5-12.5)	(norm: 2.4-12.6)	(norm: 73-506)	(norm: above 1.2)
Group I (main group) n=52	79.61±11.89*	45.1±6.89*	39.81±7.7*	0.13±0.07*
Group II (comparison group) n=28	70.82±12.85*	43.16±14.25*	46.73±14.25*	0.17±0.11*

Group III (control group) n=32	6.3±0.75	9.11±1.79	246.24±51.31	3.90±2.15

Note: *significance of differences compared to the control group ($p < 0.05$).

Ultrasound assessment of ovarian reserve and ovarian function is an important method in reproductive medicine. This non-invasive diagnostic approach provides valuable information about the structural and functional state of the ovaries and is widely used both in clinical practice and in scientific research. Ultrasound can be used to determine various parameters, including: ovarian volume (measuring ovarian volume allows us to assess their size and structure; a decrease in ovarian volume may indicate a decrease in ovarian reserve); antral follicle count (antral follicles are small fluid-filled cysts within the ovaries, each containing an immature egg; their number may be a direct indicator of ovarian reserve). These indicators together form an integral assessment of ovarian function and help clinicians evaluate the degree of follicular depletion in women with suspected reproductive disorders.

We analyzed the size and structure of the ovaries, the data we obtained are presented in Table 4. The ultrasound examination made it possible not only to quantify the follicular pool, but also to determine possible structural abnormalities that may accompany ovarian dysfunction. Such parameters as ovarian echogenicity, stromal density, and the presence or absence of dominant follicles were also taken into account, which adds additional depth to the interpretation of the obtained results. The summarized findings allow us to compare ovarian characteristics between study groups and assess how changes in ovarian morphology correlate with hormonal indicators and clinical manifestations of premature ovarian insufficiency.

Table 4

Ultrasound data of the uterus and appendages by group

Study groups	Right ovary		Left ovary	
	Volume, cm3	Antral follicle count	Volume, cm3	Antral follicle count
Group I (main group) n=52	2.49±0.04*	2.59±0.29*	2.48±0.03*	2.55±0.26*
Group II (comparison group) n=28	2.45±0.05*	2.35±0.32*	2.46±0.05*	2.42±0.35*
Group III (control group) n=32	6.66±0.04	6.87±0.27	6.67±0.05	6.87±0.29

Note: *significance of differences compared to the control group ($p < 0.05$).

According to ultrasound, both ovaries were visible in all 3 groups in 100% of cases. This indicates that the visualization quality was sufficient for reliable assessment of ovarian morphology and that no technical limitations interfered with the evaluation. The average volume of the ovaries in women with POI (in the main group and control group) did not exceed 3 cm^3. Such reduced ovarian volume is consistent with the typical ultrasound pattern of premature ovarian insufficiency and reflects a markedly diminished ovarian reserve. Ovarian atrophy and reduced stromal tissue are well-described sonographic features in this category of patients. Follicular apparatus – the number of antral follicles in the ovary did not exceed 5 pieces. A low antral follicle count is one of the most informative markers of ovarian reserve depletion and strongly correlates with impaired reproductive potential. The presence of fewer than five antral follicles per ovary is generally considered a sign of a critically reduced follicular pool, which aligns with the hormonal profile observed in women with POI. These findings, together with the reduced ovarian volume, provide a comprehensive

ultrasound confirmation of ovarian failure and support the clinical and laboratory diagnosis.

§3.2. History of COVID-19 and premature ovarian insufficiency

Of the examined women, 52 were diagnosed with COVID-19. The most common clinical manifestations were: fever (an increase in body temperature above 38 degrees), observed in all 52 (100%) patients. This universal presence of fever indicates the systemic inflammatory response characteristic of acute SARS-CoV-2 infection. Severe weakness was noted in 47 patients (90.3%), cough in 43 patients (82.6%), anosmia in 40 patients (77%), hyposmia in 12 patients (23%), loss of taste in 46 patients (88.4%), drowsiness, anxiety in 29 (55.7%), muscle pain in 33 (63.4%), shortness of breath in 38 (73%), and characteristic signs of viral lung damage on computed tomography (CT) in 43 (82.6%). These CT findings reflect the involvement of lung tissue typical for moderate to severe forms of viral pneumonia. In total, 28 women were admitted for inpatient treatment (53.8%), which demonstrates that more than half of the patients experienced a clinically significant course of the disease requiring hospital management.

Menstrual dysfunction was noted in 13 patients (25%) after 3–6 months, in 32 patients (61%) after 6–9 months, and in 7 patients (14%) for more than 9 months after contracting COVID-19 (Figure 1). This distribution shows that menstrual irregularities persisted long after the acute phase of infection, indicating a delayed or prolonged effect of COVID-19 on the hypothalamic–pituitary–ovarian axis. The fact that the majority of patients developed symptoms within 6–9 months suggests that post-infectious endocrine dysfunction may evolve gradually and manifest over an extended period. Such long-term consequences emphasize the importance of monitoring reproductive health in women after coronavirus infection, especially in those at risk for developing premature ovarian insufficiency.

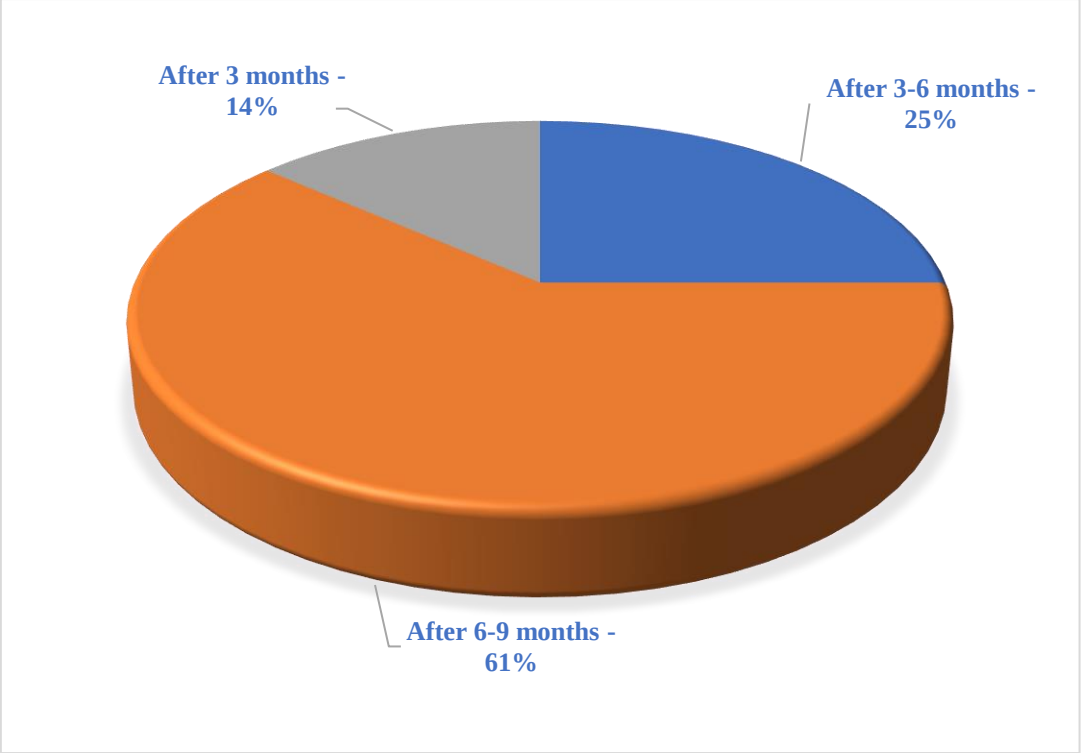


Figure 1. Period of onset of menstrual irregularities after coronavirus infection

The main complaints of women in the main group during treatment were as follows: amenorrhea in 52 patients (100%), hot flashes and sweating in 46 patients (88.4%), mood swings in 50 women (96.1%), sleep disturbances in 35 patients (67.3%), decreased or absent sexual desire in 43 women (82.6%), weight gain in 28 patients (53.8%), difficulty achieving pregnancy in 27 women (51.9%). These symptoms reflect a wide range of clinical manifestations characteristic of premature ovarian insufficiency and highlight the significant impact of this condition on the physical, emotional, and reproductive well-being of affected women.

Amenorrhea in all 52 patients (100%) represents the most prominent hallmark of ovarian failure and indicates a complete cessation of menstrual function. Hot flashes and sweating, reported by 46 women (88.4%), are typical vasomotor symptoms caused by estrogen deficiency and are often among the earliest signs of hormonal imbalance. Mood swings, present in 50 patients

(96.1%), further demonstrate the systemic effect of hypoestrogenism on the central nervous system, contributing to emotional instability, irritability, and reduced stress tolerance.

Sleep disturbances were noted in 35 women (67.3%), a common complaint associated with both hormonal changes and psychological stress linked to reproductive health concerns. Decreased or absent sexual desire in 43 patients (82.6%) reflects the decline in estrogen and androgen levels, which play an essential role in maintaining libido and overall sexual well-being. Weight gain observed in 28 women (53.8%) may be related to metabolic changes resulting from endocrine dysfunction, reduced physical activity, or emotional stress.

Difficulty achieving pregnancy in 27 women (51.9%) emphasizes the reproductive consequences of premature ovarian insufficiency and underlines the need for timely diagnosis and counseling. This symptom is particularly important because it directly impacts fertility potential and often becomes the primary reason for seeking medical care. The overall combination of these complaints demonstrates the multifactorial nature of POI and the importance of a comprehensive, multidisciplinary approach to management.

§3.3. Results of the "DASS-21" psychological status survey.

According to the results of the questionnaire, the level of anxiety was found to be significantly higher in the main group than in the comparison and control groups. This indicates that women with POI experience a markedly greater psychological burden, which may be associated with both hormonal changes and the emotional impact of reproductive dysfunction. The average level of anxiety was 17.44±0.54 in the main group, 6.32±0.43 in the comparison group, and 5.31±0.47 in the control group (data are presented in Table 5). These findings demonstrate that the anxiety scores in the main group were almost three times higher than in the comparison group and more than three times higher than in the

control group, highlighting a strong association between premature ovarian insufficiency and increased psychological distress.

The significantly elevated anxiety levels in women with POI may be explained by a combination of hypoestrogenism, stress related to menstrual dysfunction, uncertainty regarding fertility, and the chronic burden of symptoms such as hot flashes, insomnia, and mood instability. The comparatively low anxiety scores in the comparison and control groups suggest that psychological well-being remains relatively stable in women without POI, further emphasizing the importance of timely psychological assessment and supportive care in this patient population. These results underscore the need for integrating mental health evaluation into routine management strategies for women with premature ovarian insufficiency.

Table 5

Anxiety level according to the results of the DASS-21 questionnaire

Distribution by groups	Number of patients	Average anxiety level (in points)	Confidence interval for the mean value with a confidence level of 0.95	
			Upper limit	Lower limit
Group I (main group)	52	17.44*	17.98	16.90
Group II (comparison group)	28	6.32	6.74	5.89
Group III (control group)	32	5.31	5.78	4.84

Note: * The significance of differences is statistically significant compared to the comparison group and the control $p < 0.05$.

According to a comparative analysis of the scores, women in the main group had higher anxiety levels than women in the control and comparison groups (Figure 2).

