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**ТИББИЁТДА ЯНГИ КУН
НОВЫЙ ДЕНЬ В МЕДИЦИНЕ
NEW DAY IN MEDICINE**

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FERRITINNING TURLI KO'RSATKICHLARI BILAN TURMUSH QURGAN JUFTLARNI EKUGA TAYYORLASH

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✓ Rezyume

Ferritin temir resurslarining belgisi bo'lib, organizm uchun juda muhimdir. U oksidlovchi stress va yallig'lanish bilan bog'liq bo'lgan prooksidant ta'sirga ega. Ferritinning past darajasi temir tanqisligi anemiyasini ko'rsatadi va uning darajasining oshishi yallig'lanish mavjudligini va oksidlovchi stressning yuqori darajasini ko'rsatadi. Ferritin darajasining o'zgarishi bilan bog'liq bu barcha yakuniy omillar tug'ilishga sezilarli ta'sir ko'rsatadi.

Ushbu tadqiqotda biz "Siz ona bo'lasiz" EKU markazida EKU/ICSI protokoliga kiritilgan bemorlarda ferritin darajasini o'zgarishini ta'sirini baholadik. Ushbu tadqiqotning maqsadi ferritin darajasini o'zgarishini olingan oositlar soni va sifatiga, shuningdek, EKU/ICSI protokollari paytida embrionlarga ta'sirini qiyosiy tahlil qilishdir. Shunday qilib, ferritin darajasidagi o'zgarishlar oositlar va embrionlarning sifatini buzishi mumkin, natijada embrionlar o'sishi va rivojlanishi yomonlashadi va muvaffaqiyatli homiladorlik ehtimoli kamayadi.

Kalit so'zlar: EKU/ICSI, oosit va embrion sifati, ferritin, yashirin temir tanqisligi.

PREPARING COUPLES FOR IVF WITH DIFFERENT FERRITIN LEVELS

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✓ Resume

Ferritin is a marker of iron resources and is vital to the body⁶. It has a pro-oxidant effect associated with oxidative stress and inflammation⁹. A low level of ferritin indicates iron deficiency anemia, and an increase in its level indicates the presence of inflammation and high levels of oxidative stress. All of these corollary factors associated with changes in ferritin levels have a significant impact on fertility¹.

In this study, we evaluated the effect of changing the level of ferritin in patients included in the IVF/ICSI protocol at the IVF Center "Cis on Bulacis". The purpose of this study is a comparative analysis of the effect of changing the level of ferritin on the number and quality of retrieved oocytes, as well as embryos during IVF/ICSI protocols. Thus, changes in ferritin levels can impair the quality of oocytes and embryos, resulting in poor growth and development and reduced chances of a successful pregnancy.

Key words: IVF/ICSI, oocyte and embryo quality, ferritin, latent iron deficiency.

ПОДГОТОВКА СУПРУЖЕСКИХ ПАР К ЭКО С РАЗЛИЧНЫМИ ПОКАЗАТЕЛЯМИ ФЕРРИТИНА

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✓ **Резюме**

Ферритин является маркером ресурсов железа и имеет жизненно важное значение для организма. Он оказывает прооксидантное действие, связанное с окислительным стрессом и воспалением. Низкий уровень ферритина указывает на железодефицитную анемию, а повышение его уровня на наличие воспаления и высокий уровень окислительного стресса. Все эти следственные факторы, связанные с изменением уровня ферритина оказывают значительное влияние на фертильность.

В данном исследовании проведена оценка влияния изменения уровня ферритина у пациенток, вошедших в протокол ЭКО/ИКСИ в ЭКО Центре «Сиз она бўласиз». Целью данного исследования является сравнительный анализ влияния изменения уровня ферритина на количество и качества извлеченных ооцитов, а также эмбрионов во время протоколов ЭКО/ИКСИ. Таким образом, изменение уровня ферритина может ухудшить качество ооцитов и эмбрионов, что приведет к плохому росту и развитию и снижению шансов на успешную беременность.

Ключевые слова: ЭКО/ИКСИ, качество ооцитов и эмбрионов, ферритин, латентный железодефицит.

Introduction

Worldwide, reproductively active women are at high risk of iron deficiency anemia, which causes significant morbidity and infertility.¹ More than 186 million people worldwide suffer from infertility, most of whom are in developing countries². Ovulation disorders, including polycystic ovary syndrome (PCOS), hypothalamic dysfunction, ovarian failure, tubal infertility, endometriosis, uterine and cervical causes are common etiologies of female infertility³. Infertility is a clinically common condition, occurring in about 10% of women of reproductive age⁴. Research has confirmed a strong association between micronutrient deficiencies and mysterious infertility in women¹. A recent epidemiological observational study found that women who did not receive iron supplements had a higher risk of ovulatory infertility compared to women who took iron supplements⁵.

