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**RESEARCH ON SOME VARIANTS OF *FSHB*, *FSHR*, *TEX11*
AND *TEX14* IN PATIENTS WITH AZOOSPERMIA
AND SEVERE OLIGOSPERMIA OF UNKNOWN CAUSE**

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LIST OF PUBLISHED WORKS
RESEARCH RESULTS OF THE THESIS

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2. **Van Quyet Pham, The Son Trinh, Duy Bac Nguyen, Thi Thu Hien Le, Huu Viet Dinh, Ngoc Manh Ha, Huu Dinh Tran, Thuy Duong Nguyen** (2025). Reserch on the association between rs10835638 in fshb and male infertility in Vietnam, *Viet Nam Medical Journal*, 551:351-357.
3. **Van Quyet Pham, Thuy Duong Nguyen, Duy Bac Nguyen, Thi Thu Hien Le, Huu Viet Dinh, Ngoc Manh Ha, Thi Hoa Vu, Lang Phallin, Men Borall, Soeng Piseth, The Son Trinh** (2025). Research on the association between the *FSHR* rs6166 variant and semen characteristics in infertile men in Vietnam, *Journal of Military Pharmaco-medicine*, 50(9):25-34.

INTRODUCTION

Globally, the rate of male infertility is increasing across many regions. Notably, several publications have issued warnings about the progressive decline in sperm count among men, which has become increasingly evident and poses significant challenges in infertility treatment, particularly for cases of azoospermia and severe oligozoospermia.

FSH (Follicle-Stimulating Hormone) has been shown to be effective in stimulating spermatogenesis in cases of gonadotropin deficiency. However, among patients with idiopathic infertility, the treatment response varies widely, ranging from positive improvement to complete non-responsiveness. Several international studies have indicated that variants in the *FSHB* and *FSHR* may influence this therapeutic outcome.

The *TEX* (Testis Expressed) gene family comprises genes that are specifically expressed in testicular tissue. Among them, only three genes are classified as highly associated with male infertility: *TEX11*, *TEX14*, and *TEX15*. *TEX11*, located on the X chromosome, tends to express phenotypic effects more readily than *TEX15*, although both genes play essential roles in chromosome synapsis. Therefore, to investigate genes associated with male infertility, *TEX11* and *TEX14* are the most noteworthy representatives.

The *FSHB*, *FSHR*, *TEX11*, and *TEX14* are important genetic factors with strong potential associated with male infertility. However, in Vietnam, no comprehensive study has yet examined all four of these genes in relation to azoospermia and severe oligozoospermia. For this reason, we conducted the study titled “Research on some variants of *FSHB*, *FSHR*, *TEX11* and *TEX14* in patients with azoospermia and severe oligospermia of unknown cause”, with the following objectives:

1. To identify several variants of *FSHB*, *FSHR*, *TEX11* and *TEX14* in patients with azoospermia and severe oligozoospermia of unknown cause.
2. To evaluate the association between these variants of *FSHB*, *FSHR*, *TEX11* and *TEX14* and clinical as well as subclinical characteristics in patients with azoospermia and severe oligozoospermia of unknown cause.

NEW CONTRIBUTIONS OF THE THESIS

We identified the genotype frequencies and allele frequencies of the *FSHB* rs10835638, *FSHR* rs6166, *TEX11* rs4844247, and *TEX14* rs79813370 variants in 210 patients with azoospermia and severe oligozoospermia of unknown cause.

The *FSHB* rs10835638 variant was found to be associated with male infertility due to azoospermia and severe oligozoospermia; it was also associated specifically with male infertility caused by severe oligozoospermia; the GT genotype may be a risk factor contributing to reduced sperm progressive motility. The *FSHR* rs6166 variant showed no association with male infertility due to azoospermia or severe oligozoospermia; however, the AA genotype was associated with reduced sperm motility compared to the GG genotype and increased testosterone levels compared to genotypes carrying the G allele in the severe oligozoospermia group. The *TEX11* rs4844247 and *TEX14* rs79813370 variants showed no association with male infertility due to azoospermia or severe oligozoospermia.

THESIS STRUCTURE

The thesis consists of 131 pages (excluding references and appendices), divided into 4 chapters, with 36 tables and 28 figures; There are 139 references (07 Vietnamese documents and 132 English documents). Introduction: 02 pages; Literature review: 32 pages; Subjects and research methods: 24 pages; Research results: 39 pages; Discussion: 31 pages; Conclusion: 02 pages; Recommendations: 01 page

Chapter 1. LITERATURE REVIEW

1.1. Spermatogenesis

1.1.1. Stages of germ cell

Spermatogenesis takes place within the seminiferous tubules of the testes through mitosis, which increases the number of cells, and meiosis, which produces haploid chromosomes. The process includes the following stages: spermatogonia, meiosis, and the spermatid stage.

In this process, the synaptonemal complex (SC), together with recombination foci (RF) that appear on chromosomes, plays a central role in coordinating synapsis and recombination during meiotic division. When the SC or the RF is defective to varying degrees, spermatogenesis may arrest, leading to reduced sperm count or abnormalities in sperm quality.

Cells at this stage do not completely separate to form individual cells; instead, they remain connected through intercellular bridges until the spermatids differentiate into spermatozoa and detach from the seminiferous epithelium to enter the lumen of the tubule. Studies have demonstrated that these intercellular bridges are essential for completing meiosis.

1.1.2. The role of reproductive hormones in spermatogenesis

Normal FSH levels are not a reliable predictor of spermatogenesis, even in cases of non-obstructive azoospermia where FSH levels may be normal.

In the testes, FSH binds to receptors on the surface of Sertoli cells, activating them. FSH also supports the functional maturation of Sertoli cells, enabling them to nourish and assist germ cells throughout sperm production. Abnormalities in either the ligand or the receptor of FSH may disrupt spermatogenesis, resulting in decreased sperm count, including oligospermia or azoospermia.