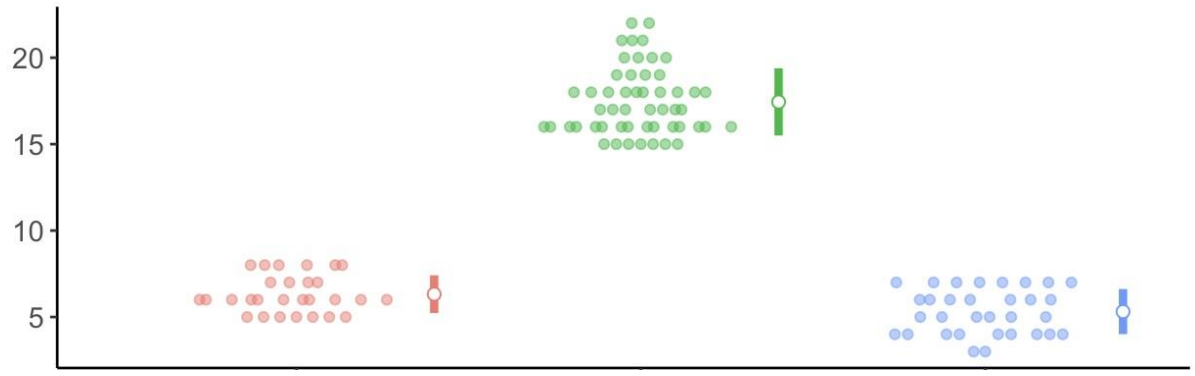


Figure 2. Distribution of anxiety levels by study group (group I – green, group II – red, group III – blue)

Next, we analyzed the stress levels of women in the three groups. According to the results of the analysis, the average score in the main group was 29.90±0.79, in the comparison group this figure was 12.07±0.64, and in the control group it was 9.65±0.64 (data are presented in Table 6). These results demonstrate a pronounced gradient in stress levels, with women in the main group exhibiting almost a threefold increase compared to the comparison group and more than a threefold increase compared to the control group. Such findings suggest that premature ovarian insufficiency is closely associated with heightened emotional tension and chronic stress, likely driven by both physiological mechanisms and the psychological burden of reproductive health concerns.

Also, the level of anxiety and stress was significantly higher in women with coronavirus infection. This observation aligns with global data indicating that COVID-19 can have long-term effects on mental well-being through mechanisms such as systemic inflammation, hormonal dysregulation, prolonged recovery, and uncertainty about health outcomes. The combined influence of premature ovarian

insufficiency and prior coronavirus infection may therefore potentiate emotional distress, creating an additive or synergistic effect on mental health parameters. These findings highlight the need for comprehensive psychological support and monitoring in this group of patients, as elevated stress levels may further exacerbate endocrine imbalance and negatively impact overall quality of life.

Table 6

Stress level according to the results of the DASS-21 questionnaire

Distribution by groups	Number of patients	Average stress level (in points)	Confidence interval for the mean value with a confidence level of 0.95	
			Upper limit	Lower limit
Group I (main group)	52	29.90 *	30.69	29.11
Group II (comparison group)	28	12.07	12.70	11.43
Group III (control group)	32	9.65	10.30	9.01

Note: * The significance of differences is statistically significant compared to the comparison group and the control $p < 0.05$.

According to the results of the study, the indicators of women in the main group were significantly different from those in the comparison and control groups. This statistically significant divergence suggests that premature ovarian insufficiency, especially when occurring after COVID-19 infection, is associated with more pronounced clinical, hormonal, and psychological changes. The obtained data indicate that the women in the main group demonstrated

consistently higher levels of emotional and physiological burden, which distinguishes them from other groups included in the analysis.

When comparing the scores, an increase in stress levels was characteristic of patients in the main group with coronavirus infection (Figure 3). This finding confirms that the combination of POI and previous SARS-CoV-2 infection may have a synergistic negative effect on stress response mechanisms. Post-COVID physiological alterations, together with hormonal dysregulation characteristic of POI, can amplify vulnerability to stress, emotional instability, and psychosomatic symptoms. The results underscore the need for targeted psychological and endocrine support for these women, as elevated stress can further worsen reproductive and metabolic outcomes.

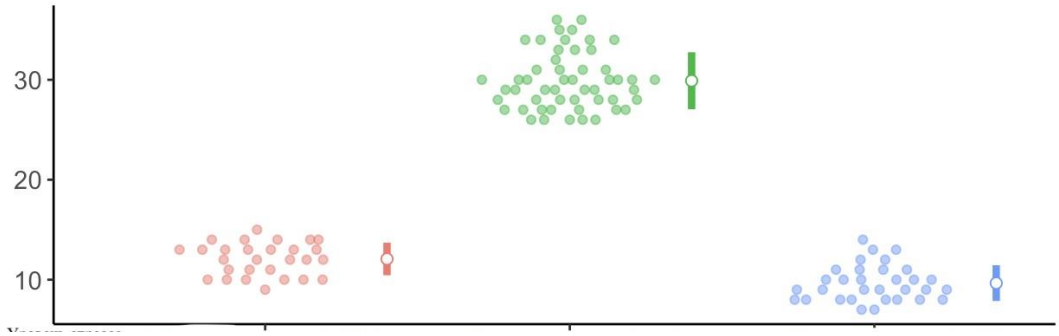


Figure 3. Distribution of stress levels by study group (group I – green, group II – red, group III – blue)

The third indicator of the DASS-21 questionnaire is the level of depression. As a result of the evaluation of the scores, we found that the level of depression was lower in the control and comparison groups. This trend indicates that women without premature ovarian insufficiency, or those with milder reproductive disturbances, experience significantly fewer depressive symptoms. The average score determining the level of depression was 23.42±0.71 in the main group, 11.92±0.61 in the comparison group, and 6.09±0.57 in the control group (data are presented in Table 7). These findings demonstrate that depressive symptoms were

almost twice as high in the main group compared to the comparison group, and nearly four times higher than in the control group.

Such a pattern suggests a strong association between premature ovarian insufficiency and increased susceptibility to depressive states. This relationship can be explained by several mechanisms, including estrogen deficiency, disruption of the hypothalamic–pituitary–ovarian axis, and the psychological burden associated with menstrual dysfunction and reduced fertility potential. The much lower depression scores in the comparison and control groups reinforce the notion that hormonal imbalance plays an essential role in emotional well-being and that women with POI are particularly vulnerable to mood disturbances.

Table 7

Level of depression according to the results of the DASS-21 questionnaire

Distribution by groups	Number of patients	Average level of depression (in points)	Confidence interval for the mean value with a confidence level of 0.95	
			Upper limit	Lower limit
Group I (main group)	52	23.42*	24.12	22.71
Group II (comparison group)	28	11.92	12.54	11.31
Group III (control group)	32	6.09	6.66	5.52

Note: * The significance of differences is statistically significant compared to the comparison group and the control $p < 0.05$.

According to the data in the table, you can see the difference in the level of depression in the three groups. The highest rates of depression were found in the main group, that is, in women with POI due to coronavirus infection (Figure 4).

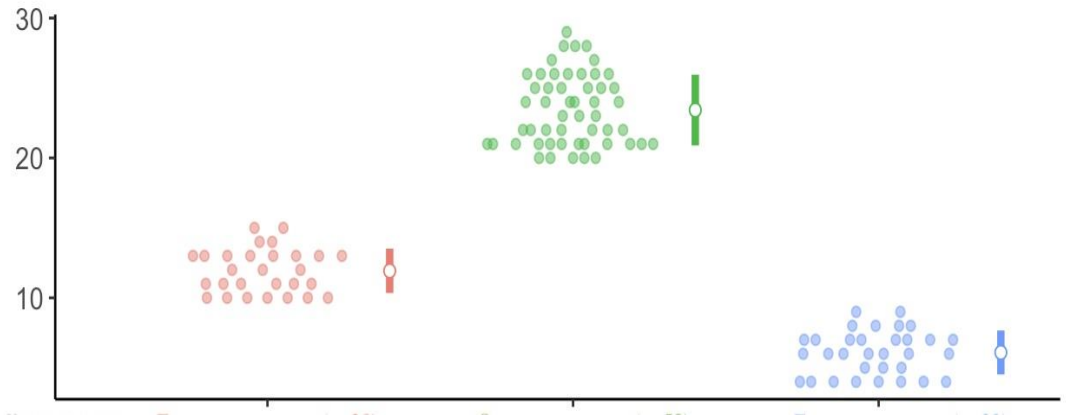


Figure 4. Distribution of depression levels by study group (group I – green, group II – red, group III – blue)

Across all three measures, women in the baseline group showed significantly more severe levels of anxiety, stress, and depression.

§3.4. Determination of neuropeptide levels (KISS1 and BDNF)

Analysis of the content of KISS1 (kisspeptin) and BDNF (brain-derived neurotrophic factor) in the blood of patients with Premature ovarian insufficiency (POI) who developed after COVID-19, and comparison with women from the control and comparison groups, is an important study that will help to understand the molecular mechanisms associated with the development of this condition. Such an analysis allows us to identify key biomarkers that may reflect alterations in neuroendocrine pathways triggered by SARS-CoV-2 infection and contributing to ovarian dysfunction. The assessment of these indicators plays a crucial role in linking clinical manifestations with underlying endocrine and neurobiological processes involved in reproductive health.

Analysis of KISS1 concentrations showed significant changes in its values in the studied groups. You can see the difference in kisspeptin concentrations in the blood in Table 8. This may indicate the important role of kisspeptin in the regulation of the reproductive system in women and its association with the development of POI after COVID-19. Kisspeptin is known for its function in

regulating the hypothalamic-pituitary-gonadal axis and the level of gonadotropic hormones. Disturbances in kisspeptin levels may reflect dysregulation of GnRH secretion, leading to disruption of ovarian function and contributing to the clinical picture of POI. The observed deviations in KISS1 concentrations may therefore represent one of the central links in the cascade of neuroendocrine changes induced by coronavirus infection.

Evaluation of BDNF values is also important, since this neurotrophic factor may be associated with neuroinflammation and may affect the function of the hypothalamus and pituitary gland. Alterations in BDNF levels can impair neuronal signaling and regulatory mechanisms essential for maintaining hormonal balance, thus influencing reproductive function. Given that COVID-19 is known to cause inflammatory processes in the central nervous system, including the hypothalamic regions responsible for reproductive control, changes in BDNF levels may provide additional insight into the pathways leading to premature ovarian insufficiency.

These results provide valuable information for understanding the pathophysiology of POI following COVID-19 and may serve as a starting point for further research and the development of potential therapeutic strategies. The combined assessment of kisspeptin and BDNF creates a more comprehensive picture of neuroendocrine alterations and opens the possibility for identifying early biomarkers that could help in predicting POI development after viral infection. By evaluating both hypothalamic regulatory peptides and neurotrophic factors simultaneously, it becomes possible to capture early stages of central dysregulation before overt clinical manifestations appear. This integrated approach may allow clinicians to identify subtle disruptions in the hypothalamic–pituitary–gonadal axis long before the onset of amenorrhea or hormonal decline.

Moreover, these findings highlight the importance of integrated neuroendocrine monitoring in women recovering from COVID-19, especially in those presenting with menstrual dysfunction or early signs of ovarian

insufficiency. Routine assessment of kisspeptin and BDNF may serve as an additional diagnostic tool alongside traditional reproductive hormone panels, offering a deeper understanding of central regulatory mechanisms affected by SARS-CoV-2. In clinical practice, incorporating these markers into follow-up protocols could facilitate early diagnosis, timely intervention, and the development of personalized strategies aimed at preserving ovarian function.

Furthermore, the observed associations suggest that therapeutic approaches targeting neurotrophic pathways or stress-related hypothalamic suppression may have potential in mitigating POI progression after COVID-19. This includes interventions aimed at reducing chronic stress, modulating HPA axis activity, or supporting neuronal resilience through lifestyle, pharmacological, or neuromodulatory therapies. Future studies may focus on longitudinal monitoring, interventional trials, and mechanistic research that further elucidates how viral neurotropism, inflammation, and psychoemotional disturbances jointly contribute to ovarian aging.

Table 8

Blood kisspeptin levels by study group

Distribution by groups	Number of patients	Average kisspeptin level (pg/ml)	Confidence interval for the mean value with a confidence level of 0.95	
			Upper limit	Lower limit
Group I (main group)	52	249.39*	256.62	242.16
Group II (comparison group)	28	352.50 *	361.10	343.89

Group III (control group)	32	439.90	448.23	431.58

Note: * The significance of differences is statistically significant compared to the comparison group and the control $p < 0.05$.

According to the results of the study, the average blood kisspeptin concentration in the main group was 249.39±7.23 pg/ml. The kisspeptin level in this group ranged from 191.4 to 298.1 pg/ml. These relatively low values indicate a pronounced suppression of kisspeptin production in women with premature ovarian insufficiency following COVID-19, which may reflect impaired regulation of the hypothalamic–pituitary–gonadal axis. The mean blood kisspeptin concentration in the comparison group was 352.50±8.61 pg/ml, with a range of 310.01 to 387.1 pg/ml. This intermediate level suggests that although POI is present in this group, the absence of coronavirus infection may mitigate the degree of neuroendocrine disruption compared with women in the main group.

