

Expression Analysis of the CRISP2, CATSPER1, PATE1 and SEMG1 in the Sperm of Men with Idiopathic Asthenozoospermia

Zohreh Heidary¹, Majid Zaki-Dizaji¹, Kioomars Saliminejad¹, Hamid Reza Khorramkhorshid^{1, 2*}

1- Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

2- Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

Abstract

Background: The purpose of this study was to analyze the expression level of CRISP2, CATSPER1, PATE1 and SEMG1 genes in the sperm of men with asthenozoospermia (AZS). AZS is a cause of infertility in men in which the motility of the sperm is reduced. So far, a few genes have been associated with AZS; however, in most of the cases, its molecular etiology is unclear.

Methods: A total of 35 subjects with idiopathic AZS and 35 fertile and healthy men as control were included. In study after total RNA extraction and cDNA synthesis, relative quantification was performed. B2M was used as the normalizer gene and fold change was calculated by $2^{-\Delta\Delta Ct}$ method. Mann-Whitney test was used to compare the expression levels between the case and control groups with significance level of $p < 0.05$.

Results: Our results showed that CRISP2 ($p=0.03$) and SEMG1 ($p=0.03$) were significantly down- and up-regulated in AZS men respectively compared to the controls. But CATSPER1 and PATE1 did not show significant changes.

Conclusion: Down-regulation of CRISP2 and up-regulation of SEMG1 were associated with AZS, which could be suggested as the potential candidate genes for the development of a diagnostic marker or potentially for more studies for treatment of AZS.

Keywords: Asthenozoospermia, CATSPER1, CRISP2, Gene expression, Male infertility, PATE1, Real-time PCR, SEMG1, Sperm motility.

To cite this article: Heidary Z, Zaki-Dizaji M, Saliminejad K, Khorramkhorshid HR. Expression Analysis of the CRISP2, CATSPER1, PATE1 and SEMG1 in the Sperm of Men with Idiopathic Asthenozoospermia. *J Reprod Infertil.* 2019;20(2):70-75.

* Corresponding Author:

Hamid Reza

Khorramkhorshid, Genetic Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

E-mail:

hrkk1@uswr.ac.ir,

h.khorramkhorshid@ari.ir

Received: Dec. 8, 2018

Accepted: Jan. 26, 2019

Introduction

Infertility of men accounts for ~50% of all infertile cases, and genetic causes are responsible for 15-30% of all male infertility (1). Asthenozoospermia (AZS) as a cause of infertility in men is defined by absent or decreasing forward sperm motility (Progressive motility <32%) (2-8). AZS could be seen as a pure isolated condition or could be coupled with additional sperm abnormalities. Isolated form of AZS is considered as one of the causes of infertility in men, approximately accounts for 20% of infertile men and in more than 60% of cases this condition is associated with decreased number of sperm (Oligoasthenozoospermia) and/or abnormal sperm morphologies (Oligo-

asthenoterato- and asthenoteratozoospermia) (9, 10).

A few studies have been reported to find the etiology of AZS (11). Differentially expressed genes including CATSPER1 (Cation Channel Sperm Associated 1) in mouse sperm (12), and PATE1 (Prostate and Testis Expressed 1), SEMG1 (Semenogelin 1), and CRISP2 (Cysteine-Rich Secretory Protein 2), in the human study are apparently related to AZS (13-15). CRISP2 is the only member of CRISP family which is expressed in the testis (16, 17) in an androgen-independent manner (18). CRISP2 protein is located in the acrosome and the outer dense fibers of the sperm tail (19-

