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CATSPER3 GENE EXPRESSION AND SPERM PARAMETERS IN INFERTILE MEN

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Abstract

Background

A seminal fluid analysis is responsible for providing information on the quantity, quality of the sperm. The CatSper cation channel is a specific calcium channel in sperm that is essential for the hyperactivation of sperm motility. Many males are infertile have down-regulation of *CatSper3* gene expression. Therefore, this study hypothesized that the expression of *CatSper3* gene may involve directly into various subnormal seminal fluid parameters.

Objective

The objective of this study is to evaluate the expression of the *CatSper3* gene in both fertile and infertile males, and to determine the relationship between the level of gene expression, seminal fluid parameters.

Materials and Methods

In the resent study semen samples were composed from 50 males. The samples were categorized into five categories depending on particular sperm criteria obtained from typical examination of the seminal fluid: sperm concentration, progressive sperm motility, and normal in structure sperm. As follows: Group1: comprised 15 oligozoospermia males. Group2: comprised 3 teratozoospermia males. Group 3: comprised 7 asthenoteratozoospermia males. Group 4: comprised 16 asthenoligoteratozoospermia males. Group 5: comprised 9 normozoospermia males. A typical analysis of the seminal fluid was done following the rules given by the World Health Organization (2021). The samples were used to evaluate the sperm chromatin immaturity test by aniline blue. In the molecular method of this study, a quantitative expression of *CatSper3* gene was executed by Real Time polymerase chain reaction. Then the percentage of sperm chromatin immaturity was related to the results of *CatSper3* gene expression to show the influence of abnormal sperm chromatin immaturity on the result of seminal fluid analysis.

Results

There was significant positive correlation between the fold of *CatSper3* gene with sperm concentration (r=0.357; p=0.011), highly significant with total sperm count (r=0.398; p=.004), progressive motility (r=0.364; p=0.009), total motility (r=0.336; p=0.017).

The findings were revealed a significant reduction in the mean of gene expression fold ($2^{-\Delta Ct}$) for *CatSper3* gene in semen samples in, teratozoospermic and asthenozoospermic patient groups as following: [(teratozoospermic group: 0.162, AT group: 0.224, AOT group: 0.359)]. The lowest folding was detected in teratozoospermic group when in comparison to other subgroups of infertile men. Additional, oligozoospermic and teratozoospermic groups were showed downregulation of *CatSper3* (0.234) while regulation expression of gene in normozoospermia group (1.000). Therefore, the results indicated a significant correlation between important factors comprised in the present study, such as semen parameters, with folding expression for *CatSper3*gene.

Conclusion:

according to result the expression of *CatSper3* gene correlate significantly with sperm concentration, motility and morphology, this study suggested that *CatSper3* gene has influence in seminal fluid parameters more beyond its effects on sperm motility. Large study for each seminal fluid categories is needed.

