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RESEARCH PAPER

Human protamine 1 gene structure and function affected by rs35576928 mutation: a bioinformatics analysis

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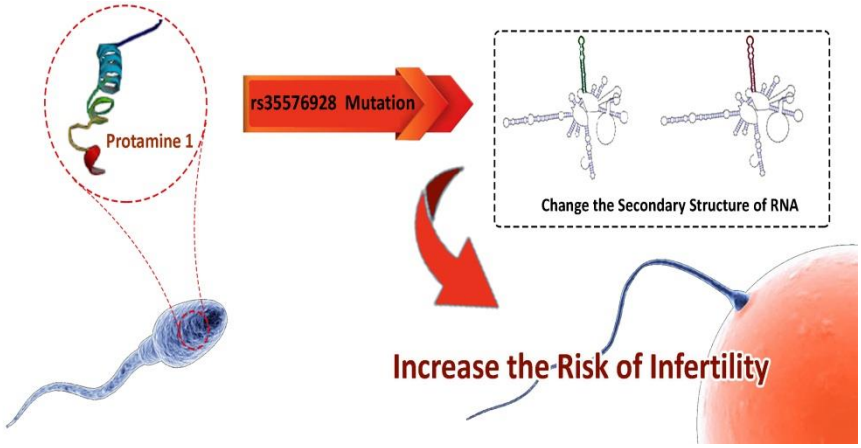
Highlights

- Protamine is a vital protein for sperm structure and function.
- There are two types of protamine in the structure of human sperm, usually in the same proportion as normal sperm.
- Protamine 1 contains several polymorphisms that can affect its structure and function.
- The polymorphism rs35576928 is an exonic variant that can affect the structure of the RNA and protein of protamine.

Graphical Abstract



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Abstract

Human sperm contain most type 1 and type 2 protamines (PRM1) and (PRM2), protamines, which have about equal proportions in normal sperm. Protamines can infertile if they change structure or function. Nucleotide polymorphisms are one cause of changes in protamine structure and function. For protamine 1 the genetic variation rs35576928 is known. In. this study bioinformatics approach was used to investigate the effects of the rs35576928 mutation on protamine 1 structure and function. matics tools including RNAsnp, NetGene2, in this study several bioinformatics tools were used to investigate how the polymorphism affects RNA structure and splicing. The tools used included RNAsnp, NetGene2, and ASAP. To evaluate the effects of rs35576928 on proteins, other tools such as SNAP and ExPASy were also used. The analyzes have also shown that this polymorphism has the primary, secondary and The results of this study have also shown that this polymorphism has primary, secondary, and the results of this study have also shown that this polymorphism has primary, secondary, and results of this study have also shown that protamine 1 has the following functions: general functions. Infertile males may be affected by rs35576928 as it has destructive effects on protamine RNA and protein structure.

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Introduction

Protamine is a protein that has many positively charged residues that form a very compact set of sperm genomes (1). Also, the presence of cysteine residues in the structure of protamines causes the formation of disulfide bridges between adjacent protamines and ultimately leads to the formation of a strong nucleoprotamine complex (2). Evidence is available that protamine is derived from histone H1 (3). High evolutionary diversity is another characteristic of protamine proteins (4). Darwinian positive choice can be one of the reasons for this rapid evolution (5). Comparison of protamine sequences from different species reinforced this hypothesis, as sequence comparisons showed that there were large amounts of non-synonymous mutations in their structure (5).

The human genome has a copy of the protamine 1 (PRM1) gene and a copy of the protamine 2 (PRM2) gene located on chromosome 16 (6). Both genes contain an intron. The gene sequences of protamine 1 and protamine 2 are located on a genomic domain along with the transition protein 2 (TNP2) gene and a gene sequence called gene 4 (7). Such special organization leads to the organized expression of these genes during the spermatogenesis process. Protamine 1 and protamine 2 together with transcription factor 2 have high expression but the expression of gene 4 is very low or sometimes not expressed at all (8). Therefore, more studies are needed to determine the possibility of gene 4 being a pseudogene. Gene 4 has been termed protamine 3, and it has been suggested that this gene may have originated from a duplication of the protamine 1 gene (9). Since the predicted amino acid sequence of genes 4, nothing like protamine, so-called protamine 3 for the gene is not common. The sequence of this gene is free of arginine and is rich in glutamic acid. Thus, the protein from gene 4 may not bind to DNA and may not be true protamine (9).

