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FEATURES OF SPERMOGRAM INDICATORS IN IDIOPATHIC INFERTILITY IN MEN

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About 15% of married couples in the world suffer from infertility. In approximately 50% of cases, the main problem lies in the male factor. Male infertility is a multifactorial syndrome that encompasses a wide range of disorders. Infertility in men without an established history is called "idiopathic infertility". The aim of the work was to compare reproductive function of men according to spermogram indicators for both a control (normozoospermia) and an experimental (idiopathic infertility) groups. The study consisted in studying spermogram indicators in patients of both groups according to WHO recommendations and comparing them with each other and with reference values. Comparison of physicochemical indicators of ejaculate revealed that the volume of ejaculate in groups II and I did not reliably differ from each other, as did the pH indicator. The indicators of the time of dilution of ejaculate in persons with idiopathic infertility significantly increased (by 1.4 times) in comparison with men of the control group. Reliable violations of the quality indicators of ejaculate, characterized by a decrease in sperm motility by 1.6 times, were revealed. The number of immobile spermatozoa was 1.7 times higher in idiopathic infertility compared to control values. A 1.8-fold decrease in the concentration of fructose in the ejaculate correlated with pronounced processes of sperm motility and viability impairment. The indicator of the concentration of citric acid, which reflects the functional state of the prostate gland and the endocrine function of the testicles, was 1.7 times higher in patients with idiopathic infertility compared to the control group. The content of zinc in seminal fluid differs most between infertile men and men of the control group, it was 3.4 times lower in men with idiopathic infertility.

Thus, the obtained data on the indicators of spermograms denote that reproductive problems in men with idiopathic infertility consist in a reliable violation of the qualitative indicators of ejaculate especially in reduced mobility. Which correlates with changes in the index of liquefaction time, which increases and biochemical indicators (decrease in the concentration of fructose, citrate and zinc) compared to ejaculate indicators in men with normozoospermia.

Key words: *idiopathic male infertility, ejaculate, seminal plasma, spermogram.*

Connection of the publication with planned research works.

The work is a fragment of the planned research topics of Danylo Halytsky Lviv National Medical University «Development of prognostic and diagnostic immunobiochemical criteria for the effects of extreme factors of various nature on the body» (state registration number 0121U100163) and «Application of mathematical methods for the study of physico-chemical processes in biotechnical systems» (state registration number 0117U001078).

Introduction.

Modern data from the literature indicate that the male factor of infertility in a married couple is approximately 50% [1-3]. In infertile couples resorting to assisted reproductive technologies, idiopathic infertility (II) is found in approximately 30% of cases of all causes of male infertility. According to the data of the European Association of Urology [3], idiopathic infertility is the inability of a couple to conceive a child, in which the reasons for impaired fertility remain unexplained. Idiopathic infertility can be both with normozoospermia [1] and accompanied by impaired sperm quality, in particular, the presence of oligoasthenoteratozoospermia [2, 3]. Idiopathic infertility is established only after excluding all possible causes of infertility, that is, when a detailed history is collected, the necessary clinical and additional studies are carried out and no etiological factors of the disease are found [3, 4].

Confirmation of idiopathic genesis of infertility in men who have not had offspring for 1 year is carried out after excluding a number of pathological conditions (absence of disorders in the endocrine and reproductive systems; presence of minor disorders in the form of asthenozoospermia or normospermia; absence of sexually transmitted diseases; absence of antisperm antibodies ; absence of genetic diseases, etc.).

In the genesis of idiopathic infertility, hidden factors that can explain male infertility can play a certain role: enzymatic disorders of spermatogenesis processes, DNA damage of spermatozoa, increased levels of indicators of peroxide oxidation of biomembrane lipids, undetected genetic pathologies [1, 3, 5-7].

The causes of idiopathic infertility in a married couple can arise due to the summation of negative factors, often unknown to science today and related not only to fertility. There are factors that increase the risk of idiopathic infertility: male sex, age older than 30 years and a certain lifestyle (presence of bad habits, professional factors, negative environmental factors, presence of frequent stress, etc.) [1, 3, 4].

The aim of the work.

To compare the reproductive function of men with idiopathic infertility with a control group with normozoospermia based on spermogram indicators.

Object and methods of research.