Introduction

Infertility is the medical disorder that impairs the body's ability to carry out the essential process of reproduction, as stated by the American Society for Reproductive Medicine (ASRM) (Sigman, 2019). Human semen comprises several crucial trace elements, such as calcium, copper, manganese, magnesium, zinc, and selenium, these elements are essential for metabolic activities, appropriate spermatogenesis, sperm development, motility, capacitation, and general sperm function (Mirnamniha et al., 2019). Eukaryotic cells facilitate the influx of Ca2+ ions by means of specific ion channel proteins located on the plasma membrane (Molina et al., 2018). CatSpers is unique channels that are defined by a structure comprising of six transmembrane-spanning repeats and a particularly pore that closely mimics the voltage-gated calcium-permeable channels (Mohammadi et al.,2013). The *CatSper3* gene has located on chromosome 5q31.1 (Lobley et al.,2003). Catsper protein location restricts in its distribution to the midpiece of sperm (Nowicka-Bauer and Szymczak-Cendlak, 2021). The CatSpers consists of four alpha subunits (*CatSper* 1-4) that form a calcium ion (Ca+2) channel, this channel comprises of a particular pore and two additional subunits CatSper (beta) and CatSper(gamma)(Wanget.al.,2009). *CatSper 1, 3*, and 4 are found in spermatids and are believed to be regulated by the same method of transcriptional regulation. *CatSper 2*, on the other hand, is present in spermatocytes during the pachytene stage (Singh and Rajender, 2015). Among CATSPER families, *CatSper 1–4* are the only ones that are fully express and primarily responsible for sperm motility and sperm activities (Jin et al., 2005; Li et al., 2007; Qi et al., 2007). *Catsper1-3* have major expression in the testis, while *Catsper4* is primarily express in the testis with less expression reported in placenta and lung tissues (Lobley et al., 2003). After undergoing differentiation, spermatozoa leave the testes and reaches the epididymis, where they mature and undergo several changes, including the development of motility (Paul et al. 2021). In the first region of the epididymis, the sperm develop the ability to move progressively and then remain in the lower part of the epididymis, where they remain unable to move (Ozkocer el al., 2021). Until the motility of sperm is increase during the process of ejaculation (Vyklicka et al., 2020). The presence of several substances in the seminal plasma facilitates this particular pattern of motility, such as (H+, Cl−, Na+, and HCO−3) (Bernardino et al. 2019). The regulated movement, referred to as activated motility, enables the sperm to migrate in a progressive move (Dey et al. 2019). Calcium, a well-known intracellular and prevalent signaling molecule, plays a crucial function in enhancing the movement, maturation, increased activity, reaction to external stimuli, directional movement, and fertilization of sperm cells (Valsa et al., 2015; Harchegani et al., 2019). In this study *CatSper 3* was studied in infertile men with oligozoospermia, teratozoospermia, asthenoteratozoospermia, and asthenoligoteratozoospermia in comparison with fertile individuals with normal sperm parameters.

Materials and methods

A total of 50 infertile and fertile men (20-52 yr.). Who were attended the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University in Baghdad-Iraq. The duration of the study was extended from October 2023 until February2024. The selected 50 men were divided, based on the type of infertility factor that investigated in the semen analysis, into (15) infertile men with oligozoospermia, (3) infertile men with teratozoospermia, (7) infertile men with asthenoteratozoospermia and (16) infertile men with asthenoligoteratozoospermia. (9) healthy fertile men as normozoospermia were also registered in this study. Applied by the WHO in the sixth addition of the manual of seminal fluid analysis. All men with azoospermia, aspermia were excluded from the study.

Methods used for *CatSper3* gene expression

Total RNA isolation

Semen samples were centrifuged by 4000 rpm/4min. The supernatant part was removed and the pellet was suspended in 1ml TransZol Up Reagent. Overnight, the samples were kept at-23ºC.For each ml of TransZol Up Reagent, added 200 µl of chloroform. The tube was vortexed gently for 30 seconds and incubated for 3 minutes at room temperature. The tube was Centrifuged at 10,000 rpm for 15 minutes at 2-8 ºC. The mixture was separated into a lower pink organic phase (50–60% of the total volume of TransZol Up), an interphase, and a colorless upper aqueous phase containing the RNA.The RNA-containing colorless upper phase transferred to a new RNase-free tube then added an equivalent volume of 96–100% ethanol then mixed gently by inverting the tube the created solution together transferred to a spin column. Centrifuged at 12,000 rpm for 30 seconds at room temperature. Removed the flow through. Added 500 µl of (CB9) to the spin column. Centrifuged at 12,000 rpm for 30 seconds at room temperature. Discarded the flow through (recurrent once). Added 500 µl of (WB9) into the spin column. Centrifuged at 12,000 rpm for 30 seconds at room temperature (Discarded the flow through recurrent once). Centrifuged the column matrix at 12,000 rpm for 2 minutes at room temperature to eliminate any leftover ethanol, and then air-dry for some mins. The spin column was positioned in a clean 1.5 mL RNase-free tube. Incubated for 1 minute at room temperature after adding

50-200 µl of RNase-free Water. The RNA was eluted by centrifugating at 12,000 rpm for 1 minute. (To improve the yield. Finally, the extracted RNA was deposited at -20°C.

