Original Article

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Estimation of Spermatid Specific Thioredoxin 3 (SPTRXR3) and Testis Expressed Protein (101) Among Infertile Males in Al-Najaf Province, Iraq

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Abstract

Background: Among women, infertility rises by 0.370%, while among men; it rises by 0.291% per year. Idiopathic variables, such as smoking, drinking, using drugs, being overweight, experiencing stress at work or at home, becoming older, and consuming certain foods, are certain of the reasons of men fertility SPTPRX3, a unique member of the thioredoxin family, specifically found in the testis and male germ line, accumulates in the external area of malfunctioning sperm cells. Subjects and Method: Two groups, totaling 80 participants, participated in this study. The first group is the patient group, which consists of 40 infertile individuals and 40 healthy control males. A specialist fertilization doctor determined the infertility of the men by samples collection at fertilization Center at Al-Sader Medical City in Al-Najaf city. Results: Infertile men had considerably higher levels of TEX-101 (3.09 ± 1.14 ng/mL) than control individuals (1.9 ± 0.67 ng/mL), according to the data ($p< 0.001$). In addition, male infertility exhibited statistically significant higher levels of SPTRXR3 (6.22 ± 1.7 pg/mL) than with healthy individuals (2.84 ± 0.6 pg/mL) ($p<0.001$). Conclusions: Confirming their importance as markers in male infertility, the measurement of TEX-101 and SPTRX3 concentrations validates their significance

Keywords:

TEX-101, SPTRX3, infertility.

Introduction

Approximately 15% of couples worldwide are said to experience infertility, which refers to the condition where they are unable to conceive even after engaging in unprotected sexual intercourse for a period of one year [1]. Out of these cases, male infertility comprises 20%, while the combined causes of female and male infertility make up 30% of the total [2, 3].

Both males and females contribute to the causes of infertility. Specifically, it has been noted that in terms of causation, 29.3% of cases are believed to be of unknown origin (idiopathic), 37.1% are attributed to a male factor, and 17.6% are caused by a combination of male and female factors.

According to estimates, between 10% and 15% of all couples struggle with infertility, making it a major issue in the majority of developed nations [4]. Male infertility's potential molecular causes, as well as the creation of novel therapy strategies, have both attracted attentions in recent years. The majority of research demonstrate that DNA damage is caused by oxidative stress in the male sperm nucleus [5].

The nuclear and mitochondrial genomes may experience DNA damage. The spermatozoa therefore maintain their ability to fertilize. This is a major discovery since it shows that spermatozoa can convey defective genes to the developing fetus [6]. SPTRX-3, an initial thioredoxin specific to the Golgi apparatus, is believed to have a potential involvement in the modification of proteins after translation, which is crucial for

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the development of acrosomes [7]. It is worth noting that SPTRX-3 is found within the cytoplasm outside of the cell and forms a wrapping around the sidepieces of the sperm tail in structurally abnormal human spermatozoa. However, it cannot be detected in fully matured, normal spermatozoa across various animal species [8]. TEX-101, a protein present in the cell membrane, is solely synthesized by germ cells in the testes and is released into the seminal plasma.

According to Fujihara et al.'s [9] study, males with disruptions in the TEX-101 gene produced spermatozoa that appeared to be normal in structure but were unable to successfully fertilize. In context of all previous investigations, TEX101 might be a reflection of the quantity of viable sperm and the role of sperm. SPTRXR3, a crucial protein involved in fertilization during the capacity process, works in contrast to TEX-101, which is involved in acrosome processes. The study of the association between seminal parameters and biochemical parameters as well as the estimation of the seminal plasma levels of SPTRXR3 and TEX101 in infertile males were the objectives of a recent study.

Materials and Method

Subject of study

Two groups, totaling 80 participants, were used in the investigation. The first group is the patient group, which is made up of 40 infertile males and 40 healthy control males. The infertile males were determined by a specialized fertilization doctor by samples collection at fertilization Center at Al-Sader Medical City in Al-Najaf city.

