



## The importance of biochemical indicators in determining male infertility

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### ABSTRACT

**Introduction and aim.** Recently, infertility has become a global problem and the frequency of the “male” factor in family infertility has reached 40–50%. The aim of the research is to investigate the role of some biochemical indicators (endocrine factors and fructose) in determining male infertility.

**Material and methods.** In the study, the spermogram of 101 men aged 20–46 with idiopathic male infertility, the concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and testosterone hormones in their blood, and the concentration of fructose in their sperm samples were analyzed, and their correlations were determined.

**Results.** The concentration of FSH in the blood serum of men with asthenozoospermia and oligozoospermia increased statistically significantly by 57.7% and 2.4 times, respectively, compared to the control. More serious endocrinological disorders were recorded in men with azoospermia. In men with non-obstructive azoospermia, the concentration of FSH is 8.8 times, that of LH is 2.9 times; while prolactin increased by 89.0% compared to the control, testosterone concentration decreased by 22.9%. The fructose concentration in the oligozoospermia group compared to the control group increased by 60.8% ( $p_{H1} < 0.001$ ), and in the non-obstructive azoospermia group by 2.0 times ( $p_{H1} = 0.001$ ). A positive correlation between FSH and LH and a negative correlation between fructose concentration and forward motility of spermatozoa were determined in both asthenospermic and oligozoospermic patients ( $\rho = 0.544$ ;  $p = 0.002$ ). In case of non-obstructive azoospermia, FSH and prolactin, in azoospermia, LH and testosterone were directly proportional.

**Conclusion.** During male infertility, there is a serious relationship between sperm indicators and endocrine disorders. An increase in the concentration of fructose is the main indicator of a decrease in the number and motility of spermatozoa. A high concentration of FSH and LH in men with azoospermia can be considered one of the important indicators in the diagnosis of non-obstructive azoospermia.

**Keywords.** FSH, LH, male infertility, prolactin and fructose

### Introduction

Currently, 14–30% of married couples suffer from infertility, and the share of male infertility in this problem is about 50%. This is explained by the increase in the harmful effects of environmental, industrial and household factors on the human body.<sup>1,2</sup> Infertility in men is multifactorial, including various endocrine diseases, damage to the testicles for any reason, sexually transmitted infections, varicocele, harmful habits, obstruction of the

genitals and their growth, obesity, environmental factors, chronic stress, sleep disorders, intense office work, physical load, deficiency, vitamin deficiency, etc.<sup>3</sup> Under the influence of these factors, the quantitative and qualitative indicators of the spermogram deteriorate, as the number of spermatozoa decreases, their mobility weakens, the number of spermatozoa with fragmented DNA and chromatin disorders increases, and pathological spermatozoa with anomalous structure are formed. Structural-func-

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tional pathology of spermatozoa has a weak ability to fertilize, resulting in infertility in men.<sup>4,5</sup> Currently, for the purpose of laboratory diagnosis of male infertility, indicators characterizing the general fertilizing ability of sperm – concentration of spermatozoa, motility, proportion of spermatozoa with normal morphology – are studied.<sup>6–8</sup> Current diagnostic methods for determining male infertility cannot determine its causes in 44–70% of cases. At this time, the treating doctor diagnoses idiopathic male infertility and cannot determine the causes of pathospermia.<sup>6,9</sup> Although the spermogram is a simple method for examining spermatozoa, it does not provide complete information about the disorders of the spermatogenesis process.<sup>10,11</sup> In most cases, pathological changes in sperm are non-specific, they cannot accurately determine the type of infertility, they only show any changes in indicators. In this regard, the parallel study of sperm and blood plasma metabolites in the development of male infertility can create greater diagnostic possibilities. In recent years, in the diagnosis of male infertility, the study of hormonal balance, determination of the level of fructose in sperm plasma has been studied more widely.

We believe that determining the level of new effective biochemical markers along with spermogram indicators can provide more accurate information in determining the type of male infertility.