In human sperm, the levels of protamine 1 and protamine 2 are equal (P1/P2 ratio is 1) but their function is different (10). Differences in the function of protamine 1 and protamine 2 include the following: 1) Protamine 2, unlike protamine 1, is a zinc-finger protein and contains a Cys2-His2 motif (11). 2) Protamine 1 is present in all mammals, while protamine 2 is present only in some mammals, which indicates a more basic and protected function of protamine 1. Changes in protamines 1 and 2 in infertile men show different effects (12). Both protamines 1 and 2 must undergo post-translational modifications to bind to DNA and form a strong complex. There are many mutations in the protamine 1 and 2 genes. Single nucleotide polymorphisms (SNPs) in protamine 1 and 2 genes can increase the risk of male infertility (13). For example, four synonymous SNPs in protamine 1 and one missense SNP (C248T) in protamine 2, as well as one SNP in the 3' region of protamines 1 and 2, are very common. C248T polymorphism in the protamine 2 gene can cause immature mRNA formation and eventually infertility. All of these mutations have little effect on male fertility (13, 14). In 2012, He et al., conducted a study on protamines in infertile men. They determined the allelic frequency for different variants of PRM1 and PRM2 in azoospermic and oligospermic individuals. Their study showed that the rs35576928 variant in the PRM1 gene was significantly associated with severe oligospermia (15). The aim of this study was to investigate the effect of rs35576928 polymorphism on the structure and function of protamine 1 through bioinformatics analysis.

Materials and Methods

Screening and characterization of the studied polymorphism

There are many single nucleotide polymorphisms in the sequence of protamine 1 and 2 genes. These polymorphisms were found in different places; some were upstream of the gene, some were introns or exons, and some were downstream of the gene. Due to the importance of non-synonymous polymorphisms, the focus was on the selection of exonic missense varieties. Common polymorphisms of protamines 1 and 2 were extracted by a systematic search in databases such as PubMed and Google Scholar. Protamine polymorphisms associated with male infertility were analyzed and finally, an exon and non-synonymous rs35576928 polymorphism was selected for the study (16).

The polymorphism was analyzed by the National Center for Biotechnology Information (NCBI) and SNPedia databases and it was found that this polymorphism could also be considered an upstream variety. The minor allele frequency (MAF) of this genetic variety ranged from 0.008 to 0.039. This polymorphism, which had the amino acid code p.Arg34Ser and the genomic code c.102G> C, was eventually nominated for bioinformatics studies.

Investigation of the effect of rs35576928 polymorphism on RNA structure and splicing

Since rs35576928 polymorphism is an exon variety, it can affect both protein structure and RNA structure and splicing process. The effect of this variety on RNA structure was investigated by RNAsnp software (https://rth.dk/resources/rnasnp). To do this, first, the protamine 1 gene sequence was obtained from NCBI and then the coding sequence (CDS) region was obtained by the relevant link. After locating the polymorphic region on the CDS, the protamine 1 sequence was presented to RNAsnp software and then the program was run. The results were obtained by specific plot and RNA schematic structure, before and after mutation.

Based on the above software algorithm, a p-value less than 0.2 was considered as a significant level. To investigate the effect of rs35576928 polymorphism on the splicing process, the protamine 1 nucleotide sequence was first obtained from NCBI and the position of the above polymorphism on this sequence was determined. The sequence was then presented to ASSP (Alternative Splice Site Predictor) and NetGene2 online software in two variants containing different polymorphism alleles. After running, the relevant diagrams were obtained and analyzed.

Investigation of the effect of rs35576928 polymorphism on protein function

As mentioned, rs35576928 polymorphism is a non-synonymous exon variety. Therefore, it can affect the structure and function of the protein. To investigate the effect of the above polymorphism on the structure and function of protamine 1, the CDS sequence of this gene was first taken from NCBI and translated into protein by ExPASy online software. The polymorphic position on the protein structure was then determined. The primary structure characteristics of the protein were investigated before and after mutation. The secondary structure of the protein after the mutation was investigated using the Chou-Fasman method. Then the mentioned protein sequence was entered into SNAP software and the destructive effects of rs35576928 polymorphism on protamine 1 structure were identified. Also, the change in hydrophobic properties of the protein after the mutation was investigated by ExPASy online software.

