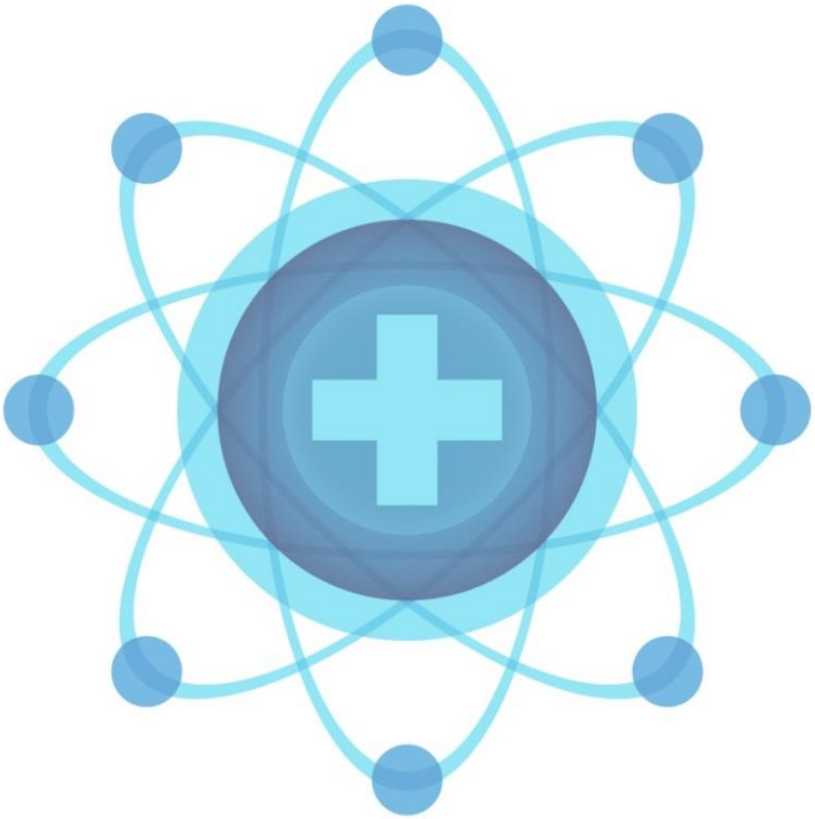

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Gly102Ser MISSENS MUTATION OF LHB GENE (rs5030774, c.304G>A, G1502A) AMONG PATIENTS WITH MALE INFERTILITY.

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Abstract: The luteinizing hormone ß-chain (LHB) gene is localized on chromosome 11p13 and contains 3 exons. The analysis of the Gly102Ser missense mutation of the LHB gene in the formation of male infertility was carried out. The study was conducted on a sample of 140 patients with various clinical forms of male infertility and 155 conditionally healthy fertile men. The obtained molecular genetic data on the G1502A marker of the LHB gene are representative. The data will complement the international database (Allele Frequency Database) on the frequency of the mutation variant G1502A of the LHB gene for various populations and ethnic groups of the countries of the world.
Keywords: missense mutation Gly102Ser of the LHB gene, male infertility, Allele Frequency Database, luteinizing hormone.

Relevance. In recent years, more and more attention of fundamental research in the field of reproductive medicine has been directed to studying the genetic basis for the development of infertility of central origin, when the fertile function is impaired at the level of the hypothalamic-pituitary-gonadal regulation of the production of sex hormones in both men and women [1,5,6 ,7,10,12,20]. The luteinizing hormone ß-chain **(**LHB) gene is localized on chromosome 11p13 and contains 3 exons. 179 SNPs are described in the gene, among them the most functionally significant are polymorphisms of the coding region of the Trp8Arg, Ile15Thr and Gly102Ser gene, leading to a decrease in the activity of luteinizing hormone (LH). The G (1502) A polymorphic variant is a substitution in the LH ß-chain gen, leading to the replacement of Gly with Ser at position 102 of the LHB protein (Gly 102 Ser, rs 5030774) [15,19].