The study of reproductive function of 73 men aged 22 to 45 years, average age 32.7±4.3 years (group I) was conducted in the urology department of the CNE «Lviv Regional Clinical Hospital» and in the «Salyutas Medical Center». The control group consisted of persons of the same age who lived in the territory of Lviv region (group II, n=56). Diagnosis of idiopathic infertility was carried out at the outpatient stage according to WHO standards [8, 9]. The study consisted in studying spermogram indicators in patients of both groups according to WHO recommendations [10] and comparing them with each other and with reference values.

Table 1 – Quantitative and qualitative indicators of the spermogram in men with idiopathic infertility

Indicator	Reference indicators [8]	Group I (idiopathic infertility, n=73)	Group II (control, normozoospermia, n=56)
Volume, mL	2,0 >	3,1±0,5	3,4±0,4
The number of spermatozoa in the ejaculate, million		107,9±12,6*	195,7±24,2
Dilution, min	< 60	54,7±4,6*	38,3±4,1
pH	7,2 – 7,8	7,8±0,3	7,3±0,3
Concentration spermatozoa, million/ml	15 >	38,42±4,73*	58,27±5,12
Live spermatozoa, %	50	63,3±3,1	68,9±3,6
Sperm motility, %	< 50	31,4±4,2*	50,7±5,2
Immotile spermatozoa, %	>10	12,1±1,3*	7,1±0,7
Morphology, %	30 > with a normal head shape	38,1±5,2*	68,8±6,9
Leukocytes, million/ml	< 1	0,46±0,08	0,28±0,07

Notes: *p<0.05 in comparison with group II (normozoospermia).

Inclusion criteria: cases of idiopathic infertility in the specified persons, which were verified in specialized health care institutions in accordance with the protocol of the Ministry of Health of Ukraine; availability of the patient's informed consent for the study. The comparison criteria were different parameters of the patients' spermogram.

Variational and statistical data processing were performed using the software package for personal computers Microsoft Excel. Basic statistical indicators such as arithmetic mean (M) and standard error (m) were determined. The results are presented as the arithmetic mean (M) ± standard error of the mean (m). The number of experiments (n) corresponds to the number of individuals studied in each case (each time used sperm obtained from one patient or a virtually healthy donor). The reliability of changes between the statistical characteristics of two alternative sets of parametric data was determined by Student's t-test. The critical levels of reliability in testing statistical hypotheses in the studies were taken as 0.95, 0.99 and 0.999.

The absolute value of the correlation coefficient r was 0.90 – 0.98. The reliability of the calculated parameters of the line was checked by Fisher's F-test: the approximation for which p≤0.05 was considered reliable.

All studies were conducted with the proper permission of the Commission on Bioethical Expertise of Danylo Halytsky Lviv National Medical University and the written consent of the patients.

Research results and their discussion.

The given data are represented by classic spermogram indicators in the form of statistically processed average values, which reflect quantitative and qualitative characteristics of men who applied to medical institutions in Lviv for fertility disorders **(table 1)**.

Comparing the physical and chemical indicators of ejaculate, it can be seen that the volume of ejaculate in groups I and II did not differ significantly ((3.1±0.5) ml and (3.4±0.4) ml, respectively), and the pH indicator in the sperm of patients of group I, it differed in pathological growth – (7.8±0.3), in contrast to individuals of group II – (7.3±0.3) ml, although this difference is within the limits of reference indicators.

Violation of the physico-chemical properties of seminal plasma consisted in a significant increase in the ejaculate dilution time in men of group I – (54.7±4.6) min, compared to men of group II – (38.3±4) min (p<0.05).

Statistically reliable violations of the quality indicators of ejaculate, characterized by a decrease in sperm motility, were revealed. In group I, this indicator was (31.4±4.2)%, and in control group II – (50.7±5.2)%, that is, in idiopathic infertility it was 1.6 times lower (p<0, 05). The number of immobile spermatozoa was 1.7 times higher in individuals of group I than in individuals of group II, respectively (12.1±1.3) and (7.1±0.7)% (p<0.05). Idiopathic infertility in individuals from the I group was characterized by a significant decrease in quality indicators of ejaculate, in particular, mobility, which was 1.6 times less than the control (p<0.05). This correlates with changes in physico-chemical (average dilution time is 1.4 times greater, p<0.05) and biochemical (decrease in fructose levels by 1.8 times, citrate – by 1.7 times; p<0.05) indicators. which characterizes the violation of male reproductive function.