Results

Effects of rs35576928 variety on RNA structure and splicing process

The rs35576928 polymorphism, although an exon variety, can also alter the splicing process and RNA structure. Analysis of the splicing process after polymorphism was performed with online software ASSP and NetGene2. After loading the nucleotide sequence of protamine 1 with different genotypes in the above software, interesting results were obtained. The results of ASSP showed that the splicing pattern in the polymorphic region is different between G and T genotypes (Figure 1). Data from NetGene2 also showed that the rs35576928 variety could change the splicing pattern in the polymorphic area (Figure 2).

The effect of rs35576928 polymorphism on RNA structure was investigated by RNAsnp online software. To perform this project, the immature RNA sequence of protamine 1 was loaded and run in the above software. The data obtained from this software showed that rs35576928 polymorphism significantly (Folding window: 2-399, Local region: 169-218, Distance: 0.2391, p-value: 0.0629) changes the secondary structure of RNA (Figure 3). A significance level less than 0.2 in this software is considered significant. The data showed that the minimum free energy for the structure containing genotype T was equal to -111.30 kcal/mol while this energy for the structure containing genotype G was equal to -109.80 kcal/mol. This increase in the minimum free energy can cause RNA instability.

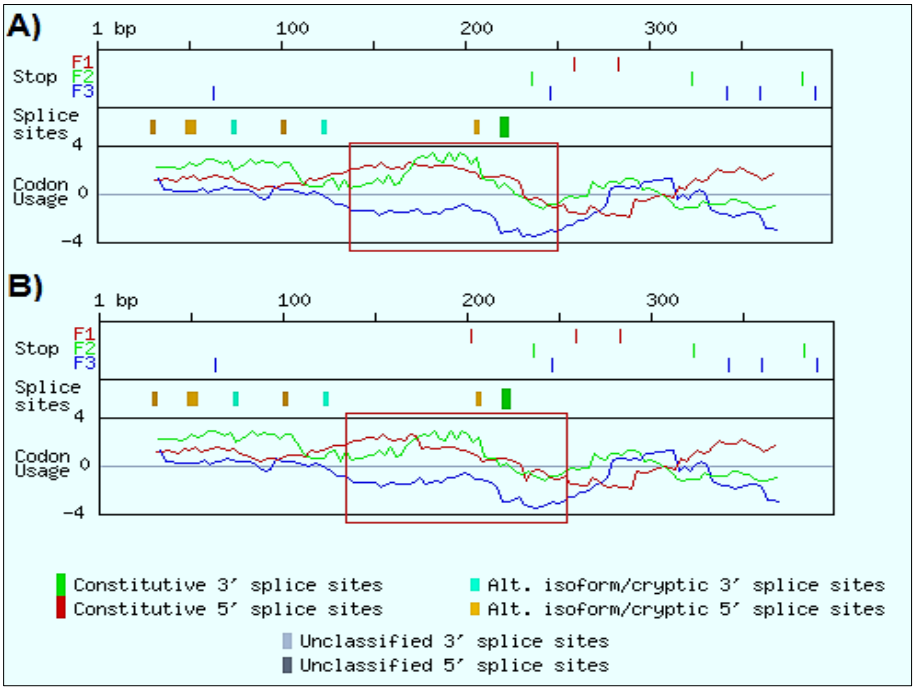

Figure 1. Results of ASSP server. The data revealed that the rs35576928 polymorphism could alter the splicing pattern in T allele (A) than G allele (B).