G(1502)A mutation of the LHB gene, which is the subject of this study, was first discovered in the Singaporean-Chinese population (Roy et al., 1996). Subsequently, this mutational variant was identified in other Asian countries, but its highest allelic frequency was in Singaporean Chinese [14,17,19]. Carriers of the LHβ

1052A allele have been found to have lower LH levels, and this polymorphism may be associated with infertility in both men and women [2,3].

Objective. Evaluation of the role of the missense mutation of the Gly 102 Ser gene LHB in the formation of male infertility.

Materials and methods. The study included 140 men with infertility. Of these: 35 (25.0%) were patients with azoospermia, 105 (75.5%) were patients without azoospermia. The control group included 155 fertile men.

Genotyping of a polymorphic locus Gly 102 Ser gene LHB was performed by real-time polymerase chain reaction (RotorGene Q, Quagen, Germany), having previously isolated genomic DNA from blood samples using the Ribo-prep reagent kit (InterLabService, Russia). The analysis of associations of this locus was carried out by comparing two samples according to the "case-control" type.

Statistical processing of the obtained results was carried out using the OpenEpi software package. V.9.2 . Estimation of deviation of distributions of genotypes of a locus Gly 102 Ser gene LHB from the Hardy–Weinberg distribution was performed using Pearson's modified chi-square test. The data were calculated using the online program "Hardy - Weinberg equilibrium calculator.

Results and discussion

Tables 1, 2 and 3 and figures 1 and 2 present the results of occurrence frequencies, calculations of the deviation of the theoretical and empirical frequencies of the distribution of alleles and genotypes of the missense mutation G1502A of the LHB gene for RHV in groups of patients with MB and population samples. As can be seen from the tables, the occurrence of the mutation variant G1502A of the LHB gene among patients was low. It was found that out of 140 patients with MB, only 1.2% were carriers of the heterozygous variant G1502A (2/140). Both carriers of the missense mutation belonged to a subgroup of patients with infertility, without azoospermia. During the work, the homozygous variant of this mutation was not identified. On the contrary, none of the 155 examined conditionally healthy individuals was a carrier of the missense mutation G1502A of the LHB gene.

In the general group of patients , the empirical-actual distribution of alleles and genotypes of the G1502A mutation in the LHB gene corresponded to theoretical-expected at RHV, (χ^2 =0.01; p=0.9, according to Fisher's exact test).

