REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

COMPARATIVE STUDY OF THE RELATIONSHIP BETWEEN SOME BIOCHEMICAL INDICATORS AND KARYOTYPE IN IDIOPATHIC MALE INFERTILITY IN AZERBAIJAN

Specialty: 2406.02 – Biochemistry

Science field: Medicine

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BAKU – 2025

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GENERAL CHARACTERISTICS OF THE RESEARCH

Relevance of the topic. Infertility is one of the major medical and social problems in healthcare. Globally, 12–15% of married couples suffer from infertility, leading to profound psychological, social, and economic challenges. Infertility accounts for 7.5% of marriage breakdowns. Among the factors contributing to infertility within marriage, the female factor accounts for 45%, the male factor for 40–50%, and combined factors from both partners account for 15%[1,2,3]. Male infertility is of multifactorial origin, with social, demographic, and environmental factors playing a significant role in its pathogenesis. This issue may arise due to various endocrine disorders, testicular injuries of any cause, sexually transmitted infections, varicocele, harmful habits, obstruction of the genital organs and their appendages, obesity, environmental factors, chronic stress, sleep disorders, prolonged sedentary work, lack of physical activity, vitamin deficiencies, etc.[4,5]

As a result of the influence of these factors, both the quantitative and qualitative parameters of the spermogram deteriorate. Thus, the number of spermatozoa decreases, their motility weakens, the number of spermatozoa with fragmented DNA and chromatin abnormalities increases, and pathological spermatozoa with abnormal structures are formed.

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Such spermatozoa have a reduced ability to fertilize the egg cell, ultimately leading to infertility in men[6,7].

Current diagnostic methods for male infertility fail to identify its causes in 30–40% of cases. In such situations, the treating physician establishes a diagnosis of idiopathic male infertility and is unable to determine the underlying causes of pathospermia[8,9]. Currently, laboratory diagnostics of male infertility primarily focus on indicators that characterize the overall fertilizing potential of semen—such as sperm count, motility, and the proportion of spermatozoa with normal morphology. Although the spermogram is the main method for examining spermatozoa, it does not provide complete information about disorders in the process of spermatogenesis[10]. From this perspective, the development of new diagnostic programs based on existing clinical and laboratory examinations may play a crucial role in addressing the problem of male infertility[10]. Determining the fluctuation ranges of biochemical indicators in ejaculate and blood plasma can help define the diagnostic potential of laboratory tests and provide both quantitative and qualitative characteristics of spermatogenesis pathologies.

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Studies show that changes in the levels of reproductive hormones in infertile men directly affect sperm production[11,12]. For this reason, it is essential to investigate the interrelated roles of hormonal (such as decreased levels of male sex hormones, increased levels of female sex hormones, and thyroid gland pathologies), genetic, and biochemical factors in the pathogenesis of male infertility. This could pave the way for the development of new diagnostic and therapeutic approaches. In recent years, the concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) have been the primary focus in the diagnosis of male infertility.Although the literature includes studies on the characteristics of the metabolic indicators of seminal plasma that ensure sperm viability, as well as the biochemical parameters of ejaculate and blood plasma, the data are fragmented. There is a pressing need for comprehensive research, as well as for the investigation and application of new markers. Parallel analysis of the ratio of metabolites in ejaculate and blood plasma may be of greater scientific interest. In this regard, extensive research is being conducted on nitric oxide levels and hormonal balance in the blood of infertile men, as well as on the level of fructose in seminal plasma.

The aim of the research was to investigate the causes of male infertility in Azerbaijan using comprehensive diagnostic methods, including biochemical, molecular, and genetic approaches.

Tasks of the research:

1. Examination of the sperm of male infertility patients using morphological methods and identifying groups with pathospermia development based on biochemical and morphological analysis of the ejaculate;
2. Investigation of hormonal changes (testosterone, LH, FSH, and prolactin) in the blood serum of individuals in the research groups;

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3. Comparative evaluation of nitric oxide levels in the blood and fructose levels in the ejaculate of individuals in the research groups;
4. Determination of molecular-genetic indicators (chromosome karyotype) in individuals in the research groups;
5. Investigation of the correlation between ejaculate indicators and biochemical parameters in different groups of pathospermia;
6. Identification of biochemical markers for different groups of pathospermia and determination of possible differential diagnostic criteria.

Research methods. Microscopic, genetic, biochemical, and immunoenzymatic (ELISA) analysis methods were used in the research.

The main points of the dissertation for defense:

1. A decrease in the number of rapid, progressive spermatozoa with normal morphology in ejaculate, along with an increase in the number of spermatozoa with mixed pathologies, can be considered key indicators in the diagnosis of male infertility.
2. Analysis of the concentrations of FSH and LH in men with idiopathic male infertility is of significant importance in the differential diagnosis of pathological forms of infertility.
3. Hyperprolactinemia plays a significant role in the etiology of male infertility, especially in non-obstructive azoospermia.
4. A direct correlation between the structural-morphological composition of spermatozoa, hormonal imbalance, and the level of nitric oxide exists in idiopathic male infertility.
5. An increase in the concentration of fructose in the ejaculate is one of the key indicators of the decrease in the number of progressive spermatozoa. Based on the concentration of fructose in the ejaculate, it is possible to identify obstructive azoospermia.
6. Chromosomal pathologies do not play a significant role in the etiology of idiopathic male infertility.

Scientific novelty of the research. For the first time, risk groups for infertility among the Azerbaijani population were identified based on age and degree of infertility using morphological evaluation of

sperm. In addition, to determine the cause of infertility, more informative immuno-biochemical and molecular genetic markers were used. Thus, by measuring the amount of fructose in the seminal fluid of the study groups, it is possible to obtain direct information about the function of the seminal vesicles and, at the same time, to detect obstructive azoospermia. Through cytogenetic examinations, it was determined whether the infertility problem in these individuals was congenital or acquired by identifying genetic defects. At the same time, it was established that chromosomal pathologies do not play a significant role in the etiology of idiopathic male infertility. It was proven that in individuals with pathospermia, sperm activity varies depending on the concentration of nitric oxide (NO). Studying the mechanisms by which immuno-molecular, hormonal, and metabolic changes, as well as karyotypic abnormalities, affect the ultrastructure of spermatozoa in infertile men may have scientific significance in elucidating the pathogenesis of asthenozoospermia.

Theoretical and practical significance of the research. Using numerous examinations, the main causes leading to male infertility were investigated, and diagnostic parameters were identified based on a comparative analysis of the obtained results. These findings make it possible to determine appropriate treatment principles for idiopathic male infertility and will assist in the development of recommendations for physicians working in this field. As an outcome of our research, a group of hormonal and metabolic factors responsible for impaired sperm motility was identified. In groups with reduced sperm motility, a high level of fructose in the ejaculate was detected. Fructose deficiency in the cell and an increase in nitric oxide levels lead to accelerated lipid peroxidation in the sperm membrane, weakened antioxidant defense, damage to nuclear and mitochondrial DNA, and impaired sperm motility. The numerous studies and literature reviews mentioned above indicate that, to date, the treatment of male infertility remains incomplete, with idiopathic infertility accounting for 30–40% of cases. Since the cause of idiopathic infertility is unknown, its treatment cannot be considered satisfactory. For this reason, our primary goal is to identify the underlying causes of infertility and

thereby contribute to the optimization of its treatment. Considering the financial burden of ineffective treatments in cases of idiopathic infertility, it can be stated with confidence that this research will have significant economic value in the future.

Approbation of the research. The main points and results of the dissertation were presented and discussed at the following conferences: the 24th Republican Scientific Conference of Doctoral Students and Young Researchers (Baku, 2021), the 4th International Congress of Multidisciplinary Studies in Medical Sciences (Turkey, 2022), the scientific-practical conference dedicated to the 80th anniversary of Professor A.M. Afandiyev (Baku, 2023), the scientific-practical conference dedicated to the birthday of National Leader Heydar Aliyev (Baku, 2024), the scientific-practical conference dedicated to the birthday of Aziz Mammadkarim oglu Aliyev (Baku, 2024), the scientific-practical conference dedicated to the 80th anniversary of Vagif Shadlinsky (Baku, 2024), the All-Russian Scientific and Practical Conference of Young Scientists with International Participation (Moscow, 2024), the International Scientific-Practical Congress on Current Issues in Medicine (Baku, 2024), and the International Gevher Nesibe Health Sciences Conference (Turkey, 2024).

Practical Application of the Results. The results of the study are being applied in the educational process and lectures of the Department of Biochemistry at Azerbaijan Medical University.

Publications. A total of 18 scientific papers have been published on the topic of the dissertation, including 5 articles and 3 abstracts in international journals, as well as 5 articles and 5 abstracts published in national journals.

Organization where the dissertation was conducted. The dissertation was carried out at the Department of Biochemistry of Azerbaijan Medical University and at the "Healthy Family" Medical Center.