Sample collection

In order to collect the blood samples for this investigation, sterile medical syringes were used to draw 5 ml of blood from the brachial vein of the patients and the controls, which was then put in a gel tube. After allowing the blood to coagulate for 30 minutes at room temperature, samples were centrifuged for 5 minutes at 3000 rpm to separate the serum from the remaining blood components. For an ELISA test to analyze hormones, the serum was removed using a micropipette, deposited in two repeaters of Eppendorf tubes, and then frozen at -20°C. During the visit to the infertility center, 80 semen samples were collected from patients through masturbation. The samples were obtained in sterile containers specifically designed for single use, following a period of sexual abstinence ranging from 3 to 7 days. Immediately after being collected, each sample collection was brought to the lab and kept in an incubator set at 37°C until the entire liquefaction process was complete.

Immediately after the process of liquefaction, the seminal plasma was separated by centrifuging the seminal fluid for 10 minutes at 3000 rpm, and it was then kept at -36° C until biochemical examination. Then, per WHO standards, the semen samples were examined under a microscope for microscopic analysis.

Immunological Assays

The ELISA kit, a biochemical assay, was utilized in the study by the Elabscience Company (USA) to quantify hormone parameters such as testosterone, FSH, LH, and prolactin. The levels of SPTRXR3, a thioredoxin reductase specific to spermatids, and TEX-101, a protein expressed in the testes, in the seminal fluid were measured using an ELISA kit obtained from My BioSource, a company based in the United States.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 25 for Windows software, developed by IBM in the US in 2017, was used to enter, organize, and analyze data of the study participants, infertile patients, and controls. Before beginning the study, all variables were examined for mistakes or inconsistencies. The seminal parameters, hormonal parameters, and biochemical parameters were all examined for statistical normality using histograms and normal distribution curves, and they all appeared to follow the statistical normal distribution. The average infertility male parameters were compared to the control group using an independent T-test. P-values equal to or less than 0.05 were considered statistically significant. Lastly, using the Windows version of Microsoft Word 2013, results and findings are presented in tables and/or figures as appropriate.

Results and Discussion

Age and body mass index distribution

The average age ± standard deviation of the individuals in the control and infertile patient groups was determined, and the results are displayed in table 1 with significant differences between the matched groups at ($p < 0.001$). The age group was divided into three groups as given in table (1) and figure (1). The findings, presented in numerical and percentage form for both study groups, revealed a substantial disparity between the patient group and the control group, indicating a significant difference ($P < 0.001$). The results of the study indicated that age groups between 31 and 40 years (42.5%) and 41 to 50 years (30%) had the highest rates of infertility. As shown in table (1) and figure (2), the findings of the present study showed that the mean body mass index of both groups of infertility patients increased significantly ($p < 0.001$) when compared to the healthy control match group. The majority of the patients in our study were normal (50%) and overweight (53%) and displayed significant (p0.05) differences from the control, as shown in table (1) and figure (2). The range of BMIs included normal, overweight, and obese. The results in agreement with previous studies [10]. Infertility that cannot be cured is a result of aging. But specifically, according to the

National Vital Statistics in America, due to postponing pregnancy, the average age of the father and mother of babies born rises by 40% every year. There is a correlation between Advanced Paternal Age (APA), which refers to being above 40 years old, and a decline in sperm quality regarding factors such as volume, motility, morphology, and an increase in SDF [11]. In their study, Brandt et al. [12] found that the quality of gametes and semen begins to diminish at ages above 40 due to the accumulation of mutations in the sperm's nucleus and mitochondria. Increased cell division, decreased DNA replication, ineffective DNA repair, and a buildup of mutagens from both internal and external sources, including oxidative stress exposure, are all consequences of the accumulation of mutations in sperm DNA [13]. The outcome of the study aligns with the conclusions drawn in previous research [14] that indicate obesity does not play a role in causing sperm DNA damage in the form of fragmentation. However, Kahn and Brannigan [15] found that obesity negatively affects male fertility, impacting parameters analyzed in semen, sperm DNA integrity, and the likelihood of successful conception.

Table (1): age groups and body mass index distribution among study population

Variable		Group				Statistical test	P. value
		Fertile men (N=40)		Infertile men (N=40)			
		No	%	No	%		
Age (years)	<30	11	27.5	22	55	X^2: 7.08	<0.001 H.S
	31-40	17	42.5	8	20		
	41-50	12	30	10	25		
	Total Mean ± SD*	33.17 ± 9.8		36.2 ± 5.4		t-test	<0.001 H.S
BMI (kg/m²)	Normal	20	50	18	45	X^2: 1.25	<0.05
	Overweight	14	35	12	30		
	Obese	6	15	10	25		
	Total Mean ± SD	26.13 ± 3.8		28.7 ± 5.17		t-test	<0.001 H.S

X^2: chi-square; *: standard deviation; **sig**: significant at <0.05; **H.S**: highly significant; **t**: independent t. test.