23). This protein may be secreted from the acrosome during the acrosome reaction (24) or be implicated in sperm-egg fusion (26) and it may modulate sperm flagellar motility (25). CATSPER1 is a voltage-gated Ca^{2+} -permeable channel specifically expressed in the sperm flagellum especially on the plasma membrane of sperm tail (27). In a murine model of AZS, up-regulation of CATSPER1 increased the sperm intracellular Ca^{2+} concentration, sperm concentration, and percentages of sperm activity and overall sperm motility (12). It seems that CATSPER1 is essential for sperm motility and hyperactivation through regulation of calcium concentration (28). PATE1 was associated with the age-related functions of testis and epididymis. Comparison of aged men and young asthenozoospermic men showed a similar expression level of PATE1. Previous studies on antibody blocking of PATE1 showed that it involved in sperm motility and sperm-egg penetration (14). SEMG1 as another candidate gene for AZS is expressed in seminal vesicles (29, 30) and has been associated with the spermatogenesis process (31). In a study by Yu et al. (2014), SEMG1 was six-fold up-regulated in AZS in comparison to fertile men (15). It appears that SEMG1 as a major structural protein of semen coagulum is involved in the semen coagulation and spermatozoa immobilization to inhibit human sperm capacitation (32). By a literature review of the previous studies in the sperm of men with AZS and healthy individuals, four candidate genes including CRISP2, CATSPER1, PATE1 and SEMG1 were selected to evaluate the expression levels of these genes in our population.

Methods

Subjects: A total of 35 subjects with idiopathic AZS were included. The semen samples were collected between May 2017 and October 2017 from Avicenna Fertility Center, Tehran, Iran. Infertile men with alcohol, drug, tobacco and substance abuse were also excluded. The subjects with known diseases such as cryptorchidism, varicocele, orchitis, epididymitis, endocrine hypogonadism, obstruction of the vas deferens, microdeletions of the Y chromosome and karyotype anomalies were excluded from the study as well. The control group involved 35 age-matched fertile healthy men whose semen analysis showed normal results based on WHO criteria, and had fathered at least one child. The study was approved by the Ethical Committee of the Avicenna Research Institute.

All subjects were informed about the purpose of sample collection and informed written consents were obtained.

The semen samples were obtained by masturbation after 3-7 days of sexual abstinence. Semen analyses were performed according to the World Health Organization (WHO) recommendations (2). The inclusion criteria were asthenozoospermic men with the concentration of $>20 \times 10^6$ sperm/ml in the asthenozoospermic group and rapid forward progressive motile sperm (Grade A) of $<25\%$, and total progressive motile sperm (Grade A+B) of $<50\%$ (2).

Total RNA extraction and cDNA synthesis: Total RNA was extracted from sperm pellets using RN easy mini kit (Qiagen, Germany) following the manufacturer's instructions. The quality and concentration of total RNA were determined by spectrophotometry using Nanodrop 2000 (Thermo Scientific, USA). The extracted RNA samples were stored at -80°C . The cDNA was synthesized from $0.5 \mu\text{g}$ of total RNA using PrimeScript RT reagent kit according to the manufacturer's instructions (TakaraBIO, Shiga, Japan).

Quantitative real-time PCR: Real-time PCR was performed using SYBR Premix DimerEraser kit gene according to the manufacturer's instructions (Takara, Shiga, Japan). All the reactions were carried out on a Rotor-Gene Q real-time PCR instrument (Qiagen Inc., Germany) according to the manufacturers' instructions. Briefly, $5 \mu\text{l}$ of SYBR Premix, 5 pmol of each primer and 50 ng of cDNA as template were used in a final volume of $10 \mu\text{l}$. The amplification reactions were thermally cycled as follows: denaturation at 95°C for 30 s , followed by 40 cycles of denaturation at 95°C for 30 s , annealing at 60°C for 10 s , and extension at 72°C for 15 s . Human beta-2-microglobulin (B2M) (33) was selected as a normalization standard and fold change in expression of each target mRNA relative to B2M was calculated based on $2^{-\Delta\Delta\text{Ct}}$ relative expression formula. The primer sets were designed in exon-junction or between two adjacent exons separated by a large intron to ensure the amplification of RNA and not genomic DNA. The primers used for detecting the expression of genes are listed in table 1.

Statistical analysis: Data are expressed as mean \pm standard deviation and median, 10–90 percentiles. Shapiro-Wilk normality test was used to analyze the distribution of the values for each variable in each group.