Volume and structure of the dissertation. The dissertation consists of 158 computer pages (total 241.580 characters). It contains Introduction (11.139 characters), Literature Review (80.440

characters). Chapter II: Materials and Methods (17,380 characters). Chapter III: Personal Research (70,959 characters), Conclusion (58,413 characters), Results (3,249 characters), Practical Recommendations (580 characters), and Bibliography. The bibliography contains 255 scientific sources, including 17 in Azerbaijani, 58 in Russian, and 180 in foreign languages. The dissertation is illustrated with 26 tables and 17 figures.

MATERIALS VƏ METHODS

Clinical Characteristics of the Patients. In the research, blood and sperm samples from 101 men aged 24-45, who were examined at the Baku Healthy Family Medical Center and treated for idiopathic male infertility, were analyzed. The study excluded patients with a history of regular steroid medication use (androgens or antiestrogens), those with chromosomal translocations, symptoms of hypogonadotropic hypogonadism, as well as those with endocrine diseases that reduce testosterone secretion, such as hypothyroidism, thyrotoxicosis (based on thyroid-stimulating hormone and free T4 analysis), patients in the decompensated stage of diabetes mellitus, hypercortisolism, kidney or liver failure, acute exacerbations of urogenital tract inflammation and infectious diseases, and those with pituitary adenomas. The study included men who had been childless for more than a year, where the female factor as a cause of infertility was ruled out, and whose spouses did not have detected antisperm antibodies. For the control group, fertile men with children and practically healthy men were selected. The control group consisted of 20 fertile men aged between 23 and 40, with an average age of 31.1±1.0. This research was conducted between 2019 and 2022 at the Clinical research laboratory of the Biochemistry Department of Azerbaijan Medical University and the Healthy Family Medical Center.

After the diagnosis of infertility was confirmed based on sperm analysis, research groups were established based on the examination. The patients included in the study were divided into three groups

according to the criteria set by the World Health Organization in 2010 and revised in 2021 [9], based on the count and motility of their spermatozoa: asthenozoospermia (patients with a normal sperm count but low progressive motility <32%) – 56 individuals; oligozoospermia (sperm count less than 15 million per ml of semen) – 30 individuals; azoospermia (absence of spermatozoa in the ejaculate) – 15 individuals. The azoospermia group was further subdivided into two groups: obstructive azoospermia (n=7) and non-obstructive azoospermia (n=8) (Table 1).

Table 1

Distribution of individuals included in the study by age and groups

Indica-tors	Groups				
	control	asthenozoo-spermia	oligozoo-spermia	non-obstructive azoospermia	obstructive azoospermia
N	20	56	30	7	8
M	31.1	31.4	32.5	31.4	30.8
Median	31.5	30.0	32.5	31.0	29.5
Q1	28.0	27.5	28.0	23.0	27.0
Q2	34.0	35.0	35.0	38.0	35.0

Note: M – mean indicator, Q1 – quartile1, Q2 – quartile2

Research methods

Microscopic examination of semen. Semen samples were collected via masturbation from men who had abstained from sexual intercourse for three to five days, and the samples were placed into dry, clean, disposable containers for morphological analysis. Phase-contrast microscopy and special staining agents were used to evaluate the structure and function of spermatozoa. In the spermogram analysis.

both macroscopic and microscopic parameters of the ejaculate were determined. Microscopic parameters included the number of spermatozoa per 1 ml of ejaculate, the total number of spermatozoa in the entire volume, the count of actively motile spermatozoa, weakly motile and immotile spermatozoa, as well as the number of morphologically normal spermatozoa and pathological spermatozoa. Smears prepared from the seminal fluid were stained using Giemsa and Diff-Quik methods.

Chromosome karyotype analysis. Karyotype analysis of infertile patients was conducted at the Genetics Genetic Diseases Diagnosis Center under a contract with the Baku Healthy Family Medical Center, within the framework of the "United Kingdom National External Quality Assessment Scheme in Clinical Cytogenetics - UKNEQAS/CONEGAS" quality program for chromosome analyses. Blood samples from the patients were obtained using standard cytogenetic techniques. From each patient, 3 ml of blood was used to prepare a cell suspension through a stepwise process, from which smears were made. A fixative solution was added to the smears, and the cells were incubated for 37 minutes to allow swelling and better visibility under the microscope. The preparations were stained with GTG (Giemsa Trypsin Giemsa) dye and kept at a temperature of 37°C for 2 days. The prepared samples were examined under a microscope using a 100X objective. The results of the chromosome analysis were evaluated after 25-30 days.

Biochemical research methods

Immunoassay analysis (FSH, LH, testosterone, and prolactin). The concentrations of FSH, LH, testosterone, and prolactin in the blood serum of infertile men included in the study and fertile men in the control group were measured using the electrochemiluminescence immunoassay principle on the Roche e411 autoanalyzer.

Analysis of fructose in seminal fluid. The concentration of fructose in the seminal fluid of infertile men included in the study and fertile men in the control group was analyzed using a biochemical method. The

concentration of fructose was determined by a colorimetric method with the help of the "Semen Fructose" reagent kit from B.I.R.D. Diagnostics (Baharafshan Institute of Research and Development).

Analysis of nitric oxide. The concentration of nitric oxide was analyzed by a colorimetric method using the "R&D Systems" reagent kit. This reagent kit (DE1500B) is designed for the determination of nitric oxide (NO_2^-/NO_3^- in various biological fluids, including blood serum. Since nitric oxide is an unstable and volatile compound, its direct measurement is challenging. The majority of NO is oxidized to nitrites (NO_2^-) and nitrates (NO_3^-). Therefore, the determination of these ions allows for the quantitative estimation of NO production. After the conversion of NO to (NO_3^-) and (NO_2^-), the concentration of nitric oxide is measured spectrophotometrically using the Griess reaction. After being converted to (NO_3^-) and (NO_2^-), the concentration of nitric oxide (NO) is measured spectrophotometrically using the Griess reaction. The principle of the reagent kit produced by R&D Systems is based on the conversion of nitrates into nitrites with the help of the enzyme nitrate reductase. Subsequently, the concentration of nitrites, which are the products of the Griess reaction, is measured at a wavelength of 540 nm using a specific dye. The concentration of NO in the sample is determined indirectly based on the levels of nitrites and nitrates. The concentration of nitrates in blood serum is calculated by subtracting the concentration of endogenous nitrites (X) from the total nitrate concentration (Y):

The concentration of nitrates = (Y-X) nmol/l

Statistical analysis methods. All data obtained during the study were statistically analyzed in accordance with modern recommendations. Based on the nature of the research, it was classified according to the type of research work – scientific (specialized); according to the design – analytical (control-cohort); according to the material – prospective; according to the volume – selective; according to the method – clinical; according to the duration – transverse; according to the location – laboratory-based [9, p. 13]. Statistical analyses were conducted using

variation, dispersion, correlation, and ROC-analysis methods with the help of the EXCEL-2019 spreadsheet processor and the SPSS-26 statistical software package.

In the variation analysis, both parametric (t-Student-Bonferroni) and non-parametric (Mann-Whitney U and Kruskal-Wallis H) methods were used. Factor analysis was carried out using dispersion methods – specifically the ANOVA test. In the ROC analysis, ROC curves were constructed to evaluate the sensitivity and specificity of the tested parameters, serving as integral indicators of diagnostic performance.

RESULTS AND DISCUSSION

Morphological characteristics of spermatozoa in men with idiopathic male infertility

The main clinical characteristics of male infertility are a decrease in the number of spermatozoa in the semen, their complete absence, or the dominance of spermatozoa with pathological forms that are incapable of fertilization. In this study, the morphological structure of spermatozoa was evaluated based on their external appearance using a microscopic method and compared with Krüger's criteria. The proportion of spermatozoa with mixed pathology, exhibiting pathological features in the neck and head, as well as morphological alterations in the neck, head, and tail, was studied in the patients included in the research. When the proportion of such spermatozoa exceeds 96%, the semen loses its ability to fertilize, and teratozoospermia occurs. This may be caused by chromosomal pathologies, enzyme defects, viral infections, etc. When the concentration of progressively motile spermatozoa is below 32%, the diagnosis of asthenozoospermia is made. The proportion of morphologically normal spermatozoa that fully meet Krüger's criteria should be no less than 4%.

The results of the spermogram analysis revealed that the median number of spermatozoa in the semen significantly decreased in comparison to the control group: in men with asthenozoospermia, it decreased by 41.1% ($p_{III}<0.001$), and in men with oligozoospermia, it decreased by 7.9 times ($p_{III}<0.001$), which is statistically significant.

According to the results of the spermogram analysis, the median proportion of progressively motile spermatozoa significantly decreased in comparison to the control group: in men with asthenozoospermia, it decreased by 73.7% ($p_{HI}<0.001$), and in men with oligozoospermia, it decreased by 3.7 times ($p_{HI}<0.001$), which is statistically significant.