The evaluation of the morphological properties of the ejaculate was carried out according to two parameters: the percentage of live spermatozoa in the ejaculate and the indicator of the shape of the head. The percentage of live spermatozoa in the groups was not significantly different: (63.3±3.1) and (68.9±3.6)%, respectively (p>0.05). The indicator of the shape of the head was 1.8 times higher in the ejaculate of individuals of group II with normozoospermia, respectively, in groups I and II this indicator was (38.1±5.2) and (68.8±6.9)% of spermatozoa with normal by the shape of the head (p<0.05). This indicates that the leading pathological process in patients with idiopathic infertility is a violation of ejaculate kinesis in the absence of probable changes in most quantitative indicators. In contrast to individuals of group I, in men of group II, ejaculate kinesograms were characterized by significantly better indicators with correspondingly higher (although not likely) quantitative indicators. In general, the spermogram indicators of men in the control group were within the reference values.

Kinesograms of ejaculate were analyzed in the context of possible violations of sperm plasma. For this purpose, a selective biochemical study of ejaculate markers (fructose, citrate, and zinc) that may cause idiopathic infertility was used **(table 2)**. Such changes in the ejaculate of men with idiopathic infertility are presented in the form of a decrease in the concentration of citric acid and fructose [2, 3]. Fructose is an indicator of the secretory function of the seminal vesicles, as well as a source of energy for ejaculate spermatozoa. Its synthesis takes place entirely in the seminal vesicles under the influence of exogenous androgens. The speed of the process of splitting fructose (fructolysis)

is related to the mobility and viability of spermatozoa.

A decrease in the concentration of fructose in the ejaculate may indicate a hypoandrogenic state, the presence of inflammatory changes, atresia or obstructive conditions in the seminal vesicles. A decrease in the concentration of fructose in the ejaculate can be permanent or stable, which leads to a decrease in the mobility and viability of spermatozoa. Citric acid is synthesized in the structures of the prostatitis vesicular complex. It acts as a factor in the dilution of ejaculate, activation of hyaluronidase and other factors that contribute to the process of penetration of the spermatozoon to the egg cell. Determination of the concentration of citric acid in semen provides information about the secretory function of the prostate gland. Its decrease is a sign of a chronic inflammatory process of both bacterial and abacterial genesis, in particular its subclinical forms, as well as a hypogonadal state of various genesis.

A biochemical study of the ejaculate showed the presence of violations of the secretion of fructose by the seminal vesicles and citric acid by the prostate gland in patients with idiopathic infertility. A 1.8-fold decrease in the level of fructose in the spermogram of patients of group I, compared to the data of patients of group II (respectively (9.8±0.7) and (17.7±2.1) mmol/l ($p<0.05$) correlated with pronounced processes of impaired sperm motility and viability. This was confirmed by the data obtained in the scientific work, in the form of an established statistically significant difference in the average indicator of the quantitative content of fructose in the studied groups of patients.

In particular, we found a positive correlation ($r=0.69$, $p<0.05$) between the fructose content in the ejaculate and sperm motility **(fig. 1)**, as well as a positive correlation ($r=0.53$, $p<0.05$) between fructose content in the ejaculate and the number of spermatozoa in the ejaculate **(fig. 2)**.

At the same time, in patients with idiopathic infertility and normozoospermic men, there was no correlation between fructose content and other indicators of the spermogram.

The average statistical indicator of the concentration of citric acid, which reflects the functional state of the prostate gland and the endocrine function of the testicles, was also significantly reduced in patients with idiopathic infertility, amounting to (19.7±1.8) mmol/l. In the II group, this indicator was (33.1±4.3) mmol/l, respectively. The difference in groups I and II was 1.7 times ($p<0.05$). Correlation dependence between the concentration of citric acid and spermogram parameters was not observed, or these dependences were statistically improbable.

Table 2 – Biochemical indicators of ejaculate of men with idiopathic infertility

Indicator	Reference indicators [8]	Group I (idiopathic infertility, n=73)	Group II (control, normozoospermia, n=56)
Fructose, mmol/l	11,9-28,8	9,8±0,7*	17,7±2,1
Citric acid, mmol/l	23,4-31,2	19,7±1,8*	33,1±4,3
Zinc, mmol/l	>2,4	1,3±0,2**	4,4±0,2

Notes: *$p<0.05$; **$p<0.001$ in comparison with group II (normozoospermia).

In turn, zinc plays an important role in the stabilization of sperm membranes and chromatin, and also exhibits antibacterial activity. Lack of zinc in many cases causes impotence.