LH stimulates Leydig cells to produce testosterone. Both abnormally low and abnormally high LH levels can impair spermatogenesis. Testosterone promotes the production of sperm through its action on Sertoli cells and plays a crucial role in the differentiation of round spermatids into mature spermatozoa.

Elevated prolactin levels reduce LH and testosterone concentrations, consequently impairing spermatogenesis. Increased estradiol levels inhibit testosterone production and disrupt the spermatogenic process.

The endocrine system regulating reproduction is highly interconnected, and any imbalance in hormone levels or abnormalities in hormone receptors may lead to impaired spermatogenesis and a reduction in sperm count.

1.2. Causes and factors affecting impaired spermatogenesis

1.2.1. Genetic causes

1.2.1.1. Abnormalities at the cellular level

Chromosomal numerical abnormalities: Klinefelter syndrome, XYY syndrome, 45,X syndrome, etc. Chromosomal structural abnormalities: AZF microdeletions on the Y chromosome, XX male syndrome, Prader-Willi syndrome, Robertsonian translocations, etc.

1.2.1.2. Abnormalities at the molecular level

Genes located on autosomes: Isolated FSH deficiency, Noonan syndrome, Laurence-Moon syndrome, Bardet-Biedl syndrome, 5 α -reductase deficiency, etc.

Genes located on the X chromosome: Kallmann syndrome, androgen receptor gene variants.

1.2.2. Non-Genetic causes

Hormonal disorders: Hyperprolactinemia, elevated glucocorticoid levels. Testicular disorders: Cryptorchidism, undescended testes, congenital anorchia, testicular trauma; bacterial or viral orchitis; varicocele, etc.

1.2.3. Influencing factors

Age, sleep quality, medications, radiotherapy, high temperature exposure, toxins, infections, environmental pollution, diet, systemic diseases such as liver cirrhosis or renal failure, stress, and free radicals—all may directly or indirectly impair spermatogenesis in the testes.

1.2.4. Types of Spermatogenic Impairment

1.2.4.1. Reduced sperm quantity

1.2.4.2. Reduced sperm quality

1.3. Azoospermia and severe oligozoospermia

Azoospermia: No sperm are observed in the semen sample, or only extremely few sperm are detected after centrifugation (cryptozoospermia). Severe oligozoospermia: Sperm concentration less than 5 million/ml of semen.

Azoospermia and severe oligozoospermia of unknown cause refer to cases in which clinical examination and conventional laboratory tests fail to identify any cause or potential contributing factors that could explain the patient's condition.

1.4. Genetic diagnosis in impaired spermatogenesis

1.4.1. Cytogenetic testing

1.4.2. Cytogenetic-Molecular testing

1.4.3. Molecular Testing

1.4.3.1. Polymerase Chain Reaction (PCR)

1.4.3.2. Sanger Sequencing

1.4.3.3. Restriction Fragment Length Polymorphism (RFLP)

1.5. Roles of the *FSHB*, *FSHR*, *TEX11* and *TEX14* in spermatogenesis

1.5.1. *FSHB*

The *FSHB* encodes the beta subunit of follicle-stimulating hormone (FSH) and is located on chromosome 11p13 (MIM 136530). It consists of 3 exons and 2 introns.

Similar to all genes, *FSHB* exhibits polymorphisms and mutations, each of which may exert different effects on male reproductive function, ranging from subtle manifestations to complete infertility. Variants of the *FSHB* can produce a wide spectrum of phenotypic outcomes, from azoospermia to various degrees of oligozoospermia.

1.5.2. *FSHR*

The human *FSHR* is located on chromosome 2p21–p16 and encodes the FSH receptor, a G protein-coupled receptor (GPCR). The gene contains 10 exons and 9 introns.

Variants of *FSHR*, including mutations and polymorphisms, can lead to impaired reproductive function. The resulting clinical manifestations reported so far range from mild to severe oligozoospermia. To date, no studies have established a direct association between *FSHR* gene defects and azoospermia.

1.5.3. *TEX11*

The *TEX11* (Testis Expressed 11) is located on the long arm of the X chromosome, consisting of 31 exons. The *TEX11* protein is an essential component of the synaptonemal complex (SC).

Defects in the formation of the SC or dysfunction within the recombination machinery may lead to meiotic arrest and infertility in humans.

1.5.4. *TEX14*

The *TEX14* is located on chromosome 17q22 and contains 33 exons. *TEX14* is required for the formation of intercellular bridges between germ cells originating from the midbody structure.

Functional impairment of *TEX14* disrupts the formation of intercellular bridges, which can lead to defective spermatogenesis and contribute to male infertility.

1.6. Research on the association between *FSHB*, *FSHR*, *TEX11*, *TEX14* variants and azoospermia/severe oligozoospermia

1.6.1. Global Research

These include both controlled descriptive studies and uncontrolled descriptive studies. Several mutations and polymorphisms in the *FSHB*, *FSHR*, *TEX11*, and *TEX14* have been identified as being associated with male infertility characterized by impaired spermatogenesis or reduced FSH levels.

The *FSHB* rs10835638, *FSHR* rs6166, and *TEX11* rs4844247 variants have been widely investigated. However, findings regarding their association with infertility remain inconsistent across countries.

Inclusion criteria in these studies: Azoospermia, oligozoospermia, normal male karyotype (46,XY), and no AZF microdeletions.

Exclusion criteria: Obstructive azoospermia, testicular diseases or trauma, sexual and ejaculatory disorders, and genetic disorders.

Genetic analysis techniques used: PCR–RFLP, Whole Exome Sequencing (WES), and Sanger sequencing.

1.6.2. Research in Vietnam

The *TEX14* rs79813370 variant has begun to be examined in Vietnam and has been reported to be associated with male infertility in general, using the PCR-RFLP genetic analysis method.

