GENETIC POLYMORPHISMS OF *CRISP2* GENE IN ASSOCIATION WITH INFERTILITY IN IRAQI PATIENTS

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ABSTRACT

The objective of this study was to evaluate the association and effect of *CRISP2* variants on the risk of asthenozoospermia, a male infertility condition marked by absent or diminished sperm motility. There are numerous reasons why individuals develop asthenozoospermia. Therefore, it is crucial to understand the molecular mechanisms underlying this condition of infertility. Furthermore, seminal plasma, a rich source of sperm quality-related biomarkers, transports the many spermatozoa pools that make up human ejaculate down the epididymis. These spermatozoa vary in size, shape, and motility. The morphology and mobility of male ejaculated spermatozoa are affected by a number of genes, including *CRISP2*. In seminal samples from 120 Iraqi infertile male patients and 40 healthy males who were matched for age, gender, and ethnicity as a control group, the connection of the *CRISP2* gene single nucleotide polymorphisms L56V, M176I, and C196R with infertility was investigated. According to statistical analysis of the genotype distribution of these three nsSNPs of the *CRISP2* gene in patients with the asthenozoospermia subgroup and the control group, there weren't detectable differences in genotype distribution between AS, OAS, OTA, and fertile men in the Iraqi research sample. Based on allele frequencies, C, T, and G were determined to be protective alleles, with OR values of 0.74, 0.64, and 2.0, respectively.

Keywords: Genotyping, allele frequencies, SNPs, Asthenozoospermia.

تعدد اشكال النيوكليوتيدة المفردة لجين *CRISP2* وعلاقتها بوهن الخصوبة لدى المرضى العراقيين

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المستخلص

تهدف الدراسة الحالية الى تقييم التأثير والعلاقه بين تغايرات المورث CRISPs وخطر وهن النطف كحالة من عدم الخصوبة عند الذكور تتميز بانخفاض أو عدم وجود حيوانات منوية حركة، ومسبباتها في البشر عوامل متعددة. وبالتالي ، فإن توضيح العمليات الجزيئية التي تسبب هذه الحالة أمر مهم. يتكون القذف البشري من تجمعات غير متجانسة من الحيوانات المنوية متفاوتة الخصائص مثل الشكل والحجم والحركة التي يتم نقلها عبر البربخ خلال البلازما المنوية ، والتي تعد مصدرًا غنيًا للعلامات الحيوية المتعلقة بجودة الحيوانات المنوية. يعد *CRISP2* واحدًا من عدة جينات تساهم في شكل وحركة الحيوانات المنوية الذكرية المقذوفة. في الدراسة تم التحقق من العلاقة بين تعدد أشكال النوكليوتيدات المفردة L56V و M176I و C196R للمورث *CRISP2* مع وهن الخصوبة في عينة السائل المنوي المأخوذة من 120 مريضًا من الذكور العراقيين المصابين و 40 من الذكور الأصحاء المتطابقين في العمر والجنس والعرق كمجموعة ضابطة. أظهرت نتائج التحليل الاحصائي لتوزيع الطرز الوراثية لهذه الأشكال الثلاثة غير المترادفة للمورث *CRISP2* في المجموعات الفرعية للمرضى بوهن النطف ومجموعة السيطرة عدم وجود اختلافات ذات دلالة احصائية في توزيع هذه الانماط بين المجاميع الفرعية المختلفة والرجال العراقيين الخصبين في عينة الدراسة. وأظهرت نتائج تحليل تكرار الأليلات C و T و G كأليلات واقية بقيم النسبة الفردية 0.74 و 0.64 و 2.0على التوالي.

كلمات مفتاحية: الطرز الوراثية، تكرار الأليلات، تعدد أشكال النوكليوتيدات المفردة، وهن النطف.