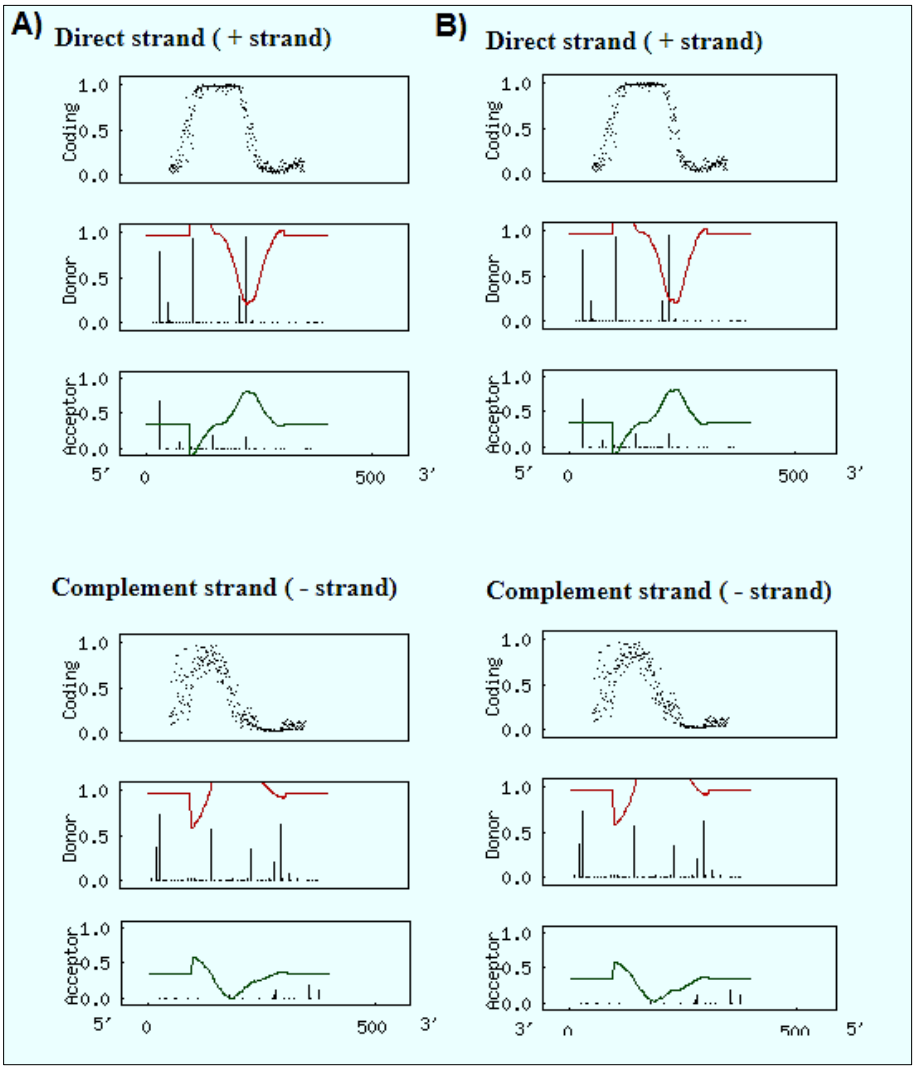

Figure 2. Results of NetGene2 server. The data from this server revealed that the pattern of splicing alters in both direct and complement strands in T allele (A) than G allele (B).