Changes in the endocrine system also play a major role in the disruption of the spermatogenesis process. One of the main causes of male infertility is endocrine disorders.<sup>12,13</sup> Hormones regulate male reproductive functions based on the principle of well-integrated complex interactions at different developmental stages. In men, sexual development and hormonal functions depend on the functional activity of a complex chain involving the hypothalamus-pituitary-gonadal (HPG) and the central nervous system. The hypothalamic-pituitary-gonadal system consists of three endocrine organs: the hypothalamus, the anterior lobe of the pituitary gland, and the testes, which secrete peptide, protein, and steroid hormones.

Gonadotropin-releasing hormone (GnRH) synthesized in the hypothalamus regulates the secretion of gonadotropins, follicle-stimulating hormone (FSH), luteinizing hormone (LH) in the anterior lobe of the pituitary gland. FSH accelerates the maturation of spermatogonia by acting on Sertoli cells. In Leydig cells, LH ensures the synthesis and release of testosterone.<sup>14–16</sup> In the presence of testosterone, FSH stimulates Sertoli cells and induces spermatogenesis.<sup>17</sup> Although many hormones play a role in the formation of sperm, testosterone is considered the most important factor in the process of spermatogenesis.<sup>18</sup>

Seminal fluid consists of complex organic and inorganic compounds, and although these substances are not of decisive importance in the fertilization process,

they play an important role in the mobility, functional activity and transportation of spermatozoa in the female reproductive organs. Among these substances, fructose is an aerobic and anaerobic energy source for sperm motility, as fructose undergoes metabolism and breaks down into lactate and pyruvate. Metabolism of fructose in spermatozoa takes place through the classical Embden-Meyer glycolysis pathway. Phosphates of hexoses, triose phosphates and pyruvic acid are intermediates in the formation of lactic acid. Lactate is the main carbohydrate source in spermatozoa, and lactic acid is broken down to carbon dioxide and water in the Krebs cycle in the presence of oxygen. Due to the generated energy, the mobility of spermatozoa is ensured. In the presence of glucose, fructose participates in the disintegration of the outer acrosomal membrane - the formation of the acrosomal reaction.<sup>1,19</sup> Fructose also plays an important role in sperm viscosity and hyaluronidase activation. Determination of fructose in the seminal fluid can allow to determine the state of the seminal vesicles, endocrine anomalies, and obstruction of the seminal ducts. This vital biochemical component not only increases the functional activity of the prostate, but also plays an important role in sperm coagulation and dilution.<sup>1,20</sup>

## Aim

The aim was to investigate the role of some biochemical indicators (endocrine factors and fructose) in determining male infertility.

## Material and methods

The study plan was implemented in accordance with the Helsinki Final Act and the protocol was approved by the Ethics Committee Azerbaijan Medical University ICE Committee on Medical Sciences (№8. 28.06.2020).

In the study were analyzed blood and sperm samples of 101 men aged 20–46 years ( $31.6 \pm 0.5$  years) with idiopathic male infertility, who had not had children for more than a year and the female cause of infertility was excluded, as well as the presence of antisperm antibodies in the couple. All subjects gave informed consent for inclusion before they took part in the study. The study was conducted in accordance with the Declaration of Helsinki. The study excluded patients with a burdened drug history (androgens or anti-estrogens), chromosomal translocations, hypogonadotropic hypogonadism, as well as other endocrine diseases leading to decreased testosterone secretion: hypothyroidism, thyrotoxicosis (determined based on the levels of thyroid-stimulating hormone, free thyroxine T4), decompensation of diabetes mellitus, hypercortisolism, as well as renal or liver failure, inflammatory and infectious diseases of the urogenital tract in the acute stage, with pituitary adenoma. We studied men who had not had children for more than a year and the reasons were only

the male factor. a female cause of infertility was excluded, as was the presence of antisperm antibodies in the couple 20 fertile men aged 23–40 years ( $31.1 \pm 1.1$  years) were included in the control group. The control group was selected among men who had children (fertile) and were practically healthy. After 3–5 days of abstinence from sexual intercourse, sperm samples were collected from each male patient by masturbation, and the samples were poured into dry, clean disposable sterile containers and analyzed morphologically after 30 minutes. Phase-contrast microscopy and special dyes were used to evaluate the structure and functions of spermatozoa. 4 days before the spermogram, alcohol consumption, including beer, as well as hot procedures – going to the bath, sauna, hot bath, unfavorable working conditions are prohibited. Motility was assessed as the proportion of sperm that gradually became motile at  $37^\circ\text{C}$  using a Makler chamber. Men were considered azoospermic if no sperm were detected or if sperm were detected only after centrifugation of the semen sample. Men with azoospermia were not included in these analyses.