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INTRODUCTION

Being an infertile male is one of the most common conditions among those between the ages of 20 and 45, making it a global public health issue that touches all societies. (26). Currently, it is one of the most pressing social problems affecting developed countries, with implications for communities and families. It affects over 186 million individuals globally (44). Infertility is described as "a disorder of the reproductive system defined by inability to achieve a clinical pregnancy after twelve months or more of regular, unprotected sexual intercourse" by the World Health Organization (47). Infertility is expected to affect 60–80 million couples annually. Regarding reproductive health, this is an important national concern. (39). Male infertility is a multifactorial disorder that involves many different factors. Each couple struggle with infertility, with male factor infertility accounting for over 50% of the reasons. (1). There is growing evidence that the quality of young, healthy men's sperm is declining globally (48). This dramatic drop is probably due to environmental factors rather than genetics (46, 38). These factors in around 15% of male infertility cases, chromosomal abnormalities, Y chromosome microdeletions, and single nucleotide polymorphisms, can be identified (28). Seminal plasma, the non-cellular liquid component of sperm that is a rich source of biomarkers related to sperm quality, transports the numerous pools of spermatozoa that make up human ejaculate through the epididymis with diverse properties such as shape, size, and motility (8, 45). Low sperm concentration, aberrant sperm morphology, or either absent or reduced sperm motility are all signs of male infertility, which is categorized as oligozoospermia, teratozoospermia, or asthenozoospermia (7), the latter being the most common (9). This condition is described as a <40% drop in overall motility and a <32% decrease in progressive motility in seminal samples (49). Asthenozoospermia can arise from a variety of pathogenic origins and routes. They could entail defective sperm motility and/or shape, along with issues of energy metabolism that are closely related to sperm motility or flawed signal transduction pathways. Consequently, despite advancements in relevant research, the molecular mechanisms behind this infertility condition are still not comprehended (42). Recent developments in genomics have improved our knowledge of how genes affect male health and fertility and expanded the number of genes linked to infertility (36, 43). It is nearly impossible to distinguish between all genetic causes of infertility since each gene has lots of possible variants (51). The human protein atlas indicates that many genes, including the *CRISP2* gene, are expressed more frequently in the testis than in other tissue types. The bulk of the matched proteins are involved in spermatogenesis when the genes with upregulation in the testis are examined (4, 11). *CRISP2* is a protein-coding gene located on chromosome 6p21.3. It spans over 21 kilobase and has ten coding exons. A 243-amino acid protein is encoded by exons 4–10 (23). Known as Cysteine-rich secretory protein 2 (CRISP2*)*. which has received a lot of attention recently (31, 52, 21). This protein belongs to the highly conserved and widely distributed Cysteine Rich Secretory Proteins (CRISPs) subfamily of the CAP superfamily, which is found in vertebrates (27). Since the CRISP2 protein, the sole member of the CRISP subfamily expressed in the mammalian testis and male germ cells, is crucial for the shape and motility of male ejaculated spermatozoa, it has gained attention. Its expression is not influenced by androgens, nor is the CRISP2 protein glycosylated (14, 53, 34). Sperm progressive motility and the acrosome reaction are both regulated by the CRISP2 protein. optimal sperm flagellar beating, interfaces with the CatSper subunit, and modulates the Ryanodine receptors (30). In fact, the research of two CRISP2-deficient mouse lines shows that CRISP2 is important in sperm motility since its absence causes reduced motility and stiff midpiece syndrome (17), which lead to poor sperm hyperactivation (6). This is predicated on CRISP2's ability to regulate calcium flow through ryanodine receptors through its ability to bind CATSPER1 (30). Aggressive motility of the flagellum is marked by a high-amplitude, asymmetric waveform. To let sperm, enter the egg coats, it occurred at the fertilization site (40). Similarly, sperm motility and fertilization

efficacy were reduced considerably in CATSPER knockout animals (37). Male infertility can arise even in the absence of any other health issues in the body because of the faulty regulation of sperm motility driven by a mutation in those proteins (18). A growing number of researchers are now focusing on the relationship between genetic variations and male infertility (50). 90 percent of the total human genetic variants are caused by single nucleotide polymorphisms (SNPs), one of the numerous mutation types. SNPs have been associated with a number of disorders, accounting for 0.1% of population variance. These changes happen when a single nucleotide (A, T, C, or G) in the genome is altered (2). The coding area contains about 50% missense and 50% silent or synonymous SNPs. Non-coding SNPs can change the stability of mRNA and the function of the promoter by adding or removing miRNA sites, which could shift gene expression and cause an up- or down-regulation of a gene. (3). Nonsynonymous SNPs (nsSNPs) are SNPs that change the encoded amino acids and may improve or negatively affect the protein's future structure and/or function. About half of the genetic changes linked to human diseases are mediated by these nsSNPs (10).