A comparative analysis of the zinc content in the sperm plasma of men with idiopathic infertility and normozoospermia was carried out. While the zinc concentration was 4.4±0.2 mmol/l in the normal range, it was 1.3±0.2 mmol/l with idiopathic infertility, i.e. it was 3.4 times lower ($p<0.001$). It is important to note that the content of zinc in the sperm plasma is closely correlated ($r=0.48$, $p<0.05$) with sperm motility, which is confirmed by a positive correlation of medium strength **(fig. 3)**.

As you know, zinc is a trace element, which in the body is a cofactor for more than 300 enzymes involved in DNA transcription and protein synthesis [11]. The presence of this trace element is necessary for cell division in general and spermatogenesis in particular. The

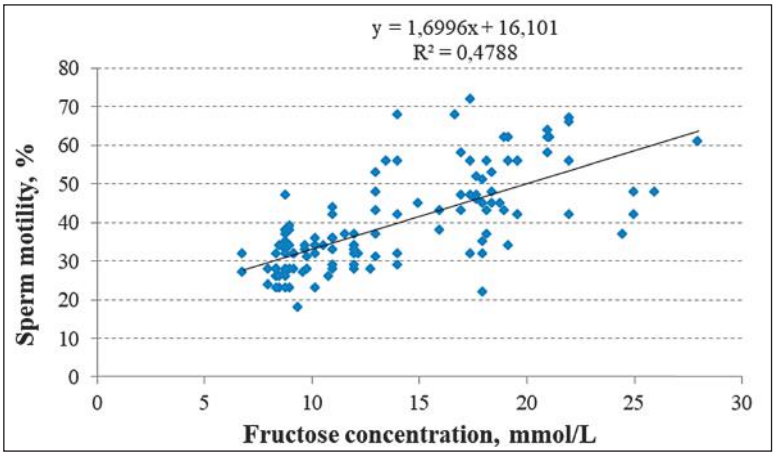

Figure 1 – Correlation between seminal fructose concentration (mmol/L) and sperm motility (%).

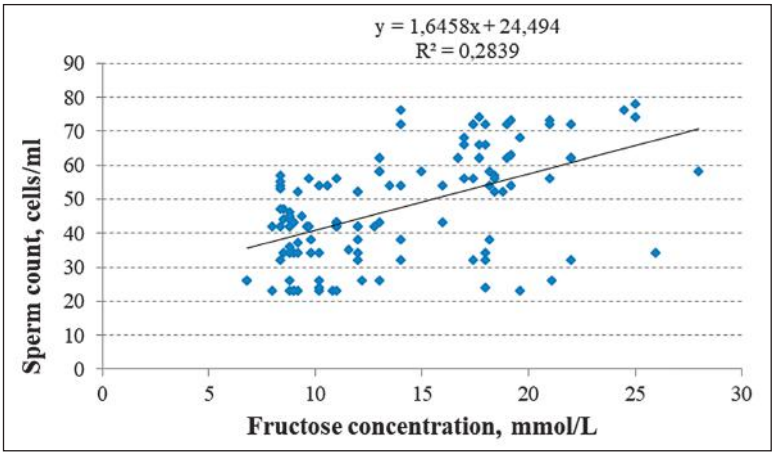

Figure 2 – Correlation between seminal fructose concentration (mmol/L) and sperm count (%).

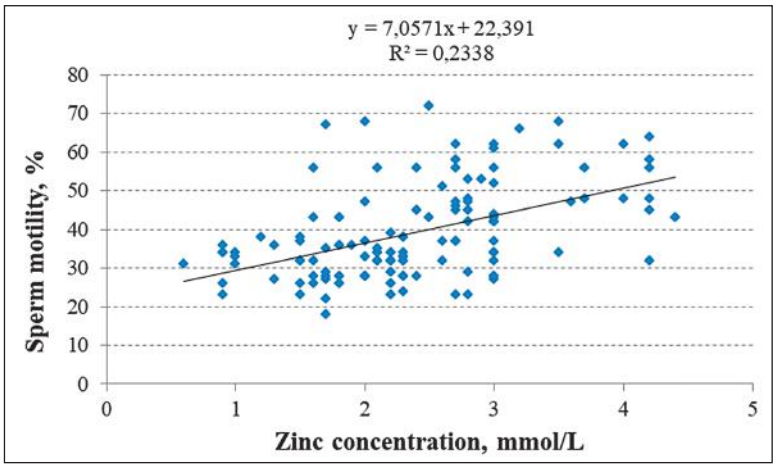

Figure 3 – Correlation between seminal zinc concentration (mmol/L) and sperm motility (%).