The number and quality of spermatozoa in the semen was analyzed. The patients included in the study were divided into 3 groups based on the criteria established by the World Health Organization in 2010 according to the number and activity of their spermatozoa: asthenozoospermia (patients with a normal number of spermatozoa and low forward motility) – 56 people; oligozoospermia (number of spermatozoa in 1 ml of sperm  $<15$  million) – 30 people; azoospermia (absence of spermatozoa in ejaculate) – 15 people.<sup>7</sup> Patients in the azoospermia group were divided into two groups: obstructive ( $n=7$ ) and non-obstructive azoospermia ( $n=8$ ). The morphological structure of spermatozoa was evaluated based on their appearance and compared with Kruger's criteria. Spermatozoa were classified into one of four morphological groups: normal, head pathology, neck pathology, mixed pathology. Thus, spermatozoa with head, neck and tail pathology can be found in the spermogram. In some cases, several pathologies are detected in anomalous cells at the same time (mixed pathology). When the ratio of such spermatozoa is higher than 96%, the sperm loses its ability to fertilize and teratozoospermia occurs. This is caused by chromosomal pathologies, enzyme diseases, viral infections, etc. can be. Asthenozoospermia is diagnosed when the concentration of moving spermatozoa is below 32%. The proportion of morphologically normal spermatozoa that fully meet the Kruger criteria should not be less than 4%.<sup>20,21</sup>

The concentration of fructose was determined by centrifuging the sperm sample at a speed of 3000 rpm for 10 minutes. The concentration of fructose in sperm fluid was determined by the colorimetric method with the help of B.I.R.D. Diagnostics (Baharafshan institute of Research and development) "semen fructose" reagent kit.

In order to examine the hormonal status, the concentration of FSH, LH, testosterone and prolactin in the blood serum of men was measured using the electrochemiluminescence immunoassay technique with the help of Roche e411 autoanalyzer. The statistical analysis of the obtained results was carried out using the Excel-2017 software package based on Mann-Whitney and Kruskal-Wallis non-parametric criteria. Statistical analysis of the obtained results was carried out using the SPSS-26 software package (IBM, Armonk, NY, USA) based on the t-Student-Bonferroni and H-Kruskal-Wallis tests. Differences between groups were considered statistically significant when  $p < 0.05$ . Correlation dependence between indicators was determined based on Spearman's statistical criterion. To identify relationships between indicators, a  $\rho$ (Rho)-Spearman correlation analysis was carried out. The statistical significance of the  $\rho$ -correlation coefficient was assessed using a two-sided test.

## Results

According to the results of sperm analysis, the number of spermatozoa decreased by 41,1% ( $p_H < 0.001$ ) in the asthenozoospermia group, and by 7.9 times ( $p_{HI} < 0.001$ ) in the oligozoospermia group compared to the control group. The number of moving spermatozoa decreased by 73,7% ( $p_H < 0.001$ ) in the oligozoospermia group, and by 3.7 times ( $p_H < 0.001$ ) in the asthenozoospermia group compared to the control. As can be seen, the total number of spermatozoa in men with oligozoospermia compared to men with asthenozoospermia decreased by 5.6 times ( $p_{H2} < 0.001$ ), and the number of moving spermatozoa decreased by 2.1 times ( $p_{H2} < 0.001$ ). According to the calculations, a statistically significant difference in the number of moving spermatozoa between the groups was determined ( $p_K < 0.001$ ).

Male infertility can be caused not only by the number of spermatozoa, but also by changes in their morphological structure. In our research work, the number ratio of spermatozoa with head and neck pathology was determined. According to the results of microscopic analysis, there was no statistically significant change in the number of spermatozoa with neck and head pathology in men with asthenozoospermia compared to the control group. In men with oligozoospermia, the ratio of spermatozoa with neck and head pathology was significantly reduced by 10.3% ( $p_{HI} = 0.01$ ) and 41,7% ( $p_{HI} = 0.003$ ), respectively, compared to the control group. A statistically significant difference was determined between the groups in terms of the proportion of spermatozoa with neck ( $p_K = 0.031$ ) and head ( $p_K = 0.005$ ) pathology.