Table 2

Spermogram indicators in idiopathic male infertility

Sperm Parameters		Groups			P_K
		control (n=20)	asthenozoo-spermia, (n=56)	oligozoospermia, (n=30)	
Count, mln./ml	M	64.6	45.9	7.2	<0.001
	Median	63.5	45.0 $p_{H1}<0.001$	8.0 $p_{H1}<0.001$; $p_{H2}<0.001$	
	Q1	61.0	30.0	4.5	
	Q2	74.0	60.0	10.0	
Progressively motile sperm, %	M	34.6	17.9	7.8	<0.001
	Median	33.0	19.0 $p_{H1}<0.001$	9.0 $p_{H1}<0.001$; $p_{H2}<0.001$	
	Q1	32.0	14.0	2.0	
	Q2	35.5	23.0	11.0	
Neck pathologies, %	M	63.3	60.0	55.2	0.031
	Median	64.0	61.0	58.0 $p_{H1}=0.010$	
	Q1	61.0	55.0	48.0	
	Q2	68.5	68.0	62.0	
Head pathologies, %	M	9.9	8.1	5.3	0.005
	Median	8.5	7.0	6.0 $p_{H1}=0.003$; $p_{H2}=0.041$	
	Q1	7.0	5.0	3.0	
	Q2	12.0	10.0	7.0	
Mixed pathology, %	M	21.2	28.2	37.3	<0.001
	Median	20.5	26.5 $p_{H1}=0.014$	33.0 $p_{H1}<0.001$; $p_{H2}=0.001$	
	Q1	17.5	21.0	28.0	
	Q2	24.0	35.0	48.0	
Preserved normal structure, %	M	5.7	3.7	2.2	<0.001
	Median	5.0	3.0 $p_{H1}<0.001$	2.0 $p_{H1}<0.001$; $p_{H2}=0.016$	
	Q1	4.0	2.0	2.0	
	Q2	7.0	5.0	2.0	

Note: M – mean value, N – number, Q1 – first quartile, Q2 – second quartile, p_{H1} – compared to the control group, p_{H2} – compared to patients with asthenozoospermia, p_{H3} – compared to patients with oligozoospermia, p_{H4} – compared to patients with obstructive azoospermia, P_K – comparison among all groups.

As shown, the proportion of progressively motile spermatozoa in men with oligozoospermia is 2.1 times ($p_{H2}<0.001$) significantly lower compared to men with asthenozoospermia.When examining the morphological changes in the structure of spermatozoa, it was found that in asthenozoospermic men, the proportion of spermatozoa with neck and head pathologies did not significantly differ from the control group, although a tendency for a decrease was observed. In oligozoospermic men, however, the proportion of spermatozoa with neck pathologies decreased by 10.3% ($p_{H1}=0.010$), and the proportion of spermatozoa with head pathologies decreased by 41.7% ($p_{H1}=0.003$) compared to the control group, which is statistically significant (Table 2).

According to the results of microscopic analyses, the proportion of spermatozoa with mixed pathology significantly increased compared to the control group in men with asthenozoospermia – 29.3% ($p_{H1}=0.014$), and in men with oligozoospermia – 61.0% ($p_{H1}<0.001$). In men with oligozoospermia, the proportion of spermatozoa with mixed pathology was also significantly higher – 24.5% ($p_{H2}=0.001$) compared to men with asthenozoospermia.

The proportion of spermatozoa with normal morphology significantly decreased compared to the control group of men with asthenozoospermia – 66.7% ($p_{H1}<0.001$), and to men with oligozoospermia – by 2.5 times ($p_{H1}<0.001$). In men with oligozoospermia, the proportion of spermatozoa with preserved normal structure was 50.0% lower ($p_{H2}=0.016$) compared to men with asthenozoospermia.

Thus, in the semen samples of men with idiopathic male infertility, the proportion of spermatozoa with head and neck abnormalities, as well as those with preserved normal structure, significantly decreases, whereas the proportion of spermatozoa with mixed pathology increases. This difference was more pronounced in men with asthenozoospermia and oligozoospermia ($p_K<0.001$).

Karyotype analysis of infertile men

Chromosomal abnormalities are observed in 2-14% of infertile men during karyotype analysis. The analysis of the chromosomal

karyotype plays an important diagnostic role in investigating the causes of male infertility.

In our study, karyotype analysis was performed on 50 patients. Of these patients, 49 had a normal chromosomal karyotype.

As seen in Table 3, only one infertile man had a chromosomal anomaly, while no changes were observed in the karyotype analysis of the other patients. In this individual, the short arm of chromosome 8 was deleted at the 11th and 23rd segments, and the short arm of chromosome 10 was deleted at the 13th segment (Table 3).

Table 3

Biochemical indicators of the patient with chromosomal karyotype changes

Chromosomal karyotype	Indicators					
	FSH, mIU/ml	LH, mIU/ml	TH, nmol/ml	PRL, mIU/l	Fructose, mg/dl	Nitric oxide mmol/l
46,XY, t(8;10) (p11.23;p13)	25.6	7.75	18.8	507.0	418.9	320.0
Control, n=20, (46.XY), M	3.2	3.3	12.4	225.1	241.1	65.8

The patient was included in the asthenozoospermia group, meaning that the sperm count was 68 million, with 14% of spermatozoa being progressively motile. The proportion of spermatozoa with head abnormalities was 69%, with 5% having neck abnormalities, and 23% having mixed pathology. The proportion of spermatozoa with normal morphological structure was 3%. In this patient, more severe hormonal changes were observed compared to the other patients. Thus, the secretion of FSH and LH in the patient's blood serum was higher compared to fertile men, and hyperprolactinemia was also present. The levels of fructose and nitric oxide were significantly elevated.

Thus, the research has revealed that among the causes leading to idiopathic male infertility, chromosomal karyotype abnormalities rank among the last.

EVALUATION OF HORMONAL IMBALANCE IN MEN WITH IDIOPATHIC MALE INFERTILITY

Investigation of gonadotropin hormone concentrations in men with idiopathic male infertility

In men, reproductive functions are primarily regulated by the complex endocrine system known as the hypothalamic-pituitary-gonadal (HPG) axis. The HPG axis consists of three endocrine organs: the hypothalamus, the anterior pituitary, and the testes, which secrete peptides, proteins, and steroid hormones. As previously mentioned, gonadotropin hormones (FSH and LH) play a major role in male infertility. FSH and LH are gonadotropins that have a crucial role in the processes of spermatogenesis and steroidogenesis. These hormones serve as functional markers of spermatogenesis and Sertoli cell activity. FSH is essential for the development of normal and functionally active Sertoli cells. LH stimulates the synthesis of androgens, especially testosterone, which are important in the development of sperm cells. FSH accelerates the maturation of spermatogonia by acting on Sertoli cells, while LH stimulates the synthesis and secretion of testosterone in Leydig cells.

In our study, a comparative analysis of the concentrations of relevant hormones (FSH, LH, testosterone, and prolactin) was conducted to explore the etiology of idiopathic male infertility. The findings revealed that the concentration of FSH in blood serum increased compared to the control group by 1.6 times (57.7%) in men with asthenozoospermia, 2.4 times in men with oligozoospermia ($p_{HI}<0.001$), 8.8 times in men with non-obstructive azoospermia ($p_{HII}<0.001$), and 2.1 times in men with obstructive azoospermia ($p_K<0.001$).

The concentration of LH did not change significantly in men with asthenozoospermia compared to the control group. However, it

increased by 1.4 times (39.3%) in men with oligozoospermia, by 2.9 times in men with non-obstructive azoospermia ($p_{H1}<0.001$), and by 1.3 times (32.1%) in men with obstructive azoospermia compared to the control group.

As the results show, more severe endocrinological disorders were recorded in men with azoospermia. In particular, the concentrations of FSH and LH in men with non-obstructive azoospermia were, respectively, 3.7 times ($p_{H2}<0.001$) and 2.1 times ($p_{H2}<0.001$) higher compared to men with oligozoospermia, and 4.2 times ($p_{H3}<0.001$) and 2.2 times ($p_{H3}=0.033$) higher compared to those with obstructive azoospermia. The highest level of LH concentration was observed in the non-obstructive azoospermia group ($p_K <0.001$).