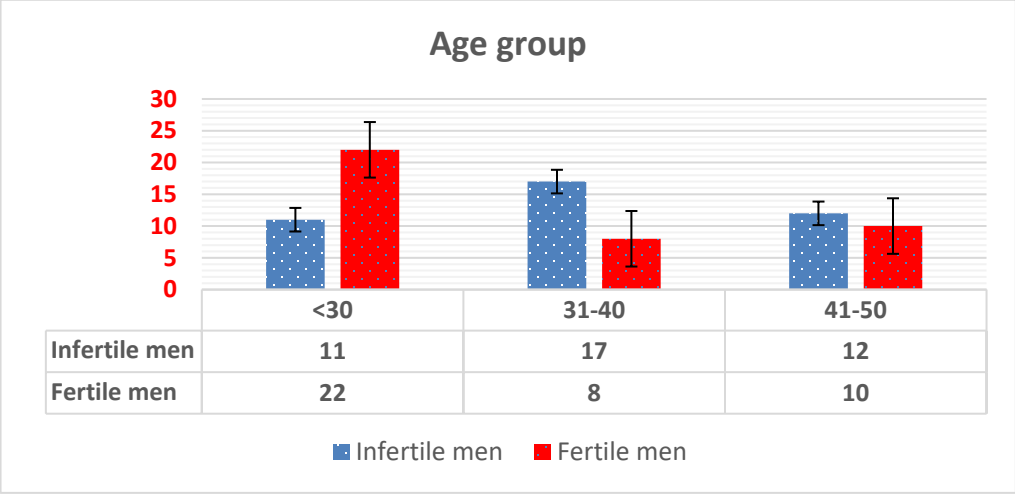

Figure (1): age groups distribution between infertile patient and control.

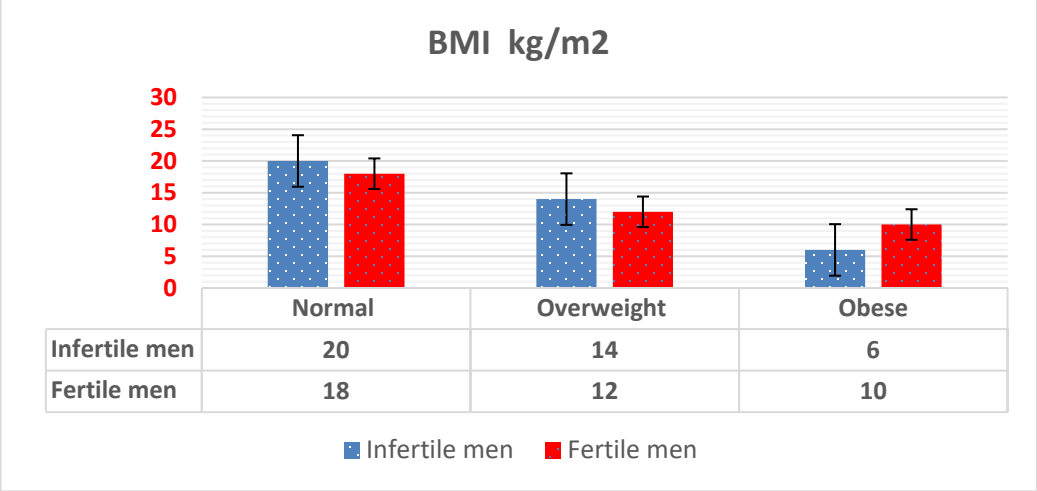

Figure (2): BMI distribution between infertile patient and control.

The special effects of obesity on male fertility are extremely negative since they raise a number of conditions that finally result in male infertility, including SDF, scrotal heat, erectile dysfunction, germ cell death, and alterations in decreasing sperm analytical parameters [16, 17].