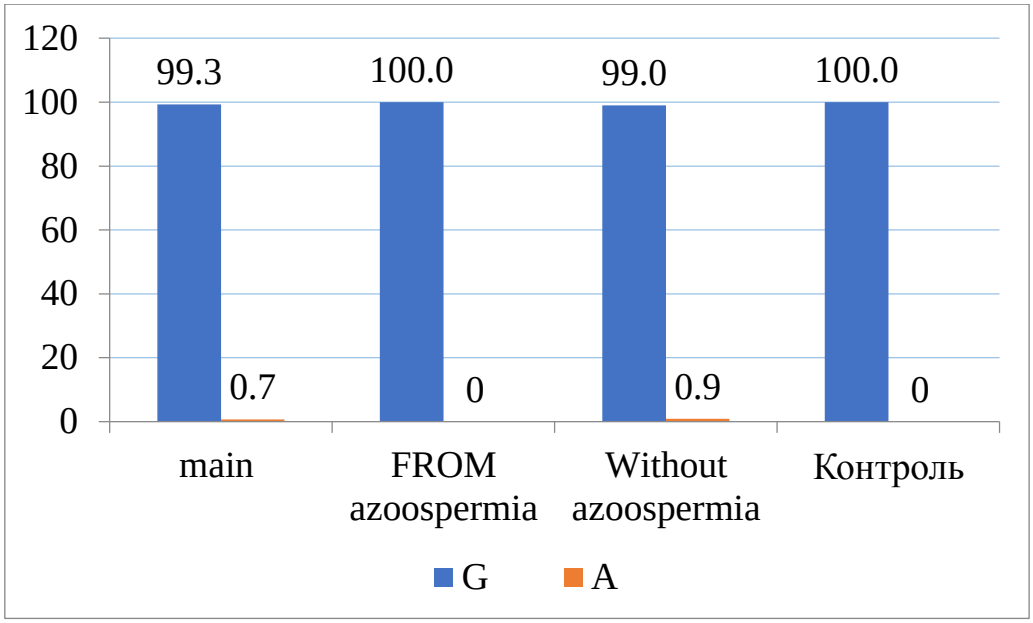

Figure 1. **Distribution of alleles of the missense mutation Gly102Ser of the LHB gene (G1502 A) in groups of patients with MB and controls**

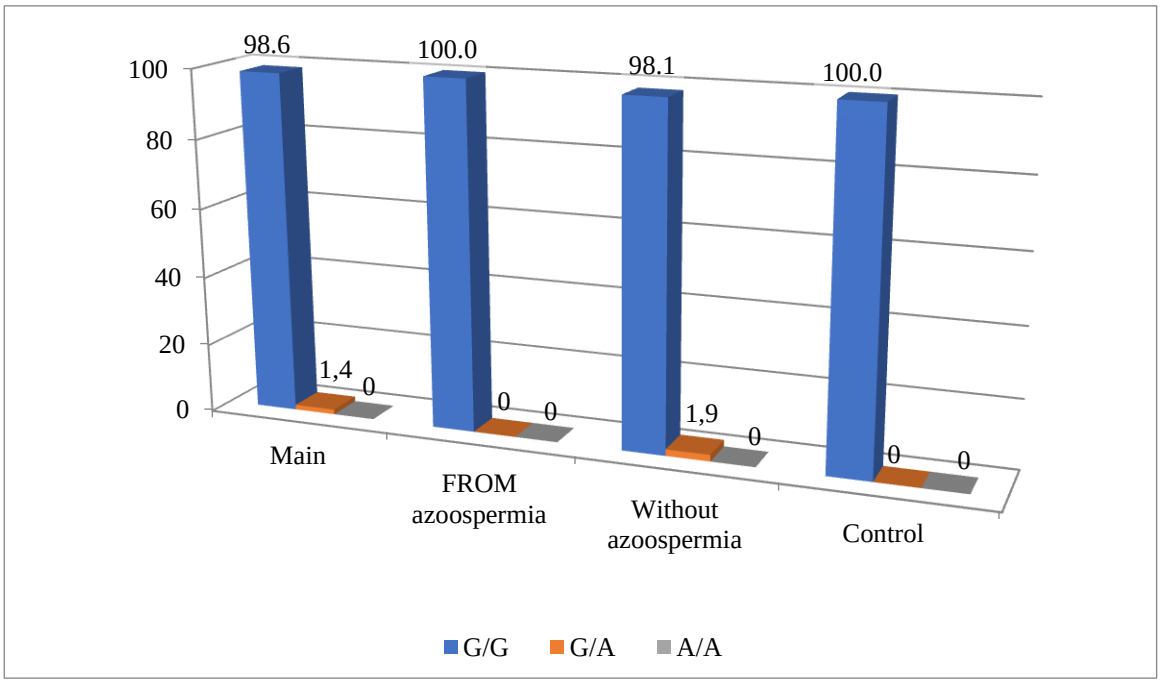

Figure 2. **The frequency of detection of genotypes of the missense mutation Gly102Ser of the LHB gene (G1502A) in groups of patients with MB and controls**

Table 1.