Chapter 2. MATERIAL AND METHODS

2.1. Study subjects

2.1.1. Inclusion criteria

- General criteria: Adult males who agree to participate in the study.
- Patient group criteria:
 - + Diagnosed with azoospermia or severe oligozoospermia (sperm concentration < 5 million/ml) of non-obstructive origin.
 - + Infertile and having no biological children conceived naturally.
 - + Normal male karyotype (46,XY), with no chromosomal abnormalities (mutations and polymorphisms) and no AZF (azoospermia factor) microdeletions on the Y chromosome.
- Control group criteria: Normal semen parameters according to the World Health Organization (WHO) 2021 standards, having at least one biological child conceived naturally, and no history of treatment using assisted reproductive technologies (ART).

2.1.2. Exclusion Criteria

- Currently using medications that affect reproductive hormones, including oral, injectable, or intravenous drugs for medical treatment.
- Cases with ectopic testes, varicocele, history of mumps orchitis, testicular trauma, or testicular cancer.
- Azoospermia or severe oligozoospermia caused by obstructive factors in the seminal pathway.
- Cases with ejaculatory disorders, retrograde ejaculation, erectile dysfunction, or acute diseases of the inguinal or genital region.
- Individuals with infectious diseases, sexually transmitted infections, HIV/AIDS, or genetic disorders.

2.2. Research Methods

2.2.1. Study Design

Cross-sectional descriptive study with a comparative control group.

2.2.2. Study Indicators

- * Demographic, clinical, and subclinical characteristics
 - Infertility status, duration of infertility, history of treatment with assisted reproductive technologies (ART), number of biological children, and method of conception.
 - Age, height, weight; smoking and alcohol consumption habits.
 - Semen analysis parameters: Semen volume, pH; sperm concentration, total count, motility, viability, and morphology.
 - Reproductive hormones: FSH, LH, testosterone, prolactin, estradiol.
 - Testicular measurements: Right testicular volume, left testicular volume, and total testicular volume.
- * Genetic characteristics of *FSHB* rs10835638, *FSHR* rs6166, *TEX11* rs4844247, *TEX14* rs79813370 variants: Genotypes/alleles and genotype and allele frequencies of the variants.

2.2.3. Sample Size

Sample size formula: In this study, we applied the sample size calculation formula for cases where the population size is unknown, proposed by Yamane Taro (1967).

$$n = Z^2_{1-\alpha/2} \frac{p(1-p)}{e^2}$$

According to the research results of Wu Q. (2017): For the *FSHR* rs6166 variant, the frequency of allele A was 0,684.

For the *FSHB* rs10835638 variant, the frequency of allele T was 0,078.

In the study by Zhang (2015) on *TEX11* rs4844247, the frequency of allele T was 0,193.

In the study by Huu Dinh Tran on *TEX14* rs79813370, the frequency of allele T was 0,1044.

We selected the lowest p value, $p = 0.078$, and chose $\varepsilon = 0.05$. Substituting these values into the sample size formula yielded $n = 111$. Thus, the minimum required sample size for each study group was 111 participants.

Within the scope of this project, the selected study population included 210 subjects in the patient group (170 with severe oligozoospermia and 40 with azoospermia) and 200 subjects in the control group.

2.2.4. Research Sites and Duration

- Research sites:

- + Sample collection: Military Clinical Embryology Institute - Vietnam Military Medical University. Hanoi Andrology and Fertility Hospital. Viet-Bi Andrology and Fertility Hospital

- + Genotyping analysis was conducted at the Institute of Biology, Vietnam Academy of Science and Technology.

- Research duration: From January 2023 to May 2025.

2.2.5. Clinical Examination Forms, Equipment, Instruments, and Reagents Used in the Study

- Clinical examination forms: Developed specifically for this study and used during examinations performed by physicians at the Military Clinical Embryology Institute for participants who had not yet undergone treatment at the institute.

- Equipment and instruments: Genetic testing instruments and equipment from the Institute of Biology, Vietnam Academy of Science and Technology were used.

2.2.6. Research procedures

2.2.6.1. Clinical examination procedure for selecting study participants

Eligible subjects included men presenting for reproductive function evaluation or wishing to have more children (control group); seeking fertility treatment (patient group) at medical facilities.

Both retrospective and prospective cases were included, in which the severe oligospermia patient group and the control group were collected from 2022. All participants underwent examination, testing, and reproductive health assessment following a standardized procedure (*costs paid by the participants for the purpose of medical examination and treatment*).

Final determination of reproductive health status (after excluding subjects who did not meet the study criteria):

- Control group: Individuals with normal semen analysis parameters according to WHO 2021 standards.

- Patients with azoospermia of unknown cause:

- + No sperm found during microTESE.

- + No sperm or extremely few sperm in semen after centrifugation, with FSH ≥ 1.5 IU/L, semen pH ≥ 7.2 , semen volume ≥ 1.4 ml, and normal liquefaction.

- + Physical exam: palpable vas deferens bilaterally, no abnormalities of the epididymis, and soft epididymis after ejaculation.

- Patients with severe oligozoospermia of unknown cause:

- + Sperm concentration < 5 million/ml in at least two tests, each test approximately three months apart.

- + FSH level may be elevated or normal (≥ 1.5 IU/L); semen pH ≥ 7.2 ; semen volume ≥ 1.4 ml; normal liquefaction.

- + Physical exam: palpable vas deferens bilaterally, no abnormalities of the epididymis, and soft epididymis after ejaculation.

2.2.6.2. *Collection of clinical and subclinical data*

2.2.6.3. *Blood sample collection*

After obtaining written informed consent, blood samples were collected for genetic analysis. All genetic tests were provided free of charge to participants.

2.2.6.4. *DNA extraction and quantification*

2.2.6.5. *PCR amplification of gene fragments containing the variants*

2.2.6.6. *Identification of gene variants using the RFLP method*

PCR products of each gene fragment meeting quality requirements were combined with the corresponding RFLP restriction enzyme, reaction buffer, and deionized water, then incubated at 37°C-65°C for 4 hours to digest the DNA at the target variant sites.

After the 4-hour incubation, PCR-RFLP products were electrophoresed on 3% agarose gel for *FSHB*, *FSHR* and *TEX14* variants, and 3.5% agarose gel for the *TEX11* variant.