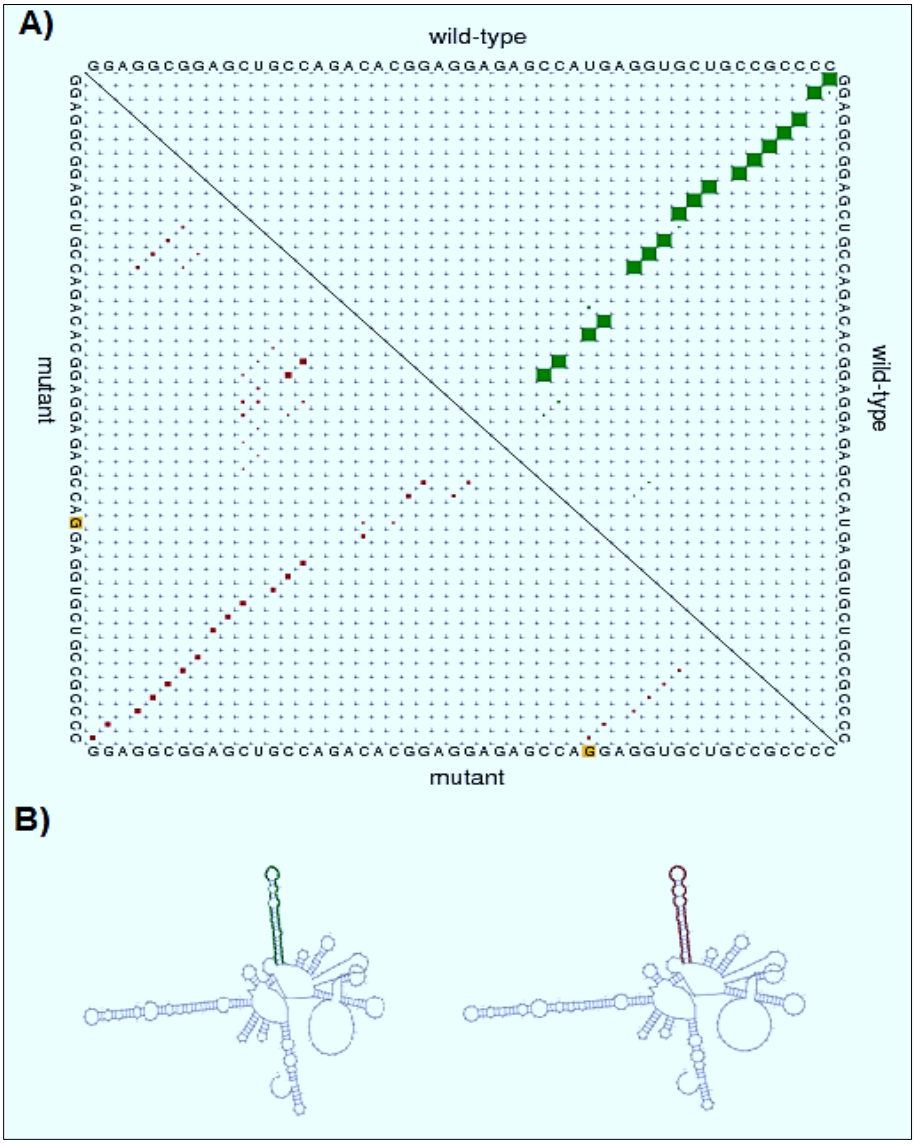

Figure 3. Data from RNAsnp server. Data revealed that the rs35576928 polymorphism could change the plot (A) and secondary structure (B) of RNA.

Effects of rs35576928 variety on PRM1 protein structure

The effects of rs35576928 polymorphism on the primary, secondary and overall structures of protamine 1 were evaluated. After translating the nucleotide sequence of protamine into peptide sequence, the effects of the above polymorphism on the first structure of the protein were evaluated by ExPASy software. The results showed that the genotype containing arginine contains 51 amino acids and its molecular weight is equal to 6822.99 dalton. The theoretical pI of this variety was 12.08 and its half-life was 30 hours. Its instability index was 167.66 while its aliphatic index was 3.92. But the genotype containing serine amino acid had a molecular weight of 6753.88 and a theoretical pI of 12.04. Its instability index was estimated at 157.59 and its aliphatic index was 3.92. The half-life of this variety was estimated at 30 hours.

Analysis of the secondary structure of the protein based on the Chou-Fasman algorithm showed that the secondary structure of the protein changes after the mutation. Also, hydrophobic analysis of proteins based on the Kyte & Doolittle method showed that after the replacement of serine amino acid in the polymorphic region, protein hydrophobicity increases in this region. The overall effect of rs35576928 polymorphism on PRM structure was analyzed using SNAP software. The data obtained from this software showed that the replacement of the amino acid serine with the amino acid arginine in codon 34 causes significant changes in the structure and function of the protein. As shown in Table 1, this replacement with a score of 53 and Expected Accuracy of 75% has detrimental effects on protein structure.

Table 1. SNAP results at codon 34 of protamine 1.

Wild type	Amino Acid Position	Variant Amino Acid	Predicted Effect	Score	Expected Accuracy (%)
R	34	A	effect	58	75
R	34	R	neutral	-99	97
R	34	N	effect	58	75
R	34	D	effect	74	85
R	34	C	effect	59	75
R	34	Q	effect	48	71
R	34	E	effect	69	80
R	34	G	effect	71	85
R	34	H	effect	52	75
R	34	I	effect	57	75
R	34	L	effect	61	80
R	34	K	effect	32	66
R	34	M	effect	59	75
R	34	F	effect	63	80
R	34	P	effect	71	85
R	34	S	effect	53	75
R	34	T	effect	44	71
R	34	W	effect	77	85
R	34	Y	effect	59	75
R	34	V	effect	56	75