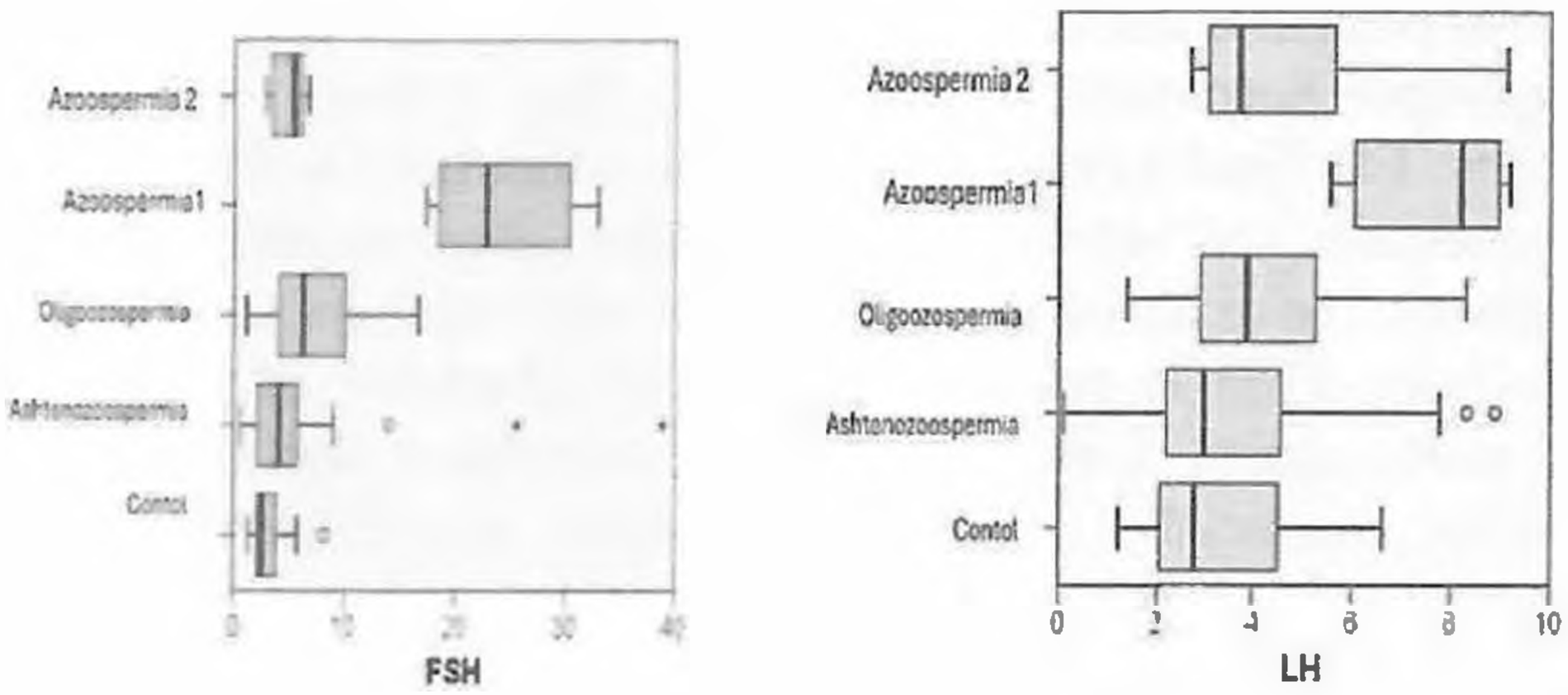


Figure 1. Changes in FSH and LH concentrations across different groups of male infertility (compared to the control group)

As seen, no significant differences in FSH and LH concentrations were observed in patients with obstructive azoospermia compared to men with oligozoospermia. Based on the obtained results, it can be noted that elevated levels of FSH and LH in the azoospermia group may be used in the differential diagnosis of the non-obstructive form of azoospermia. An increase in FSH and LH concentrations, along with a decrease in testosterone levels, may be considered key

indicators of testicular failure in cases of non-obstructive azoospermia. However, it should be taken into account that in some cases of obstructive azoospermia, hypospermatogenesis and elevated FSH levels may also occur. The results of our study are consistent with existing scientific evidence in this field (Figure 1).

Thus, it was established that the concentrations of FSH and LH in men with non-obstructive azoospermia were significantly higher compared to the corresponding values in men with asthenozoospermia, oligozoospermia, and obstructive azoospermia.

As previously mentioned, the normal course of spermatogenesis depends not only on gonadotropin hormones but also on the levels of sex hormones. One of the most important indicators of male infertility is a significant decrease in testosterone levels, both in the blood and in the seminal vesicles.

In our study, while the concentration of testosterone in the blood serum of men in the asthenozoospermia group did not change significantly compared to the control group, it was found to be 17.0% higher in men with oligozoospermia. In contrast, a decrease in testosterone levels was observed in the azoospermia group compared to the control group—22.9% in men with non-obstructive azoospermia and 10.3% in those with obstructive azoospermia. The changes in testosterone concentrations across all groups were not statistically significant. As the results show, the testosterone levels in both azoospermia groups decreased compared to men with asthenozoospermia and oligozoospermia. The increase in testosterone concentration in men with oligozoospermia leads to a weakening of gonadotropin secretion through a feedback mechanism, which in turn explains the decrease in sperm count in these patients.

The decrease or maintenance of normal testosterone levels in the context of elevated FSH and LH concentrations during azoospermia proves the disruption of the spermatogenesis process in these patients.

One of the important endocrine factors in male infertility is prolactin. Recently, the significance of prolactin in the synthesis and secretion of sex hormones has been particularly emphasized.

In the conducted study, the concentration of prolactin in the asthenozoospermia group did not change significantly compared to the control group. However, in men with oligozoospermia, prolactin levels were 31.7% lower compared to the control group. In men with non-obstructive azoospermia, prolactin concentration in the blood serum increased by 89.0%, and in men with obstructive azoospermia, it increased by 43.8% compared to the control group. Literature sources also indicate that hyperprolactinemia is observed in approximately 11% of men with oligozoospermia. Under the influence of hyperprolactinemia, a decrease in testosterone levels leads to a disruption in the spermatogenesis process and a sharp reduction in spermatozoa count, which plays a significant role in the pathogenesis of azoospermia.

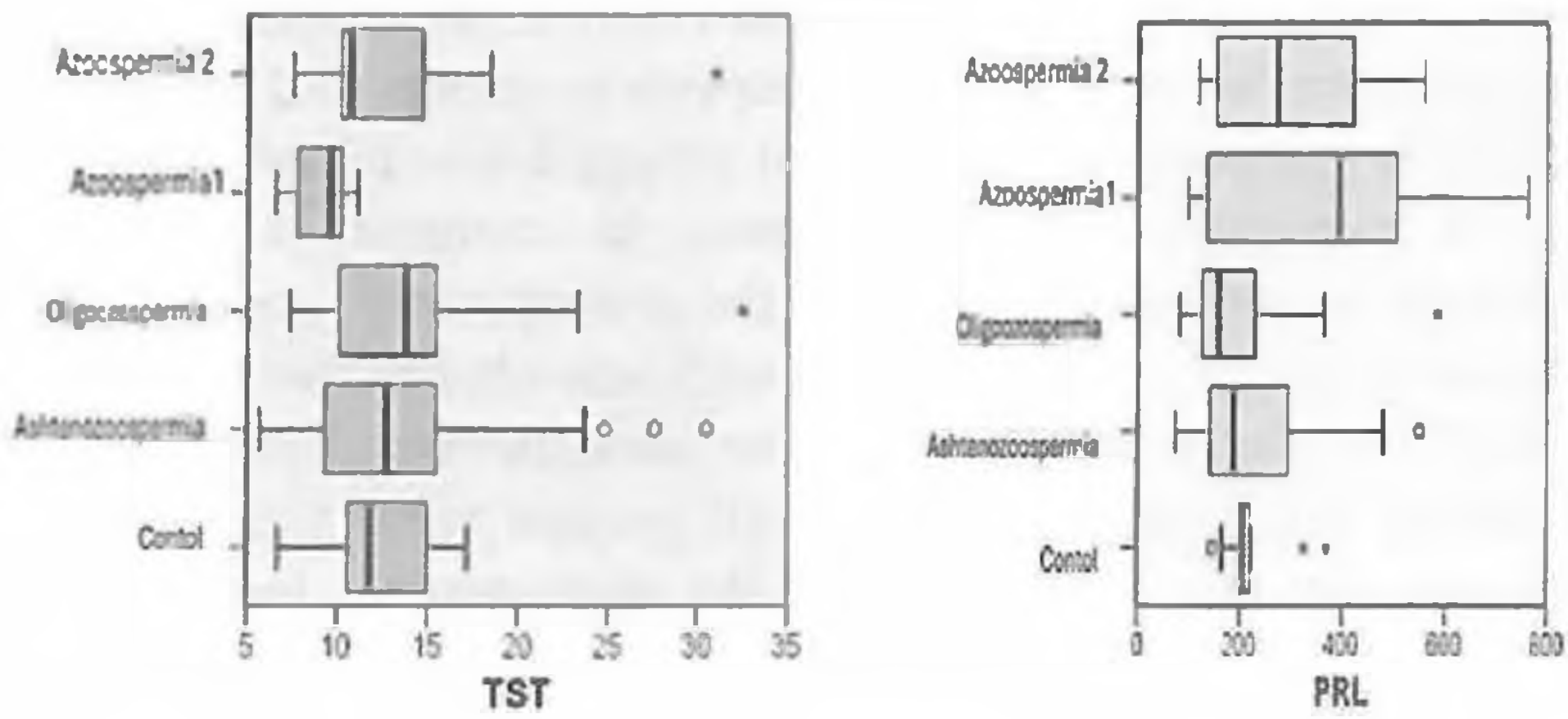


Figure 2. Concentration of testosterone (TST) and prolactin (PRL) in different groups of male infertility (compared to the control group)

The higher levels of prolactin in the azoospermia group compared to the asthenozoospermia and oligozoospermia groups prove the role of hyperprolactinemia in the disruption of the spermatogenesis process. It is likely that the decrease in prolactin levels in men with oligozoospermia leads to the disruption of protamination in the testes

— the process of spermatozoa maturation — and consequently to a reduction in spermatozoa count (Figure 2).