The highest mean blood kisspeptin concentration was found in the control group – 439.90±8.32 pg/ml. These values represent normal physiological levels and are consistent with preserved ovarian reserve and stable reproductive function. The marked gradient observed across the three groups—from lowest in the main group, higher in the comparison group, and highest in the control group—illustrates the strong association between kisspeptin deficiency, ovarian dysfunction, and the potential aggravating impact of SARS-CoV-2 infection.

The level of kisspeptin in the blood was lowest in women with coronavirus infection. This finding emphasizes the possible role of COVID-19 in suppressing kisspeptin synthesis or secretion, which may contribute to the development of premature ovarian insufficiency. Given the central role of kisspeptin in regulating GnRH pulsatility, gonadotropin release, and overall reproductive endocrine

function, reduced kisspeptin concentrations may be a key mechanistic link in the post-COVID pathogenesis of POI.

The difference in blood concentrations of kisspeptin in the main group was significant compared to the control and comparison groups (Figure 5). This statistically significant variation confirms the relevance of kisspeptin as a sensitive biomarker reflecting neuroendocrine alterations and highlights its potential utility in the early detection of reproductive dysfunction following coronavirus infection.

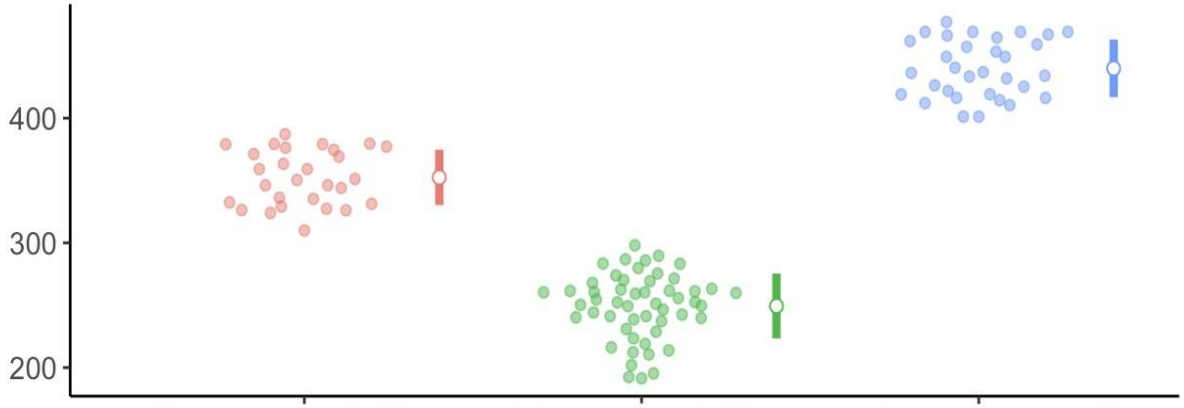


Figure 5. Comparison of kisspeptin concentrations in the blood of the women examined by groups

(group I – green, group II – red, group III – blue)

Blood BDNF levels were measured in all three groups. The mean BDNF concentration in the main group was significantly lower than in women in the control and comparison groups. This reduction in BDNF levels suggests a possible impairment of neurotrophic regulation associated with premature ovarian insufficiency, particularly when it develops after COVID-19 infection. Given that BDNF plays an essential role in neuronal survival, synaptic function, and hypothalamic regulation of endocrine processes, decreased concentrations may reflect neuroinflammatory changes or disrupted signaling pathways affecting reproductive function.

Table 9 presents the data from the study of serum BDNF levels. The differences observed between the groups demonstrate a clear and consistent trend, with the lowest BDNF levels detected in the main group, intermediate values in the comparison group, and the highest concentrations in healthy controls. This gradient may indicate the combined impact of ovarian dysfunction and post-COVID neuroendocrine alterations, further reinforcing the association between SARS-CoV-2 infection and long-term disturbances in neurobiological markers. The findings highlight the potential significance of BDNF as a supplementary biomarker for assessing neuroendocrine involvement in the pathogenesis of POI following coronavirus infection.

Table 9

Blood BDNF levels by study group

Distribution by groups	Number of patients	Average BDNF level (pg/ml)	Confidence interval for the mean value with a confidence level of 0.95	
			Upper limit	Lower limit
Group I (main group)	52	229.32*	236.35	222.29
Group II (comparison group)	28	336.14*	343.26	329.02
Group III (control group)	32	428.58	435.80	421.37

Note: * The significance of differences is statistically significant compared to the comparison group and the control $p < 0.05$.

In patients with POI that developed as a result of coronavirus infection, the level of BDNF varied from 171.04 pg/ml to 278.3 pg/ml. This relatively wide range reflects individual variability in the degree of neurotrophic impairment

among affected women, likely influenced by the severity of infection, duration of amenorrhea, and underlying endocrine dysfunction. According to the test results, the average level of BDNF concentration in the blood in the main group was 229.32±7.03 pg/ml. Such reduced values indicate a significant decline in neurotrophic support in women with POI following COVID-19, which may be linked to inflammatory processes, hypothalamic dysregulation, or impaired neuronal signaling pathways.

The serum level of BDNF in the comparison group was 336.14±7.12 pg/ml and ranged from 310.66 to 368.61 pg/ml. These intermediate values demonstrate that although women in this group also had premature ovarian insufficiency, the absence of coronavirus infection resulted in less pronounced reduction of BDNF levels. This suggests that COVID-19 may serve as an additional aggravating factor contributing to neuroendocrine disruption.

The BDNF concentration level in the control group was the highest: 428.58±7.21 pg/ml. These values are consistent with normal physiological ranges and reflect healthy neurotrophic function in women without ovarian pathology or exposure to SARS-CoV-2 infection. The clear progression—from lowest levels in the main group, higher in the comparison group, and highest in the control group—demonstrates a strong association between reduced BDNF levels, ovarian dysfunction, and post-COVID physiological disturbances.

According to the results of this study, it can be said that the level of BDNF, as well as the level of kisspeptin in the blood, was the lowest in women with coronavirus infection. This parallel decline emphasizes the combined disruption of both neurotrophic and neuroendocrine pathways in post-COVID POI and suggests that these biomarkers may jointly contribute to the pathophysiology of ovarian failure.

The difference in the blood level of BDNF in the main group was significant compared to the two groups (Figure 6). This statistically significant difference reinforces the hypothesis that COVID-19 may intensify the

mechanisms leading to POI and highlights the potential diagnostic and prognostic importance of BDNF measurements in women with post-infectious reproductive dysfunction.

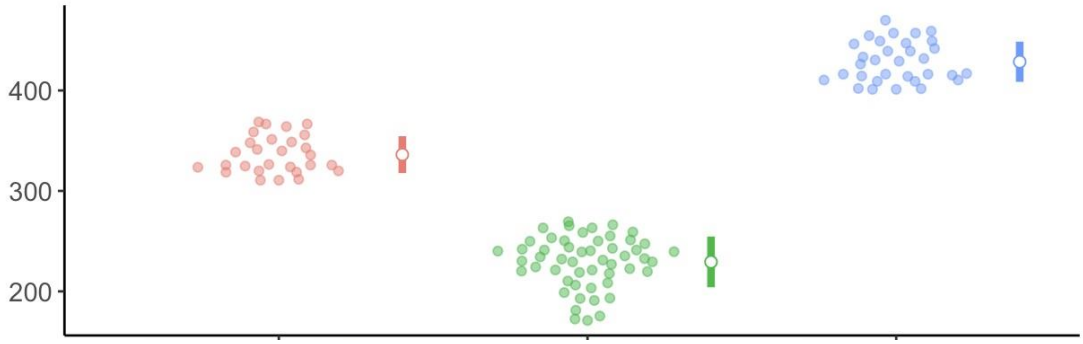


Figure 6. Comparison of BDNF concentrations in the blood of the women examined by groups

(group I – green, group II – red, group III – blue)

Thus, in the process of analyzing survey data, it was found that women change their psycho-emotional state after suffering from COVID-19, which in turn leads to an increase in the level of stress, anxiety and depression. These findings indicate that SARS-CoV-2 infection has long-term consequences not only for physical health, but also for mental and emotional well-being. Elevated levels of psychological distress may reflect a combination of post-viral fatigue, chronic inflammatory response, social stressors related to illness, and the emotional burden associated with menstrual and reproductive dysfunction.

In turn, they lead to a decrease in the secretion of kisspeptin and brain-derived neurotrophic factor, which leads to a disruption in the regulation of the female reproductive system. Given that kisspeptin is a key regulator of the hypothalamic–pituitary–gonadal axis, and BDNF plays an essential role in neuroendocrine signaling and neuronal function, reductions in these biomarkers may serve as important mechanistic links between psychological stress, neuroinflammation, and impaired ovarian function. This cascade of changes illustrates the interconnected nature of the neuroendocrine and emotional systems

in women, highlighting how alterations in emotional state can translate into measurable disruptions in reproductive regulation.

§3.5. Correlation analysis of the obtained data

In fact, a correlation coefficient that is different from 0 in the sample does not mean that the correlation in the population is significantly different from 0. This must be checked using a hypothesis test known as a correlation test. The null and alternative hypotheses for a correlation test are:

- *H0*:ρ= 0 (this means there is no linear relationship between the two variables)
- *H1*:ρ≠ 0 (means there is a linear relationship between the two variables)

Using this correlation test, the following statements are tested:

- There is sufficient evidence in the sample to reject the null hypothesis and conclude that the correlation coefficient is not equal to 0, so there is a relationship in the population.
- or conversely, there is not enough evidence in the sample that the correlation coefficient is not equal to 0, so in this case we do not reject the null hypothesis that there is no relationship between the variables. the general population.

As a result of the tests, we conducted a correlation analysis between hypothalamic markers (serum KISS1 and BDNF levels) and the psychoemotional state of women (stress level, anxiety and depression) in patients with POI and healthy women in interaction with the presence of COVID-19 in history. This analysis allowed us to identify the interrelationships between neuroendocrine regulation, emotional well-being, and reproductive function, providing a comprehensive view of how COVID-19 may influence multiple physiological systems simultaneously.

We found a significant association between kisspeptin levels and brain-derived neurotrophic factor and psychoemotional state indicators in women with coronavirus infection. A strong positive correlation was found in the overall sample (n=112), with a correlation coefficient of 0.93 ($p<0.001$). This high level of significance demonstrates that changes in neuroendocrine markers are closely linked to alterations in emotional state, confirming the integral relationship between stress-related pathways and reproductive function after COVID-19.

In patients with POI after coronavirus infection (main group), a negative correlation of moderate strength was found between kisspeptin levels and anxiety ($r = –0.79$, $p < 0.001$), and a moderate negative correlation was also found between kisspeptin levels and stress ($r = –0.86$, $p < 0.001$). These results indicate that lower kisspeptin concentrations are associated with higher levels of stress and anxiety, suggesting that kisspeptin suppression may be one of the mechanisms linking emotional dysregulation with ovarian dysfunction in the post-COVID period.

In addition, there was a strong negative correlation between BDNF levels and depression ($r = –0.85$, $p < 0.001$) in women in the main group. This finding highlights the important role of BDNF in maintaining emotional stability and suggests that reduced BDNF levels may contribute to the development of depressive symptoms in women with POI following COVID-19.

A strong positive correlation was found between stress and anxiety levels, with the correlation coefficient being 0.93 ($p < 0.001$) in women with POI due to COVID-19 (Figure 7). This close relationship confirms that emotional disturbances in this group tend to occur simultaneously and reinforce each other, forming a cluster of psychoemotional symptoms that are significantly more pronounced than in other groups.

Together, these results indicate a complex, multidirectional interaction between neuroendocrine markers and psychoemotional state. They provide strong evidence that COVID-19 can exacerbate both hormonal imbalance and

emotional dysregulation, ultimately accelerating the development of premature ovarian insufficiency.