The gel, after fluorescent staining, was placed in a gel documentation system for automatic imaging. Genotypes were determined based on the number of DNA bands and band sizes corresponding to digestion patterns at variant sites.

To validate the PCR-RFLP genotyping results, 5% of all samples were randomly selected for confirmation using Sanger sequencing.

2.2.8. Data Processing

Data were processed using SPSS version 26.0. p -value < 0.05 was considered statistically significant.

2.3. Ethical Considerations

The study was approved by the Ethics Council of the Vietnam Military Medical University, Approval Certificate No. 13/2022/CNChT-HĐĐĐ, dated December 30, 2022.

Chapter 3. RESEARCH RESULTS

3.1. Demographic characteristics, semen analysis, reproductive hormones, and testicular size

3.1.1. Demographic characteristics

The study population included 210 patients (40 with azoospermia and 170 with severe oligozoospermia) and 200 controls.

There was no significant difference in mean age between the patient and control groups ($p = 0.066$). Similarly, there was no difference between the proportions of subjects aged <40 versus ≥ 40 years ($p=0,158$).

There was no significant difference in smoking status between the two groups ($p>0.05$). However, alcohol consumption was significantly higher in the control group compared with the patient group ($p=0,028$).

In this study, there were no differences in height, weight, or BMI between the patient and control groups ($p>0.05$). When BMI was categorized into underweight, normal weight, overweight, and obese, no significant differences were observed ($p=0,153$).

Patients in the disease group had infertility durations ranging from 12 to 180 months, with an average of approximately 48 months. Additionally, 50,5% of patients had previously undergone one or more assisted reproductive technology (ART) treatments.

3.1.2. Semen Analysis Characteristics

Participants had an abstinence period of 2–7 days before semen collection. There were significant differences between the control group and the severe oligozoospermia subgroup in sperm count, motility, and morphology ($p < 0,001$). However, no significant differences were found in semen pH or volume between the groups ($p > 0,05$).

3.1.3. Reproductive Hormone Characteristics

The mean concentrations of FSH and LH in the patient group were significantly higher than those in the control group ($p < 0,001$). However, there were no significant differences in mean testosterone and prolactin levels between the two groups, with p -values of 0,933 and 0,391, respectively.

3.1.4. Testicular Size Characteristics

A comparison of testicular volume between the patient and control groups showed a statistically significant difference ($p < 0,001$).

3.2. Identification of *FSHB*, *FSHR*, *TEX11*, *TEX14* variants

3.2.1. *FSHB* rs10835638 variant

Table 3.7. Allele frequency, genotype frequency of the rs10835638 variant of *FSHB*

Group	Genotype			Allele frequency	
	GG n (%)	GT n (%)	TT n (%)	G n (%)	T n (%)
Azoospermia (n=40)	37 (92,5)	3 (7,5)	0 (0)	77 (96,25)	3 (3,75)
Severe oligozoospermia (n=170)	157 (92,35)	13 (7,65)	0 (0)	327 (96,18)	13 (3,82)
Patient (n=210)	194 (92,38)	16 (7,62)	0 (0)	404 (96,19)	16 (3,81)
Control (n=200)	194 (97)	6 (3)	0 (0)	394 (98,5)	6 (1,5)

For the azoospermia group ($n = 40$), 92,5% carried the GG genotype and 7,5% carried the GT genotype; no TT genotype was observed. The frequencies of the G and T alleles were 96,25% and 3,75%, respectively.

For the severe oligozoospermia group ($n = 170$), 92,35% carried the GG genotype and 7,65% carried the GT genotype; no TT genotype was observed. The frequencies of the G and T alleles were 96,18% and 3,82%, respectively.

When combining both patient groups (azoospermia and severe oligozoospermia), 92,4% were identified with the GG homozygous genotype and 7,6% with the GT heterozygous genotype, while no TT homozygous genotype was detected.

3.2.2.2. *FSHR* rs6166 variant

Bảng 3.8. Allele frequency, genotype frequency of the rs6166 variant of *FSHR*

Group	Genotype			Allele frequency	
	GG n (%)	GA n (%)	AA n (%)	G n (%)	A n (%)
Azoospermia (n=40)	7 (17,5)	17 (42,5)	16 (40)	31 (38,75)	49 (61,25)
Severe oligozoospermia (n=170)	18 (10,59)	72 (42,35)	80 (47,06)	108 (31,76)	232 (68,24)
Patient (n=210)	25 (11,9)	89 (42,38)	96 (45,71)	139 (33,1)	281 (66,9)
Control (n=200)	23 (11,5)	88 (44)	89 (44,5)	134 (33,5)	266 (66,5)

For the azoospermia group (n = 40), the frequencies of the GG, GA, and AA genotypes were 17,5%, 42,5%, and 40%, respectively; the G and A allele frequencies were 38,75% and 61,25%, respectively. For the severe oligozoospermia group (n = 170), the frequencies of the GG, GA, and AA genotypes were 10,59%, 42,35%, and 47,06%, respectively; the G and A allele frequencies were 31,76% and 68,24%, respectively.

Across the entire patient group (azoospermia and severe oligozoospermia), 25/210 (11,91%) individuals carried the GG genotype, 89/210 (42,38%) carried the GA genotype, and 96/210 (45,71%) carried the AA genotype.

3.2.2.3. *TEX11* rs4844247 variant

In the azoospermia group (n = 40), 82,5% carried the C allele and 17,5% carried the T allele. In the severe oligozoospermia group (n = 170), 87,06% carried the C allele and 12,94% carried the T allele.

Across both patient groups combined, 86.19% of alleles were C and 13.81% were T.

3.2.2.4. *TEX14* rs79813370 variant

In the azoospermia group (n = 40), 77,5% carried the GG genotype and 22,5% carried the GT genotype; no TT genotype was observed. The frequencies of the G and T alleles were 88,75% and 11,25%, respectively. In the severe oligozoospermia group (n = 170), the frequencies of the GG, GT, and TT genotypes were 84,12%, 14,71%, and 1,18%, respectively; the G and T allele frequencies were 91,47% and 8,53%, respectively.

