International Journal of Clinical and Experimental Medicine Research, 2025, 9(6), 684-691
https: //www.hillpublisher.com/journals/ijcemr/
ISSN Online: 2575-7970
ISSN Print: 2575-7989

Expression of Seminal Plasma TEX101 Protein in Male Infertility Patients and Predictive Value of IVF-ET Fertilization Rate

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How to cite this paper: Lili Liao, Bo Chen, Tong Pan, Daxiong Huang. (2025) Expression of Seminal Plasma TEX101 Protein in Male Infertility Patients and Predictive Value of IVF-ET Fertilization Rate. *International Journal of Clinical and Experimental Medicine Research*, 9(6), 684-691.
DOI: 10.26855/ijcemr.2025.11.020

Received: October 31, 2025
Accepted: November 29, 2025
Published: December 30, 2025

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Abstract

Objective: To investigate the expression of the seminal plasma TEX101 protein in male infertility patients and its predictive value regarding the fertilization rate in in vitro fertilization-embryo transfer (IVF-ET). **Methods:** A total of 71 patients were selected and grouped according to whether the deoxyribonucleic acid break index (DFI) was greater than 30%. The relevant parameters, in vitro fertilization rate and embryo development of the two groups, were recorded and compared. **Results:** The high DFI group exhibited lower sperm concentration, total number, PR, IVF fertilization rate, blastocyst formation rate, available embryo rate, high-quality embryo rate, and TEX101 protein concentration in comparison to the normal DFI group. Additionally, the sperm malformation rate was found to be higher in the high DFI group ($P < 0.05$). No correlation was observed between TEX101 protein concentration and semen volume. However, a positive correlation was identified with sperm concentration, total sperm count, sperm motility rate, PR, and IVF fertilization rate ($P < 0.05$). The logistic regression analysis indicated that TEX101 protein concentration was a favorable factor affecting fertilization ($P < 0.05$). An 817.40 ng/ml threshold was established as the predictive critical value, resulting in an area under the ROC curve of 0.732, a sensitivity of 91.5%, and a specificity of 45.8%. **Conclusions:** TEX101 protein is associated with sperm quality. Its expression is higher in the seminal fluid of normal DFI infertility patients. This protein is found to have predictive value for IVF fertilization rates and is expected to become a new index for the evaluation of male fertility and IVF fertilization rates.

Keywords

Male infertility; Seminal plasma TEX101 protein; IVF fertilization rate; Sperm DNA fragmentation rate

1. Introduction

According to the World Health Organization (WHO), currently, 10% to 15% of couples worldwide face infertility issues, with male factors contributing to about half of these cases [1]. Azoospermia (OA) and non-obstructive azoospermia (NOA) are found in 5% to 20% of male infertility cases [2]. In the case of NOA, Microdissection testicular sperm extraction (MD-TESE) stands out as the most effective method for sperm extraction [3]. The Sperm retrieval rate (SRR) is approximately 40% [4]. In vitro fertilization-embryo transfer (IVF-ET) has successfully addressed

fertility problems in NOA patients. Therefore, it is crucial to identify a sensitive, specific, cost-effective, straightforward, and non-invasive biochemical indicator to assess male fertility and predict IVF fertilization rates. Diamandis discovered that seminal plasma proteins exhibit high tissue-specific concentrations, making them a valuable source of biomarkers for diagnosing male reproductive system diseases [5]. Drabovich suggests that seminal plasma could serve as a novel biomarker and a specimen for non-invasive diagnosis of reproductive system diseases [6]. TEX101 is a prominent seminal plasma protein that is specifically expressed in germ cells, excreted from the sperm surface in the epididymal duct, and T cells play a role in the production of CD4 protein [7]. Current research on the TEX101 protein in seminal plasma primarily focuses on its role as a biomarker for male infertility, distinguishing azoospermia types, and differentiating NOA subtypes. Studies have confirmed that the TEX101 protein in seminal plasma can distinguish between NOA and OA [8, 9]. Seminal plasma TEX101 levels also differentiate NOA subtypes from Hypospermatogenesis (HS), Maturation arrest (MA), and Sertoli cell-only syndrome (SCOS) [10]. In this study, we compare the expression of the TEX101 protein in seminal plasma between infertile patients with normal DFI to explore its relationship with male infertility and its predictive value for IVF fertilization rates.