The frequency of distribution of alleles and genotypes of the missense mutation Gly 102 Ser of the LHB gene (G1502A) in groups of patients with MB and controls

No.	Group	Distribution frequency:									
		alleles				genotypes					
		G		A		G/G		G/A		A/A	
		n	%	n	%	N	%	n	%	n	%
1	Main group (n=140)	278	99.3	2	0.71	138	98.6	2	1.4	0	0.0
a	FROM azoospermia	70	one hundre	0	0	35	one hundr	0	0	0	0.0

	(n=35)		d				ed				
b	Without azoospermia (n= 105)	208	99.0	2	0.9	103	98.1	2	1.9	0	0.0
2	Control group, (n=155)	310	100.0	0	0.0	155	100.0	0	0.0	0	0.0

The frequencies of the ancestral wild G1502 and minor mutation 1502A alleles, respectively, were 0.99/0.01 in the group of patients with MB (Table 2).

table 2

Expected and observed frequencies of distribution of locus genotypes by RHB of polymorphism G1502A in the LHB gene

Main group					
alleles	Allele frequency				
G	0.99				
A	0.01				
Genotypes	Genotype frequency		$\chi 2$	p	df
	observable	expected			
G/G	0.99	0.99	0		
G/A	0.01	0.01	0		
A/A	0	0	0.01		
Total	1.0	1.0	0.01	0.889	1

The empirically observed and theoretical frequencies of the G/G, G/A, and A/A genotypes of the Gly 102 Ser of the LHB gene were 0.99/0.99, 0.01/0.01, and 0.0/0.0, respectively, and the difference did not differ significantly from the equilibrium significance level for RCM, which was at level by 5%.

It should be emphasized that among patients with impaired fertility or in the studied populations of the world, the homozygous variant A 1502A of the Gly 102 Ser polymorphism of the LHB gene, which has a high risk of developing a severe form of reproductive disorders, was also not detected (theoretically, it is extremely rare).

Our data on this mutation indicate the low frequencies of detected actual heterozygotes, and, accordingly, the extremely low level of not only the expected, but also the observed heterozygosity of this locus ($H_o = 0.01$) in our population.

Table 3 .

The difference between the expected and observed frequencies of heterozygosity of the G1502A polymorphic marker in the LHB gene

Groups	Ho _	H_e	D*
Main group	0.01	0.01	0.01
Without azoospermia	0.02	0.02	0.01

Note : D= (Ho - He)/He

The results of the population analysis show that the empirical distribution of the Gly 102 Ser genotypic variants of the LHB gene corresponded to the theoretically expected one, i.e., in this case, RCM is performed in the group of patients. However, this missense mutation was characterized by low frequencies of unfavorable 1502A

and heterozygosity in the studied group of patients with impaired fertility, therefore, a low level of genetic variability of this mutation in the Uzbek population.

Based on the data we obtained and the available world literature data, we conducted a comparative analysis of the Gly 102 Ser mutation of the LHB gene and the risk of male infertility. Original population and association studies were searched using the NCBI, Scopus , Google Academia and HuGE Navigator databases, etc. The main criteria for inclusion in the comparative analysis were the following conditions:
- the study should have a case-control design (case- control model) ;
- analysis of the prevalence and assessment of the association between the Gly 102 Ser mutation of the LHB gene and the development of male infertility.

Table 4 shows a comparative analysis of our results on the Gly 102 Ser polymorphism of the LHB gene with data from the world population.

Table 4 .

Comparative analysis of the frequency of the G1502A mutation in the LHB gene

Population/country Total number of examined	Number of examined patients with and without mutation				Authors
	-		+		
	n	%	n	%	
Egypt: n =300	195	65.0	105	35.0	Nagat S. Mohamad et all, (2012); Hashad D et all, (2012)
Chinese/ Singapore n =145	140	96.6	five	3.4	Ramanujam LN, et all (2000) Ramanujam et al., 1999 Ramanujam et al., 1998
Asia, n =170	165	97.1	five	2.9	Roy AC . et all (1996)
Uzbekistan: n =140	138	98.6	2	1.4	Authors:
Europeans: n =205 Finns: n =60 Danes : n =145	250	100.0	-	-	Lamminen T . et all (2002)
Bengalis from Asia n =78	78	100.0	-	-	Lamminen T . et all (2002)
South Korea: n =95	95	100.0	-	-	Lee S. et all (200 3)
Africans/ Rwanda n =100	100.0	100.0	-	-	Lamminen T . et all (2002)