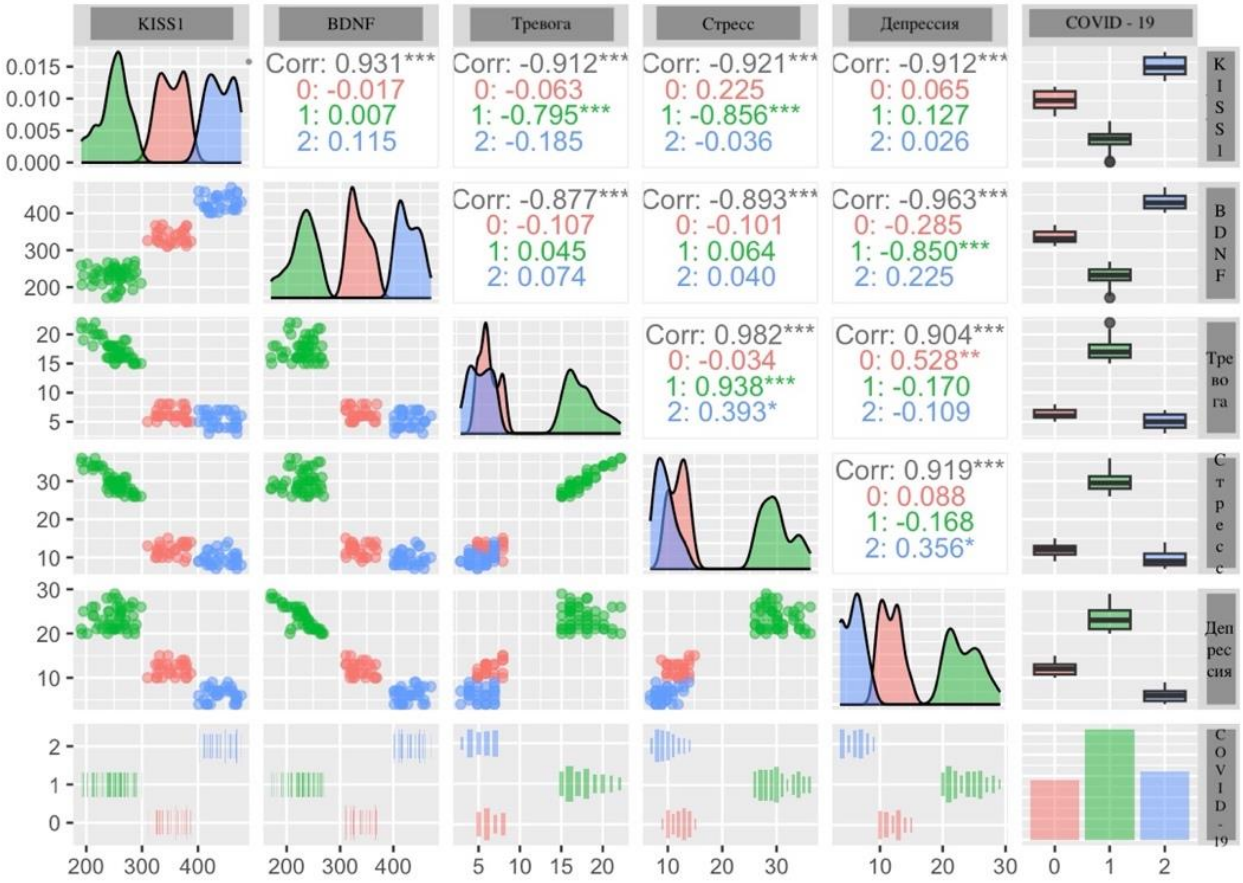


Figure 7. Pairwise statistical analysis of KISS1, BDNF, stress, anxiety, depression indicators by study groups

Note: The result of the computer program calculation produces the following significance levels (α= (0.1, 0.05, 0.01, 0.001, < 0.001) and the corresponding symbols (' ', ' . ', '*', '**', '***') depending on the p-value. Green represents the main group, blue represents the comparison group, and red represents the control group.

Many studies rely on the use of a statistic called a p-value to assess whether we can reject or not the null hypothesis. Given the assumption that the null hypothesis is accepted, the p-value is defined as the probability of obtaining an extreme result that is equal to or more than the one actually observed in the data. The p-value for a given set of data is determined by performing a statistical test. This p-value is then compared to a predetermined valueα. If the p-value for the test is less than the alpha value, the null hypothesis is rejected. If the p-value is greater than or equal to alpha, the null hypothesis is accepted.

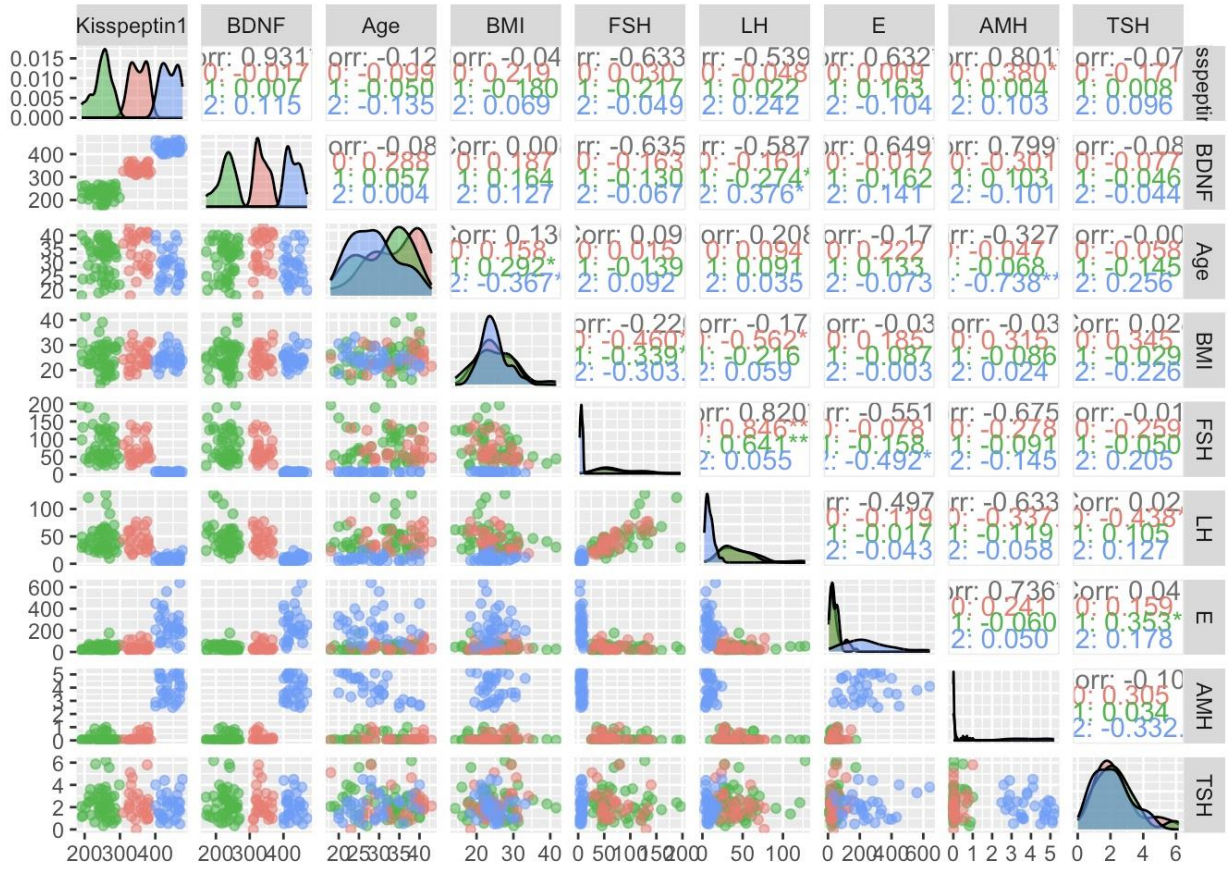


Figure 8. Pairwise statistical analysis of KISS1, BDNF, age, BMI, FSH, LH, Estradiol, AMH, TSH indicators by study groups

Note: The result of the computer program calculation produces the following significance levels (α= (0.1, 0.05, 0.01, 0.001, < 0.001) and the corresponding symbols (' ', ' . ', '*', '**', '***') depending on the p-value. Green represents the main group, blue represents the comparison group, and red represents the control group.

Among other indicators, the results of the correlation analysis did not reveal a strong correlation between LH and FSH, as well as between age and AMH in the control group ($r = –0.74$, $p < 0.001$), which means that other variables may not have linear relationships with each other in the given data sample (Figure 8). This suggests that in healthy women, the regulatory mechanisms of the reproductive system may function independently of age-related changes within the observed range, and that gonadotropin levels remain relatively stable in the absence of ovarian pathology. The lack of strong correlations in the control group emphasizes that significant associations seen in women with POI arise only in the context of pathological processes, particularly those triggered by COVID-19.

A positive correlation was found between FSH and LH in the total sample (n = 112) with a correlation coefficient of 0.82. This indicates a synchronous increase in both gonadotropins in response to ovarian insufficiency, reflecting the classic pattern of hypergonadotropic hypogonadism. That is, a change in one variable is not accompanied by a systematic change in the other variables. This fact supports the conclusion that while gonadotropins are tightly linked, other reproductive markers such as AMH or emotional state indicators follow their own independent trajectories.

Thus, the data from the correlation analysis confirm the existence of a relationship between the psychoemotional state of a woman and the level of hypothalamic symptoms in women with POI due to coronavirus infection. These findings highlight the multidimensional nature of post-COVID reproductive dysfunction, in which emotional disturbances, neuroendocrine impairment, and ovarian insufficiency are interconnected through overlapping physiological pathways. The results underscore the importance of comprehensive assessment—including hormonal, psychological, and neurobiological evaluation—in women who developed POI following SARS-CoV-2 infection.

§3.6. Correlation and regression analysis of the results obtained

To achieve the best results, certain conditions (Gauss-Markov conditions) must be met.

The mathematical expectation of the random error for any observation must be zero, i.e. $E(si)=0(i=1,2,\ldots n)$.

The variance of the random error for all observations must be constant, i.e. $D(si)=s2(i=1,2,\ldots n)$.

The random errors must be statistically independent, i.e. $E(si*sj)=0(i\neq j)$.

If the Gauss-Markov conditions are met, the model can be called a classical normal linear regression model, with random errors normally distributed, i.e. ssi $=N(0,1)$.

Based on the results of the correlation analysis, we decided to predict the indicators for which the correlation coefficient was very significant. We conducted a regression analysis of kisspeptin levels with the severity of anxiety and stress, as well as neurotrophic factor levels with the degree of depression in the main group of women. This approach made it possible not only to quantify the strength of associations but also to create predictive models capable of estimating hormonal and neurotrophic changes based on psychoemotional parameters. Such modeling is especially important in understanding the mechanisms of POI development after COVID-19, where psychological and neuroendocrine disturbances appear to be closely intertwined.

The distribution of data in the main group had a bell-shaped shape, indicating that we could proceed to linear regression analysis. This distribution confirms that the assumptions of parametric modeling—normality and homoscedasticity—were largely met, making linear regression an appropriate and valid analytical tool. The bell-shaped distribution also suggests that the studied markers (kisspeptin, BDNF, stress, anxiety, depression) vary in a predictable manner within the population of women with POI, reflecting consistent biological responses to stress and viral neuroinvasion.

The linear regression model in the main group for kisspeptin and anxiety levels is shown in Table 10. This model provides a quantitative assessment of how changes in anxiety severity influence kisspeptin concentrations, thereby clarifying the extent to which emotional dysregulation affects hypothalamic function. By examining the regression coefficients, confidence intervals, and significance levels, we are able to evaluate the predictive accuracy of the model and determine how reliably anxiety levels can serve as a surrogate marker for kisspeptin suppression. These findings offer important insights into the neuroendocrine pathways involved in the development of premature ovarian insufficiency after COVID-19.