Semen parameters among infertile and control groups:

The parameters of the semen analysis findings are presented in (Table 2). Comparing the sperm count to the control, the results in this table showed a significantly significant (P <0.001) decreased. Additionally, there was a valuable increase in non-progressive motility (11.3 ± 3.6) in comparison to control, showing that progressive motility was significantly decreased (34.12 ± 10.3) as compared to control (54.2 ± 15.3). The findings showed that, in comparison to control, there was a highly significant (P <0.001) rise in immotile (55.7 ± 14.2) and a substantial decrease in total motility (45.2 ± 12.9). While grade activity (1.66 ± 0.6) decreased significantly (P <0.001) as compared to the control group. The difference between the normal and abnormal morphologies of the semen was very significant (P <0.001), with the normal morphology showing a decrease (15.8 ± 4.3) in comparison to the control, and the abnormal morphologies showing an increase (P <0.001) in comparison to the control. This results in agreement with previous studies [18]. There has been evidence that the metabolic syndrome (MS) and male infertility may be related. Male hypogonadism is correlated with obesity and (MS). The semen of infertile men who are obese was found to contain insulin and leptin, which are crucial hormones that regulate male reproductive functions by influencing the hypothalamus-pituitary-testes (HPT) axis [19]. Leptin is one such hormone that originates from adipose tissue and is best known for controlling how much food is consumed and how much energy is expended through hypothalamic actions [20]. The development of lower androgen levels in obese males may be significantly influenced by excess leptin. These results were consistent with previous studies that showed men who were having difficulties conceiving pregnancy had lower sperm counts, reduced sperm motility, and sperm with different morphologies in their semen than healthy control participants [21]. Additionally, the current study supports earlier research on infertile men conducted in Tikrit [22].

Table (2): semen parameters comparison among infertile and control groups

Semen parameters		Infertile males Mean ± SD* (N.40)	Control Mean ± SD (N.40)	P-value
Sperm count (million/ml)		40.4 ± 8.2	± 10.6 46.3	<0.001
Motility (%)	Progressive	± 10.3 34.12	± 15.3 54.2	<0.001
	Non-progressive	11.3 ± 3.6	± 3.6 9.2	<0.001
	Immotile	± 14.2 55.7	± 9.8 36.47	<0.001
	Total	± 12.9 45.2	± 16.2 64.15	<0.001
Grade activity		± 0.6 1.66	± 0.3 2.25	<0.001
Morphology	Normal	± 4.3 15.8	± 19.2 41.2	<0.001
	Abnormal	± 18.2 83.9	± 10.2 58.2	<0.001

Hormonal parameters among infertile and control groups

The findings of sex hormone testing in infertile males showed a considerable (P 0.001) reduction in blood testosterone concentration while no significant variations in follicular stimulating hormone (FSH) in comparison to control.

In comparison to the control group, the serum concentrations of luteinizing hormone (LH) and prolactin (PRL) significantly increased (Table 3).

Table (3): hormonal parameters among study group

Hormone	Infertile men Mean ± SD (No. 40)	Fertile men Mean ± SD (No. 40)	P-value
Testosterone ng/ml	± 1.6 3.5	± 0.98 5.8	< 0.001
L.H mIIU/mL	± 2.1 6.7	± 1. 3 4.82	< 0.001
F.S.H mIU/mL	4.48 ± 1.77	± 1.9 4.28	> 0.05 N.S
Prolactin ng/mL	± 5.4 12.04	± 3.7 10.5	< 0.05

By examining abnormal hormone levels in men experiencing male infertility, it is possible to identify issues related to spermatogenesis. Several studies have indicated that sex hormones can be used as a potential treatment for male infertility. The malfunctioning of the hypothalamus, pituitary, and testis is what causes the hormonal anomalies in infertile males. Male infertility hormonal variables must be identified and characterized for both diagnostic and possible pharmacogenetic treatment objectives.

The preservation of spermatogenesis depends on intra-testicular testosterone. 25% of urologists who responded to a recent conduct survey said they treat patients with male infertility who have low testosterone levels with exogenous testosterone. The anterior pituitary gland's gonadotropic cells create and release FSH and hLH. Leydig cells in men may be stimulated to produce more testosterone as a result of the interaction between hLH and FSH, which would increase the generation of sperm [10]. It is unclear exactly how FSH influences the level of male testosterone. An estimated 20% to 30% of patients with male infertility have low testosterone levels or excessive LH levels. Intra testicular testosterone serves as a mediator for the impact of LH-stimulated

spermatogenesis. Testosterone has the ability to prevent the release of FSH and LH in gonadotropic cells, which ultimately prevents spermatogenesis.