Discusion

Infertility occurs in 10 to 15% of couples worldwide and about half of the causes of infertility are in men (15). With the advancement of molecular biology, many genes involved in infertility have been identified. Defects in genes involved in spermatogenesis can cause infertility (17). Polymorphisms in autosomal genes such as CFTR, BRCA2, etc. have been shown to play an important role in male infertility (18). Another gene that has been studied extensively is the POLG gene, which encodes DNA polymerase gamma this protein is involved in the replication and modification of mitochondrial DNA, which has been shown to replicate the variable CAG at the N-terminus of the protein, altering enzyme function and thereby altering infertility in men (19, 20). Another gene that has been studied extensively is the FSHR gene, which is the product of the FSH hormone receptor gene. Two SNPs have been identified in exon 7 of this gene. Studies show that these SNPs are associated with infertility in men (21-23).

Other genes in which polymorphism can increase the risk of infertility is the protamine 1 and 2 genes. Protamines are proteins that have many positively charged amino acids, which results in the formation of a highly compact set of genomic DNA (24). In addition, the presence of the amino acid cysteine in the structure of protamines causes disulfide bridges between adjacent protamines and forms a strong nucleoprotamine complex (24). Therefore, any inappropriate change in the structure of the protamine gene can disrupt the nucleoprotein complex and result in infertility. There are many polymorphisms in the structure of protamines 1 and 2 that may affect the function of these genes. In this study, we evaluated the effect of rs35576928 polymorphism on the structure of PRM1 using a bioinformatics approach.

The results of our study showed that the above polymorphism can change both RNA structure and splicing pattern and can affect the primary, secondary and overall structure of the protein. Therefore, the pathological effects of this polymorphism in infertile men may be due to these results. There are some studies that have examined the effect of this polymorphism on different types of male infertility (25).

For example, a study by the He et al., 2012 determined the allelic frequency for different variants of PRM1 and PRM2 in individuals with azoospermia and oligospermia (15). Their study showed that the rs35576928 variant in the PRM1 gene was significantly associated with severe oligospermia. However, no significant relationship was observed between this SNP and azoospermic individuals. Jodar et al., reported in 2011 that rs35576928 may be an important risk factor for male infertility. However, their study did not find a significant relationship between rs35576928 and azoospermia in Spanish and Swedish men (26). Other studies have shown that variant rs35576928 in PRM1 is not associated with oligospermia and azoospermia in men (14, 26-28).

Contradictory results in different studies can be due to racial and geographical differences, epigenetic differences, and differences in the diet of the studied populations. Protamine protection is vital in mammals. Small changes in the coding and non-coding regions of protamine genes can severely affect the stability and expression of this gene. The molecular mechanisms involved in the abnormal expression of protamine protein and its association with spermatogenesis alteration has not yet been clearly identified, but the possible reasons could be as follows: first, abnormal varieties of protamine 1 and 2 are more common in infertile individuals than in fertile individuals (14, 26, 29, 30). Second, patients with abnormal protamine expression show severe defects in their semen quality (13, 31, 32). However, animal models with deficient protamine expression show problems with spermatogenesis. Protamine expression may act as a control point during spermatogenesis. Therefore, any change in it can disrupt the sperm production process. Abnormal expression of protamine can lead to increased levels of apoptosis (30, 33). Alteration of seminiferous tubules or incomplete apoptosis can lead to heterogeneous clinical features such as azoospermia and oligospermia (29, 34). Further studies are needed to determine the biological effects of PRM1 and PRM2 variants on spermatogenesis.

Conclusion

Protamine is a vital protein in the sperm structure that can cause the sperm genome to be tightly packaged. This protein has two types, 1 and 2, which are usually equal in proportion to normal sperm. Based on the importance of this protein in sperm structure, any change in their structure and function can affect sperm function and the male reproductive system. One of the factors that can change their structure and function is single nucleotide polymorphisms. There are many single nucleotide polymorphisms in the structure of protamines, one of the most important is the rs35576928 polymorphism in the PRM1 sequence. This genetic variant can alter RNA structure, splicing process, and the structure and function of protamine 1. Therefore, the pathological effects of this variety in infertile men may be due to these reasons. However, for more accurate results, further tests, including in vitro and in vivo analysis, are needed.

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