According to the results of microscopic analysis, spermatozoa with mixed pathology prevailed in men with infertility. In asthenozoospermic men, the percentage of spermatozoa with mixed pathology increased by 29.3% ( $p_{HI} = 0.014$ ), and in oligozoospermic men by 61%

( $p_{H1} < 0.001$ ), compared to the control group, statistically significantly increased. The percentage of spermatozoa with mixed pathology in men with oligozoospermia was 24.5% ( $p_{H2} = 0.001$ ) higher than in men with asthenozoospermia. A statistically significant difference in the number of spermatozoa with mixed pathology was determined between the groups ( $p_K < 0.001$ ).

**Table 1.** Indicators of sperm during idiopathic male infertility\*

Sperm index	Groups			p		
	Kontrol (n=20)	Asthenozoospermia (n=56)	Oligozoospermia (n=30)			
Number, (millions)	M	64.6	45.9	7.2	<0.001	
	Median			8		
		63.5	45	$p_{H1} < 0.001$		$p_{H2} < 0.001$
	Q1	61	30	4.5		
	Q2	74	60	10		
Forward movement, (million)	M	34.6	17.9	7.8	<0.001	
	Median			9		
		33	19	$p_{H1} < 0.001$		$p_{H2} < 0.001$
	Q1	32	14	2		
	Q2	35.5	23	11		
Neck pathology, %	M	63.3	60	55.2	0.031	
	Median			58		
		64	61	$p_{H1} = 0.01$		
	Q1	61	55	48		
	Q2	68.5	68	62		
Head pathology, %	M	9.9	8.1	5.3	0.005	
	Median			6		
		8.5	7.0	$p_{H1} = 0.003$		$p_{H2} = 0.041$
	Q1	7	5	3		
	Q2	12	10	7		
Mixed pathology, %	M	21.2	28.2	37.3	<0.001	
	Median			33		
		20.5	26.5	$p_{H1} < 0.001$		$p_{H2} = 0.001$
	Q1	17.5	21	28		
	Q2	24.0	35	48		
Normal, %	M	5.7	3.7	2.2	<0.001	
	Median			2		
		5	3	$p_{H1} < 0.001$		$p_{H2} = 0.016$
	Q1	4	2			
	Q2	7		2		

\* M – arithmetic mean, n – number, Q1 – quartile1, Q2 – quartile2,  $p_{H1}$  – compared to control,  $p_{H2}$  – compared to patients with asthenozoospermia, p – compared between all groups

In men with asthenozoospermia, the percentage of spermatozoa with normal structure is 66.7% ( $p_{H1} < 0.001$ ); in men with oligozoospermia – 2.5 times ( $p_{H1} < 0.001$ ) statistically significantly decreased compared to the control group. The proportion of morphologically normal spermatozoa in men with oli-

gozoospermia was 50% ( $p_{H2} = 0.016$ ) lower than in men with asthenozoospermia. A statistically significant difference was determined between the groups in terms of the proportion of morphologically normal spermatozoa ( $p_K < 0.001$ ) (Table 1).

Thus, during male infertility, the proportion of morphologically normal spermatozoa in sperm samples is significantly reduced, while the number of spermatozoa with mixed pathology is on the contrary increased. This difference was more pronounced in men with oligozoospermia.

Serious disorders in the endocrine system have been identified during male infertility. In the study, the concentration of FSH in the blood of men with asthenozoospermia increased by 57.7% compared to the control, but the result was not statistically significant. There was no significant change in the concentration of LH, testosterone and prolactin in this group compared to the control group.

The concentration of FSH in the blood serum of men with oligozoospermia was statistically significantly increased by 2.4 times ( $p_{H1} = 0.046$ ), the concentration of LH and testosterone tended to increase compared to the control by 39.3% and 17%, respectively, the concentration of prolactin and decreased by 31.7%.