Table 1. Primer sequences and their related PCR product sizes used for real-time RT-PCR

Gene	Forward primer (5'--->3')	Reverse primer(5'--->3')	Size (bp)
CRISP2	TGCCATTATTGTCCTGCTGGT	CATGTTACAGCCAGTTGTATTCT	187
CATSPER1	AAGGGCAATTTTCAGAAACGCA	TCAAAGGCCAAGGATTGGGTTA	157
PATE1	TCTGCTGCTTTAGGGCGTTAT	GGTGGCACATCCTACACTGA	120
SEMG1	CCAGACACCAACATGGATCTCA	TGAGGTCAACTGACACCTTGATA	179
B2M	CGAGATGCTTTGCTCCGTG	TCCATTCTCTGCTGGATGAGG	118

The differences of mRNAs expression in the ejaculated sperm between astheno- and normozoospermic men were determined by a Mann-Whitney test. The $p < 0.05$ were considered significant. To assess relevant sensitivity and specificity of expression assay, the receiver Operating characteristic (ROC) curve analysis, and area under the curve (AUC) were used. The analyses were performed by SPSS software version 23.0 (Chicago, IL, USA).

Results

Descriptive analysis of samples showed that the mean ages in the AZS and control groups were 37 ± 6 and 35 ± 4 years, respectively. The mean sperm count in the AZS men and control group were 33.8 ± 12 and 43.2 ± 16 million *sperm/ml*, respectively.

The mRNA expression levels of CRISP2, CATSPER1, PATE1, and SEMG1 gene were examined by qRT-PCR in the ejaculated sperm samples from the AZS and control groups. Our results showed that no significant differences in CATSPER1, and PATE1 mRNA expression were found between the two groups. On the other hand, CRISP2 ($p=0.03$) and SEMG1 ($p=0.03$) were significantly down- and up-regulated respectively in the semen samples from asthenozoospermic men compared to normozoospermic controls (Table 2).

ROC curve analysis of two differently expressed genes (SEMG1 and CRISP2), alone or combined is displayed in figure 1. SEMG1 and CRISP2 ex-

Table 2. Expression levels (Fold change) of CATSPER1, PATE1, CRISP2, and SEMG1 mRNAs in sperm of asthenozoospermia men and control groups

Genes	p-value	Patients Mean \pm SD	Controls Mean \pm SD
SEMG1	0.03	6.89 \pm 8.53	4.00 \pm 5.77
CATSPER1	0.58	1.88 \pm 2.52	2.24 \pm 2.43
CRISP2	0.03	3.72 \pm 2.01	4.81 \pm 2.24
PATE1	0.67	2.65 \pm 2.99	1.96 \pm 2.06

pression, alone or combined, showed a poor sensitivity and specificity for identification of AZS phenotype between case and control groups.

Discussion

AZS, a cause of infertility in men, could be caused by dysfunction of energy metabolism or structural defects in the sperm-tail proteins and the sperm motility proteins. Despite the advances in etiology of male infertility, the molecular mechanisms that impair sperm motility in most cases are unclear (34). The mRNA expression analysis of four candidate genes including CRISP2, CATSPER1, SEMG1 and PATE1 in the sperm of men with AZS and control groups showed that down-regulation of CRISP2 ($p=0.036$) and up-regulation of SEMG1 ($p=0.03$) were associated with AZS.

Zhou et al. demonstrated that miR-27b and miR-27a negatively regulate CRISP2 protein expression in AZS and asthenoteratozoospermia, respectively (13, 35). Accordingly, high miR-27b and miR-27a expression or low CRISP2 protein ex-

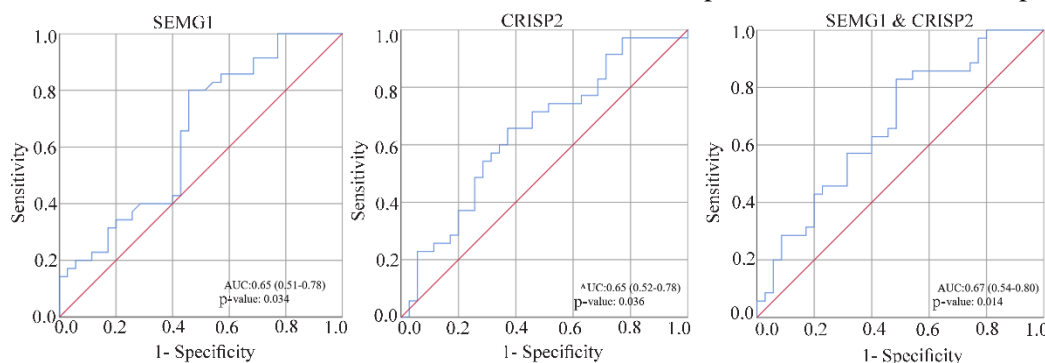


Figure 1. ROC curve analysis of expression data. ROC curve comparing sensitivity and specificity of expression data for detection of AZS patients. ROC curve analyses suggest that expression of SEMG1 and CRISP2 have poor diagnostic value for AZS detection