MATERIALS AND METHODS

The patient group consists of men who do not have children and are having difficulty conceiving due to poor seminal fluid quality. In this study, 120 Iraqi men with primary infertility (40 AS, 40 OAS, and 40 OTA) were enrolled, and 40 fertile, healthy men of the same ethnicity without any systemic conditions were considered the control group. All the patients and control subjects are aged between 20 and 51 years. The patients were recruited from Al Jazeera Private Laboratory, Baghdad, alghadeda- Baghdad, Iraq. During the period of October, 2019 to September, 2020. Patients who met the exclusion criteria were those with secondary infertility, an aberrant karyotype, obstructive azoospermia, and varicocele. Chronic diseases in men, like cardiovascular, diabetes, and hypertension, were forbidden. The healthy, fertile men were volunteers with at least one child. Prior to the patients' enrollment in the trial, the semen samples were collected with their consent. The Ethics Committee, Department of Biotechnology, College of Science, and University of Baghdad all gave their approval to this work.

Semen collection

Semen was collected immediately into a clean, dry, and sterile disposable plastic Petri dish from all patients and the control group's freshly ejaculated samples in a room designated specifically for this purpose. The sample was directly moved to the semen examination after 30 to 60 minutes. Each semen sample was allowed to liquefy following the WHO recommended procedures. The semen was analyzed macroscopically and microscopically when it had fully liquefied. Based on the semen quality analysis, the samples were categorized into four groups: Normozoospermia (control) Asthenozoospermia (AS), Oligoasthenozoo-spermia (OAS), and Oligoteratoasthenozoo-spermia (OTA), (49).

Semen samples

One ml of whole freshly ejaculated human samples from all patients and the control group's semen were centrifuged (1 min/12000 rpm or 5min/5000 rpm). The supernatant was removed, and the pellet was resuspended in 500 µl of TE buffer and stored at 4°C until DNA extraction within a week.

Genotyping of *CRISP2* SNPs

The EasyPure® Genomic DNA Kit (TransGen, biotech. EE101-01) was used to extract the genomic DNA from preserved semen pellets that were suspended in 500 µl of TE buffer. Purity and concentration assessments were then conducted. According to their reference sequences (rs) in the NCBI (National Center for Biotechnology Information) database, a pair of primer sequences for the *CRISP2* SNPs (L59V rs1765509750, M176I rs533319863, and C196R rs36069724) were designed using Primer 3plus, V4, and the University Code of Student Conduct (UCSC) programs, and then synthesized by Alpha DNA Ltd. (Canada) and kept in lyophilized form. The primer sequences used in this study's experiments are shown in Table 1. In a PCR amplification process with a final volume of 25 µl, these forward and reverse sequence primers were utilized. The PCR mixture contains 3 µl of

DNA sample (100 ng), 1 µl of forward primer (10 M), 1 µl of reverse primer (10 M), 12.5 µl EasyTaq® PCR SuperMix, and 7.5 µl nuclease-free distilled water. Following a series of optimization steps, the PCR conditions were set as follows: One cycle of initial denaturation at 94°C for three minutes was followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. one cycle of a final extension step at 72°C for five minutes was performed. To check amplification, the amplified PCR fragments were identified by electrophoresis on a 2% (w/v) agarose gel. PCR products were then sent for sequencing by the Sanger method using an automated DNA sequencer (Macrogen Corporation, South Korea). After alignment with a reference sequence in the Gene Bank from NCBI, Bioedit software revealed the genotypes.

Statistical analysis

ANOVA statistical analyses were performed using GraphPad Prism 8 for Windows. P values <0.05 were applied to evaluate whether differences were statistically significant. After checking for Hardy-Weinberg equilibrium (HWE) agreement, allele and genotype frequencies were expressed as percentage frequencies, and differences were evaluated using Pearson's Chi-square test (https://www.genecalculators.net/). Odds ratios (OR) with confidence intervals (CI estimates at 95%) were employed to represent the relationship between *CRISP2* SNPs and infertility.