According to statistics, almost 25% of the world's population has an iron deficiency⁶. In women, iron deficiency anemia occurs 6 times more often than in men, and the likelihood of becoming pregnant in iron deficiency states is reduced by 60%⁷. The World Health Organization estimated that non-pregnant women were the largest group affected by anemia (468 million), and this raises the possibility of considering anemia as a possible risk factor for reduced female fertility⁸.

Ferritin is an intracellular iron storage protein that is a vital marker of iron resources, while iron is an essential redox metal that is also vital to humans⁶. It has a pro-oxidant effect associated with oxidative stress and inflammation⁹. Low ferritin levels may indicate iron deficiency anemia, which can have a significant impact on fertility¹. Iron deficiency (iron deficiency anemia) and infertility are directly related, because of the process of egg maturation and ovulation is disrupted⁹.

In recent years, many authors have examined the relationship between follicular fluid ferritin levels and in vitro fertilization (IVF) outcomes in their researches, including embryo quality, clinical pregnancy rates, and live birth rates¹⁰. In Vitro Fertilization (IVF) is a widely used assisted reproductive technology (ART) procedure that helps couples who are struggling with infertility to conceive a child¹¹. IVF involves the fertilization of an egg with a sperm cell in the laboratory, and then the transfer of the resulting embryo back into the uterus⁶. Follicular fluid is the fluid that surrounds the developing oocyte in the follicle and provides essential nutrients and growth factors for the oocyte and the developing embryo. Follicular fluid is the fluid that surrounds the developing oocyte in the follicle and provides essential nutrients and growth factors for the oocyte and the developing embryo.³ The quantity and quality of directly harvested oocytes represents ovarian responses and is considered the most important clinical indicator of oocyte production, apart from their ability to fully mature and successfully survive¹⁰. Egg quality is an important factor in determining the success of IVF. Poor egg quality can lead to a lower chance of conception, lower quality embryos and a higher risk of miscarriage¹². The researchers found that with a lack of this microelement, oxygen supply to tissues and cells deteriorates, which negatively affects the functioning of the ovaries, leads to a deterioration in the quality of eggs, as well as to the outcome (IVF) in infertile women who are at the stage of the IVF cycle¹⁰.

The authors state that women who do not get enough iron may have problems with ovulation and likely poor ovulatory health, which can prevent pregnancy at a rate up to 60% higher than in women

with adequate blood levels of iron¹³. However, studies are still ongoing, some of which have shown that amenorrhea (absence of menstruation) may be caused by iron deficiency anemia.¹. This is due to the fact that the female body does not want to lose iron anymore, so it stops from menstruating. In fact, researchers estimate that amenorrhea occurs in about 50% of women of childbearing age with iron deficiency anemia¹.

Thus, ferritin levels can impair the health of the embryo, leading to poor growth and development and reduced chances of a successful pregnancy. In this study, we assessed the effect of the ferritin level in patients included in the IVF protocol at the IVF Center "Siz Ona Bo`lasiz". The purpose of this study is to compare the effect of ferritin levels on the number of received oocytes, metaphase II (MII) oocytes and viable embryos during IVF protocols.

Methods

In this research paper were enrolled 101 patients who were examined and prepared for in vitro fertilization at the IVF Center "Siz Ona Bo`lasiz" from November 2021 to December 2022. Patients were fully informed about the research work and had a choice to leave it whenever they want. Enrolled patients were divided into three groups according to the WHO Guidelines for the use of ferritin levels to assess iron status in individuals and populations from 30 to 70 µg/L8:

Group 1 consisted of n=46 (45.5%) patients with normal levels of ferritin in the blood;

Group 2 consisted of n=30 (29.8%) patients with low levels of ferritin in the blood;

Group 3 consisted of n=25 (24.7%) patients with high levels of ferritin in the blood.

There were no significant differences between groups in baseline characteristics, IVF indications, prior IVF failures, and baseline hormonal profile. The levels of AMH in the study groups were the same 1.6±0.4. Exclusion criteria were: 1) age < 19 or > 40 years; 2) male factor 3) PCOS; 4) severe endometriosis, 5) abnormal uterine cavity (submucosal fibroids, endometrial polyps, uterine septum) or 6) prior ovarian surgery.