Table 10

Linear regression analysis assessing the relationship between kisspeptin levels and anxiety levels

The parameters	Evaluation	CI (confidence interval)	p-value
Estimate	434.49	394.09-474.90	<0.001
Anxiety level	-10.61	-12.91 - -8.31	<0.001
R2/R2adj (coefficient of determination)	0.631/0.624		
Number of patients	52		

Table 10 shows the estimates for the model parameters—the value of the free coefficient (in this case 434.49) and the estimated effect of anxiety level on kisspeptin (–10.61). The free coefficient reflects the predicted kisspeptin concentration when the anxiety level is zero, indicating that in the absence of anxiety, kisspeptin levels remain within the physiological range typical of healthy women. This aligns with the baseline levels observed in the control group and supports the biological plausibility of the model.

The regression coefficient of –10.61 shows that with each one-point increase in anxiety scores, kisspeptin levels decrease by an average of 10.61 pg/ml. This represents a substantial decline, suggesting that anxiety has a strong inhibitory effect on hypothalamic kisspeptin-producing neurons. Given the central role of kisspeptin in GnRH pulsatility and reproductive hormonal regulation, such reductions may contribute directly to the mechanism by which anxiety exacerbates reproductive dysfunction in women after COVID-19.

This represents the diagnosticity of the model—the most important thing to consider is the p-value (here it is 2.02e-12, or almost zero), which indicates whether the model is reliable or not and whether it fits the data. An extremely small p-value indicates a high degree of statistical significance, confirming that

the observed relationship between anxiety and kisspeptin is robust, reproducible, and not due to random variation. This reliability strengthens the argument that anxiety is an important predictor of neuroendocrine disruption in women with POI.

Based on these results, we can say that there is a significant negative relationship between anxiety level and kisspeptin (p-value < 0.001), with kisspeptin levels changing by –10.61 units (+/– 0.01) for each unit increase in anxiety level. The narrow confidence interval around this estimate demonstrates high precision of the model and reinforces the stability of the predicted values. Biologically, this finding supports the concept that anxiety-induced activation of the hypothalamic–pituitary–adrenal axis suppresses kisspeptin secretion, thereby impairing reproductive hormone regulation and potentially accelerating the development of premature ovarian insufficiency.

The linear regression model in the main group for kisspeptin and stress levels is shown in Table 11.

Table 11

Linear regression analysis assessing the relationship between kisspeptin levels and stress levels

The parameters	Evaluation	CI (confidence interval)	p-value
Estimate	482.46	442.46-522.68	<0.001
Stress level	-7.79	-9.13 - -6.46	<0.001
R2/R2adj. (coefficient of determination)	0.732/0.727		
Number of patients	52		

ccording to Table 11, the model parameters are estimated — the value of the free coefficient (in this case 482.46) and the estimated effect of stress level on kisspeptin (–7.79). The free coefficient reflects the predicted kisspeptin concentration when the stress level is zero, indicating that in the absence of stress, kisspeptin levels would remain within the physiologically normal range typical of

healthy women. This value aligns with the baseline kisspeptin levels observed in the control group, reinforcing the internal validity of the model.

According to the data, the p-value (here it is equal to 6.43e–16, or almost zero) indicates the level of reliability of the model. Such an extremely small p-value demonstrates a very high degree of statistical significance and confirms that the relationship observed between stress and kisspeptin is not accidental. This reliability suggests that stress plays a major and consistent role in predicting kisspeptin levels in women with POI after COVID-19.

Based on these results, according to the data, it can be said that there is a significant negative relationship between stress level and kisspeptin (p value < 0.001), with kisspeptin levels changing by –7.79 units (+/– 0.01) for each unit increase in stress level. The precision of this estimate, reflected by the narrow confidence interval, shows that the model produces stable and reproducible predictions. Biologically, a decrease of nearly 8 pg/ml in kisspeptin concentration per each incremental rise in stress score is considerable, given the central role of kisspeptin in regulating GnRH secretion and reproductive function.

This statistical pattern confirms that stress exerts a strong inhibitory effect on kisspeptin-producing neurons, likely mediated by activation of the hypothalamic–pituitary–adrenal (HPA) axis and cortisol elevation during prolonged stress exposure. Thus, chronic psychoemotional tension may significantly suppress hypothalamic signaling, contributing to impaired reproductive hormone regulation and the progression of premature ovarian insufficiency.

Overall, these regression results support the conceptual framework in which stress acts as a critical neuroendocrine disruptor, reducing kisspeptin levels and accelerating the development of ovarian insufficiency in women affected by COVID-19.

According to the analysis, we concluded that we can predict kisspeptin levels based on anxiety levels using the following formula:

$$Kisspeptin1 = 434.5 - 10.61 * anxiety\ level$$

Correlation-regression analysis revealed a relationship between these parameters, with a coefficient of determination of 0.6241. This means that 62.4% of the variation in kisspeptin is explained by changes in anxiety levels, while the remaining 37.6% is explained by other factors. Thus, anxiety represents an important predictor of kisspeptin concentration, although its influence is somewhat lower compared to the effect of stress. Nevertheless, the fact that more than half of the variance in kisspeptin levels can be accounted for by anxiety underscores the substantial role of emotional dysregulation in hypothalamic imbalance among women who developed POI after COVID-19.

The relationship between kisspeptin and anxiety levels is moderate and negative with a correlation coefficient of –0.795, i.e. an increase in anxiety levels leads to a decrease in kisspeptin (Figure 9(a)). This inverse association suggests that heightened anxiety is accompanied by suppression of kisspeptin signaling, reflecting the well-established inhibitory influence of anxiety-related activation of the hypothalamic–pituitary–adrenal (HPA) axis on the hypothalamic–pituitary–gonadal (HPG) axis. Elevated anxiety increases cortisol secretion, which interferes with the activity of kisspeptin neurons, leading to decreased GnRH pulsatility and subsequent reproductive dysfunction.

The moderate strength of the correlation indicates that anxiety is an important but not the only factor affecting kisspeptin levels. Other contributors may include chronic stress, neuroinflammatory changes following SARS-CoV-2 infection, metabolic disturbances, sleep alterations, and individual biological susceptibility. This multifactorial influence highlights the complexity of neuroendocrine interactions in the post-COVID period.

Nevertheless, the regression analysis confirms that anxiety has a measurable and biologically relevant impact on kisspeptin concentrations, supporting the concept that emotional and psychological disturbances contribute directly to the development and progression of premature ovarian insufficiency. As illustrated in Figure 9(a), higher anxiety scores consistently correspond to

lower kisspeptin values, reinforcing the hypothesized mechanism through which psychoemotional burden disrupts reproductive regulation at the hypothalamic level.

To estimate kisspeptin levels based on stress levels, you can use the following formula:

$$Kisspeptin1 = 482.5 - 7.794 * stress\ level$$

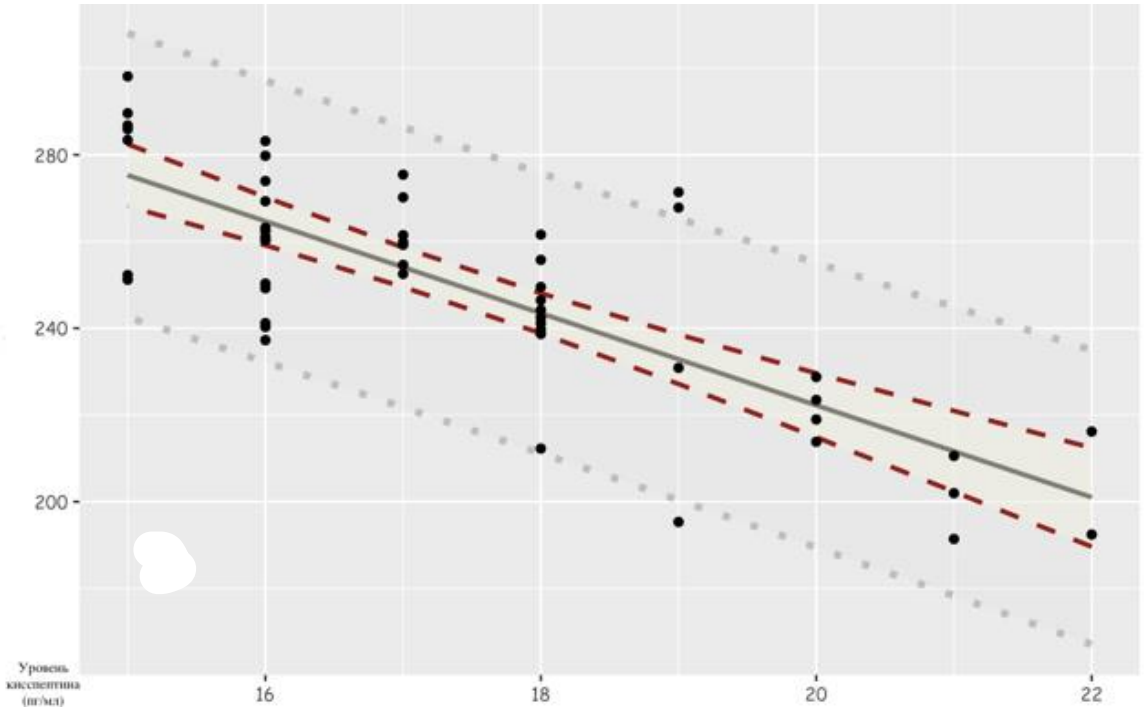
Also, according to the correlation-regression analysis, it became clear that there is a relationship between these indicators, the coefficient of determination is 0.726. This means that 72.6% of the changes in kisspeptin are explained by changes in stress levels, and the remaining 27.4% are explained by other factors. Such a high value of the coefficient of determination indicates that stress is one of the key predictors influencing kisspeptin secretion in women with POI after COVID-19. The model captures the majority of variability in kisspeptin levels, which confirms the close neuroendocrine connection between stress reactivity and hypothalamic regulation.

The correlation coefficient is –0.856, so the relationship between the parameters under consideration is strong and inverse, and an increase in stress levels leads to a decrease in kisspeptin levels and vice versa, as can be seen in the graph (Figure 9 (b)). This strong negative correlation indicates that elevated stress suppresses kisspeptin secretion, which is consistent with the known inhibitory effect of chronic stress on the hypothalamic–pituitary–gonadal axis. As stress increases, activation of the hypothalamic–pituitary–adrenal (HPA) axis leads to increased cortisol production, which can inhibit kisspeptin neurons and disrupt GnRH pulsatility. Consequently, a decrease in kisspeptin levels may serve as a biological mediator linking psychological stress to reproductive dysfunction and the development of POI.

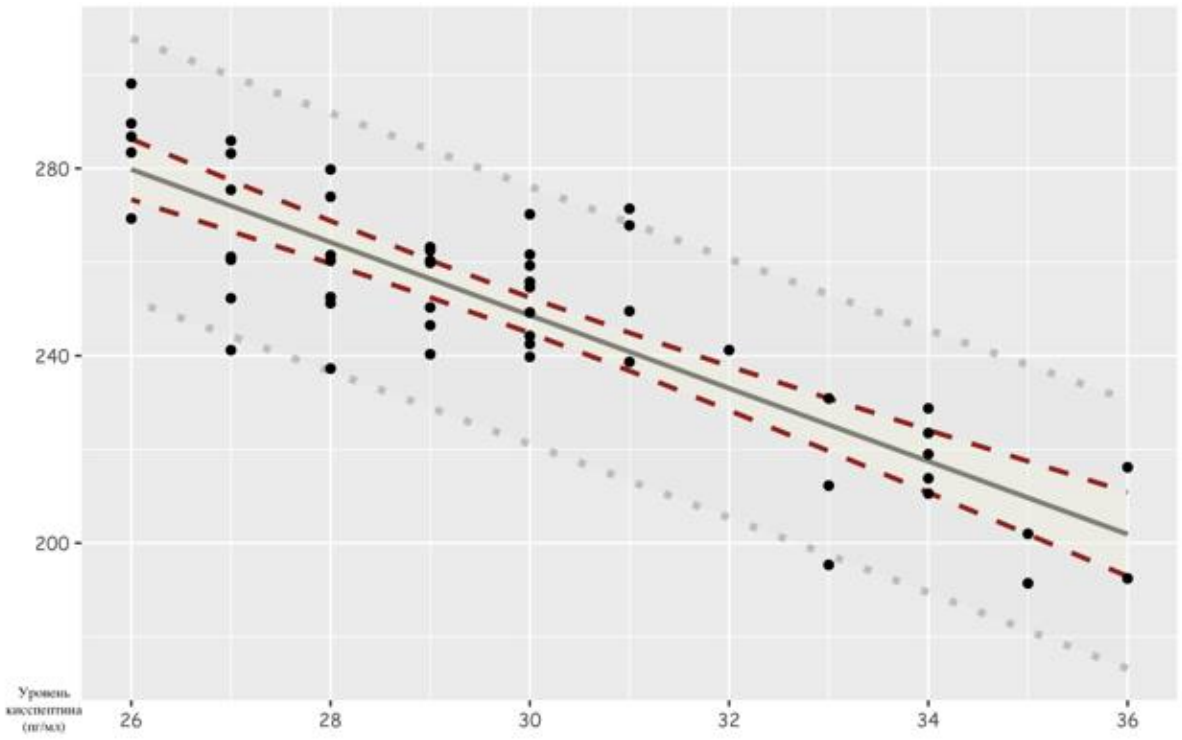
The regression results further support the interpretation that stress-induced suppression of kisspeptin plays a central role in the pathogenesis of ovarian insufficiency following COVID-19. Since kisspeptin is essential for the initiation

and maintenance of reproductive hormonal regulation, its decline under chronic stress conditions represents a plausible mechanistic explanation for menstrual dysfunction and ovarian failure observed in these women. Additionally, the unexplained 27.4% of variability suggests that other factors—such as neuroinflammation, direct viral effects on the hypothalamus, metabolic status, and individual susceptibility—may also contribute to kisspeptin dysregulation.