Table 1. Designed primers used in the study

Primer	Sequence (5′→3′ direction)
	CRISP2 (SNP Genotyping) L59V
Forward	**GGAAAGCAGTCTCTCCACCT**
Reverse	**CTCCTTACAGCACTGCCTCT**
	CRISP2 (SNP Genotyping) M176I
Forward	**ACTGCCCTTAGAGTATAAACAGT**
Reverse	**GTTGGTACGGGGTATTCTTT**
	CRISP2 (SNP Genotyping) C196R
Forward	**CTGCATAGTCCTTTGTCAC**
Reverse	**AGAATACCCCGTACCAACAAG**

RESULTS AND DISCUSSION

The expression of *CRISP2* within the testis was further confirmed with previously published data; this study sought to define the frequency of L59V, M176I, and C196R polymorphisms in the *CRISP2* gene among Iraqi infertile patients. To determine whether there may be a correlation between any of these polymorphisms and a particular element of sperm function as a cause of male infertility in humans, we used direct DNA sequencing following PCR amplification using specific primers. 120 infertile men with distinct asthenozoospermia subgroups had their nucleotide sequence variants in two exons of the *CRISP2* gene screened. The results were compared with sequences from 40 control men. Patients were stratified on the basis of sperm density, motile sperm, or normal morphology percentages as described using WHO criteria. Results from Tables 2, 3, and 4 indicate that there was no significant correlation, indicating that these polymorphisms in a heterozygous condition are not a significant factor in sperm function. Human *CRISP2* gene SNPs were obtained from the dbSNP database (dbSNP NCBI: https://www.ncbi.nlm.nih.gov/snp/?term=CRISP2). It comprised 15846 SNPs in total, 260 of which were missense (nsSNP), making up just 1.64% of all SNPs identified in the human *CRISP2* gene. It also contained 757 non-coding transcripts, 92 synonymous SNPs, 14471 intronic SNPs, 1 initiator codon variant, 1 inframe insertion, 1 inframe indel, and 4 inframe deletions. L59V, M176I, and C196R are the only three *CRISP2* nsSNPs that were picked to be Studied regarding their impact on protein-coding sequences. The SNP leucine 59 valine (L59V) of the *CRISP2* gene (G/C rs1765509750; at position +175 located on chromosome 6: 49700676 bp in exon 5) was presented with three genotypes (GG, GC, and CC) that were related to two alleles (G and C). The *CRISP2* gene's Methionine 176 Isoleucine (M176I) SNP (C/T rs533319863; at position +528 found on chromosome 6: 49695912 bp in exon 9) has three genotypes (CC, CT, and TT),

each of which corresponds to two alleles (C and T). Furthermore, the *CRISP2* gene's SNP Cysteine 196 Arginine (C196R) (A/G rs36069724; at position +586 on chromosome 6: 49695854 bp in exon 9) was found to have three genotypes (AA, AG, and GG) that matched two alleles (A and G). as shown in Table (2).

Table 2. Characteristics of *CRISP2* variants involved in this study

CH//SNPS	L59V	M176I	C196R
RS	rs1765509750	rs533319863	rs36069724
POSITION	Ch. 6:49700676 bp G>C*	Ch. 6:49695912 bp C>T*	Ch. 6:49695854 bp A>G
LOCATION	ex5 bp 109	ex9 bp 13	ex9 bp 71
TYPE	Conservative change (non-polar hydrophobic)	Conservative change (non-polar hydrophobic)	Loss of disulphide bond
ALTERNATE RESIDUES	aa 59 L→ V	aa 176 M→I	aa 196 C→ R
NOTE	*Novel variants/SNPs leucine to valine both hydrophobic aliphatic	*Novel variants/SNPs Methionine to Isoleucine both hydrophobic aliphatic	Cysteine to Arginine hydrophilic polar uncharged to hydrophilic basic

The patient's genotype frequencies, as shown in tables 3, 4, and 5, reflect that the wild-type genotype and allele had been used as a reference. In Table 3, the distribution of genotype and allele frequency analyses for the L59V is shown compared with the control group for the asthenozoospermic subgroups. Out of 120 infertile Iraqi men, 99 were homozygous, giving a total frequency of 82.5% (99/120). With a frequency of 11.6% (14/120), 14 men were heterozygous. A homozygous mutant was identified in seven men with a frequency of 5.9% (7/120). These genotypes' frequencies were similar to those of the control group (31/40, 77.5%), (6/40, 15.0%), and (3/40, 7.5%). The odd ratio and % CI for the GC and CC genotypes in the L59V polymorphism were 0.73 (0.2-2.0) and 0.73 (0.1-2.9), respectively. No statistically significant differences were observed (p = 0.55 and 0.66). indicating that the risk of infertility was close to that of the 59 GG wild type for these genotypes. As seen in Table (3), the 59 C allele's OR and 95% CI were 0.74 (0.3–1.5); p = 0.43 with no noticeable difference from the control.