zinc content in the seminal fluid of a healthy man is 30 times higher than that in the blood. It is the content of zinc in the seminal fluid that differs the most in healthy and infertile men compared to the corresponding indicators for other trace elements. In a large number of works, the influence of zinc on the state of sperm was investigated: the level of this trace element in the seminal fluid was reliably correlated with the number of spermatozoa (in the case of zinc deficiency, the density of spermatozoa was < 20 million in 1 ml of ejaculate) and affected the volume of ejaculate [11]. After ejaculation, zinc contained in the seminal fluid binds to the plasma membrane of the sperm and stabilizes its structure.

Zinc deficiency affects the functioning of the pituitary gland and leads to inhibition of the production and release of luteinizing (takes a direct part in the regulation of testosterone levels due to its effect on the interstitial Leydig cells) and follicle-stimulating (regulates and stimulates spermatogenesis in the convoluted tubules) hormones, which play an important role in sexual and reproductive functions of a man [11].

Conclusions.

1. It was established that the level of general indicators of the spermogram is reliably worse in men with idiopathic infertility compared to the indicators of men with normozoospermia.

2. Reproductive problems in men with idiopathic infertility consist in a significant violation of the quality indicators of the ejaculate, the mobility is 1.6 times lower ($p<0.05$), which correlates with changes in the liquefaction time indicator, which increases by 1.4 times ($p<0$ .05) and biochemical indicators (decrease in fructose levels by 1.9 times, citrate by 1.7 times, zinc by 3.4 times, $p<0.001$-0.05), compared to ejaculate indicators in men with normozoospermia.

Prospects for further research.

In the future it is planned to study the effect of the influence of rheumatoid arthritis, as a concomitant pathology of male infertility, on spermogram indicators in idiopathic infertility.

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ОСОБЛИВОСТІ ПОКАЗНИКІВ СПЕРМОГРАМИ ПРИ ІДІОПАТИЧНІЙ НЕПЛІДНОСТІ ЧОЛОВІКІВ

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Резюме. За даними Європейського товариства репродуктології та ембріології в Україні неплідцям страждають біля 1 млн подружніх пар, тобто 15-17%. Однак, в дійсності цей показник є вищим оскільки, за результатами соціологічних опитувань кількість випадків неплідця значно перевищує число звернень за медичною допомогою з цього приводу. Згідно зі статистичними даними неплідцям у світі страждають 15% подружніх пар, а частка чоловічого фактора у структурі загального неплідця становить близько 50% та має тенденцію до зростання. У безплідних парах, що вдаються до допоміжних репродуктивних технологій, ідіопатичне неплідця представлено близько 30% випадків від усіх причин чоловічого неплідця. Згідно даних Європейської асоціації урологів (2022), ідіопатичне неплідця – це нездатність пари зачати дитину, при якому причини порушення фертильності залишаються нез'ясованими. Метою роботи було порівняти

репродуктивну функцію у чоловіків з ідіопатичним непліддям та контрольної групи з нормозооспермією на основі показників спермограми.

Діагностика ідіопатичного неплідця чоловіків (n=73) віком 22-45 років, проводилася на амбулаторному етапі згідно зі стандартами ВООЗ (група І). Групу контролю складали особи такого ж віку, які мешкали на території Львівської області (група ІІ, n=56). Дослідження полягало у вивченні показників спермограми у пацієнтів обох груп згідно рекомендацій ВООЗ і порівнянні їх між собою та з референтними значеннями.