pression was significantly associated with low sperm motility, abnormal morphology, and infertility in asthenoteratozoospermic men (13, 35, 36). Consistent with these studies, CRISP2 expression was downregulated in AZS samples, but this downregulation was found in mRNA. Similar to our finding, Jing et al. reported downregulation of CRISP2 mRNA (37) and protein (38) or both (39, 40) in AZS. CRISP2 protein that has been localized in the acrosome and sperm tail and involved in sperm-egg fusion, is a candidate gene in men infertility; however, analysis of CRISP2 variations in asthenozoo- and/or teratozoospermia failed to find a significant association (41). It is exciting that Agarwal et al. found CRISP2 is uniquely expressed in the spermatozoa of infertile men with unilateral varicocele and it is absent in fertile men (42). These findings suggest the involvement of CRISP2 in the infertility may be the result of impairing sperm motility and development of varicocele. Varicocele is a risk factor for sperm motility and it can significantly affect it although the pathophysiologic mechanisms are not yet completely known.

Consistent to our finding, previous studies in AZS samples have shown SEMG1 levels increase and remain bound to spermatozoa (15, 43, 44), and their mRNA is overexpressed in spermatozoa (15). Legare et al. found over-expression of SEMG1 in infertile men and men who failed to fertilize eggs during IVF procedures (45). The high expression of SEMG1 in AZS patients could cause the accumulation of Sg1, and this might increase the concentration of Sg1 and decrease the motility of sperm in AZS men (15). The Sg1, the predominant secreted protein in semen, is contributed in the formation of a gel matrix which encases the ejaculated spermatozoa. After ejaculation, Sg1 is broken down into smaller peptides by PSA. This process liquefies the semen coagulum and allows the sperm to be more motile (15, 46). Interestingly, some SEMG1 variations (Such as p.Tyr315His and p.Gly400Asp) are most likely affecting molecular interactions or protein activity and possibly leading to hyperviscosity and AZS (47).

In contrast to previous reports, no association was found between the differential expression of CATSPER1 and PATE1 with the AZS which might be related to sample size. CATSPER1 protein is a calcium channel which particularly acts in the plasma membrane of the sperm tail (27). Low, lack of or mislocalized expression of CATSPER1 protein in spermatozoa may be involved in

developing asthenozoospermic phenotype and low hyperactivated motility (48-50). This low or absent expression of CATSPER1 was seen in mRNA level (49, 50). Also, some polymorphisms and mutations of CATSPER1 are associated with AZS (49, 51, 52). PATE1 protein in sperm seems to mediate sperm-egg interactions. According to a previous report, a defect in sperm PATE1 protein was revealed in both aged and young AZS men. The antibody of PATE1 blocking can decrease the motility of human sperm and zona-free hamster oocyte penetration (14). Recently, it was shown that PATE1 variant (A1423G) was possibly one of the genetic risk factors for idiopathic AZS (53).

Conclusion

In conclusion, although our samples were limited, our finding suggested that down- and up-regulation of the CRISP2 and SEMG1 respectively was associated with the idiopathic AZS infertile men.

Acknowledgement

We would like to thank all the participants in this study. The study was supported by the Avicenna Research Institute, Tehran, Iran.

Conflict of Interest

The study was supported by the Avicenna Research Institute, Tehran, Iran.