Table 3. Genotype and allele frequencies detected by hardy-weinberg equilibrium law of *CRISP2* gene polymorphism rs1765509750 (L59V) between patient group and control group

Groups		*CRISP2* gene at position +175 (dbSNP-ID: rs1765509750) Genotypes			Alleles	
		GG	GC	CC	*G*	*C*
Patients (No. =120)	No.	(99)	(14)	(7)	(212)	(28)
	%	82.5 %	11.6 %	5.9 %	88.3 %	11.7 %
Controls (No. = 40)	No.	(31)	(6)	(3)	(68)	(12)
	%	77.5 %	15.0 %	7.5 %	70.0 %	30.0 %
OR		1.00	0.73	0.73	1.00	0.74
95% (C.I.)		(Reference)	(0.2-2.0)	(0.1-2.9)	(Reference)	(0.3-1.5)
P value		----	0.55	0.66	----	0.43

In comparison to controls, the results of genotyping and allele frequency studies for M176I in asthenozoospermic subgroups of patients are displayed in table 4. 108 out of 120 (90.0%) samples had homozygous. Eight were heterozygous, at a frequency of 6.6% (8/120). Only four men were found to be homozygous mutants, representing a frequency of 3.4% (4/120). The frequencies of these genotypes (34/40, 85.0%), (4/40, 10.0%), and (2/40, 5.0%) respectively, did not statistically differ from the control group. For the CT and TT genotypes in the M176I polymorphism, the odd ratio and 95% CI were 0.62 (0.1-2.2) and 0.62 (0.1-3.5), respectively. since the differences still aren't statistically significant (p = 0.47 and p = 0.60, respectively). There was neither significant difference from the control in the OR and 95% CI for 176 T alleles, which means that these genotypes did not have a higher risk of infertility than the wild-type CC.

Table 4. Genotype and allele frequencies detected by hardy-weinberg equilibrium law of *CRISP2* gene polymorphism rs533319863 (M176I) between patient group and control group

Groups		*CRISP2* gene at position +528 (dbSNP-ID: rs533319863) Genotypes			Alleles	
		CC	CT	TT	*C*	*T*
Patients (No. =120)	No.	(108)	(8)	(4)	(224)	(16)
	%	90.0 %	6.6 %	3.4 %	93.3 %	6.7 %
Controls (No. = 40)	No.	(34)	(4)	(2)	(72)	(8)
	%	85.0 %	10.0 %	5.0 %	90.0 %	10.0 %
OR		1.00	0.62	0.62	1.00	0.64
95% (C.I.)		(Reference)	(0.1-2.2)	(0.1-3.5)	(Reference)	(0.26-1.5)
P value		----	0.47	0.60	----	0.33

Table 5 compares the genotype and allele frequencies among patients in the asthenozoospermic subgroup to controls for the C196R variant. It was observed that 111 of the 120 infertile Iraqi men were homozygous, for a total frequency of 92.5%. Only six men, or 5.0% (6/120), were heterozygous. A homozygous mutant with a frequency of 2.5% (3/120) was found in three men. The frequencies of these genotypes did not differ significantly from the control group (38/40, 95.0%), (2/40, 5.0%), and (0/40, 0.0%), respectively. The odd ratio and 95% CI for the AG and GG genotypes in the C196R polymorphism were 1.0 (0.1–5.3) and 2.4 (0.1–47.8), respectively. with no statistically significant differences (p = 0.97 and p = 0.56). Indicating that these genotypes did not have a higher risk of infertility than the wild-type AA, the OR and 95% CI for the 196 G allele were 2.0 (0.4–9.3); p = 0.35, with no significant difference from the control, as indicates in Table 5.