Complementary DNA Synthesis

the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (AE311) was applied for cDNA synthesis. was reverse-transcribed to complementary DNA (cDNA). Reverse transcriptase reactions including RNA specimens with purified total RNA, Anchored Oligo (dT)18 Primer (0.5 µg / µl), Random Primer (0.1 µg / µl), 2x ES Reaction Mix, EasyScript ® RT/RI Enzyme Mix, gDNA Remover and RNase-free Water the total volume 20 µl. The specimens were put into thermal cycler and incubated at 25ºC for 10 min, at 42ºC for 15 min, and 85ºC for 5 sec and were preserved at 4ºC. The consisting cDNA specimens were kept at-20ºC.

cDNA Amplification with Real Time Polymerase Chain Reaction (RT-PCR)

For every sample on ice taken 2 µl cDNA samples of were put into the tube and added 10 µl master mix from TransStart® Top Green qPCR Super Mix (TransGen, biotech. AQ131-01), 2 µl primers and 6 µl N.F.Water. Gene expression levels were assessed by using Qiagen roter gene Q Real-Time PCR system. GAPDH was applied as the control gene. The reactions were kept in a tube at 94oC for 1 min, followed by 30 cycles of 94oC for 10 sec, 58oC for 15 sec and 58oC for 20 sec. All reactions were repeated 2 times.

Statistical analysis:

SPSS for Windows, version 22 (SPSS Inc., Chicago, Illinois, United States) was used to perform statistical analysis on the data. The data was presented in a mean, standard deviation (SD). A Shapiro–Wilk normality test was used to determine whether the studied parameters followed a Gaussian distribution. The P value for all tests was judged significant if less than 0.05.

Results

The study included (41) infertile [oligo. 15 (30.00%), Terato. 3(6.00%), AT 7(14.00%), AOT 16(32.00%)] and (9) fertile men (18.00%). By comparison of the control with all of the abnormal sperm parameter groups, table (4-3) demonstrates the Pearson correlation between *CatSper3* gene expression fold and sperm parameters in this study regardless the type of seminal fluid characteristics group. There was non-significant negative correlation with liquefaction time (r=-0.183; p=0.204), non-progressive motility (r=-0.114; p=0.432), percentage of immotile sperms (r=-081; p=0.576). While there was significant positive correlation between the fold of *CatSper3* gene with sperm concentration (r=0.357; p=0.011), highly significant with total sperm count (r=0.398; p=.004), progressive motility (r=0.364; p=0.009), total motility (r=0.336; p=0.017). Correlation is significant at the 0.01 level of fold.

Table (4-3): Pearson Correlations between the expressions of *CatSper3* gene with seminal fluid parameters

		Fold
Liquefaction Time	R=	-.183
	P=	.204
sperm concentration million/ml	R=	.357*
	P=	.011
Total sperm count million/ejaculate	R=	.398**
	P=	.004
progressive motile sperm (A+B) %	R=	.364**
	P=	.009
non progressive motile sperm%	R=	-.114
	P=	.432
Total motility%	R=	.336*
	P=	.017
immotile sperm%	R=	-.081
	P=	.576

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
R= person correlation=, p-value= Sig. (2-tailed).

Normalization of the *CatSper3* gene's Ct (cycle threshold) values. A quantitative RT-PCR test was used in the comparative study to evaluate *CatSper*3 expression and compare it between the oligo, Terato. AT, AOT, and fertile control groups. The relative quantification equation was used to determine gene expression fold change (Livak and Schmittgen, 2008). This is based on the normalizing of Ct values of the *CatSper3* cDNA to the GAPDH (which is the ΔCt). The mean of ΔCt (normalization Ct values) for each group of studies is obvious in Table (4-5). The ΔCt means were (11.048), (11.57), (11.1065), (10.42), (8.96) in the oligo, terato, AT, AOT and control groups, respectively. The $2^{-\Delta Ct}$ values have been used by each research group to determine the *CatSper3* gene's expression. Table 4–5 displays the outcomes. Every group's $2^{-\Delta Ct}$ results have been compared to the control group. The $2^{-\Delta Ct}$ mean values were 0.00047, 0.00033, 0.00045and 0.00072, respectively, in the Oligo, terato. AT, and OAT groups, while the control group's $2^{-\Delta Ct}$ mean value was 0. 00202.The fold of *CatSper3* gene expression were (0.234), (0.162), (0.224), (0.359), respectively, in the Oligo, terato. AT, and OAT groups, while the control group was (1.000). Showed in Table (4-5).