In men with non-obstructive azoospermia, the concentration of FSH – 8.8 times ( $p_{H1} < 0.001$ ), LH – 2.9 times ( $p_{H1} < 0.001$ ); while prolactin increased by 89% compared to the control, testosterone concentration decreased by 22.9%.

In men with obstructive azoospermia, the concentration of FSH – 2.1 times, LH – 32.1%; while prolactin increased by 31.8% compared to the control, testosterone concentration decreased by 10.3%. These results were not statistically significant.

As can be seen from the obtained results, serious endocrine disorders were not recorded in asthenozoospermic patients, unlike other groups, compared to fertile men, only the concentration of FSH increased significantly. In patients with oligozoospermia, compared to men with asthenozoospermia, both FSH (51%) and LH (30%) concentrations increased more, and testosterone (8.7%), prolactin (19.6%) concentrations tended to decrease.

More serious endocrinological disorders were recorded in men with azoospermia. Thus, in men with non-obstructive azoospermia, the concentration of FSH, LH and prolactin was 3.7 times ( $p_{H3} < 0.001$ ), 2.1 times ( $p_{H3} < 0.001$ ) and 2.5 times ( $p_{H3} = 0.024$ ), respectively, compared to men with oligozoospermia. has increased significantly. Testosterone concentration in this group decreased by 43.8% compared to men with oligozoospermia. At the same time, in men with non-obstructive azoospermia, compared to obstructive azoospermia, the concentration of FSH, LH and prolactin increased by 4.2 times ( $p_{H4} < 0.001$ ), 2.2 times ( $p_{H4} = 0.033$ ) and 43.8%, respectively. A statistically sig-

nificant difference was determined between the groups according to the concentration of FSH ( $p_K < 0.001$ ) and LH ( $p_K = 0.001$ ) (Table 2).

**Table 2.** Changes in the concentration of FSH, LH, testosterone, prolactin hormones in blood, fructose in sperm during idiopathic male infertility\*

Index	Groups					p	
	Check (n=20)	Asthenozoospermia (n=56)	Oligozoospermia (n=30)	Azoospermia (non-obstructive) (n=7)	Azoospermia (obstructive) (n=8)		
Age	M	31.1	31.4	32.5	31.4	30.8	0.862
	Median	31.5	30	32.5	31	29.5	
	Q1	28	27.5	28	23	27	
	Q2	3	35.	35	38	35	
FSH mIU/ml	M	3.2	5.2	7.3	24.4	4.9	<0.001
	Median	2.6	4.1	6.2	22.8	5.4	
				$p_{H1} = 0.046$	$p_{H1} < 0.001$	$p_{H2} < 0.001$	
					$p_{H3} < 0.001$	$p_{H4} < 0.001$	
	Q1	2.1	2.2	4	17.5	3.4	
Q2	3.8	5.7	10	32.7	6.1		
LH mIU/ml	M	3.3	3.9	4.3	8.3	4.6	0.001
	Median	2.8		3.9	8.2	3.7	
					$p_{H1} < 0.001$	$p_{H2} < 0.001$	
					$p_{H3} = 0.001$	$p_{H4} = 0.033$	
	Q1	2.1	2.3	2.9	5.6	3.1	
Q2	4.5	4.6	5.3	9.2	5.6		
TST nmol/ml	M	12.4	13.1	14	9	13.7	0.167
	Median	11.8	12.7	13.8	9.6	10.7	
	Q1	10.6	9.3	10.1	6.7	10.2	
	Q2	15	15.5	15.5	10.3	14.7	
PRL mIU/l	M	225.1	228.6	188.1	379.8	300.8	0.196
	Median	208	189	158	394	274	
					$p_{H3} = 0.024$		
	Q1	191	141	127	136	155	
	Q2	225	301	230	507	422	
Fructose, mg/dl	M	241.1	300.5	388.6	412.7	241.8	<0.001
	Median	238.8	284.8	384	476	194.0	
				$p_{H1} < 0.001$	$p_{H1} = 0.001$		
				$p_{H2} < 0.001$	$p_{H2} = 0.024$		
				$p_{H4} < 0.001$	$p_{H4} = 0.004$		
Q1	232.1	244.2	356	408	45.1		
Q2	245.5	350.5	406	496	429		