Table 5. Genotype and allele frequencies detected by hardy-weinberg equilibrium law of *CRISP2* gene polymorphism rs36069724 (C196R) between patient group and control group

Groups		*CRISP2* gene at position +586 (dbSNP-ID: rs36069724) Genotypes			Alleles	
		AA	AG	GG	*A*	*G*
Patients (No. =120)	No.	(111)	(6)	(3)	(228)	(12)
	%	92.5 %	5.0 %	2.5 %	95.0 %	5.0 %
Controls (No. = 40)	No.	(38)	(2)	(0)	(78)	(2)
	%	95.0 %	5.0 %	0.0 %	97.5 %	2.5 %
OR		1.00	1.00	2.4	1.00	2.0
95% (C.I.)		(Reference)	(0.1-5.3)	(0.1-47.8)	(Reference)	(0.4-9.3)
P value		----	0.97	0.56	----	0.35

Genetic polymorphisms are prevalent in the human genome. Due to their widespread distribution across a given genome and inexpensive cost relative to other molecular markers, SNPs are the most frequently utilized in genetic disease investigations (35). SNPs can be silent, change the encoded amino acids, or appear in non-coding areas. They can impact messenger RNA configuration (stability), proteins, and promoter activity, which affects gene expression. Therefore, detecting and examining numerous gene variations may result in a greater comprehension of their impact on gene function and a person's health. (33). Three CRISPs in humans are integrated into sperm either during its synthesis in the testis or during epididymal maturation (15). Two distinct domains, the CAP and CRISP domains, are linked by a hinge region in all CRISPs, which contain 16 entirely conserved cysteines that create intramolecular disulfide bonds (19). The human developing acrosome and sperm tail accessory structures include the testis-enriched protein CRISP2. It interacts with gametogenetin 1 (GGN1) in the tail and mitogen-activated protein kinase kinase 11 in the acrosome to modulate the Ca+2-gating of ryanodine receptors. The epididymal lumen, where CRISP1 connects with sperm membranes, is where these proteins are mostly made (32). The epididymis likewise contains

CRISP3, which is more widely expressed, especially in the salivary gland and B-cells (29), and it is upregulated in a variety of diseases, such as prostate cancer and pancreatitis (5). In addition to the mammalian reproductive tract, CRISPs are also found in the venom of various toxic snake and lizard species, the Xenopus laevis hatching gland (12), and the buccal gland of the parasitic lamprey (22). Additionally, it has been shown that the CRISP domain of CRISP2 interacts with a number of binding partners, including MAP3K11 and GGN1 (13, 25). A number of ion channels are known to be variably regulated by CRISP domains in the venom of reptiles and the secretions of the lamprey buccal gland (22). and in mice can influence Ca2 via RyR channels (16). In the human male population, CRISP2 is a highly variable gene; it is mainly expressed in the testis and forms in the haploid germ cell compartment, where it is localized to the sperm acrosome and tail. This is the first research on CRISP polymorphisms in an Iraqi community of fertile and infertile men and the second investigation of a human population. Our attention is drawn to three distinct CRISP2 polymorphisms, all of which change the amino acid sequence in a different way. The findings show no discernible difference between these SNPs in infertile and fertile men. These results show that none of the protein-coding SNPs resulted in a dominant form of male infertility (all three SNPs were only ever found in a heterozygous state). These results are in line with Jamsai et al.'s study of human CRISP2 polymorphisms in Australian males, which involved screening coding regions in 92 infertile Australian men with asthenozoospermia and 176 control men using denaturing HPLC and sequencing (24). They detected 21 polymorphisms, of which three were picked for our study. All were only found in fertile men and were only found in heterozygous form. Jamsai's research reveals an interaction between the COOH end of GGN1 (gametogenetin 1) in the sperm tail and the ion channel regulation region (ICR), a sub-region within the CRISP domain of CRISP2. The results imply that there is no meaningful association between these polymorphisms and male infertility in Australians, indicating that these polymorphisms in a heterozygous form are not a powerful determinant of sperm function. Although none of the reported polymorphisms causes a dominant type of infertility, evidence suggests that SNPs linked to complex traits like infertility can accept amino acid alterations in less-essential regions of the protein. The combination of a number of the complex trait-associated SNPs, distributed across numerous genes, may subsequently result in a phenotypic trait (41), or, in extremely rare circumstances, they may occur in a homozygous form that directly causes infertility. This suggests that, when coupled with SNPs in other genes, some of the detected CRISP2 SNPs may contribute to male infertility. On the other hand, a report on the Hanoverian warmblood horse's CRISP SNPs has been made. The study discovered a non-synonymous E208K polymorphism that was statistically associated with reduced male fertility in heterozygotes (20). This evidence supports the idea that CRISPs are male fertility regulators.

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