Порівняння фізико-хімічних показників еякуляту виявило, що об'єм еякуляту у чоловіків із ідіопатичним непліддям та в групі контролю достовірно не відрізнявся між собою, як і показник pH. Достовірно збільшувались показники часу розрідження еякуляту в осіб із ідіопатичним ненпліддям (в 1,4 раза) у порівнянні з чоловіками контрольної групи. Виявлено достовірні порушення якісних показників еякуляту, що характеризуються зменшення рухливості сперматозоїдів в 1,6 раза. Кількість нерухомих сперматозоїдів була в 1,7 раза більшою при ідіопатичному неплідді щодо контрольних значень. Зменшення концентрації фруктози в еякуляті в 1,8 раза корелювало з вираженими процесами порушення рухливості та життєздатності сперматозоїдів. Показник концентрації лимонної кислоти, яка відображає функціональний стан передміхурової залози і ендокринної функції яєчок, був у 1,7 раза у пацієнтів з ідіопатичним непліддям у порівнянні з контрольною групою. Вона виступає фактором розрідження еякуляту, активації гіалуронідази та інших факторів, що сприяють процесам проникнення сперматозоїда до яйцеклітини. Вміст цинку в сім'яній рідині найбільше відрізняється у безплідних чоловіків і чоловіків контрольної групи, у чоловіків з ідіопатичним непліддям він був в 3,4 раза нижчим. Слід відмітити, цинк є кофактором для більш ніж 300 ензимів, що беруть участь у транскрипції ДНК і синтезі білка. Наявність цього мікроелемента обов'язкова для поділу клітин загалом і зокрема сперматогенезу.

Таким чином, отримані дані щодо показників спермограм свідчать, що репродуктивні проблеми у чоловіків з ідіопатичним непліддям полягають у достовірному порушенні якісних показників еякуляту, рухливість є меншою, що корелює із змінами показника часу розрідження, який зростає та біохімічними показниками (зниження концентрації фруктози, цитрату та цинку) порівняно з показниками еякуляту в чоловіків із нормозооспермією.

Ключові слова: ідіопатичне неплідця чоловіків, еякулят, сім'яна плазма, спермограма.

FEATURES OF SPERMOGRAM INDICATORS IN IDIOPATHIC INFERTILITY IN MEN

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Abstract. According to the European Society of Reproductive and Embryological Society, about 1 million couples in Ukraine suffer from infertility, i.e. 15-17%. However, in reality, this indicator is higher because, according to the results of sociological surveys, the number of cases of infertility significantly exceeds the number of requests for medical help in this regard. According to statistical data, infertility affects 15% of married couples in the world, and the share of the male factor in the structure of total infertility is about 50% and has a growing tendency. In infertile couples resorting to assisted reproductive technologies, idiopathic infertility represents about 30% of all causes of male infertility. According to the data of the European Association of Urology (2022), idiopathic infertility is the inability of a couple to conceive a child, in which the reasons for impaired fertility remain unclear. The aim of the work was to compare reproductive function in men with idiopathic infertility and a control group with normozoospermia based on spermogram indicators.

Diagnosis of idiopathic infertility in men (n=73) aged 22-45 was carried out at the outpatient stage according to WHO standards (group I). The control group consisted of persons of the same age who lived in the Lviv region (group II, n=56). The study consisted in studying spermogram indicators in patients of both groups according to WHO recommendations and comparing them with each other and with reference values.

Comparison of physicochemical parameters of ejaculate revealed that the volume of ejaculate in men with idiopathic infertility and in the control group did not differ significantly from each other, as did the pH indicator. The indicators of the time of dilution of ejaculate in persons with idiopathic infertility significantly increased (by 1.4 times) in comparison with men of the control group. Reliable violations of the quality indicators of ejaculate, characterized by a decrease in sperm motility by 1.6 times, were revealed. The number of immobile spermatozoa was 1.7 times higher in idiopathic infertility compared to control values. A 1.8-fold decrease in the concentration of fructose in the ejaculate correlated with pronounced processes of sperm motility and viability impairment. The indicator of the concentration of citric acid, which reflects the functional state of the prostate gland and the endocrine function of the testicles, was 1.7 times higher in patients with idiopathic infertility compared to the control group. It acts as a factor in the dilution of ejaculate, activation of hyaluronidase and other factors that contribute to the process of penetration of the spermatozoon to the egg cell. The content of zinc in seminal fluid differs most between infertile men and men of the control group, it was 3.4 times lower in men with idiopathic infertility. It should be noted that zinc is a cofactor for more than 300 enzymes involved in DNA transcription and protein synthesis. The presence of this trace element is necessary for cell division in general and spermatogenesis in particular.

Thus, the obtained data on the indicators of spermograms indicate that reproductive problems in men with idiopathic infertility consist in a reliable violation of the quality indicators of ejaculate, namely, mobility is lower, which correlates with changes in the index of dilution time, which increases and biochemical indicators (decrease in the concentration of fructose, citrate and zinc) compared to ejaculate indicators in men with normozoospermia.

Key words: idiopathic male infertility, ejaculate, seminal plasma, spermogram.

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Conflict of interest:
The authors declare no conflict of interest.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article.

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