References

1. Coutton C, Fissore RA, Palermo GD, Stouffs K, Toure A. Male infertility: genetics, mechanism, and therapies. *Biomed Res Int*. 2016;2016:7372362.
2. WHO Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World health organization. 2010. 286 p.
3. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. *Reprod Biomed Online*. 2007;14(6):734-45.
4. Tüttelmann F, Röpke A. Genetics of Male Infertility. In: Simoni M, Huhtaniemi IT, editors. *Endocrinology of the Testis and Male Reproduction*. Cham: Springer International Publishing; 2017. p. 1029-49.
5. Miyamoto T, Minase G, Shin T, Ueda H, Okada H, Sengoku K. Human male infertility and its genetic causes. *Reprod Med Biol*. 2017;16(2):81-8.
6. Hargreave TB, Elliott D. Genetics and male infertility. In: Schill WB, Comhaire FH, Hargreave TB, editors. *Andrology for the clinician*. Berlin, Heidelberg: Springer; 2006. p. 462-80.

7. Neto FT, Bach PV, Najari BB, Li PS, Goldstein M. Genetics of male infertility. *Curr Urol Rep.* 2016;17(10):70.
8. Walsh TJ, Pera RR, Turek PJ. The genetics of male infertility. *Semin Reprod Med.* 2009;27(2):124-36.
9. Asero P, Calogero AE, Condorelli RA, Mongioi L, Vicari E, Lanzafame F, et al. Relevance of genetic investigation in male infertility. *J Endocrinol Invest.* 2014;37(5):415-27.
10. Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi Segovia LM, et al. Asthenozoospermia: analysis of a large population. *Arch Androl.* 2003;49(5):343-9.
11. Heidary Z, Saliminejad K, Zaki-Dizaji M, Khorram Khorshid HR. Genetic aspects of idiopathic asthenozoospermia as a cause of male infertility. *Hum Fertil (Camb).* 2018:1-10.
12. Luo S, Jia J, Hu H, Ma W, Jiao Y, Dong J. A Chinese herbal decoction can increase the intracellular Ca²⁺ concentration and CatSper1 expression in mouse sperm tails. *Mol Med Rep.* 2013;7(1):195-200.
13. Zhou JH, Zhou QZ, Lyu XM, Zhu T, Chen ZJ, Chen MK, et al. The expression of cysteine-rich secretory protein 2 (CRISP2) and its specific regulator miR-27b in the spermatozoa of patients with asthenozoospermia. *Biol Reprod.* 2015;92(1):28.
14. Liu FJ, Liu X, Han JL, Wang YW, Jin SH, Liu XX, et al. Aged men share the sperm protein PATE1 defect with young asthenozoospermia patients. *Hum Reprod.* 2015;30(4):861-9.
15. Yu Q, Zhou Q, Wei Q, Li J, Feng C, Mao X. SEMG1 may be the candidate gene for idiopathic asthenozoospermia. *Andrologia.* 2014;46(2):158-66.
16. Mizuki N, Sarapata DE, Garcia-Sanz JA, Kasahara M. The mouse male germ cell-specific gene *Tpx-1*: molecular structure, mode of expression in spermatogenesis, and sequence similarity to two non-mammalian genes. *Mamm Genome.* 1992;3(5):274-80.