Thus, in both groups of azoospermic patients, prolactin concentration increased and testosterone concentration decreased compared to patients with asthenozoospermia and oligozoospermia.

The significance of nitric oxide and fructose in men with idiopathic infertility

A number of studies have highlighted the significant role of nitric oxide (NO) in the functional activity of spermatozoa. It is well known that many parameters of semen, including sperm count, motility, and morphological structure, are highly sensitive to the effects of free radicals. The influence of nitric oxide on spermatogenesis and the fertilization process is associated with a dual mechanism. Thus, it is considered both a cytotoxic agent and an essential factor in the normal functional activity of spermatozoa (when its concentration is below 1 μmol/L). Considering the significant role of nitric oxide in the functional activity of spermatozoa, the concentration of nitric oxide in the blood of men with idiopathic male infertility was analyzed. The concentration of nitric oxide was determined based on the levels of nitrate and nitrite. The study revealed that the concentration of nitric oxide in men with asthenozoospermia was 2.8 times higher (p_{H1}=0.007), and in men with oligozoospermia — 2.1 times higher compared to the control group. As can be seen, the concentration of nitric oxide in the blood of men with asthenozoospermia was 31.2% higher than the corresponding indicator in men with oligozoospermia (Table 4).

Thus, the increase in nitric oxide levels in cases of idiopathic male infertility is one of the key indicators of a significant rise in the proportion of morphologically abnormal, pathological, and less motile spermatozoa in the asthenozoospermia group.

Alongside nitric oxide, fructose has also been identified as playing an important role in the metabolism and activity of spermatozoa. Fructose is one of the key energy sources necessary for the functional activity of spermatozoa. Carbohydrates play a crucial role in sperm

metabolism. While glucose serves as an energy source throughout the entire functional activity of spermatozoa, fructose ensures their rapid and swift movement during the acrosomal reaction phase, facilitating contact with the oocyte.

Table 4

Changes in nitric oxide concentration in male infertility

Sperm parameters		Groups			P_K
		control (n=20)	asthenozoo-spermia, (n=56)	oligozoo-spermia (n=30)	
Nitric oxide, mmol/L (in blood serum)	N	16	55	13	0.020
	M	65.8	135.1	108.1	
	Median	51.6	144.3 p_{HI}=0.007	110.0	
	Q1	43.8	27.4	96.0	
	Q2	86.5	210.3	118.0	

In the study, the concentration of fructose in the seminal fluid of men with asthenozoospermia showed a slight increase of 19.3% compared to the control group. However, in men with oligozoospermia, it increased significantly by 60.8% (p_{H1}<0.001). In men with non-obstructive azoospermia, it doubled (p_{H1}=0.001) compared to the control. In contrast, in men with obstructive azoospermia, the fructose concentration tended to decrease by 23.1% compared to the control group.

In men with oligozoospermia, the fructose concentration was 34.8% higher (p_{H2}<0.001) compared to men with asthenozoospermia. In the non-obstructive azoospermia group, the fructose concentration was 2.5 times higher (p_{H3}=0.004) compared to men with obstructive azoospermia. This suggests that the spermatogenesis process is not impaired in obstructive azoospermia.

As the results show, it is possible to differentiate the groups of azoospermia based on the fructose concentration in the seminal fluid. Specifically, the fructose concentration is significantly higher in men with non-obstructive azoospermia compared to the obstructive azoospermia group (Table 5).

Table 5

Changes in fructose concentration in the ejaculate of male infertility patients

Sperm Parameters		Groups					P_K
		Control (n=20)	Asthenozoospermia (n=56)	Oligozoospermia (n=30)	Azoospermiya		
					Non-obstructive	Obstructive	
Fructose, mg/dL (in seminal fluid)	M	241.1	300.5	388.6	412.7	241.8	<0.001
	Median	238.8	284.8	384.0 p_{H1}<0.001 p_{H2}<0.001 p_{H4}<0.001	476.0 p_{H1}<0.001 p_{H2}=0.024 p_{H4}=0.004	194.0	
	Q1	232.1	244.2	356.0	408.0	45.1	
	Q2	245.5	350.5	406.0	496.0	429.0	

In the study, correlation relationships between clinical-morphological indicators of spermatozoa and hormonal disturbances, as well as certain biochemical markers (nitric oxide and fructose), were identified in male infertility groups. In the control group of practically healthy men, a negative correlation was found between the level of testosterone and the percentage of progressively motile spermatozoa (ρ=-0.460; p=0.041), which proves the significant role of testosterone in sperm motility. The results of the statistical correlation analysis showed that the progressive motility of spermatozoa with mixed pathology is significantly weakened (ρ=-0.454; p=0.044).

Correlation dependence between clinical-morphological indicators of spermatozoa and hormonal and biochemical parameters in idiopathic male infertility

In men with asthenozoospermia, a positive correlation (ρ=0.544; p<0.001) between FSH and LH levels was identified, indicating the

interdependence of the synthesis and secretion of both hormones. In this group, the high concentration of FSH leads to a decrease in the total sperm count (ρ=-0.388; p=0.003).

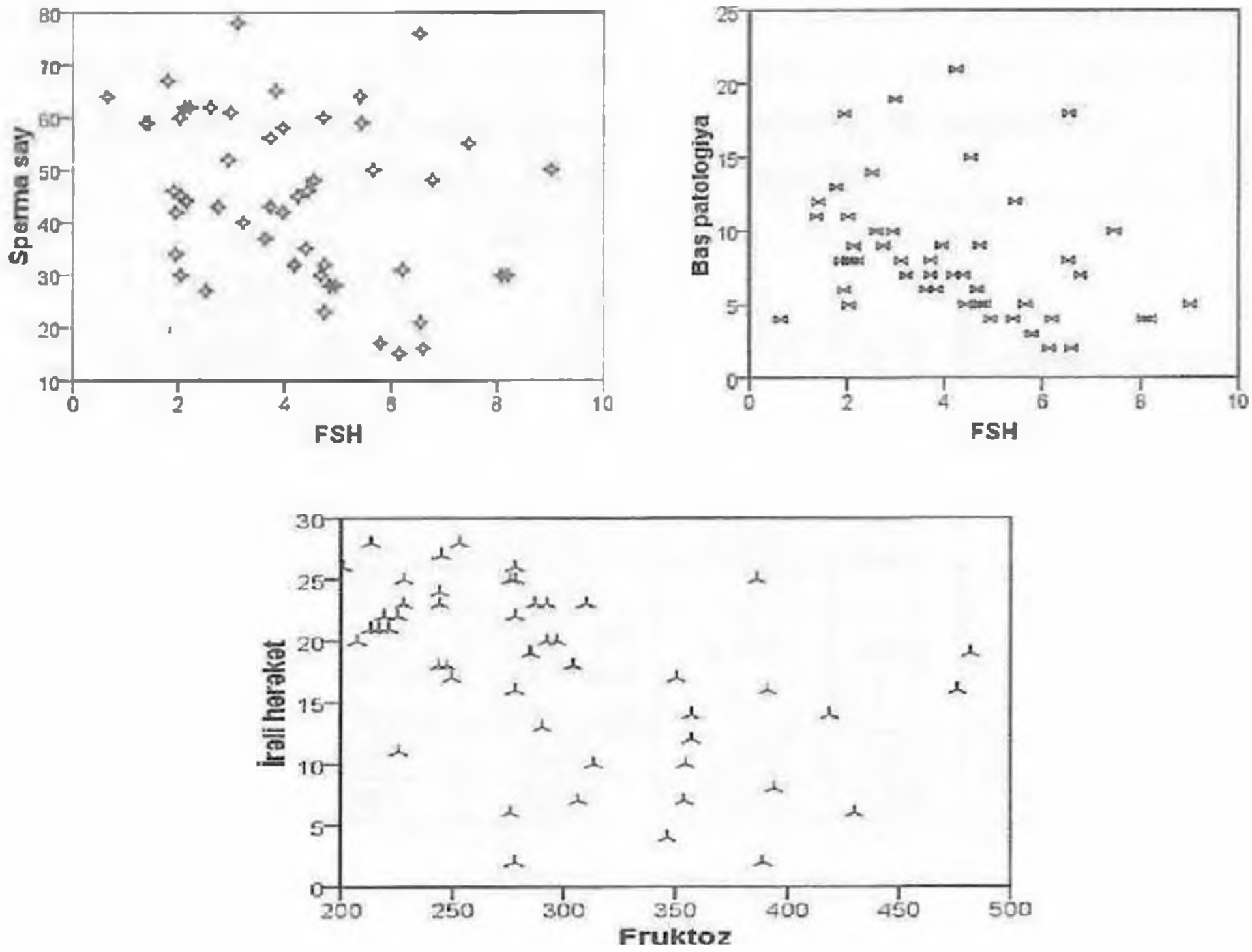


Figure 3. Correlation between FSH and total sperm count, as well as the ratio of spermatozoa with head morphology abnormalities, in the asthenozoospermia group; the correlation between fructose and the ratio of progressively motile spermatozoa

A positive correlation (ρ=0.604; p<0.001) was found between the concentrations of nitric oxide and fructose, while a negative correlation (ρ=-0.691; p<0.001) was observed with the progressive motility of spermatozoa.