Together, these findings highlight the importance of evaluating stress levels as part of the diagnostic and prognostic assessment of women at risk for POI after SARS-CoV-2 infection. The strong inverse association shown in Figure 9(b) visually confirms the mathematical relationship, demonstrating a consistent downward trend in kisspeptin concentrations as stress severity increases.



(a)



(b)

Figure 9. Linear regression of blood kisspeptin levels and graphical representation of anxiety (a) and stress (b) levels in women in the main group

Based on paired correlation analysis, anxiety and stress levels have a strong positive correlation (r = 0.938). This means that we can use either of these indicators to predict kisspeptin levels after COVID-19. In other words, increases in one psychoemotional parameter are almost always accompanied by proportional increases in the other, confirming that anxiety and stress function as tightly interrelated components of the same psychophysiological response system. Their strong collinearity also indicates that the emotional burden experienced by women after COVID-19 is multidimensional and accumulative, amplifying its impact on neuroendocrine regulation and, consequently, on kisspeptin secretion.

Next, a linear regression analysis of BDNF levels and depression levels was performed. The observational data in the main group were bell-shaped. This indicates that the distribution of the data approximates normality, which is an important requirement for applying parametric statistical models such as linear

regression. The bell-shaped distribution suggests that the neurotrophic responses of women with POI after COVID-19 follow a predictable pattern, with most values clustering around the mean and fewer extreme outliers, thus supporting the accuracy and reliability of subsequent modeling.

After determining that our data were consistent with our predictions, we moved on to linear regression analysis to assess the relationship between brain-derived neurotrophic factor and depression levels. This analytical approach allowed us to quantify not only the strength and direction of the relationship, but also the magnitude of the change in BDNF concentration associated with variations in depressive symptoms. The regression model enabled us to determine how emotional dysregulation may translate into measurable neurobiological changes, providing further evidence for the involvement of central nervous system pathways in the development of premature ovarian insufficiency after COVID-19.

The use of both correlation and linear regression analyses has made it possible to interpret the complex interactions between psychological stress, neurotrophic markers, and reproductive endocrine function in an integrated fashion. These findings support the growing hypothesis that POI after SARS-CoV-2 infection may emerge from the combined impact of emotional stress responses, hypothalamic dysfunction, and neuroinflammatory processes.

Table 12

Linear regression analysis of the relationship between BDNF levels and depression levels

The Prophets	Evaluation	CI (confidence interval)	p-value
Free coefficient	427.89	392.71-463.07	<0.001
Stress level	-8.48	-9.97 - -6.98	<0.001

R2/R2adj. (coefficient of determination)	0.722/0.717
Number of patients	52

As can be seen from Table 12, the estimates for the model parameters are the value of the free coefficient (in this case 427.89) and the estimated effect of depression level on BDNF level (–8.48). The free coefficient represents the expected BDNF value when the level of depression is zero, meaning that in the absence of depressive symptoms, the predicted BDNF concentration would be approximately 427.89 pg/ml. This value is consistent with the physiological range observed in healthy women from the control group and therefore confirms the internal validity of the model.

The estimated regression coefficient of –8.48 indicates that with each one-point increase in depression scores, the level of BDNF decreases by an average of 8.48 units. This effect size suggests not only a statistically significant, but also a clinically meaningful reduction in BDNF levels as emotional distress intensifies. Such a pattern supports the hypothesis that depressive symptoms may contribute to neurotrophic depletion, which in turn disrupts hypothalamic signaling and reproductive regulation.

We can see that the p value (here it is 1.62e-15, or almost zero) indicates that the model fits the data well. A p-value this small demonstrates an extremely high level of statistical significance, confirming that the relationship between depression and BDNF is not due to random variation, but reflects a robust and consistent trend across the study population.

Based on these results, we can say that there is a significant negative relationship between depression level and BDNF (p value < 0.001). For each unit increase in depression level, we can estimate a change in BDNF level of –8.48 units (+/– 0.01). This narrow confidence interval indicates high precision in the estimated effect. From a biological standpoint, such a strong negative slope

suggests that depressive processes—likely mediated by chronic stress, inflammation, dysregulation of the HPA axis, and reduced neurogenesis—have a direct and measurable impact on neurotrophic support within the central nervous system.

Thus, the regression analysis confirms that depression severity is a key predictor of reduced BDNF levels, highlighting an important mechanistic link between psychoemotional state and neuroendocrine pathways involved in the development of premature ovarian insufficiency, particularly in the context of post-COVID physiological alterations.

Based on the results of the regression analysis, we determined a formula for predicting BDNF levels based on depression levels:

$$BDNF=427.9-8.477* \text{ depression level}$$

According to the results of the correlation-regression analysis, the correlation between the level of depression and BDNF parameters was found to be 0.7167. It can be said that 71.6% of the changes in BDNF levels are explained by depression, and the remaining 38.4% are due to other factors. This indicates that depressive symptoms contribute substantially to the variability in BDNF concentrations, making depression one of the strongest psychometric predictors of neurotrophic decline in women with POI, especially in the post-COVID period. Such a high explanatory value suggests that emotional and neurobiological mechanisms are tightly interconnected and jointly modulate hypothalamic function and ovarian regulation.

The relationship between BDNF and the level of depression is high and negative with a correlation coefficient of –0.850, i.e. an increase in the severity of depression leads to a decrease in brain-derived neurotrophic factor (Figure 10). This very strong inverse association indicates that worsening depressive symptoms are accompanied by a pronounced reduction in neurotrophic support. From a biological standpoint, reduced BDNF reflects impaired synaptic plasticity, weakened neuronal resilience, and altered hypothalamic signaling

pathways under chronic psychological stress. These mechanisms can disrupt the hypothalamic–pituitary–gonadal axis, contributing to hormonal imbalance, impaired folliculogenesis, and ultimately accelerating the development of premature ovarian insufficiency.

The strong negative correlation also implies that BDNF can serve as a sensitive biomarker for detecting early neuroendocrine disturbances associated with emotional dysregulation. Given that depression and chronic stress are known to activate inflammatory pathways, reduce neurogenesis, and suppress trophic factors in the brain, the observed decrease in BDNF may represent a key mechanistic link between psychosocial stress and reproductive dysfunction after COVID-19 infection.

Overall, the findings confirm that the psychoemotional state—particularly depression—plays a crucial role in modulating neurotrophic signaling, and that BDNF levels may reflect the combined impact of stress, mood disturbances, and viral neuroinvasion on ovarian function. These relationships underscore the need for integrated assessment of mental health and neuroendocrine biomarkers in women at risk of POI.

Based on the survey data, we can say that if the level of kisspeptin in the blood serum is below 350 pg/ml, then there is a risk of developing POI (since the level of kisspeptin in the control group of women ranged from 401.16 to 477.10 pg/ml). The lower the level of kisspeptin in the blood, the higher the risk of developing POI. This threshold value can be considered an important prognostic marker, enabling the early identification of women whose hypothalamic–pituitary–gonadal axis is at risk of developing dysregulation. Given that kisspeptin plays a central role in maintaining GnRH pulsatility, a decline below this cut-off likely reflects a critical reduction in hypothalamic signaling that precedes overt hormonal and clinical manifestations of ovarian insufficiency.

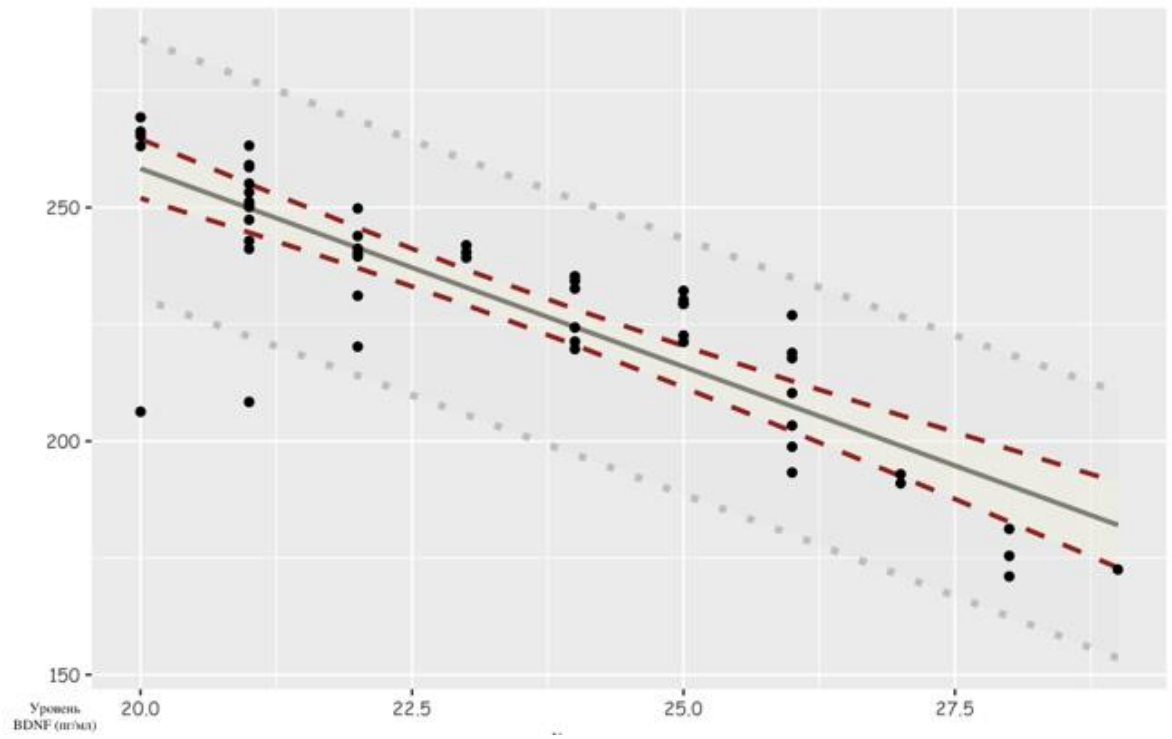


Figure 10. Linear regression of blood BDNF levels and graphic representation of depression levels in women in the main group

Also, according to the level of brain-derived neurotrophic factor, i.e. below 350 pg/ml (in healthy women, the level of BDNF ranged from 401.16 to 469.91 pg/ml), it indicates the risk of developing POI. Since BDNF is a key neurotrophic molecule essential for hypothalamic neuronal integrity, synaptic regulation, and ovarian follicle survival, its reduction may represent the earliest neurobiological sign of progressive reproductive dysfunction. These findings suggest that BDNF, similar to kisspeptin, serves as a sensitive biomarker reflecting central nervous system involvement in the pathogenesis of POI, especially in patients who have undergone COVID-19 infection.