Seminal level of (SPTRXR3) and (TEX-101)

The levels of TEX-101 (3.09 ± 1.14 ng/mL) in infertile individual were considerably high than those of the control group (1.9 ± 0.67 ng/mL), as shown in Table 4 and figures 3 and 4 below (p <0.001). Additionally, male infertile had statistically higher levels of SPTRXR3 (6.22 ± 1.7 pg/mL) than healthy individuals (2.84 ± 0.8 pg/mL) (p 0.001).

Table (4): level of SPTRXR3 and TEX101 among infertile men in comparison with control.

P-value	Mean ± SD	Study group	Variable
SPTRXR3 (pg/ml)	Infertile men No. (40)	± 1.7 6.22	<0.001
	Control No. (40)	± 0.8 2.84	
TEX101 (ng/ml)	Infertile men No. (40)	± 1.14 3.09	<0.001
	Control No. (40)	± 0.67 1.9	

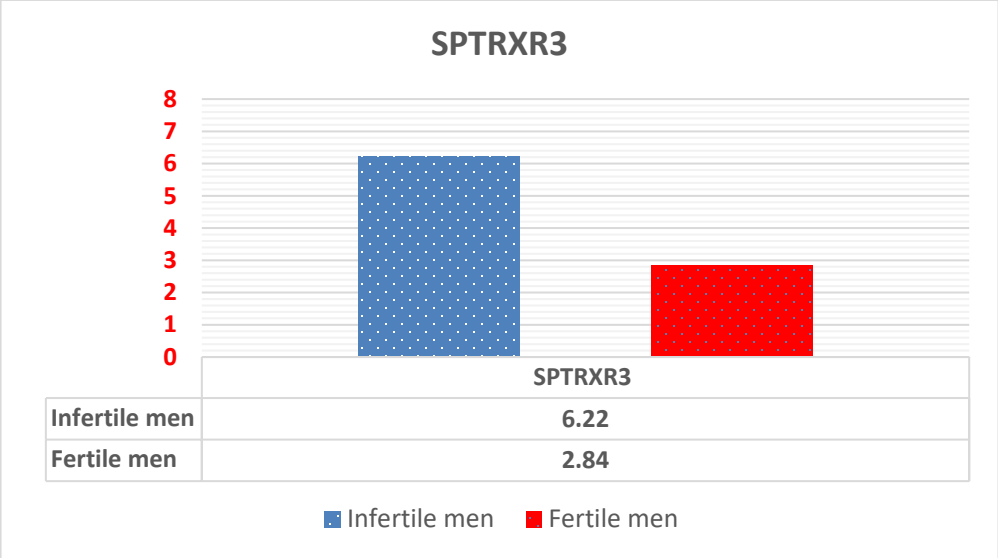

Figure (3): seminal level of SPTRXR3 among infertile male and control.