17. Kratzschmar J, Haendler B, Eberspaecher U, Roosterman D, Donner P, Schleuning WD. The human cysteine-rich secretory protein (CRISP) family. Primary structure and tissue distribution of CRISP-1, CRISP-2 and CRISP-3. *Eur J Biochem.* 1996;236(3):827-36.
18. Haendler B, Habenicht UF, Schwidetzky U, Schuttker I, Schleuning WD. Differential androgen regulation of the murine genes for cysteine-rich secretory proteins (CRISP). *Eur J Biochem.* 1997;250(2):440-6.
19. O'Bryan MK, Sebire K, Meinhardt A, Edgar K, Keah HH, Hearn MT, et al. *Tpx-1* is a component of the outer dense fibers and acrosome of rat spermatozoa. *Mol Reprod Dev.* 2001;58(1):116-25.
20. Busso D, Cohen DJ, Hayashi M, Kasahara M, Cua-snicu PS. Human testicular protein TPX1/CRISP-2: localization in spermatozoa, fate after capacitation and relevance for gamete interaction. *Mol Hum Reprod.* 2005;11(4):299-305.
21. Gibbs GM, Bianco DM, Jamsai D, Herlihy A, Ristevski S, Aitken RJ, et al. Cysteine-rich secretory protein 2 binds to mitogen-activated protein kinase kinase 11 in mouse sperm. *Biol Reprod.* 2007;77(1):108-14.
22. Jamsai D, Bianco DM, Smith SJ, Merriner DJ, Ly-Huynh JD, Herlihy A, et al. Characterization of gametogenetin 1 (GGN1) and its potential role in male fertility through the interaction with the ion channel regulator, cysteine-rich secretory protein 2 (CRISP2) in the sperm tail. *Reproduction.* 2008;135(6):751-9.
23. Jamsai D, Rijal S, Bianco DM, O'Connor AE, Merriner DJ, Smith SJ, et al. A novel protein, sperm head and tail associated protein (SHTAP), interacts with cysteine-rich secretory protein 2 (CRISP2) during spermatogenesis in the mouse. *Biol Cell.* 2009;102(2):93-106.
24. Nimlamool W, Bean BS, Lowe-Krentz LJ. Human sperm CRISP2 is released from the acrosome during the acrosome reaction and re-associates at the equatorial segment. *Mol Reprod Dev.* 2013;80(6):488-502.
25. Harper CV, Barratt CL, Publicover SJ. Stimulation of human spermatozoa with progesterone gradients to simulate approach to the oocyte. Induction of [Ca²⁺]_i oscillations and cyclical transitions in flagellar beating. *J Biol Chem.* 2004;279(44):46315-25.
26. Da Ros VG, Munoz MW, Battistone MA, Brukman NG, Carvajal G, Curci L, et al. From the epididymis to the egg: participation of CRISP proteins in mammalian fertilization. *Asian J Androl.* 2015;17(5):711-5.
27. Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, et al. A sperm ion channel required for sperm motility and male fertility. *Nature.* 2001;413(6856):603-9.
28. Sanchez-Cardenas C, Montoya F, Navarrete FA, Hernandez-Cruz A, Corkidi G, Visconti PE, et al. Intracellular Ca²⁺ threshold reversibly switches flagellar beat off and on. *Biol Reprod.* 2018;99(5):1010-21.
29. Ueda Y, Yamaguchi R, Ikawa M, Okabe M, Morii E, Maeda Y, et al. PGAP1 knock-out mice show