The identification of these threshold values for kisspeptin and BDNF is particularly important because they represent measurable biochemical indicators that can be used for screening and risk stratification. In clinical practice, the detection of decreased levels of these markers can help identify women who require closer monitoring, early diagnostic assessment, lifestyle modification,

stress management interventions, or timely hormonal evaluation. From a research perspective, such biomarkers can also serve as endpoints for evaluating new therapeutic approaches targeting hypothalamic and neurotrophic signaling pathways.

Thus, the use of these mathematical models makes it possible to predict the development of premature ovarian insufficiency. Establishing predictive cut-off values and integrating kisspeptin and BDNF into diagnostic algorithms may significantly improve early detection and prevention strategies. Predictive modeling based on these markers could help identify vulnerable women long before clinical symptoms become apparent, thereby enabling personalized reproductive health management and potentially slowing or preventing the onset of POI in high-risk populations.

CHAPTER IV. DISCUSSION

The virus itself, as well as the measures taken to reduce its spread, have had a significant impact on the lives of the world's population. The pandemic has had a significant impact on the mental health of many members of the population, leading to loneliness, social isolation, financial hardship, as well as anxiety and fear and uncertainty about contracting the virus tomorrow. These consequences were observed worldwide and were not limited to those who became ill: even healthy individuals experienced chronic stress due to lockdowns, changes in lifestyle, disruption of social support systems, and prolonged uncertainty about the future. As a result, the COVID-19 pandemic acted not only as an infectious threat but also as a powerful psychosocial stressor, influencing multiple aspects of physical and mental well-being.

It is known that stress and psychological distress affect female reproductive function. Stress factors can affect the hypothalamic-pituitary-gonadal (HPG) axis and alter the neuromodulatory cascade that controls the regulation of gonadotropin-releasing hormone (GnRH) [42]. Chronic activation of stress pathways, particularly the hypothalamic–pituitary–adrenal (HPA) axis, leads to increased secretion of cortisol, which suppresses GnRH pulsatility, disrupts folliculogenesis, and may result in menstrual irregularities, anovulation, and decreased fertility. Dysmenorrhea has been shown to be associated with high levels of stress and emotional instability [3]. These observations demonstrate that the menstrual cycle is a sensitive indicator of psychophysiological well-being. Clinically, pathological changes in the post-stress period appear some time after the stress and can persist in the future and even be quite persistent. This reflects the concept of “biological memory” of stress exposure, where neuroendocrine mechanisms remain altered long after the initial trigger.

From an endocrinological perspective, menstrual dysfunction (IMF) can be viewed as an allostatic load [2] — a cumulative burden placed on the body

by chronic stressors that disrupt homeostasis and impair normal regulatory systems. The higher the allostatic load, the more vulnerable the reproductive axis becomes to external perturbations, including infections. Thus, even before the emergence of COVID-19, researchers noted a strong bidirectional link between emotional health and menstrual cyclicity.

As the pandemic affects the functions of all organs, more and more questions are being raised about the female reproductive system, especially fertility issues, and there is a need to clarify the possible link between COVID-19 and reproductive disorders in women. Reports from various regions of the world indicate an increase in cases of menstrual irregularities, worsened premenstrual symptoms, exacerbation of endocrine diseases, and even emerging signs of diminished ovarian reserve following SARS-CoV-2 infection.

Understanding the interaction between infection, stress, and the neuroendocrine axis has therefore become a priority for modern reproductive medicine. Recent studies suggest that COVID-19 may disrupt the hypothalamic–pituitary–ovarian (HPO) axis not only through systemic inflammation but also via neurotropic effects, given the virus's demonstrated ability to enter neural tissues through the olfactory pathway. This mechanism may impair central regulatory processes that maintain normal menstrual cyclicity and ovarian function.

Furthermore, the pandemic has brought attention to the broader issue of reproductive vulnerability: even after the acute viral threat diminishes, women remain exposed to various types of ongoing psychosocial stress, including economic instability, caregiving burdens, chronic anxiety, altered work–life balance, and persistent fear related to health and future reproductive plans. Therefore, the study of the long-term effects of stress and COVID-19 on female reproductive health is not only timely but urgently needed for

developing preventive strategies, screening tools, and therapeutic approaches to protect women's reproductive potential in the post-pandemic era.

Based on the above, we defined the goal of the dissertation work: to determine the role of hypothalamic markers such as KISS1 and BDNF in the development of early insufficiency in women after COVID-19. This goal reflects the urgent need to understand not only the clinical manifestations of premature ovarian insufficiency (POI), but also the deeper molecular and neuroendocrine mechanisms that may be disrupted in the post-COVID period. Identifying these mechanisms can contribute to early diagnostics, risk stratification, development of preventive strategies, and targeted therapeutic approaches for women of reproductive age.

To achieve this goal, 112 women aged 18–40 years were divided into three groups. The main group consisted of 52 women who developed POI after COVID-19 (mean age 31.05 ± 1.78 years) and the comparison group consisted of 28 women with POI diagnosed before COVID-19 (mean age 34.28 ± 2.56 years). The control group consisted of 32 healthy women (mean age 28.68 ± 2.1 years). This grouping allowed us to differentiate between the direct influence of SARS-CoV-2 on ovarian function and POI cases that developed independently of the infection. To determine the role of kisspeptin and brain-derived neurotrophic factor in the pathogenesis of POI due to coronavirus infection, the functional state of the HPA axis was assessed, the Women's Mental State Questionnaire, blood content of kisspeptin and brain-derived neurotrophic factor, as well as a specialized questionnaire "POIQoLS". This comprehensive methodology ensured a multidimensional assessment of both neuroendocrine and psychological parameters.

There is a theoretical hypothalamic route of SARS-CoV-2 infection, in which the virus may use the olfactory epithelium to enter the host [28]. Anosmia is a common symptom of COVID-19, and axonal transport through the olfactory nerve has been proposed as the mechanism. Thus, the virus

damages the hypothalamus. This is particularly important because the hypothalamus serves as the central regulator of reproductive function, integrating signals from neurotransmitters, peripheral hormones, and environmental stressors. Disruption of hypothalamic neurons involved in GnRH pulsatility may lead to destabilization of the entire hypothalamic–pituitary–gonadal axis. It is worth noting that all women in the main group had complaints related to the sense of smell: 40 patients had anosmia (77%) and 12 patients (23%) had hyposmia. This observation further supports the possibility of neuroinvasion and functional impairment of hypothalamic pathways in these patients.

As mentioned above, stress factors have a significant impact on mental health, which in turn affects the reproductive system. The COVID-19 pandemic intensified pre-existing stressors and introduced new sources of psychological distress related to health concerns, social distancing, economic instability, and unpredictability of the situation. Many studies have been conducted on the psychological state of women during the pandemic. Studies by Ahorsu et al. (2020), Lathabhavan et al. (2021) and Maslakci et al. (2022) showed the results of the "DASS-21" questionnaire, in which women had moderate to severe levels of anxiety, stress and depression. These findings demonstrate that women were disproportionately affected by the psychological consequences of the pandemic, possibly due to gender-specific stress responses and higher emotional labor burdens.

The data we present are consistent with the results of these studies. The severity of the anxiety level in women after coronavirus infection was 17.44 ± 0.54 points, a state above 15 points was considered a severe level of anxiety. A strong level of stress was also detected in women in the main group (mean score 29.90 ± 0.79 points). Women with POI had significantly higher levels of depression after COVID-19, with a mean score of 23.42 ± 0.71, which corresponds to a severe level. These results indicate that POI in the post-

COVID period is accompanied by a multifaceted deterioration of psychoemotional well-being, forming a complex syndrome that includes both hormonal and psychological components.

Although it is well known that increased psychosocial stress can lead to menstrual irregularities, this is one of the first studies to assess menstrual irregularities in the context of the COVID-19 pandemic and to determine which such changes are associated with perceived stress. The pandemic provided a unique global "natural experiment" allowing researchers to observe how widespread and prolonged stress exposure affects menstrual health. Our data are consistent with a study by Phelan et al., in which 46% of women reported an increase in menstrual irregularities during the COVID-19 pandemic [30]. This widespread prevalence suggests that COVID-related stress, inflammation, and neurohormonal dysregulation have substantial reproductive consequences, which merit further investigation.

These findings collectively underscore the importance of studying hypothalamic markers such as KISS1 and BDNF—as their fluctuations may represent the molecular bridge between stress, viral neuroinvasion, and the development of premature ovarian insufficiency in women after COVID-19.

Some studies have suggested that abnormalities in BDNF expression may be associated with the development of POI. The association between BDNF and follicular function and loss has been previously demonstrated [40]. BDNF is present in follicular fluid, where it stimulates oocyte maturation into preimplantation embryos [24]. This demonstrates that BDNF plays not only a neurotrophic, but also a direct intraovarian regulatory role, supporting cytoplasmic and nuclear maturation of the oocyte. Paracrine release of LH stimulates follicular BDNF production in granulosa cells, including NTKR2T1 receptors. This mechanism, together with the stimulation of the KISS1R receptor by kisspeptin, ensures the survival of oocytes and their subsequent development [13]. The interaction between BDNF and kisspeptin signaling

appears to form a coordinated network essential for folliculogenesis, maintaining oocyte viability, and supporting successful reproductive outcomes.

Dorfman et al. demonstrated that mice lacking the neurotrophic receptor tyrosine kinase 2 receptor (NTRK2) or KISS1R exhibited oocyte fragmentation and oocyte cell death. This process induced the POI phenotype in mice. Such experimental models provide compelling mechanistic evidence that both BDNF and kisspeptin are indispensable components of the ovarian survival pathway. There was also a positive correlation between the number of mature oocytes and the concentration of BDNF in follicular fluid [13]. These findings collectively suggest that insufficient BDNF signaling leads to impaired follicular development and accelerated follicle depletion.

Gaitan et al. [18] demonstrated that abnormal signaling between the kisspeptin and BDNF signaling pathways resulted in the progressive loss of all classes of follicles in the ovary, leading to premature menopause. Gonadotropin secretion was initially maintained but then increased, mimicking the hormonal profile of POI [11]. This biphasic pattern indicates that the hypothalamic–pituitary–ovarian axis attempts to compensate for declining ovarian function until depletion becomes irreversible. Therefore, disturbances in BDNF and kisspeptin signaling may lie at the core of the pathophysiology of both POI and early menopause.

Plasma BDNF concentrations have already been studied in women. Begliuomini et al. studied plasma BDNF levels in women after natural menopause and amenorrhea of various etiologies compared with women with regular menstruation. According to the results of the study, BDNF levels decreased in both groups, and the lowest BDNF levels were observed in women with amenorrhea. Indeed, women with regular ovulatory cycles have higher BDNF levels than women with amenorrhea or postmenopause [7]. This suggests that circulating BDNF may serve as a sensitive peripheral indicator

of ovarian status, reflecting the integrity of follicular and neuroendocrine function.

According to these results, our data showed significant differences in the mean scores of the three groups. The mean blood BDNF level was significantly lower in women in the main group (229.32 ± 7.03 pg/ml) compared to the control group (428.58 ± 7.21 pg/ml). In the comparison group, women with POI had lower levels of brain-derived neurotrophic factor (336.14 ± 7.12 pg/ml) than healthy women. Based on this, we can conclude that BDNF levels in women with Premature ovarian insufficiency are lower than in healthy women during coronavirus infection. The magnitude of BDNF depletion in the main group suggests that SARS-CoV-2 infection may exacerbate underlying neuroendocrine vulnerabilities or trigger new central mechanisms that accelerate ovarian decline.

This is explained by the fact that direct infection of SARS-CoV-2 from the respiratory tract to the central nervous system or brain increases local levels of angiotensin II by suppressing ACE2. The interaction of angiotensin II with AT1 receptors increases kynurenine metabolites, causing prooxidant and proinflammatory effects, resulting in increased oxidative stress and reduced BDNF levels [9]. SARS-CoV-2–induced neuroinflammation also disrupts the trophic support of hypothalamic neurons, altering the production of key neuromodulators that regulate reproductive function. The combined effect of oxidative damage, excitotoxicity, and dysregulated renin–angiotensin signaling may therefore significantly suppress BDNF synthesis and release.