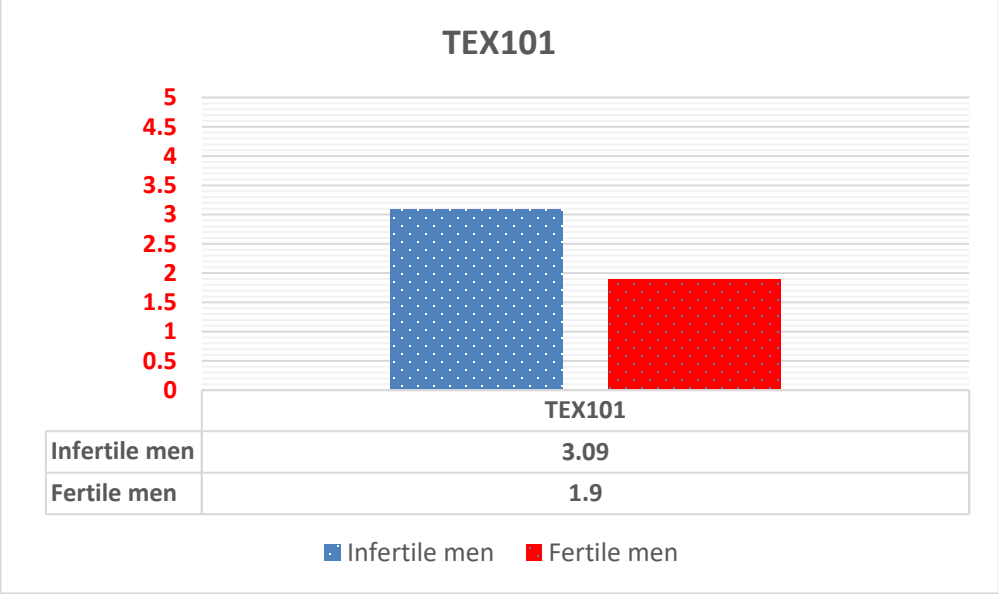

Figure (4): seminal level of TEX101 among infertile male and control

TEX-101 is one of the proteins that is released from the sperm surface and binds to GPI (glycosylphosphatidylinositol), which is then present in the seminal fluid [23]. Although there is evidence indicating that TEX-101 enzymatically escapes from the surface of epididymal sperm cells [24], Up until recently, the enzyme responsible for this process was unclear [25]. It is interesting to note that GPI-linked protein is expressed as testicular angiotensin-converting enzyme (tACE) in the sperm membrane of the testicles, and that

this leads to the sperm membrane of the testicles maturing into the epididym [26]. In addition to its influence on the catalysis of GPI-bound proteins from the surface of sperm cells, tACE (testis-specific angiotensin-converting enzyme) also plays a role in facilitating the attachment of eggs and sperm during fertilization [27]. These results prompted the hypothesis that the release of TEX-101 is caused by the tACE's epididymal transport [28]. According to Nagdas et al., [29], the plasma membrane of bovine testicular and epididymal sperm contains the TEX101 protein it is widely recognized that seminal plasma is a combination of secretions from various male glands, such as the testes, epididymis, prostate, seminal vesicles [30], and Cowper's gland, and it contains numerous proteins. In cases where no sperm are generated, the level of TEX-101 is determined to be zero. An important discovery that signifies the existence of fully developed sperm cells within the testis is the attainment of TEX-101 levels beyond a certain threshold [31]. In their research, [32, 33] observed a mere association between the total sperm count and TEX-101. According to the study conducted by Smith et al. [34], there was no indication that the simultaneous inactivation of these isoforms of thioredoxin domain-containing proteins (Txndc) had any impact on spermatogenesis, the maturation of sperm in the epididymis, or fertility [35]. The absence of Txndc in spermatozoa, on the other hand, led to age-related changes characterized by an accelerated loss of motility, increased rates of DNA damage, elevated levels of reactive oxygen species (ROS) [36], and inadequate protamination of sperm chromatin. As per the research, thioredoxins specific to sperm cells play a vital role in safeguarding against the heightened oxidative stress induced by advanced paternal age in these cells. In line with Buckman et al.'s [37] findings, males categorized as idiopathically infertile, with a presence of less than 15% SPTRX3-positive spermatozoa, constitute 51% of all infertile males and 20% of couples classified with unexplained infertility [38]. Furthermore, it was observed that 14% of men belonging to couples with a background of female-only infertility exhibit heightened levels of SPTRX3. Their research demonstrated that couples with elevated SPTRX3 levels exhibited a reduced pregnancy rate and produced a smaller number of two-pronuclear zygotes. Due to its unique localization pattern within spermatozoa and its well-established status as a germline-specific protein, SPTRX3 serves as a distinctive marker. The measurement of SPTRX3 levels is believed to have the potential to validate the clinical diagnosis of male infertility and uncover cases of undetected male infertility in situations of idiopathic infertility [39].

Conclusion

Infertile males had high quantities of TEX-101 and SPTRX3, which is evidence that these are crucial factors. The noteworthy aspect of the study's results lies in the demonstration of the functional role of the TEX-101 protein within semen, directly correlating its presence with the presence of sperm exhibiting sufficient potential for fertilization. Our research, in our opinion, indicates that SPTRXR3 is connected to the generation of defective sperm and that those pathways are involved in the biochemical processes causing male infertility. The relationships between these proteins and semen characteristics, in our opinion, are crucial

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