\* FSH – follicle-stimulating hormone, LH – lutein-stimulating hormone, TST – testosterone, PRL – prolactin, M – mathematical average, n – number, Q1 – quartile1, Q2 – quartile2,  $p_{H1}$  – compared to control,  $p_{H2}$  – compared to patients with asthenozoospermia,  $p_{H3}$  – compared to patients with oligozoospermia,  $p_{H4}$  – compared to patients with obstructive azoospermia, p – compared between all groups

The obtained results showed that the concentration of fructose did not change significantly in the groups

of asthenozoospermia (19.3% tended to increase) and obstructive azoospermia (23.1% tended to decrease) to the control group. A statistically significant increase of fructose concentration in the oligozoospermia group compared to the control group was observed by 60,8% ( $p_{H1} < 0.001$ ), and in the non-obstructive azoospermia group by 2 times ( $p_{H1} = 0.001$ ). In patients with non-obstructive azoospermia, the concentration of fructose in sperm is 23.9% ( $p_{H2} = 0.024$ ) higher than in patients with asthenozoospermia, and 2.6 times higher ( $p_{H4} = 0.004$ ) than in patients with obstructive azoospermia. A statistically significant difference was determined between the groups for the level of fructose ( $p_K < 0.001$ ).

In the study, correlations between clinical-morphological indicators of spermatozoa, hormonal disorders and fructose concentration were determined in patients diagnosed with male infertility. Based on statistical calculations, it was determined that there was a positive correlation between FSH and LH ( $\rho = 0.544$ ;  $p < 0.001$ ) in the blood of men with asthenozoospermia, which indicates the interdependence of the secretion of both hormones. Thus, LH stimulates spermatogenesis, while FSH plays an important role in the completion of the spermatogenesis process by ensuring the maturation of spermatozoa. A decrease in the total number of spermatozoa ( $\rho = -0.388$ ;  $p = 0.003$ ) and the percentage of spermatozoa with head pathology ( $\rho = -0.492$ ;  $p < 0.001$ ) was observed in this group against the background of an increase in the concentration of FSH. There is a negative relationship between fructose concentration and forward motility of spermatozoa in men with asthenozoospermia ( $\rho = -0.542$ ;  $p < 0.001$ ). Although the number of spermatozoa decreased in this group, the percentage of spermatozoa with mixed pathology increased among them ( $\rho = -0.455$ ;  $p = 0.001$ ). A negative correlation was shown between the ratio of spermatozoa with mixed pathology and the ratio of spermatozoa with neck ( $\rho = -0.784$ ;  $p < 0.001$ ) and head ( $\rho = -0.356$ ;  $p = 0.008$ ) pathology. As the number of spermatozoa with mixed pathology increased, the number of normal spermatozoa decreased ( $\rho = -0.769$ ;  $p < 0.001$ ).

The same trend was recorded in the oligozoospermia group. In the oligozoospermia group, a positive correlation ( $\rho = 0.525$ ;  $p = 0.025$ ) was shown between the age limit of patients and the proportion of spermatozoa with neck pathology. A positive correlation between FSH and LH was determined in this group as well ( $\rho = 0.544$ ;  $p = 0.002$ ). Was found between testosterone levels and sperm count ( $\rho = 0.514$ ;  $p = 0.004$ ) a positive correlation. There is a negative correlation between sperm count and fructose ( $\rho = -0.872$ ;  $p < 0.001$ ). This also shows that when the number of spermatozoa decreases, the consumption of fructose in the seminal fluid decreases. Decreased consumption of fructose results in higher concentration of fructose in sperm. Between the

proportion of sperm with cervical pathology and the proportion of sperm with mixed pathology ( $\rho=-0.919$ ;  $p<0.001$ ) was observed a negative correlation. In case of non-obstructive azoospermia, a direct correlation between FSH and prolactin was determined ( $\rho=0.990$ ;  $p=0.017$ ). That is, of non-obstructive azoospermia an increase in the level of prolactin accelerates the synthesis and secretion of FSH. In obstructive azoospermia, was found between direct correlation between LH and testosterone ( $\rho=0.714$ ;  $p=0.047$ ).