Our data confirm the findings of Czyzyk et al., who found that plasma BDNF concentrations in the group of patients with POI were significantly lower than in healthy controls in the late follicular phase. Furthermore, BDNF levels in the study group were within a very wide range, which allowed the detection of a negative correlation between time since last menstruation and BDNF concentrations [11]. This suggests that prolonged amenorrhea may

accelerate neurotrophic depletion, further impairing hypothalamic–ovarian communication. Given the role of BDNF in both neuronal and ovarian health, its reduction may contribute to a self-perpetuating cycle of declining follicular function.

After determining psychometric parameters, kisspeptin levels, and brain-derived neurotrophic factor, a comparison was made between anxiety, stress, and depression indicators and blood kisspeptin levels and brain-derived neurotrophic factor. Correlation analysis was performed. This approach made it possible to evaluate not only isolated physiological or emotional changes, but also their dynamic interrelationships, thereby identifying integrated neuroendocrine mechanisms that may contribute to the development of POI after COVID-19. By combining biochemical markers with psychometric indicators, we were able to assess the functional coherence between the hypothalamus, emotional regulation centers, and ovarian function.

We found a significant negative correlation between kisspeptin and anxiety scores in the main group ($r = –0.61$, $p < 0.001$), as well as a moderate negative correlation between kisspeptin levels and stress ($r = –0.68$, $p < 0.001$). These findings suggest that as levels of kisspeptin decrease, emotional distress intensifies, indicating that kisspeptin may play an essential modulatory role in stress responsiveness. Since kisspeptin is a key stimulator of GnRH secretion, its reduction may result not only from ovarian dysfunction but also from central stress-induced suppression. Consequently, diminished kisspeptin levels may be both a marker and a mediator of stress-related reproductive impairment.

There was also a strong negative correlation between BDNF levels and depression ($r = –0.93$, $p < 0.001$) in the main group of women. This exceptionally strong association indicates that lower BDNF concentrations reflect more severe depressive symptoms. BDNF is well known for its role in neuronal plasticity, emotional resilience, and stress adaptation. Therefore,

decreased BDNF levels in women with POI after COVID-19 may be indicative of profound neuroinflammatory or neurodegenerative changes affecting the hypothalamus and limbic system. Reduced BDNF may impair synaptic function and hypothalamic signaling pathways responsible for coordinating reproductive hormone secretion, thus further contributing to ovarian insufficiency.

A strong positive correlation was found between stress and depression levels, with a correlation coefficient of 0.93 in women with POI due to COVID-19. This suggests that the emotional disturbances in these patients are not isolated phenomena but rather represent interconnected components of a unified psychoemotional syndrome triggered by COVID-19 infection. Such synergy between stress and depression may amplify dysregulation of the HPA and HPG axes, thereby exacerbating reproductive dysfunction.

Kisspeptin and brain-derived neurotrophic factor signaling are novel markers of stress neurocircuitry involved in reproductive dysfunction. Their simultaneous decline, accompanied by intensified anxiety, stress, and depression, suggests that COVID-19 may disrupt the central neuroendocrine hubs responsible for maintaining reproductive homeostasis. The combination of reduced hypothalamic neuromodulators and heightened psychophysiological stress creates a “double hit” mechanism—one in which both central neuroinflammation and emotional overload synergistically impair ovarian function.

Our results suggest the feasibility of further investigating the effects of kisspeptin and brain-derived neurotrophic factor on the regulation of the female reproductive system. These markers may serve not only as diagnostic tools but also as potential therapeutic targets. Improving kisspeptin and BDNF signaling pathways—whether through pharmacological agents, lifestyle interventions, stress reduction techniques, or neuroprotective therapies—may help alleviate or slow the progression of POI in women after viral infections.

Thus, based on the study results and literature data, it is possible to propose a mechanism for the development of POI in women infected with COVID-19 (Figure 11). This mechanism likely includes:

- SARS-CoV-2 neuroinvasion via the olfactory pathway, leading to hypothalamic dysfunction;
- neuroinflammation and oxidative stress, reducing BDNF synthesis and impairing neuronal plasticity;
- stress-mediated suppression of the HPA and HPG axes, resulting in decreased kisspeptin production;
- disruption of GnRH pulsatility, leading to altered gonadotropin secretion;
- accelerated follicular apoptosis due to impaired BDNF/KISS1 signaling;
- progressive ovarian insufficiency, consistent with the hormonal and clinical phenotype of POI.

This proposed model integrates psychological, neurobiological, viral, and endocrine components, offering a comprehensive explanation of how COVID-19 may contribute to the development of premature ovarian insufficiency.

It is important to remember that even when the pandemic subsides, women are vulnerable to various types of acute and chronic stress. Modern lifestyle factors, psychosocial pressures, environmental conditions, and metabolic challenges continue to affect the hypothalamic–pituitary–gonadal axis. Therefore, understanding how stress interacts with neuroendocrine markers—both during and beyond the COVID-19 pandemic—remains critical. Continued research in this area will help develop more targeted healthcare policies, screening tools and therapeutic approaches that can protect women's reproductive health in the face of future epidemics, stressors, and global health challenges.

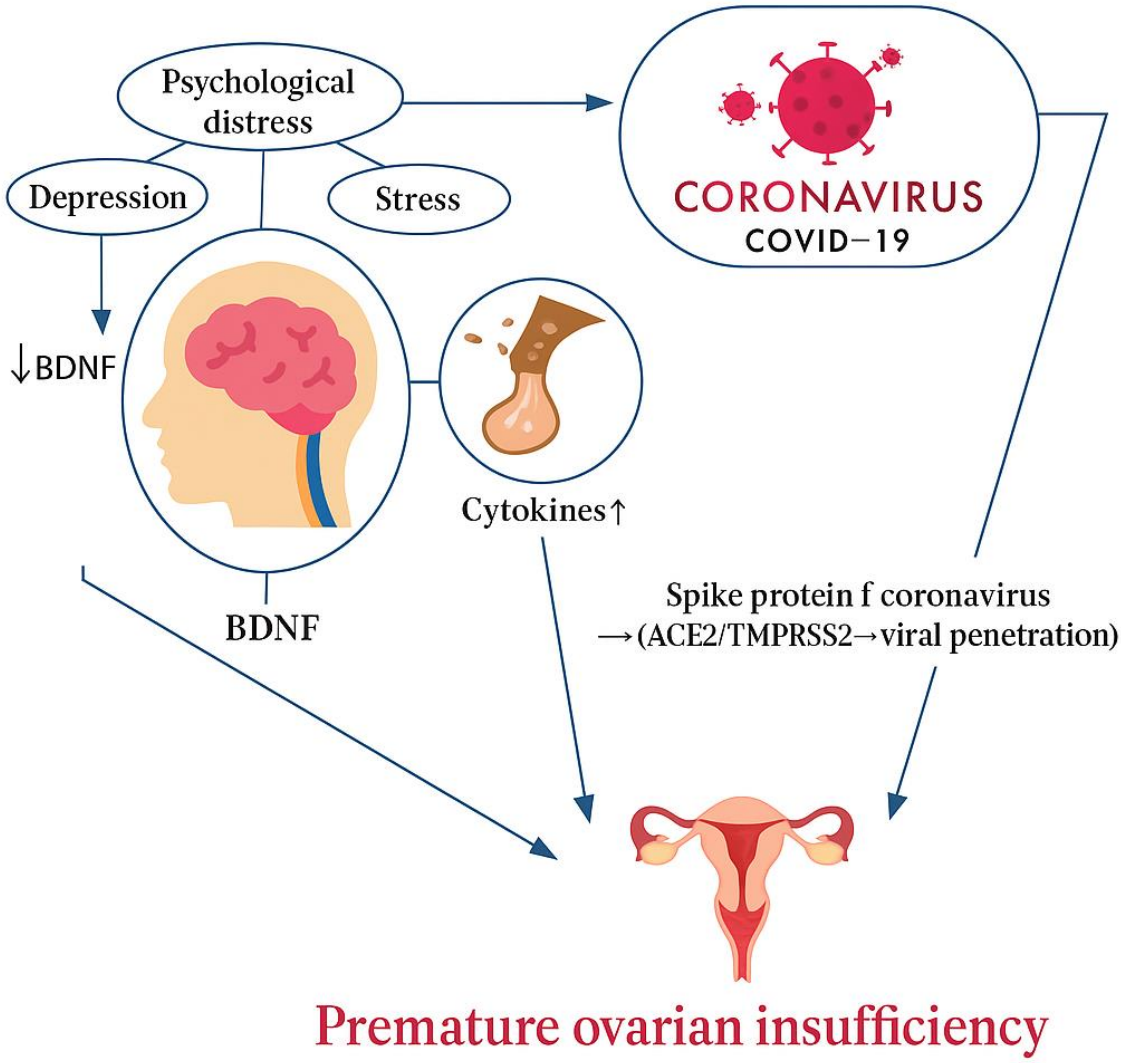


Figure 11. Possible mechanism of POI development in women due to COVID-19

Future work should focus on identifying women at higher risk of developing POI from these hypothalamic markers. This direction is especially relevant given the demonstrated associations between kisspeptin, BDNF and psychoemotional stress, suggesting that these biomarkers may serve not only as diagnostic indicators, but also as early predictors of reproductive dysfunction. Early identification of vulnerable women would allow clinicians to initiate timely monitoring, personalized interventions and preventive strategies aimed at preserving ovarian function and improving long-term reproductive health outcomes.

CONCLUSIONS

1. Among the diseases of the female reproductive system due to COVID-19, the proportion of women who developed Premature ovarian insufficiency was 2.1%. All 52 women (100%) did not complain of menstrual irregularities before COVID-19.

2. In the study of clinical and laboratory parameters of women with POI in the post-COVID period, statistically significant changes were observed in kisspeptin and brain-derived neurotrophic factor levels. Kisspeptin levels were 249.39±7.23 pg/ml, which was 1.24 times lower than in the comparison group (352.50±8.61 pg/ml) and 1.76 times lower than in the control group (439.90±8.32 pg/ml). In women with POI due to COVID-19, brain-derived neurotrophic factor levels were 229.32±7.03 pg/ml, which was 1.86 times lower than in the control group (428.58±7.21 pg/ml) and 1.27 times lower than in the comparison group (336.14±7.12 pg/ml).

3. According to the indicators of the psychoemotional state of women with COVID-19, the level of anxiety was 2.7 times higher (17.44 ± 0.54), the level of stress was 2.4 times higher (29.90 ± 0.79), and the level of depression was 1.9 times higher (23.42 ± 0.71) compared to women with POI without COVID-19.

4. Kisspeptin levels in women with POI due to COVID-19 were negatively correlated with anxiety ($r=-0.79$, $p<0.001$) and stress ($r=-0.86$, $p<0.001$). Brain-derived neurotrophic factor levels in women with POI after coronavirus infection were negatively correlated with depression levels ($r=-0.85$, $p<0.001$).

5. A mathematical method was developed to predict the development of POI in the post-Covid period as a result of multivariate analysis. Anxiety, stress, depression levels were found to be significant prognostic indicators in relation to kisspeptin (coefficient of determination $R2 = 0.72$) and brain-derived

neurotrophic factor (coefficient of determination R2 = 0.71) for the development of POI in women with previous COVID-19.

6. According to the results of the specialized survey “POIQoLS”, women in the comparison group had a lower health-related quality of life (58.89±1.15,$p<0.05$) than the women of the main group (64.9 ± 0.63,$p<0.05$This is explained by the fact that POI developed relatively earlier in the women in the comparison group.

PRACTICAL RECOMMENDATIONS

1. General practitioners and primary care physicians may use the psychological questionnaire and the corresponding calculation formula as part of the algorithm to assess the risk of premature ovarian insufficiency (POI) in women during the post-COVID period.

2. It is recommended to use the DASS-21 questionnaire, which allows to assess the psychoemotional state of women with menstrual dysfunction, which is clearly associated with hypothalamic markers (KISS1 and BDNF) and allows for early diagnosis of POI.

3. To assess the effectiveness of therapy, it is necessary to assess the quality of life of women diagnosed with POI, according to the POIQoLS survey.

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