In our study, a negative correlation between nitric oxide concentration and sperm motility in patients with asthenozoospermia

proves that high nitric oxide levels significantly affect sperm motility and viability. Thus, it can be concluded that an increase in nitric oxide may lead to a reduction in sperm motility and an increase in the concentration of fructose in semen. The increase in fructose concentration is primarily related to its decreased utilization. Specifically, fructose concentration is inversely proportional to the number of motile spermatozoa (ρ=-0.542; $p<0.001$), which indicates that only motile spermatozoa utilize fructose after ejaculation. In this regard, fructose concentration may be an important indicator in determining sperm motility (Figure 3).

In the oligozoospermia group, a positive correlation (ρ=0.544; p=0.002) between FSH and LH was also identified. The reduced secretion of testosterone led to a decrease in the total sperm count (ρ=0.511; p=0.004). The decrease in sperm count causes a reduction in the utilization of fructose, resulting in an increase in the concentration of fructose in the seminal fluid (ρ=-0.406; p=0.026). The direct correlation between prolactin levels and nitric oxide (ρ=0.699; p=0.008) suggests that an increase in prolactin synthesis and secretion may lead to higher nitric oxide levels in the blood, enhanced oxidative stress, and, consequently, structural and functional changes in spermatozoa. In patients with oligozoospermia, a direct correlation was found between fructose and nitric oxide (ρ=0.744; p=0.004), while an inverse correlation was observed with sperm count (ρ=-0.872; $p<0.001$). As mentioned earlier, the increase in nitric oxide concentration leads to the formation of pathological spermatozoa, disruption of the spermatogenesis process, and, consequently, a reduction in the number of mature spermatozoa. In non-obstructive azoospermia, a direct correlation (ρ=0.990; p=0.037) between FSH and prolactin was identified, proving the stimulatory effect of prolactin on FSH synthesis and secretion in this group. In obstructive azoospermia, however, a direct correlation (ρ=0.714; p=0.047) between LH and testosterone was found, indicating that the high concentration of LH accelerates testosterone secretion in this group. In other words, no significant changes in hormonal balance are observed in obstructive azoospermia.

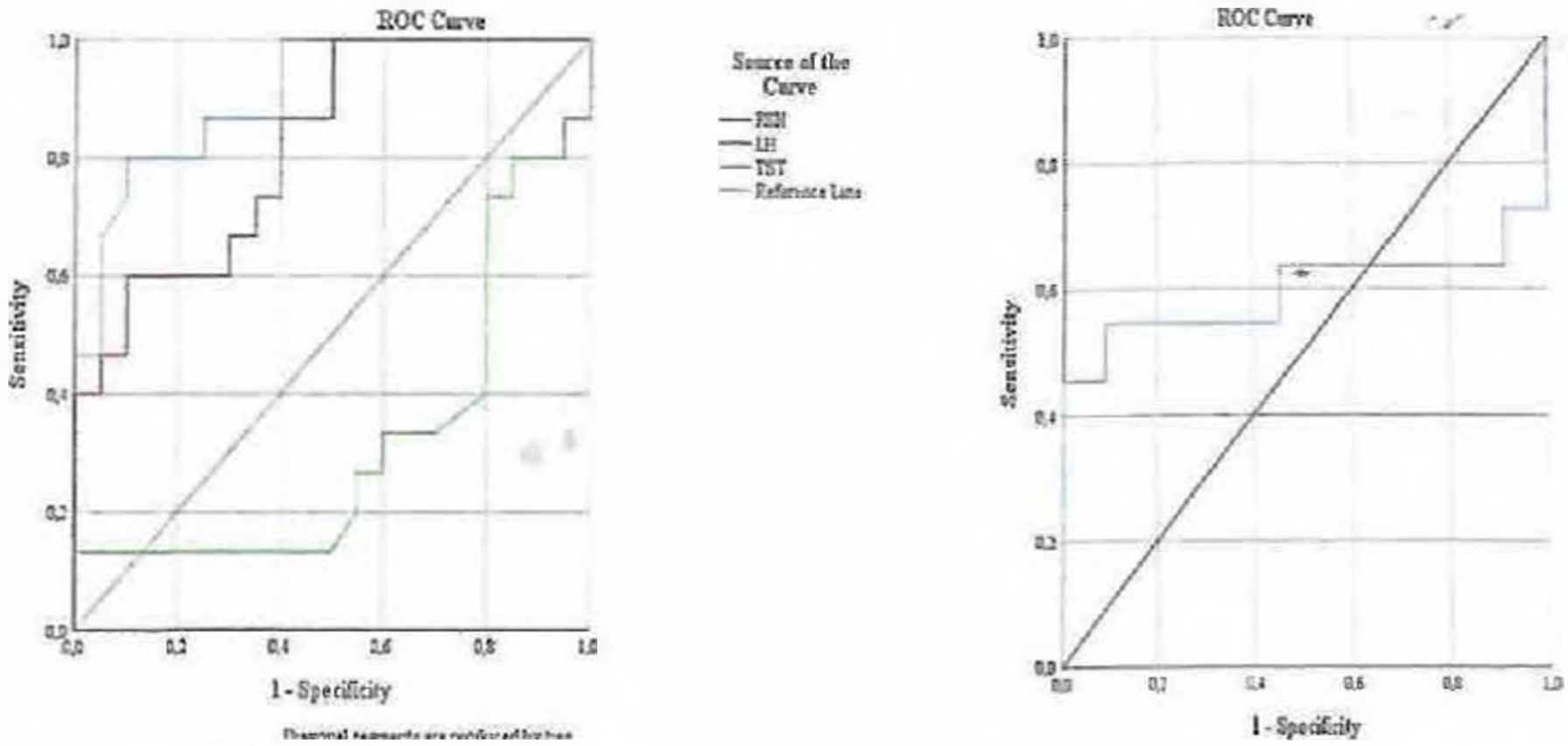
In the study, the informativeness, specificity, and sensitivity of the structural-morphological characteristics of spermatozoa, as well as hormonal and biochemical markers, in different groups of idiopathic male infertility were determined based on the results of ROC statistical analysis and the ANOVA test.

In the diagnosis of asthenozoospermia based on the ROC curves, the number of spermatozoa ($p<0.001$), the ratio of progressively motile spermatozoa ($p<0.001$), spermatozoa with mixed pathology ($p=0.002$), and spermatozoa with normal structure ($p<0.001$) are indicators with high specificity and sensitivity.

Moreover, according to the results of the ROC analysis, in the diagnosis of oligozoospermia, alongside the total number of spermatozoa ($p<0.001$), the percentage of progressively motile spermatozoa ($p<0.001$), head abnormalities ($p=0.001$), neck abnormalities ($p=0.011$), mixed pathology ($p<0.001$), and morphologically normal spermatozoa ($p<0.001$) can be considered indicators with high specificity and sensitivity.

As mentioned in previous sections, the disruption of hormonal balance plays a significant role in the pathogenesis of male infertility. According to the ROC curves and ANOVA test results, in asthenozoospermia, FSH (AUC=0.645; cut-off point >3.56 miU/ml; $p=0.055$) is diagnostically significant in terms of specificity and positive predictive value (87.2±5.4), but it does not have practical significance due to its low sensitivity and negative predictive value (40.5±8.1). In oligozoospermia, based on the results of ROC analysis, FSH (AUC=0.813; cut-off point >3.63 miU/ml; $p<0.001$) is an indicator with high specificity and sensitivity (Figure 4).

As shown in the results of the ANOVA test, FSH sensitivity and the negative predictive value (78.9±9.4) have significant diagnostic importance. In men diagnosed with oligozoospermia, although LH (cut-off point >2.66 miU/ml) does not have any practical significance in terms of specificity and the positive predictive value (71.4±7.6), it may be diagnostically significant in terms of sensitivity and the negative predictive value (66.7±12.1).



Area Under the Curve					
Changes in test results	Area (AUC)	Standard error	P-value	95% confidence interval	
				Lower bound	Upper bound
FSH	0.908	0.048	0.000	0.813	1.000
LH	0.820	0.070	0.001	0.683	0.957
TH	0.318	0.098	0.069	0.127	0.509
PRL	0.595	0.139	0.450	0.323	0.867

Figure 4. ROC curves of hormones in the azoospermia group

The practical significance of prolactin (cut-off point <189 miU/ml) has been evaluated as sufficient in terms of both specificity and the positive predictive value (90.5±6.4), as well as sensitivity and the negative predictive value (50.0±11.8).