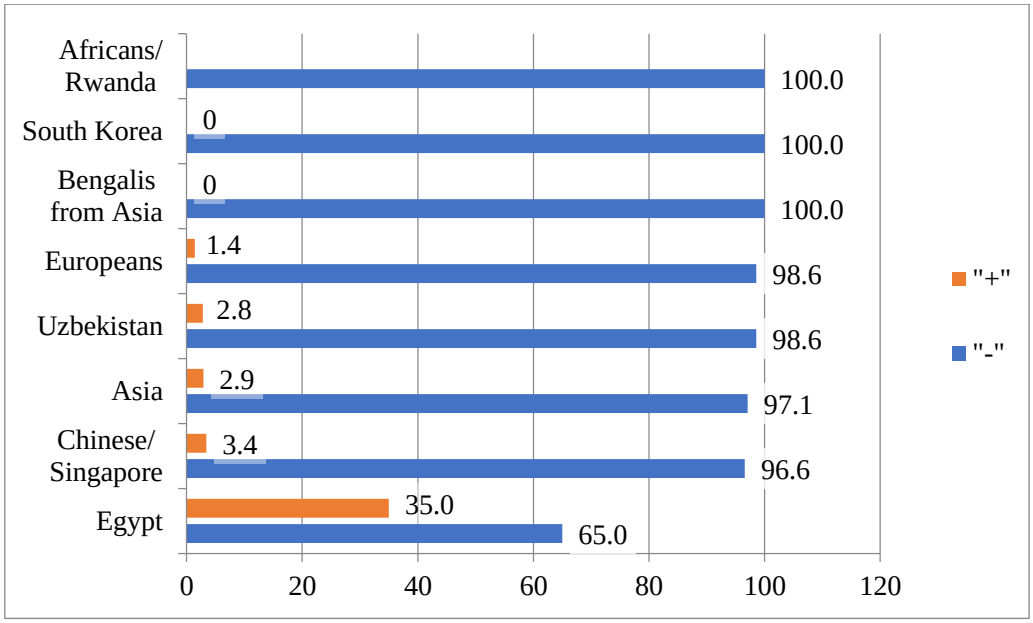

Figure 3. **Comparative analysis of the frequency of the G1502A mutation in the LHB gene**

As can be seen from the table, the number of analyzed samples of patients with MB varied from *n* = 78 (Lamminen T. et all (2002) up to *n* =300 (Nagat S. _ Mohamad et all, (2012); Hashad D. et all, (2012)). The frequency of missense mutations, i.e., the genotypic variant G1502A of the LHB gene strongly varied between these countries. Population frequency of the heterozygous variant G1502A of the LHB gene ranged from 0 in European, African and Korean populations (Lamminen T. et all, 2002; Lee S. et all, 2003) up to 0.35 (35.0%) up to 100.0 in patients from Egypt (Nagat S. Mohamad et all, 2012; Hashad D. et all, 2012).

It should be emphasized that our data on the frequency of occurrence of the heterozygous G1502A variant of the LHB gene (1.4%) were comparable to Roy A.C. et all (1996) (0.014 vs. 0.029, respectively; χ^2 =0.8; p=0.4) and Ramanujam L.N. et all (2000) (0.014 vs. 0.034, respectively; χ^2 =1.2; p=0.3), and all differences were not statistically significant (Tables 5 and 6).