## Discussion

As can be seen from the obtained results, only the concentration of FSH increased significantly in asthenozoospermic patients compared to fertile men. In patients with oligozoospermia, although the concentration of FSH increased statistically significantly, the concentration of LH tended to increase, while the concentration of testosterone and prolactin tended to decrease. More serious endocrinological disorders were recorded in men with azoospermia. In both groups of patients with azoospermia, compared to patients with asthenozoospermia and oligozoospermia, the concentration of prolactin increased, while the concentration of testosterone tended to decrease. In the group of non-obstructive azoospermia, the concentration of FSH, LH and prolactin significantly increased compared to all groups.

It is known that FSH and LH are synthesized in the anterior part of the pituitary gland, and their level increases primarily in the gonads – indicating functional disorders of the male gonads, hypogonadism as a result of primary damage to the testicles, as their secretion is regulated based on the feedback mechanism with the level of testosterone. When the level of testosterone in the blood increases, the concentration of gonadotropic hormones decreases. If the production of sperm in the testicles decreases, more FSH is synthesized in the pituitary gland in order to restore the normal function of the testicles.<sup>22</sup> Thus, a very high level of FSH indicates an abnormality in the initial stages of spermatogenesis. In addition, elevated levels of FSH during azoospermia and severe oligozoospermia cause damage to the seminiferous tubules. Apparently, in men with non-obstructive azoospermia, an increase in the level of LH, FSH, and a decrease in testosterone, on the contrary, leads to a violation of the spermatogenesis process and a sharp decrease in spermatozoa. It is known that under physiological conditions, high levels of LH and FSH stimulate the secretion of testosterone in Leydig and Sertoli cells, accelerating spermatogenesis. However, at a certain level, they negatively affect the hypothalamus-pituitary-gonadal system and influence the secretion of testosterone through a feedback mechanism. At this time, the concentration of testosterone remains either low or within the norm.<sup>22</sup> The increase in the level of FSH is more con-

sistent with the hypothesis of an increase in the secretion of inhibin in the process of spermatogenesis. The increase of LH explains its inhibitory effect on the initial stages of spermatogenesis through a feedback mechanism. However, there are possibilities that feedback factors can inhibit the secretion of LH and FSH.<sup>23</sup>

In men with asthenozoospermia, while the concentration of LH, prolactin and testosterone is normal compared to men with normozoospermia, an increase in the level of FSH only indicates the syndrome of Sertoli cells, that is, damage to cells that produce sperm. This is usually recorded more often during serious damage to the testicles and anomalies at the initial stage of spermatogenesis.<sup>24,25</sup> The results of the study showed that, although the total number of spermatozoa decreases in the oligozoospermia group compared to asthenozoospermia patients, the number of spermatozoa with mixed pathology increases. It is known that with oligozoospermia the number of sperm is predominantly reduced, and in men with asthenozoospermia the number of sperm moving forward is reduced. One of the main reasons for the violation of the forward movement of sperm is a violation of their morphological structure. Patients with asthenozoospermia have many sperm with mixed pathology.

If the level of gonadotropins is elevated ( $FSH>LH$ ) when the sperm count is low or zero, the patient should be investigated for the causes of primary hypogonadism. Deficiency in the first stages of spermatogenesis is observed in such patients.<sup>24</sup> In these cases, hypergonadotropic hypogonadism develops, the low level of testosterone leads to a violation of the feedback mechanism and an adequate increase in the concentration of gonadotropins. According to our results, in men with non-obstructive azoospermia, more hypergonadotropic hypogonadism symptoms are observed – high level of gonadotropins, testosterone tends to decrease.