- otocephaly and male infertility. *J Biol Chem.* 2007; 282(42):30373-80.
30. Jensen-Seaman MI, Li WH. Evolution of the hominoid semenogelin genes, the major proteins of ejaculated semen. *J Mol Evol.* 2003;57(3):261-70.
 31. Lundwall A. A locus on chromosome 20 encompassing genes that are highly expressed in the epididymis. *Asian J Androl.* 2007;9(4):540-4.
 32. Zhou QZ, Guo XB, Zhang WS, Zhou JH, Yang C, Bian J, et al. Expressions of miR-525-3p and its target gene SEMG1 in the spermatozoa of patients with asthenozoospermia. *Andrology.* 2018.
 33. Moreno LI, Tate CM, Knott EL, McDaniel JE, Rogers SS, Koons BW, et al. Determination of an effective housekeeping gene for the quantification of mRNA for forensic applications. *J Forensic Sci.* 2012;57(4):1051-8.
 34. Cao X, Cui Y, Zhang X, Lou J, Zhou J, Bei H, et al. Proteomic profile of human spermatozoa in healthy and asthenozoospermic individuals. *Reprod Biol Endocrinol.* 2018;16(1):16.
 35. Zhou JH, Zhou QZ, Yang JK, Lyu XM, Bian J, Guo WB, et al. MicroRNA-27a-mediated repression of cysteine-rich secretory protein 2 translation in asthenozoospermic patients. *Asian J Androl.* 2016;19(5):591-5.
 36. Li Y, Li M, Liu Y, Song G, Liu N. A microarray for microRNA profiling in spermatozoa from adult men living in an environmentally polluted site. *Bull Environ Contam Toxicol.* 2012;89(6):1111-4.
 37. Wang H, Zhou Z, Xu M, Li J, Xiao J, Xu ZY, et al. A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. *J Mol Med (Berl).* 2004;82(5):317-24.
 38. Zhou J, Xue K, Chen M, Zhou Q, Yang J, Bian J, et al. [Expression of cysteine-rich secretory protein 2 in patients with asthenozoospermia and its clinical significance]. *Nan Fang Yi Ke Da Xue Xue Bao.* 2014;34(10):1528-33. Chinese.
 39. Jing XW, Xing RW, Zhou QZ, Yu QF, Guo WB, Chen SM, et al. [Expressions of cysteine-rich secretory protein 2 in asthenospermia]. *Zhonghua Nan Ke Xue.* 2011;17(3):203-7. Chinese.
 40. Du Y, Huang X, Li J, Hu Y, Zhou Z, Sha J. Human testis specific protein 1 expression in human spermatogenesis and involvement in the pathogenesis of male infertility. *Fertil Steril.* 2006;85(6):1852-4.
 41. Jamsai D, Reilly A, Smith SJ, Gibbs GM, Baker HW, McLachlan RI, et al. Polymorphisms in the human cysteine-rich secretory protein 2 (CRISP2) gene in Australian men. *Hum Reprod.* 2008;23(9):2151-9.
 42. Agarwal A, Sharma R, Durairajanayagam D, Ayaz A, Cui Z, Willard B, et al. Major protein alterations in spermatozoa from infertile men with unilateral varicocele. *Reprod Biol Endocrinol.* 2015;13:8.
 43. Martinez-Heredia J, de Mateo S, Vidal-Taboada JM, Balleca JL, Oliva R. Identification of proteomic differences in asthenozoospermic sperm samples. *Hum Reprod.* 2008;23(4):783-91.
 44. Terai K, Yoshida K, Yoshiike M, Fujime M, Iwamoto T. Association of seminal plasma motility inhibitors/semenogelins with sperm in asthenozoospermia-infertile men. *Urol Int.* 2010;85(2):209-15.
 45. Legare C, Droit A, Fournier F, Bourassa S, Force A, Cloutier F, et al. Investigation of male infertility using quantitative comparative proteomics. *J Proteome Res.* 2014;13(12):5403-14.
 46. Robert M, Gagnon C. Semenogelin I: a coagulum forming, multifunctional seminal vesicle protein. *Cell Mol Life Sci.* 1999;55(6-7):944-60.
 47. Marques PI, Fonseca F, Carvalho AS, Puente DA, Damião I, Almeida V, et al. Sequence variation at KLK and WFDC clusters and its association to semen hyperviscosity and other male infertility phenotypes. *Hum Reprod.* 2016;31(12):2881-91.
 48. Tamburrino L, Marchiani S, Vicini E, Muciaccia B, Cambi M, Pellegrini S, et al. Quantification of CatSper1 expression in human spermatozoa and relation to functional parameters. *Hum Reprod.* 2015;30(7):1532-44.
 49. Shu F, Zhou X, Li F, Lu D, Lei B, Li Q, et al. Analysis of the correlation of CATSPER single nucleotide polymorphisms (SNPs) with idiopathic asthenospermia. *J Assist Reprod Genet.* 2015;32(11):1643-9.
 50. Wang YN, Wang B, Liang M, Han CY, Zhang B, Cai J, et al. Down-regulation of CatSper1 channel in epididymal spermatozoa contributes to the pathogenesis of asthenozoospermia, whereas up-regulation of the channel by Sheng-Jing-San treatment improves the sperm motility of asthenozoospermia in rats. *Fertil Steril.* 2013;99(2):579-87.
 51. Visser L, Westerveld GH, Xie F, van Daalen SK, van der Veen F, Lombardi MP, et al. A comprehensive gene mutation screen in men with asthenozoospermia. *Fertil Steril.* 2011;95(3):1020-4.e1-9.
 52. Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K, et al. Human male infertility caused by mutations in the CATSPER1 channel protein. *Am J Hum Genet.* 2009;84(4):505-10.
 53. Zhang S, Wang QM, Ding XP, Wang T, Mu XM, Chen ZY. Association of polymorphisms in PATE1 gene with idiopathic asthenozoospermia in Sichuan, China. *J Reprod Immunol.* 2016;118:54-60.