Ferritin was measured using an automatic platform electrochemiluminescent immunoassay (Cobas C311, Germany). The reaction includes four stages: incubation of the sample with monoclonal insulin-ferritin, biotinylated and ruthenium-labeled antibody, sandwich binding (antibody-analyte complex) with a solid phase coated with biotin-streptavidin, then placing the reaction mixture inside the measuring unit, where the microparticles are magnetically held on an electric surfaces. By applying a voltage to the electrode, the photomultiplier measures the chemiluminescent radiation.

Groups of patients with low and high ferritin levels underwent complex therapy to normalize ferritin levels. For patients of the 2nd group were used iron preparations (18 mg/day), proteins (10-20 g/day), vitamins of group B. For the patients of the 3rd group were carried out additional laboratory tests to identify foci of inflammation, and based on the results of the analyzes we prescribed them the complex therapy to normalize the level of ferritin and eliminate foci of inflammation. The drugs were used regardless of the day and phase of the menstrual cycle. The reception interval ranged from 1 to 3 months. Embryological variables were compared between the three groups. Within 3 months after the treatment patients had a repeated laboratory tests.

Data analysis was performed using RStudio. Data were normally distributed and expressed as mean and standard deviation (mean ± standard deviation). The significance of differences in different means (quantitative data from different groups and control group) was tested using analysis of independent Student's t-test for differences between the two means. When the P value is less than 0.05, then it is considered statistically significant. While it is considered a significant increase when the P value is less than 0.01.

Results and discussion

In this paper were enrolled n=101 patients, who entered the IVF protocol at the IVF Center "Siz Ona bo`lasiz". Patients were divided into three groups according to the level of ferritin in their blood. The main group consisted of n=46 (45.5%) patients with normal level of ferritin, group 2 consisted of n=30 (29.8%) patients with low level of ferritin, and group 3 consisted of patients with high ferritin levels in the blood.

The main characteristics of the patients are shown in [Table 1](#). The difference in the age of patients in the three groups was insignificant: (28.7 ± 0.7), (29.5 ± 0.5) and (29.9 ± 0.9) years, respectively. There were no significant differences between the three groups in baseline characteristics, risk factors, IVF

indications, prior IVF failures, and baseline hormonal profile. A significant difference was made by patients of group 2 with idiopathic infertility (0.1 ± 0.05) relative to group 1 (0.3 ± 0.1) and group 3 (0.2 ± 0.1) ($p=0.001$). Menstrual irregularities were observed more in the groups with normal and low levels of ferritin.

Table 1. Key characteristics of enrolled patients undergoing IVF.

Characteristics	Group 1 (n = 46)	Group 2 (n = 30)	Group 3 (n = 25)
Age (years), mean $\pm$ standard deviation	$28,7 \pm 0,7$	$29,5 \pm 0,5$	$29,9 \pm 0,9$
BMI (kg/m ²), mean $\pm$ standard deviation	$23,5 \pm 0,9$	$23,11 \pm 0,8$	$22,6 \pm 0,9$
Infertility duration (years), mean $\pm$ standard deviation	$4,1 \pm 0,5$	$4,9 \pm 0,7$	$4,6 \pm 0,8$
Primary infertility, n (%)	$1,3 \pm 0,1$	$1,3 \pm 0,1$	$1,4 \pm 0,1$
Tube factor	$0,5 \pm 0,1$	$0,6 \pm 0,1$	$0,5 \pm 0,1$
Idiopathic infertility	$0,3 \pm 0,1$	$0,1 \pm 0,05^*$	$0,2 \pm 0,1$
Decreased ovarian reserve	$0,1 \pm 0,1$	$0,1 \pm 0,1$	$0,2 \pm 0,1$
Menstrual irregularity	$0,1 \pm 0,04$	$0,1 \pm 0,05$	$0,05 \pm 0,05$
Previous IVF failures, n (%)	$0,1 \pm 0,04$	$0,1 \pm 0,05$	$0,0 \pm 0,0$

Note: * - significant difference ($p<0.05$) compared to the Group with normal blood ferritin (NORMAL).

All patients underwent laboratory tests before the start of treatment, which included a complete blood count, a biochemical blood test and a ferritin level in the blood. Data from these studies are shown in Table 2.