Hyperprolactinemia is one of the main endocrinopathies associated with male infertility. Hyperprolactinemia affects sperm motility in many studies.<sup>12,26</sup> However, in our study, the effect of hyperprolactinemia on sperm motility was not determined, the concentration of prolactin in men with asthenozoospermia did not change significantly compared to the control group. A significant increase in the level of prolactin in men with azoospermia compared to other groups confirms the important role of hyperprolactinemia in the development of male infertility. As a result of experimental experiments, it was determined that there are prolactin receptors in the prostate gland, and a high concentration of prolactin inhibits the growth of the prostate gland. In addition, the expression of prolactin receptors in the choroidal sheath and hypothalamus proves the role of this hormone in male fertility. Acute hyperprolactinemia inhibits the synthesis of testosterone by

inducing the hypersecretion of corticoids in the adrenal glands or by inhibiting the secretion of GnRH due to the prolactin receptors present in dopamine neurons in the hypothalamus and can cause spermatogenesis disruption.<sup>12,26-28</sup> In our study, a slight decrease in the level of testosterone against the background of hyperprolactinemia was observed in men with non-obstructive azoospermia. It has been established that endocrine disorders also play an important role in the formation of non-obstructive azoospermia. With non-obstructive azoospermia, a decrease in testosterone levels is also observed. Studies have shown that prolactin also plays a role in reducing testosterone levels, since prolactin suppresses the secretion of GnRH and suppresses testosterone synthesis, resulting in a decrease in testosterone levels leading to suppression of FSH expression.

Fructose and prolactin levels decrease with age in men with obstructive azoospermia. The decrease in the synthesis of LH in this cup leads to the weakening of the synthesis and secretion of testosterone, as a result, the formation of spermatozoa is disrupted, and their number decreases. A decrease in the number of spermatozoa also leads to a decrease in fructose consumption and an increase in its level in sperm.

Fructose is synthesized in seminal vesicles and is considered the main catabolizable energy substrate of seminal fluid.<sup>1</sup> While glucose ensures long-term motility of spermatozoa during their functional activity, fructose is important in their fast and rapid movement in the phase of interaction with the egg cell and in the acrosomal reaction phase.<sup>19</sup> In an environment with high activity of normal spermatozoa, the concentration of fructose decreases due to the high energy demand.<sup>29</sup> The concentration of fructose increases as a result of the decrease in the number of spermatozoa. This is primarily due to the fact that fructose is an energy reservoir for spermatozoa. Since only motile spermatozoa use fructose, the determination of fructose is important in the diagnosis of patients with asthenozoospermia. Thus, during idiopathic asthenozoospermia, the number of spermatozoa with mitochondrial defects increases. The increase in the level of fructose in the oligozoospermia and non-obstructive azoospermia group is primarily explained by the decrease in the number of spermatozoa, their anomalous morphology, and their mobility. A decrease in the level of fructose in sperm impairs the movement and coagulation of spermatozoa, which may be associated with inflammation of the genital tract. In patients with asthenozoospermia and oligozoospermia, the ratio of spermatozoa with mixed pathology significantly increases compared to the control group, while the ratio of normal spermatozoa decreases. Pathological spermatozoa consume a small amount of fructose because they are weakly motile or completely immobile.<sup>1,29,30</sup>

## Conclusion

Thus, an increase in the concentration of FSH, LH and prolactin in the blood serum, and a decrease in the concentration of testosterone causes the disruption of the spermatogenesis process, causing male infertility. A high concentration of FSH and LH in men with azoospermia can be considered one of the important indicators in the diagnosis of non-obstructive azoospermia. An increase in the concentration of fructose is the main indicator of a decrease in the number and motility of spermatozoa. Determination of FSH, LH, prolactin, testosterone and fructose concentrations can be of great importance in the differential diagnosis and prediction of male infertility, and can be used to determine the process of spermatogenesis and the biological characteristics of sperm.

## Declarations

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### Author contributions

Conceptualization, G.E.N.; Methodology, G.E.N.; Software, G.E.N.; Validation, G.E.N.; Formal Analysis, G.E.N.; Investigation, G.E.N.; Resources, G.E.N.; Data Curation, G.E.N.; Writing – Original Draft Preparation, G.E.N.; Writing – Review & Editing, G.E.N.; Visualization, G.E.N.; Supervision, X.X.; Project Administration, G.E.N.

### Conflicts of interest

The author assert that they have no conflicts of interest.

### Data availability

The author can provide the data upon request.

### Ethics approval

Permission to conduct the study was obtained from the Ethics Committee. The study plan was implemented in accordance with the Helsinki Final Act and the protocol was approved by the Ethics Committee Azerbaijan Medical University ICE Committee on Medical Sciences (№8. 28.06.2020).

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