Across the entire patient group (azoospermia and severe oligozoospermia), 82,86% were identified with the GG homozygous genotype, 16,19% with the GT heterozygous genotype, and 0,95% with the TT homozygous genotype.

3.3. Investigation of the association between *FSHB*, *FSHR*, *TEX11* and *TEX14* variants and clinical/subclinical characteristics

3.3.1. Evaluation of the genetic characteristics of the patient and control groups in relation to male infertility due to azoospermia and severe oligozoospermia

3.3.1.1. *FSHB* rs10835638 and *FSHR* rs6166 variants

* Group with male infertility due to azoospermia and severe oligozoospermia

Table 3.11.1. Association between *FSHB* and *FSHR* variants and male infertility due to azoospermia and severe oligozoospermia

Gene/SNP	Model	Control group (n = 200)	Patient group (n = 210)	OR	95% CI	p-value	
						χ^2	OR
<i>FSHB</i> / rs10835638	Additive						
	GG	194 (97%)	194 (92,38%)	1,000		0,038	
	GT	6 (3%)	16 (7,62%)	2,619	1,043-7,551		0,045
	Allele						
	G	394 (98,5%)	404 (96,19%)	1,000		0,041	
	T	6 (1,5%)	16 (3,81%)	2,556	1,030-7,302		0,048
<i>FSHR</i> / rs6166	Additive						
	GG	23 (11,50%)	25 (11,90%)	1,000		0,946	
	GA	88 (44,00%)	89 (42,38%)	0,93	0,491-1,762		0,825
	AA	89 (44,50%)	96 (45,71%)	0,992	0,526-1,874		0,981
	Recessive						
	GG+GA	111 (55,50%)	114 (54,3%)	1,000		0,805	
	AA	89 (44,50%)	96 (45,7%)	1,05	0,712-1,55		0,805
	Dominant						
	GG	23 (11,50%)	25 (11,9%)	1,000		0,899	
	GA+AA	177 (88,50%)	185 (88,1%)	0,962	0,526-1,757		0,899
	Allele						
	G	134 (33,50%)	139 (33,1%)	1,000		0,902	
	A	266 (66,50%)	281 (66,9%)	1,018	0,762-1,362		0,902

For the *FSHB* rs10835638 variant, there was a significant difference between the two groups. Specifically, the frequencies of the GT genotype and the T allele were higher in the patient group compared with the control group, with statistically significant p-values from the χ^2 test of 0,038 and 0,041, respectively. Additionally, both the GT genotype and the T allele were associated with infertility, with p-values from the OR analysis of 0,045 and 0,048, respectively.

Comparative analysis of the *FSHR* rs6166 variant showed that, under the additive, recessive, dominant, and allelic models, neither the genotypes nor the alleles demonstrated any statistically significant differences between the patient group and the control group.

* Group with male infertility due to severe oligozoospermia

Table 3.11.2. Association between *FSHB* and *FSHR* variants

And male infertility due to severe oligozoospermia

Gene/SNP	Model	Control group (n = 200)	Severe oligozoospermia (n = 170)	OR	95% CI	p-value	
						χ^2	OR
<i>FSHB</i> / rs10835638	Additive						
	GG	194 (97%)	157 (92,35%)	1,000		0,044	
	GT	6 (3%)	13 (7,65%)	2,677	0,995-7,205		0,051
	Allele						
	G	394 (98,5%)	327 (96,18%)	1,000		0,046	
	T	6 (1,5%)	13 (3,82%)	2,611	0,981-6,945		0,055
<i>FSHR</i> / rs6166	Additive						
	GG	23 (11,5%)	18 (10,59%)	1,000		0,879	
	GA	88 (44%)	72 (42,35%)	1,045	0,524-2,086		0,900
	AA	89 (44,5%)	80 (47,06%)	1,149	0,578-2,282		0,693
	Recessive						
	GG+GA	111 (55,5%)	90 (52,94%)	1,000		0,622	
	AA	89 (44,5%)	80 (47,06%)	1,109	0,735-1,671		0,622
	Dominant						
	GG	23 (11,5%)	18 (10,59%)	1,000		0,781	
	GA+AA	177 (88,5%)	152 (89,41%)	0,911	0,474-1,752		0,781
	Allele						
	G	134 (33,5%)	108 (31,76%)	1,000		0,616	
	A	266 (66,5%)	232 (68,24%)	1,082	0,795-1,473		0,616

For the *FSHB* rs10835638 variant, there was a significant difference between the two groups; specifically, the frequencies of the GT genotype and the T allele were higher in the group with male infertility due to severe oligozoospermia compared with the control group, with statistically significant $p(\chi^2)$ of 0,044 and 0,046, respectively. However, neither the GT genotype nor the T allele was associated with male infertility due to severe oligozoospermia, as indicated by $p(\text{OR}) > 0,05$.

For the *FSHR* rs6166 variant, no significant differences were observed in genotype or allele frequencies between the severe oligozoospermia group and the control group, with χ^2 p-values $> 0,05$.

* Azoospermia group

When analyzed separately by comparing the azoospermia group with the control group, both *FSHB* rs10835638 and *FSHR* rs6166 showed no significant differences in genotype or allele frequencies between the two groups, with $p(\chi^2) > 0,05$.

3.3.1.2. *TEX11 rs4844247, TEX14 rs79813370 variants*

Both variants showed no significant differences in genotype or allele frequencies between the control group and the groups with male infertility due to azoospermia and severe oligozoospermia/male infertility due to severe oligozoospermia/azoospermia, with $p(\chi^2) > 0,05$.

3.3.2. *Multivariable logistic regression model between variants, clinical characteristics, and male infertility due to azoospermia and severe oligozoospermia*

Multivariable binary logistic regression was applied, in which clinical risk factors included age and BMI ($p < 0,2$), alcohol consumption ($p < 0,05$), together with all genetic variants examined in the study.