Table (4-5): Fold of CATSPER3 expression Depending on 2-ΔCt Method

	Means Ct of CATSPER3	***Means Ct of GAPDH***	***ΔCt (Means Ct of CATSPER3)***	$2^{-\Delta Ct}$	***experimental group/ Control group***	***Fold of gene expression***
Oligozoospermia	23.535	12.4875	11.047	0.00047	0.00047/0.00202	0.234
Teratozoospermia	24.01	12.44	11.57	0.00033	0.00033/0.00202	0.163
Asthenoteratozoospermia(AT)	23.41	12.30	11.1066	0.00045	0.00045/0.00202	0.224
Asthenoligoteratozoospermia (AOT)	23.04	12.61	10.43	0.00072	0.00072/0.00202	0.359
Normal	21.47	12.52	8.95	0.00202	0.00202/0.00202	1.000

The means Ct of *GAPDH* amongst all research groups; [(oligo. (12.48), terato. (12.44), AT (12.30), AOT (12.61), normal (12.52)] showed in table (4-2) with the obvious slight variances in gene fold expression throughout all study groups, the *GAPDH* gene was utilized as a suitable reference gene (Robert et al., 2005). It has been found that using *GAPDH* as a normalization method in qRT-PCR was a highly accurate method to conduct clinical research. The amplification plots and dissociate curves of the *GAPDH* gene were shown in Figure (4-3; A, B).

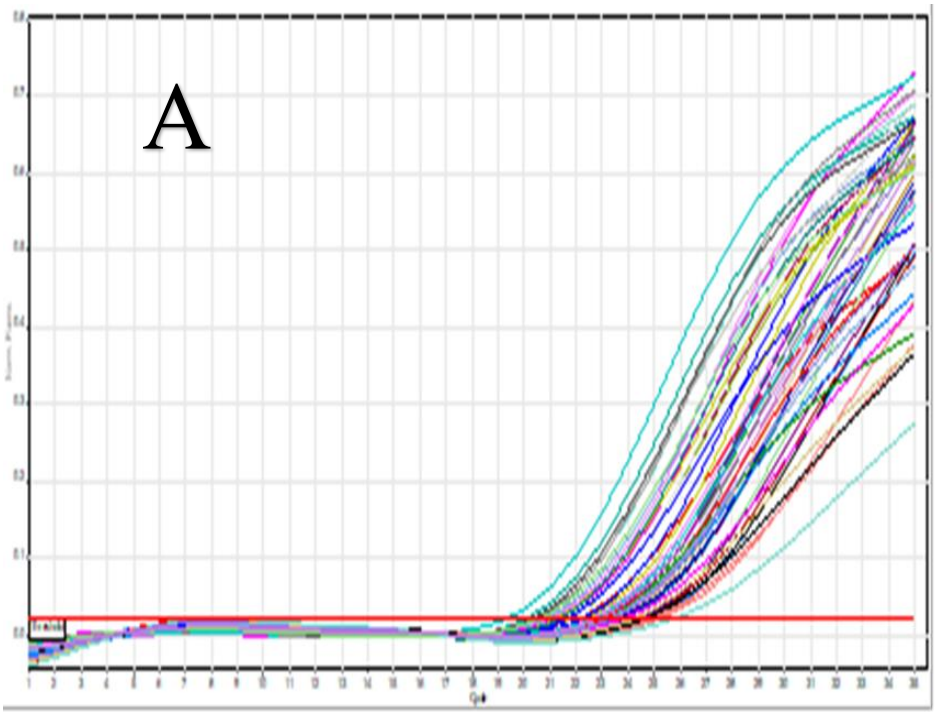

Figure (4-2): A- *CatSper3* QPCR samples were contained all study groups were utilized to plot gene amplification. The CT values varied between 25 and 27.