Table 5

Differences in the frequency of the mutation variant G1502A of the LHB gene

G1502A LHB gene	Number of alleles				χ^2	R
	Uzbekistan		Asia			
	n	%	n	%		
-	138.0 _	98.6	165	97.1	0.8	0.4
+	2.0	1.4 _	five	2.9		

Table 6

Differences in the frequency of the mutation variant G1502A of the LHB gene

G1502A LHB gene	Number of alleles				χ^2	R
	Uzbekistan		Chinese/Singapore			
	n	%	n	%		

-	138.0 _	98.6	140	96.6	1.2	0.3
+	2.0	1.4 _	5.0	3.4		

Contrary, high interpopulation differences in the frequency of the mutation variant G1502A of the LHB gene were noted in comparison of our data with data obtained in the Egyptian population. Comparative analysis revealed a significant 37 - fold increase in the 1502A mutant allele in the Egyptian population, compared with the local population studied in our study (35.0% vs. 1.4%, respectively, at $\chi 2 = 58.4$; $p<0.05$; OR =37.4; 9.018-153.1) (Table 7).

Table 7

Differences in the frequency of the mutation variant G1502A of the LHB gene

G1502A LHB gene	Number of alleles				χ^2	R
	Uzbekistan		Egypt			
	n	%	n	%		
-	138	98.6	195	65.0	58.4	<0.05
+	2	1.4 _	105	35.0		

Analysis of associations of G1502A polymorphic loci of the LHB gene with the development of male infertility was carried out using the " case - control " model only in a subgroup of patients without azospermia and in the control group.

Table 8

Differences in the frequency of allelic and genotypic variants of the Gly 102 Ser polymorphism in the LHB gene in groups of patients

Alleles and genotypes	Number of examined alleles and genotypes				$\chi 2$	p
	Without azoospermia		Control group			
	n	%	n	%		
G	208	99.0	310	100.0	3.0	0.08
A	2	1.0	0	0.0	3.0	0.08
G/G	103	98.1	155	100.0	3.0	0.08
G/A	2	1.9	0	0.0	3.0	0.08

In order to identify diagnostic efficiency in Uzbekistan, we conducted a comparative analysis of the frequency distribution of allelic and genotypic variants of the Gly 102 Ser missense mutation in the LHB gene in a subgroup of patients without azoospermia (n=105) and among individuals in the control sample (n=155). The analysis revealed a trend towards a statistically significant difference between the compared subgroups of patients and the group of conditionally healthy donors (χ^2 =3.0 and p=0.08) (Table 8). As expected, in both groups, the G1502 allele was predominant in frequency of occurrence , occurring in 99.0% of cases among patients and 100.0% in the control group. The unfavorable allele 1502A a associated with a decrease in the expression of lutinizing hormone was found in a subgroup of patients in 1.0% of cases (χ^2 =3.0; p=0.08). Mutational heterozygous genotype Gly 102 Ser of the LHB gene met in a subgroup of patients in 1.9% of cases (χ^2 =3.0; p=0.08). This

variant was not found in the control group. This means that in men with the presence of this mutational genotype in the genotype, the risk of developing male infertility is statistically significantly increased.

Conclusions: Thus, the obtained molecular genetic data on the G1502A marker of the LHB gene are representative. The mutational genotypic variant of the G1502A polymorphism of the LHB gene is unevenly distributed within the studied populations and is mainly represented in the sample from representatives of the Middle East and Asia. In other studied populations (Europe, Africa, some countries of Southeast Asia), this mutation was not found. These data also indicate a possible founder effect and genetic drift of the G1502A mutation variant of the LHB gene. in the populations of the Middle East and Asia.

The data obtained by us will complement the international database (Allele Frequency Database) according to the frequency of the mutation variant G1502A of the LHB gene for various populations and ethnic groups of the countries of the world. The introduction of this marker into the clinical practice of the Republic will make it possible to clarify the cause of infertility of central origin and in the future will improve not only the diagnostic and therapeutic effectiveness of male infertility, but also more accurately predict the outcomes of ART programs.

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