Table 3.13. Multivariable logistic regression analysis of the association between *FSHB*, *FSHR*, *TEX11*, *TEX14* variants and selected clinical characteristics with male infertility

Characteristics	OR	95% CI	<i>p</i> -value
Age ≥ 40	0,617	0,358-1,065	0,083
Drink alcohol	0,603	0,354-1,025	0,062
BMI underweight	0,405	0,062-2,633	0,344
BMI overweight	0,694	0,435-1,106	0,125
BMI obesity	0,688	0,409-1,158	0,159
<i>FSHB</i> rs10835638 GT	3,087	1,132-8,416	0,028
<i>FSHR</i> rs6166 GA	0,866	0,449-1,672	0,669
<i>FSHR</i> rs6166 AA	0,930	0,487-1,778	0,827
<i>TEX11</i> rs4844247 T	0,974	0,539-1,760	0,930
<i>TEX14</i> rs79813370 GT	0,824	0,482-1,408	0,478
<i>TEX14</i> rs79813370 TT	0,860	0,118-6,285	0,882

Table 3.14. Multivariable logistic regression analysis of the association between *FSHB*, *FSHR*, *TEX11*, *TEX14* variants and selected clinical characteristics with male infertility due to severe oligozoospermia

Đặc điểm	OR	95% CI	Giá trị <i>p</i>
Age ≥ 40	0,682	0,386-1,204	0,187
Drink alcohol	0,621	0,355-1,084	0,094
BMI underweight	0,286	0,029-2,837	0,285
BMI overweight	0,563	0,343-0,923	0,023
BMI obesity	0,610	0,353-1,053	0,076
<i>FSHB</i> rs10835638 GT	3,103	1,099-8,757	0,032
<i>FSHR</i> rs6166 GA	1,010	0,495-2,062	0,978
<i>FSHR</i> rs6166 AA	1,097	0,542-2,220	0,796
<i>TEX11</i> rs4844247 T	0,949	0,502-1,793	0,872
<i>TEX14</i> rs79813370 GT	0,714	0,397-1,284	0,261
<i>TEX14</i> rs79813370 TT	1,031	0,141-7,551	0,976

The analysis showed that the *FSHB* rs10835638 GT genotype is associated with infertility due to azoospermia or severe oligozoospermia, with OR = 3,087; $p = 0,028$; and it is also associated with infertility due to severe oligozoospermia, with OR = 3,103; $p = 0,032$.

3.3.3. Evaluation of the association between genetic characteristics and semen parameters, reproductive hormones, and testicular volume in the patient group

3.3.3.1. Association with semen parameters in the patient group

* *FSHB* rs10835638 variant

There was a difference in sperm concentration among the genotypes; specifically, individuals with the GT genotype had a lower sperm concentration compared with those with the GG genotype (median = $1,64 \times 10^6/\text{ml}$ vs. $3,29 \times 10^6/\text{ml}$). However, this difference did not reach statistical significance ($p = 0,12$).

Meanwhile, regarding sperm motility, the *FSHB* rs10835638 GT genotype was associated with a decreased proportion of rapidly progressive sperm, with $p=0,024$.

* *FSHR* rs6166 variant

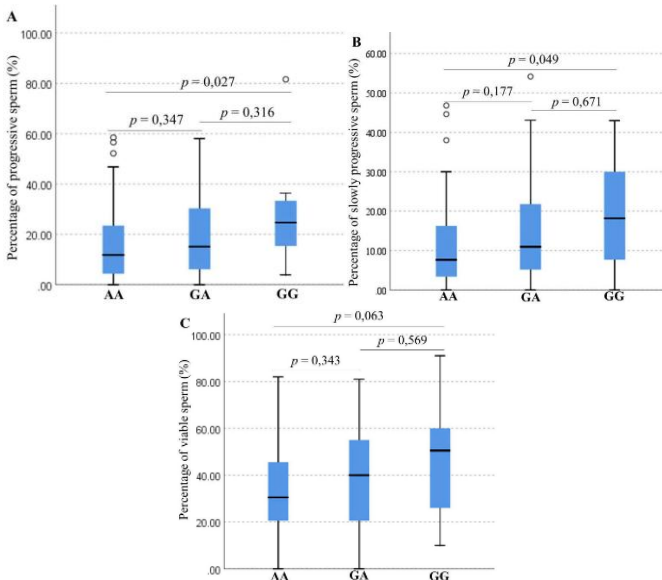


Figure 3.11. Association between the rs6166 variant of *FSHR* and semen parameters in the severe oligozoospermia group

Caption: A: Percentage of progressive sperm;

B: Percentage of slowly progressive sperm; C: Percentage of viable sperm.

The comparative analysis of genotype pairs in the severe oligozoospermia group showed that the AA genotype was significantly associated with reduced sperm motility compared with the GG genotype-specifically, a lower proportion of rapidly progressive sperm ($p=0,027$) and a lower proportion of slowly progressive sperm ($p=0,049$).

* *TEX11* rs4844247 variant

In the severe oligozoospermia group, there was no difference in semen parameters between carriers of the C and T alleles ($p > 0,05$).

* *TEX14* rs79813370 variant

In the severe oligozoospermia group, no differences in semen parameters were observed among the different genotypes of this variant.

3.3.3.2. Association between genetic characteristics and reproductive hormone concentrations in the patient group

* *FSHB* rs10835638 and *FSHR* rs6166 variants

- Infertility due to azoospermia or severe oligozoospermia:

There was no association between the genotypes of *FSHB* rs10835638 or *FSHR* rs6166 and the concentrations of FSH, LH, testosterone or prolactin.

- Infertility due to severe oligozoospermia:

+ The *FSHB* rs10835638 variant, no association was observed with FSH, LH, testosterone or prolactin concentrations ($p>0,05$).

+ Similarly, the *FSHR* rs6166 variant showed no association with FSH, LH or prolactin concentrations. However, a significant difference was observed in testosterone concentrations among the GG, GA, and AA genotypes.