According to the results of the ROC analysis and ANOVA test during azoospermia, FSH (AUC=0.908; cut-off point >4.95 miU/ml; p<0.001) and LH (AUC=0.820; cut-off point >5.26 miU/ml; p=0.001) are diagnostic indicators with high specificity and sensitivity.

Table 6

The informativeness of FSH, LH, and testosterone in the azoospermia group

NN	FSH	LH	TH
n	35	35	35
min	1.37	1.22	6.55
max	33	14.6	31
cut off point	>4.95	>5.26	<10.5
n+	15	15	15
++	12	9	9
Sn	80.0	60.0	60.0
±mp	10.3	12.6	12.6
n-	20	20	20
--	18	18	16
Sp	90.0	90.0	80.0
±mp	6.7	6.7	8.9
ODV	30	27	25
%	85.7	77.1	71.4
±mp	5.9	7.1	7.6
pPV	85.7	81.8	69.2
±mp	9.4	11.6	12.8
nPV	85.7	75.0	72.7
±mp	7.6	8.8	9.5
LR+	8.00	6.00	3.00
	good	good	sufficient
LR-	0.22	0.44	0.50
	sufficient	sufficient	sufficient

Note: Sn — sensitivity, pPV – positive predictive value, nPV – negative predictive value, Sp — specificity, LR+ — positive likelihood ratio, LR– — negative likelihood ratio

FSH (85.7±9.4) and LH (81.8±11.6) have significant diagnostic importance in terms of specificity and positive predictive value. The practical significance of testosterone has been evaluated as sufficient in terms of both specificity and the positive predictive value (69.2±12.8), as well as sensitivity and the negative predictive value (72.7±9.5). As the results show, in the diagnosis of both oligozoospermia and azoospermia, the diagnostic value of FSH has been higher compared to other hormones (Table 6).

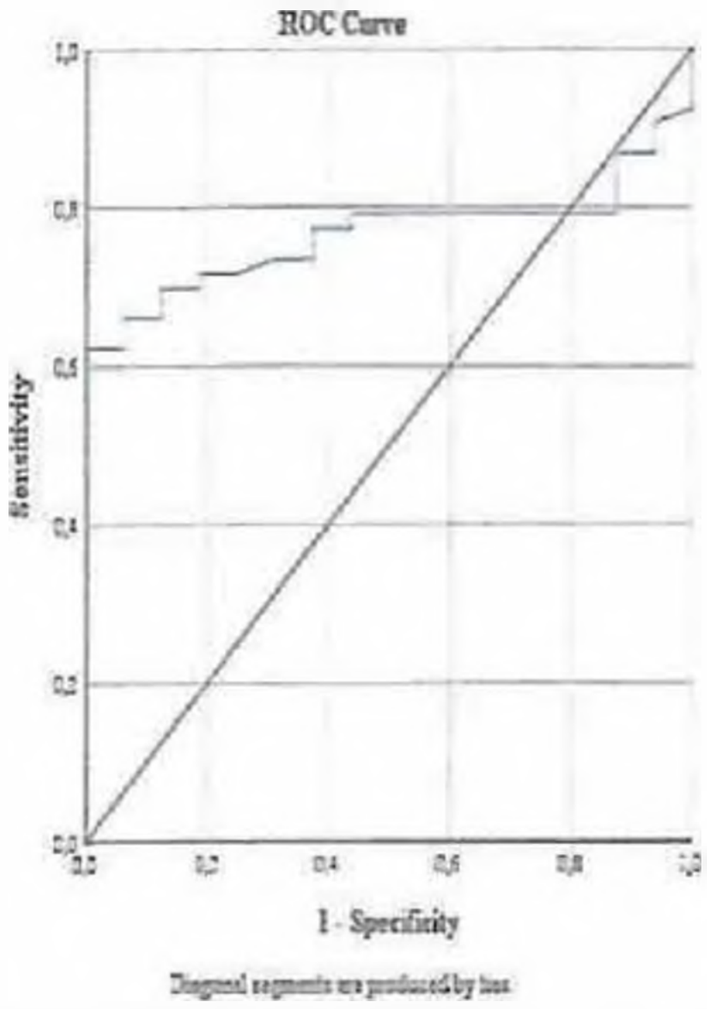
According to WHO recommendations, the analysis of fructose in semen plays an important role in the diagnosis of male infertility. In men with asthenozoospermia, the results of ROC analysis show that fructose (AUC=0.767; cut-off point >277.5 mg/dl; p=0.001) and nitric oxide (AUC=0.674; cut-off point >130.2 nmol/ml; p=0.035) are diagnostic indicators with high specificity and sensitivity. Fructose (100.0±0.0) and nitric oxide (100.0±0.0) have significant diagnostic value due to their specificity and positive predictive value. In this group, fructose has high diagnostic and practical significance due to its specificity, sensitivity, and both positive and negative predictive values (100.0±0.0). During oligozoospermia, the overall diagnostic values of fructose and nitric oxide are higher compared to asthenozoospermia. This indicates that a

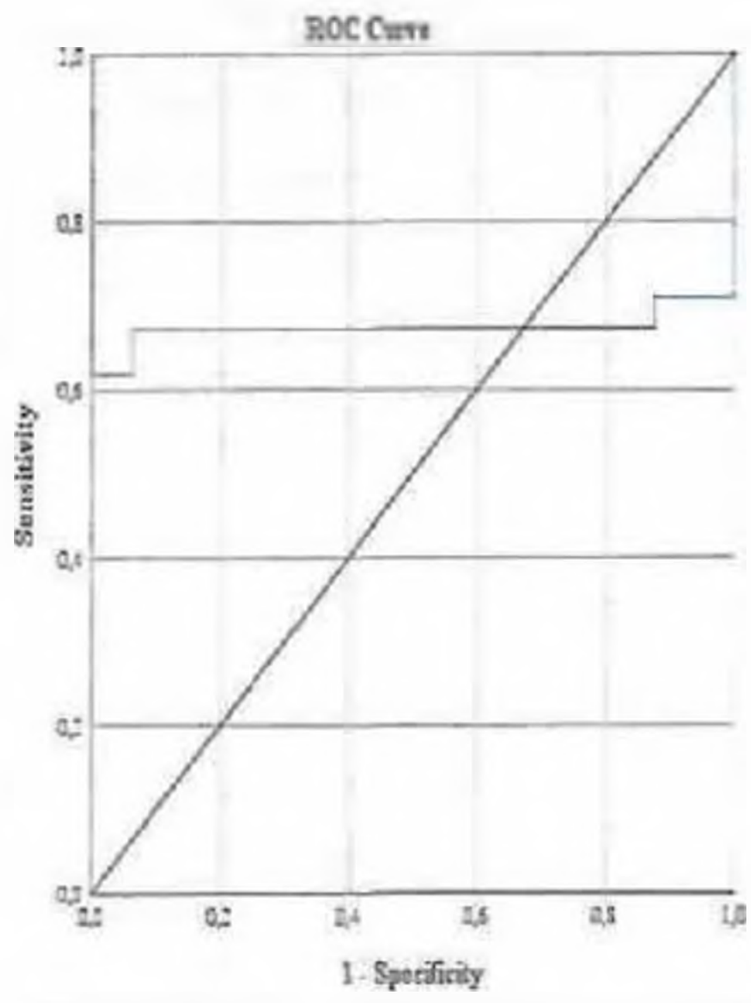
significant increase in fructose levels in seminal fluid and nitric oxide levels in blood serum primarily suggests the likelihood of oligozoospermia. In the oligozoospermia group, fructose demonstrates substantial diagnostic and practical value due to its specificity and positive predictive value (100.0±0.0), as well as its sensitivity and negative predictive value (100.0±0.0). In this case, nitric oxide shows practical significance only in terms of its sensitivity and negative predictive value (100.0±0.0) (Figure 5).

According to the results of the ROC analysis in the azoospermia group, fructose showed very low specificity and sensitivity (AUC=0.662; p=0.123; 95% CI: 0.425–0.900). In other words, fructose in seminal fluid cannot be used as a specific and sensitive indicator for the diagnosis of azoospermia.

The informative value of gonadotropic hormones and testosterone in male infertility is also supported by data from the literature.

It has been shown that there is an inverse relationship mechanism between the concentrations of FSH, LH, and testosterone and sperm motility. In other words, an increase in the levels of these hormones leads to a decrease in the number of motile spermatozoa. In addition, there is also an inverse correlation between high levels of LH and FSH and the proportion of spermatozoa with normal morphology. It is believed that LH is mainly indirectly associated with sperm motility, which confirms the role of LH in sperm maturation. The obtained results once again prove that FSH and LH are highly informative indicators in the diagnosis of male infertility.