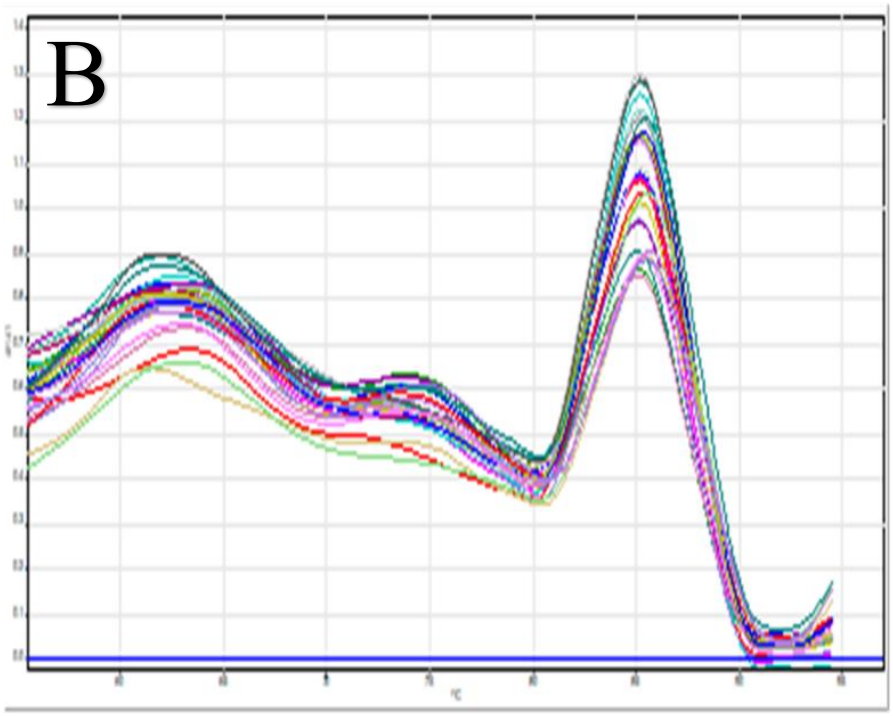

Figure (4-2): B-Disassociation curves of the *CatSper*3 gene were obtained utilizing qPCR samples that encompassed all research groups. Melting temperatures deviated from 83°C to 91°C. The images were obtained utilizing the Qiagen Rotor Gene Q qPCR device.

Discussion

The relationship between *CatSper3* gene expression and sperm parameters, specifically sperm motility is important to assess because CatSper is a crucial Ca2+ channel that plays a significant role in fertilization and affects sperm motility. It is regulated by intracellular pH, Ca2+, and voltage. Additionally, CatSper may also have an impact on the acrosome reaction (AR) Darszon et al. (2022).

The expression of about genes, such as the CatSper member genes, encode calcium channels in the sperm membrane and are an important effect on sperm hyperactivity, acrosomal reaction, and sperm diffusion into the oocyte Singh (2019). *CatSper3* gene is lower levels of mRNA expression in sperm shows poor sperm quality and primary men infertility. In current study, *CatSper3* gene is significantly downregulate in teratozoospermia and asthenoteratozoospermia patients, based on real-time PCR data, same results are report by Jalalabadi et al. (2023). There is significant positive correlation between the fold of *CatSper3* gene with sperm concentration (r=0.357; p=0.011), highly significant with total sperm count (r=0.398; p=.004), progressive motility (r=0.364; p=0.009), total motility (r=0.336; p=0.017). One cause for impair sperm function in patients with teratozoospermia and asthenoligoteratozoospermia groups is down-regulation in *CatSper3* gene expression based on recently study. Williams et al. (2015) is discovered that a lack of CatSper function impairs human spermatozoa's capability to fertilize. Qi et al. (2007) is recognized in the mice that the damage of any CatSper subunits reasons the channel loss to be completely in mature spermatozoa.

The results are in line with previous research in individuals with poor sperm quality, which demonstrates significantly lesser CatSper concentrations and mRNA expression in men with faulty spermatogenesis Wang et al. (2021). This gene expression is observed to be reduced in spermatozoa from patients with oligozoospermia, teratozoospermia, asthenoteratozoospermia (AT) and asthenoligoteratozoospermia (AOT), a disorder that comprises oligozoospermia (low number of sperm), asthenozoospermia and teratozoospermia (irregular sperm shape) (Carkci et al.2017, Silva et al.2022).

Conclusion:

According to the study, observed a significant correlation between the expression of the *CatSper3* gene and sperm concentration, motility, and morphology. These results indicate that the *CatSper3* gene has an influence on seminal fluid parameters beyond exclusively its effects on sperm motility, including sperm morphology, concentration, and count. A thorough evaluation is required for every group of seminal fluid.

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