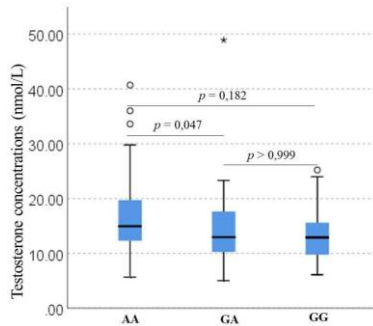


Figure 3.12. Association between the rs6166 variant of *FSHR* and testosterone concentrations in the severe oligozoospermia group.

Analysis of the detailed genotype comparisons within the severe oligozoospermia subgroup showed that the *FSHR* rs6166 GA genotype differed significantly from the AA genotype in testosterone concentrations ($p=0,047$). Specifically, the AA genotype was associated with higher testosterone concentrations compared with the GA genotype.

- Azoospermia subgroup:

No association was found between the genotypes of *FSHB* rs10835638 or *FSHR* rs6166 and the concentrations of FSH, LH, testosterone, or prolactin in the azoospermia group ($n = 40$).

* *TEX11* rs4844247 and *TEX14* rs79813370 variants:

There was no association between these variants and FSH, LH, testosterone, or prolactin concentrations in the overall patient group ($n=210$)/the severe oligozoospermia group ($n=170$)/ the azoospermia group ($n=40$).

3.3.3.3. Association between genetic characteristics and testicular volume in the patient group

The results showed that, in all groups (overall patients: $n = 210$; severe oligozoospermia: $n = 170$; azoospermia: $n = 40$), there was no association between *FSHB* rs10835638, *FSHR* rs6166, *TEX11* rs4844247, or *TEX14* rs79813370 variants and the volume of the right testis, left testis, or total testicular volume.

Chapter 4. DISCUSSION

4.1. Variants of the *FSHB*, *FSHR*, *TEX11* and *TEX14* and their association with infertility due to azoospermia and severe oligozoospermia

4.1.1. *FSHB* variant

In the group of 210 patients with azoospermia or severe oligozoospermia, the genotype frequencies were 92.38% for GG and 7.62% for GT. The TT genotype was not observed in any individual (Table 3.7).

Several studies examining the *FSHB* -211G>T variant in men have reported all three genotypes (GG, GT, TT), although the TT genotype is consistently rare. In a cohort of 255 patients with unexplained azoospermia or oligozoospermia in China, Wu Q. (2025) identified only one individual carrying the TT genotype (0.4%). Similarly, Grigorova M. (2011) reported only 14 TT carriers (1.3%) among 1,054 Estonian men.

A study on female infertility by Polyzos N.P. (2021) also found that the TT genotype appeared only in European populations (1.2%) and was absent in Asian populations, including Vietnam.

These findings suggest that the *FSHB* -211TT genotype is extremely rare in Asian populations, and likely explains why it was not detected in our sample.

Our results show that the GT genotype and the T allele are associated with infertility due to azoospermia or severe oligozoospermia (Tables 3.11.1 and 3.13), as well as infertility due to severe oligozoospermia (Tables 3.11.2 and 3.14). Similar associations were reported by Zanganehnejad Z. (2025) in a cohort of 120 infertile men with impaired spermatogenesis and 120 healthy controls.

However, some studies present inconsistent findings. For example, Neslihan Hekim (2022) suggested that the wild-type *FSHB* -211GG genotype was associated with azoospermia and oligozoospermia in Turkish men. Meanwhile, Wu Q. (2017), in a

cohort of 255 Chinese men with unexplained infertility, found no association between -211G>T and infertility overall or infertility due to azoospermia/severe oligozoospermia.

4.1.2. *FSHR* variant

The frequencies of the GA and AA genotypes were higher than the wild-type GG genotype. Several studies in men with impaired spermatogenesis have reported similar genotype distributions for the *FSHR* 2039G>A variant.

In our study, *FSHR* 2039G>A was not associated with infertility due to azoospermia or severe oligozoospermia. This finding aligns with previous reports in various populations, such as those by Pengo M. (2006), Ghirelli-Filho M. (2012), and Wu Q. (2017).

However, Ali A.R. (2021) reported that the A allele reduced the risk of non-obstructive azoospermia, whereas Balkan M. (2010) found that the A allele was associated with impaired spermatogenesis. Some studies also link the A allele to testicular cancer, while no such associations have been reported for the G allele.

Clinical trials have also produced contradictory observations: Simoni M. suggested that the AA genotype responds better to FSH stimulation, whereas Selice R. reported a better response among genotypes carrying the G allele.

These inconsistencies suggest that the role of the *FSHR* 2039G>A variant may be population-dependent, influenced by ethnic or geographic factors. In our study, the variant may exhibit a codominant pattern between the G and A alleles.

4.1.3. *TEX11* variant

Among the patients, allele C accounted for 86,19% and allele T for 13.81%. The T allele frequency was considerably lower than that of the C allele, consistent with findings by Zhang X. (2015) in 316 Chinese infertile men.

Analysis revealed no association between this variant and infertility due to azoospermia or severe oligozoospermia. This aligns

with studies in European men (Aston K.I., 2010) and Chinese men (Zhang X., 2015). However, Kolmykov (2021) reported that the T allele was associated with male infertility in a Russian population.

4.1.4. *TEX14* variant

Genotype frequencies were 82,86% for GG, 16,19% for GT, and 0,95% for TT. These are similar to findings by Tran Huu Dinh et al. (2024), who reported an association between *TEX14* rs79813370 and impaired spermatogenesis at various levels. However, in our study focusing specifically on azoospermia and severe oligozoospermia, no significant associations were observed.

4.2. Associations between *FSHB*, *FSHR*, *TEX11*, *TEX14* variants and semen parameters, reproductive hormones, and testicular volume

4.2.1. *FSHB* variant

In severe oligozoospermia, the GT genotype was associated with reduced progressive sperm motility, consistent with findings by Zanganehnejad Z. (2025) and Bang A.K. (2021).