Area Under the Curve				
Changes in test results	Area (AUC)	Standard error	P value	95% confidence interval
				lower bound
Nitric oxide	0.674	0.062	0.035	0.552
Fructose	0.767	0.055	0.001	0.659

Figure 5. ROC curves of nitric oxide and fructose in the asthenozoospermia group

According to the ROC curves, although FSH has high sensitivity and specificity in the diagnosis of asthenozoospermia, it can be considered of practical significance only due to its specificity. In the diagnosis of oligozoospermia, FSH has significant practical importance due to its high sensitivity and ODV. Prolactin may be considered practically significant for the diagnosis of oligozoospermia due to its specificity, while LH may be considered significant due to its sensitivity. It should be noted that LH does not have practical significance as a specific marker in the diagnosis of oligozoospermia. In men with azoospermia, FSH has been evaluated as a marker with high specificity and sensitivity, while LH is considered a marker with high specificity. Thus, in the diagnosis of non-obstructive azoospermia, FSH and LH have practical significance as markers with high specificity. Moreover, a decrease in testosterone concentration in these patients may also be considered diagnostically significant.

It is known that while non-obstructive azoospermia is caused by disruption of the spermatogenesis process, no such disturbances are observed in obstructive azoospermia, which is characterized by obstruction of the efferent ducts. From this perspective, endocrine pathologies play a significant role in the etiology of non-obstructive azoospermia and lead to impairment of the spermatogenesis process.

The obtained results have demonstrated that hyperprolactinemia also plays an important role in the etiology of male infertility, particularly azoospermia. Prolactin is secreted by the pituitary gland and, through a negative feedback mechanism in the hypothalamus, suppresses the pulsatile secretion of GnRH, thereby reducing the synthesis of FSH, LH, and testosterone. This may be one of the main causes of impaired spermatogenesis, decreased sperm motility, and the formation of spermatozoa with abnormal morphology. According to the results of ROC analysis, a decrease in prolactin levels may be one of the predictors of oligozoospermia.

An increase in the concentration of nitric oxide in the blood leads to the disruption of spermatozoa integrity and a reduction in their motility. As sperm activity decreases, the consumption of fructose in the seminal fluid also declines. Consequently, the concentration of

fructose in the seminal fluid increases. The results of the study further confirmed that fructose is the main energy substrate for spermatozoa. and there is an inverse relationship between its concentration and sperm activity. The primary reason for this dependence is that active spermatozoa consume more energy. Therefore. fructose can be considered a key indicator of the functional activity of the seminal vesicles and plays an important role in fertility issues. It is recommended to determine the levels of fructose and nitric oxide in infertile men with any abnormalities in their spermogram.

Thus. changes in the synthesis and secretion of hormones of the hypothalamic-pituitary-gonadal axis—namely FSH. LH. and testosterone—are among the key indicators in the assessment of male infertility. In men with normozoospermia. there is no need to analyze these hormones. Evaluating biochemical markers such as nitric oxide and fructose levels for identifying the biological characteristics of semen may help reveal new and more accurate criteria for diagnosing male infertility.

Among the causes of idiopathic male infertility. hormonal changes in the hypothalamic-pituitary-gonadal system and nitrosylation reactions involving nitric oxide play a significant role.

CONCLUSIONS

1. The analysis of the ejaculate revealed that in men with asthenozoospermia and oligozoospermia. the number of progressive spermatozoa decreased by 1.8 times and 3.7 times. respectively; the ratio of spermatozoa with normal morphology decreased by 1.7 times and 2.5 times compared to fertile men. while the ratio of spermatozoa with mixed pathology increased by 1.3 times and 1.6 times. respectively [6]. [17].
2. In the groups of asthenozoospermia and oligozoospermia. the concentration of FSH increased by 1.6 times and 2.4 times. respectively. while the concentrations of LH. testosterone. and prolactin did not change significantly. In the non-obstructive azoospermia group. the concentrations of FSH. LH. and prolactin

increased by 8.8 times. 2.9 times, and 1.9 times. respectively, while the concentration of testosterone did not change significantly. In the obstructive azoospermia group. the concentration of FSH increased by 2.1 times. while the concentrations of LH. prolactin. and testosterone did not change significantly. According to the obtained results. FSH. LH. and prolactin should be used as differential diagnostic criteria in the identification of non-obstructive azoospermia [6]. [7]. [12].

3. In men with asthenozoospermia. the concentration of nitric oxide increased by 2.8 times. while fructose did not change significantly. In men with oligozoospermia, the concentrations of nitric oxide and fructose increased by 2.1 times and 1.6 times. respectively. In men with non-obstructive azoospermia. the concentration of fructose increased by 2.0 times compared to fertile men. but in men with asthenozoospermia and obstructive azoospermia. there was no significant change [3]. [5]. [8]. [10].
4. In the etiology of idiopathic male infertility. chromosomal karyotype abnormalities are not significant and rank among the last causes.
5. In men with asthenozoospermia (ρ=0.544; $p<0.001$) and oligozoospermia (ρ=0.544; p=0.002), there was a direct correlation between FSH and LH; a direct correlation between fructose and nitric oxide (ρ=0.604; $p<0.001$; ρ=0.744; p=0.004). In men with oligozoospermia. there was an inverse correlation between the number of spermatozoa and fructose concentration (ρ=-0.406; p=0.026) and between nitric oxide and spermatozoa count (ρ=-0.872; $p<0.001$). In non-obstructive azoospermia. a direct correlation was found between FSH and prolactin (ρ=0.900; p=0.037). while in obstructive azoospermia. there was a direct correlation between LH and testosterone (ρ=0.714; p=0.047) [10].
6. It is possible to monitor ejaculate quality based on the biochemical and hormonal indicators of blood and ejaculate. According to the ROC curves. in the diagnosis of asthenozoospermia. FSH has high specificity; in the diagnosis of oligozoospermia. FSH is of significant practical importance due to its high sensitivity.

prolactin due to its specificity, and LH due to its sensitivity. In men with azoospermia, FSH has been evaluated as a practically significant marker with high specificity and sensitivity, while LH has high specificity and practical significance [16], [18].

PRACTICAL RECOMMENDATIONS

1. In the diagnosis of idiopathic male infertility, in addition to the sperm count in the ejaculate, the study of their morphological structure and motility has significant clinical importance.
2. In patients with idiopathic male infertility, the differential diagnosis of various pathological forms of infertility should be conducted based on the concentrations of FSH, LH, prolactin, and testosterone hormones in the blood.
3. The analysis of fructose concentration in the ejaculate and nitric oxide in the blood is used as biochemical predictors of sperm count and motility.
4. In the diagnosis of oligozoospermia, FSH and fructose; in the diagnosis of asthenozoospermia, the ratio of progressively motile and morphologically normal spermatozoa; and in the diagnosis of azoospermia, FSH and LH are considered indicators having high specificity and informativeness.

List of published scientific works related to the dissertation topic

1. Asgarova, T.A., Nazarova. G.E. Endocrinological factors and the role of nitric oxide in idiopathic male infertility [T.A. Asgarova. G.E. Nazarova] // Metabolism Journal, – Baku: – 2021. No. 4, – pp. 26–31.
2. Nazarova, G.E. Determination of FSH, LH and prolactin hormone levels in the blood serum of individuals with oligozoospermia and azoospermia // Proceedings of the XXIV Republican Scientific Conference of Doctoral Students and Young Researchers, – Baku: – 2021, – p. 34–36.
3. Nazarova, G.E. The role of fructose – the main energy substrate in male infertility [G.E. Nazarova] // Medical News. – 2022. no. 7(334), – p. 62–63.
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LIST OF ABBREVIATIONS

RNS	– reactive nitrogen species
LB	– lower bound at 95% confidence interval
NO	– nitric oxide
HCG	– human chorionic gonadotropin
ROS	– reactive oxygen species
FSH	– follicle-stimulating hormone
LH	– luteinizing hormone
GnRH	– gonadotropin-releasing hormone
NOS	– nitric oxide synthase
M	– mean value
ME	– median value
±m	– standard error
min	– minimum
max	– maximum
ROC	– receiver operating characteristic
TH	– testosterone
ODV	– overall diagnostic value
US	– ultrasound examination
WHO	– World Health Organization
σ	– standard deviation
UB	– upper bound at 95% confidence interval

The defense of the dissertation will be held on "21" may 2025 at 14⁰⁰ at the meeting of the Dissertation Council FD 2.07 operating under Azerbaijan Medical University.

Address: AZ 1022, Baku city 14 A. Gasimzade Street, (conference hall).

The dissertation is available for review at the library of Azerbaijan Medical University.

The electronic versions of the dissertation and the abstract have been posted on the official website of the Azerbaijan Medical University (www.amu.edu.az).

The abstract was sent to the required addresses on "21" aprel 2025.

Signed for print: 15.04.2025
Paper format: 60×841/16
Volume: 37195 characters
Number of hard copies: 20 copies
"Tabib" publishing house