Table 2 Laboratory analyzes of enrolled patients undergoing IVF

Characteristics	Group 1 (n = 46)	Group 2 (n = 30)	Group 3 (n = 25)
RBC	$4,3 \pm 0,1$	$8,8 \pm 4,5$	$8,3 \pm 3,8$
HBG	$120, 7 \pm 1,64$	$115,9 \pm 4,5$	$119,4 \pm 5,7$
MCV	$87,1 \pm 0,9$	$82,8 \pm 2,6$	$90,1 \pm 0,1$
MCH	$27,7 \pm 0,4$	$42,2 \pm 12,3$	$28,8 \pm 0,5$
MCHC	$312,1 \pm 7,9$	$301,7 \pm 15,8$	$306,6 \pm 13,1$
General bilirubin	$14,0 \pm 0,8$	$13,9 \pm 1,2$	$16,4 \pm 3,0$
Direct bilirubin	$6,3 \pm 3,2$	$3,5 \pm 0,6$	$3,0 \pm 0,3$
not direct bilirubin	$11,4 \pm 0,9$	$11,2 \pm 1,2$	$11,0 \pm 1,2$
total protein	$75,0 \pm 1,1$	$68,7 \pm 4,1$	$72,2 \pm 3,2$
Ferritin	$51,6 \pm 1,7$	$19,7 \pm 1,4^*$	$114,3 \pm 12,6^*$

Note: * - significant difference ($p<0.05$) compared to the Group with normal blood ferritin (NORMAL).

The average level of ferritin in the group with normal levels was 51.6 ± 1.7 mcr/l, in the group with low levels 19.7 ± 1.4 mcr/l, and in the group with high levels $114.3 \pm 12, 6$ μ g/l. As a result of treatment, such indicators as the number of erythrocytes, hemoglobin level, MCV, MCH, MCHC and ferritin level increased statistically significantly. After the treatment, patients had in vitro fertilization process. IVF data are shown in Table 3.

As shown in Table 3, the mean numbers of retrieved oocytes and metaphase II oocytes in group 3 were higher than in 1 and 2 groups ($p<0.05$). As for the average percentage of the quality of mature oocytes, in patients of 1 and 2 groups, the indicators are significantly higher than in patients with a high level of ferritin in the blood ($p=0.001$).

The difference in the average numbers of high-quality embryos in the main and control groups was even more significant: 3.3 ± 0.4 in group 1, 3.5 ± 0.7 in group 2 and 2.5 ± 0.7 in group 3 ($p=0.001$). The average ratio of high-quality embryos to the number of mature MII oocytes in groups 1 (46.5 ± 4.6) and 2 (45.3 ± 5.9) is almost twice as high as compared to group 3 ($27.1 \pm 5, 6$) ($p<0.05$).

As shown in Table 3, the average number of all three groups of oocytes, metaphase II oocytes, embryos did not differ significantly, but we have seen how important a normal blood ferritin level is in obtaining quality embryos. From the data in the table it can be seen that in the percentage of high-quality embryos in the group with normal and not high levels of ferritin in the blood, there is a slight statistically insignificant improvement than in the group with a high level of ferritin.

Table 3. Characteristics of the cycle of controlled ovarian stimulation in IVF cycles

Characteristics	Group 1 (n = 46)	Group 2 (n = 30)	Group 3 (n = 25)
Received oocytes (n)	14,9 ± 1,3	13,9 ± 2,0	16,4 ± 2,8
Oocytes MII (n)	10,7 ± 0,8	10,4 ± 1,6	11,6 ± 2,7
Fertilized oocytes 2PN(n)	9,4 ± 0,8	9,0 ± 1,2	9,7 ± 1,6
Average Percentage quality of mature oocytes	73,6 ± 2,7	74,7 ± 4,1	58,6 ± 6,2*
Average number of high quality embryos	3,3 ± 0,4	3,5 ± 0,7	2,5 ± 0,7
Average number of middle quality embryos	1,2 ± 0,2	1,1 ± 0,3	0,8 ± 0,2
Average number of low quality embryos	1,5 ± 0,3	1,0 ± 0,3	1,3 ± 0,3
Average ratio of high-quality embryos to the number of mature MII oocytes	46,5 ± 4,6	45,3 ± 5,9	27,1 ± 5,6*
Average ratio of middle-quality embryos to the number of mature MII oocytes	16,2 ± 3,3	16,6 ± 5,3	12,3 ± 4,1
Average ratio of low-quality embryos to the number of mature MII oocytes	21,9 ± 4,3	17,9 ± 5,2	27,8 ± 6,7

Note: * - significant difference ($p < 0.05$) compared to the Group with normal blood ferritin (NORMAL).

Conclusion

Based on the study, we have identified that the level of ferritin in women is a significant factor negatively affecting the effectiveness of IVF programs and the pregnancy rate in IVF cycles. Women preparing for the IVF program should check the level of ferritin and the presence of an inflammatory focus. Complex therapy to normalize the level of ferritin can provide an increase in the effectiveness of the IVF program and a woman's chances for a healthy pregnancy and the birth of a healthy child.

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