Although some studies reported an association between the T allele and reduced spermatogenesis, we did not observe a statistically significant difference in sperm concentration between the GG and GT genotypes ($p=0,12$), even though GT carriers tended to have lower sperm counts. The wide variation in abstinence time (2–7 days) across different centers may have affected semen concentration measurements.

Additionally, while we excluded men currently using fertility-related medications, 50.5% had undergone prior assisted reproductive treatments, which may have altered hormone levels. This may explain why we did not observe lower FSH concentrations among GT carriers, despite prior studies indicating such associations when confounding factors were better controlled.

Previous studies linking the T allele with reduced testicular volume may also reflect differences in FSH levels, which were not evident in our cohort.

4.2.2. *FSHR* variant

In the severe oligozoospermia group (n=170), the AA genotype was associated with reduced sperm motility compared with the GG genotype, including progressive motility ($p=0,027$) and slow progressive motility ($p=0,049$) (Figure 3.11).

Regarding reproductive hormones, the AA genotype was associated with higher testosterone concentrations than GA (statistically significant) and higher than GG (not statistically significant) (Figure 3.12).

These findings suggest that the AA genotype may influence later stages of spermatogenesis, particularly spermatid differentiation and tail formation-processes heavily dependent on testosterone. Therefore, according to endocrine physiological mechanisms, patients carrying the AA genotype may exhibit higher testosterone levels as a compensatory response to suppress abnormalities in sperm tail formation.

There was no association between genotype and testicular volume, likely due to the similar FSH concentrations across all genotypes.

4.2.3. *TEX11* variant

No significant differences in semen parameters were observed between carriers of allele C and allele T. This contrasts with findings from Kolmykov S. in 157 Russian men, where the T allele was associated with lower sperm count, concentration, motility, and normal morphology.

We also found no associations between the *TEX11* rs4844247 variant and reproductive hormone levels or testicular volume.

4.2.4. *TEX14* variant

Analysis revealed no significant associations between *TEX14* rs79813370 genotypes (GG, GT, TT) and semen parameters, reproductive hormone concentrations (FSH, LH, testosterone, prolactin) or testicular volume. Thus, this variant does not appear to contribute to azoospermia or severe oligozoospermia in our study population.

CONCLUSION

Based on the analysis of *FSHB*, *FSHR*, *TEX11* and *TEX14* variants in 410 individuals includes 210 patients (40 azoospermia, 170 severe oligozoospermia) and 200 controls, the following conclusions can be drawn:

1. Genotype and allele frequencies were determined for *FSHB*, *FSHR*, *TEX11* and *TEX14* variants in 210 patients with azoospermia or severe oligozoospermia of unknown cause:

- *FSHB* rs10835638 variant consisted of 92,38% GG and 7,62% GT genotypes, with no TT genotype detected. Specifically, in the severe oligozoospermia subgroup, 92,35% carried GG and 7,65% carried GT, with no TT genotype; in the azoospermia subgroup, 92,5% carried GG and 7,5% carried GT, also with no TT genotype.

- *FSHR* rs6166 variant included 11,9% GG, 42,38% GA, and 45,71% AA genotypes. In the severe oligozoospermia group, the respective frequencies were 10,59% GG, 42,35% GA, and 47,06% AA. In the azoospermia group, the frequencies were 17,5% GG, 42,5% GA, and 40% AA.

- *TEX11* rs4844247 variant, allele C accounted for 86,19% and allele T for 13,81%. In the severe oligozoospermia subgroup, allele C was 87,06% and allele T was 12,94%. In the azoospermia subgroup, allele C was 82,5% and allele T was 17,5%.

- *TEX14* rs79813370 variant included 82,86% GG, 16,19% GT, and 0,95% TT genotypes. In the severe oligozoospermia subgroup, 84,12% were GG, 14,71% GT, and 1,18% TT. In the azoospermia subgroup, 77,5% were GG, 22,5% GT, and no TT genotype was observed.

2. Assessment of the association between the genetic variants and the clinical and paraclinical characteristics of the patient group

- *FSHB* rs10835638 variant was associated with male infertility due to azoospermia and severe oligozoospermia, with higher frequencies of the GT genotype and T allele in the patient group compared with controls: GT genotype: OR=2,619 (95% CI: 1,043-

7,551; $p=0,038$); T allele: OR=2,556 (95% CI: 1,030-7,302; $p=0,041$). It was also associated with infertility due to severe oligozoospermia: GT genotype: OR=2,667 (95% CI: 0,995-7,205; $p=0,044$); T allele: OR = 2,611 (95% CI: 0,981-6,945; $p=0,046$). The GT genotype may be a risk factor for reduced rapid progressive sperm motility. Sperm concentration in GT carriers was lower than in GG carriers, although not statistically significant ($p=0,12$). The GT genotype showed no association with FSH, LH, testosterone, prolactin, or testicular volume.

- *FSHR* rs6166 variant was not associated with male infertility due to azoospermia or severe oligozoospermia. However, in the severe oligozoospermia group, the AA genotype was associated with reduced sperm motility compared with the GG genotype and increased testosterone levels with genotypes carrying the G allele (significant compared with GA, but not with GG). No associations were observed between this variant and concentrations of FSH, LH, prolactin, or testicular volume.

- *TEX11* rs4844247 and *TEX14* rs79813370: Both variants showed no significant associations with infertility due to azoospermia or severe oligozoospermia. Likewise, neither variant was associated with semen parameters, FSH, LH, testosterone, prolactin, or testicular volume.

RECOMMENDATIONS

1. Studies with larger sample size focusing specifically on azoospermia are necessary to better evaluate associations between the *FSHB*, *FSHR*, *TEX11* and *TEX14* variants and azoospermia.

2. It is advisable to recruit study participants from the same medical facility and to narrow the range of abstinence periods prior to semen analysis. In addition, only patients who have never undergone any assisted reproductive treatment should be included, in order to better control confounding factors that may affect clinical and paraclinical characteristics.