2. Materials and Methods

2.1 Data and methods

2.1.1 General Information

2.1.1.1 Research object

Data from 71 patients who underwent IVF-ET at our hospital's Reproductive Medicine Center between January 2020 and September 2021 were retrospectively analyzed. Among these patients, 36 cases with sperm DFI ≥30% were included, and they were aged between 23 and 51 years (with an average age of 34.6±5.6 years). Additionally, 35 cases with sperm DFI < 30% were included, and their ages ranged from 26 to 46 years (with an average age of 32.6±4.4 years). No statistically significant difference in age was found between the two groups ($P > 0.05$).

(1) Inclusion criteria

a. IVF-ET was administered to male patients of childbearing age with normal sexual activity who did not employ contraceptive methods, and their wives failed to conceive naturally due to exclusively male factors.

b. Female partners were under 35 years of age and had no conditions such as fallopian tube blockage, endometriosis, immune-related infertility, or ovulation disorders, and they had no special medical history.

(2) Exclusion criteria

a. A history of high fever within the last 3 months (maximum body temperature ≥39.0°C).

b. A history of inflammation or tumors of the reproductive system.

c. Retrograde ejaculation.

This study was approved by the Ethics Committee of The First People's Hospital of Foshan. Written informed consent was obtained from all patients.

2.2 Experimental methods

2.2.1 Specimen collection

In accordance with the "WHO Laboratory Manual for the Examination and Treatment of Human Semen (5th Edition)," semen samples were collected by masturbation after 3 to 7 days of abstinence. The samples were collected in a clean measuring cup and were liquefied on a temperature-controlled plate at 37°C.

2.2.2 Detection of related parameters

Computer-assisted sperm analysis (CASA) was performed using the Hamilton Thorne Biosciences IVOS sperm analysis system to record sperm concentration, motility, and other parameters. Sperm morphology was assessed using modified Pap staining, and the sperm DNA Fragmentation Index (DFI) was determined through the sperm chromatin diffusion test. The concentration of TEX101 protein in seminal plasma was measured in accordance with the procedure outlined in the TEX101 kit.

2.2.3 Outcome of IVF treatment

The fertilization rate and embryo development outcomes of the two groups were monitored and recorded. The IVF fertilization rate was calculated as follows: IVF fertilization rate = (2 pronuclei oocytes + 1 pronuclei oocytes + polypronuclei oocytes)/Total number of fertilized oocytes in IVF × 100%. The blastocyst formation rate was

determined as the number of blastocysts formed divided by the number of blastocysts cultured. The available embryo rate was defined as the number of transferable embryos divided by the number of normally cleaved embryos. Lastly, the good embryo rate was calculated as the number of good embryos divided by the number of normally cleaved embryos.

2.2.4 Statistical methods

Statistical analysis was carried out using IBM SPSS 20.0 software. The concentration of TEX101 protein in seminal plasma, semen volume, sperm concentration, total sperm count, progressive motility rate (PR), sperm motility rate, malformation rate, fertilization rate, and other parameters were presented as mean ± standard deviation ($\bar{x} \pm s$). The data from each group were derived from independent samples and exhibited a normal distribution. Pairwise comparisons were performed for the indices between the high DFI group and the normal DFI group. To ensure the homogeneity of variances, the Levene test was employed, and for the homogeneity test, the t-test was utilized. Inconsistent test results were evaluated using the t-test. The χ^2 test was applied to assess the fertilization rate, blastocyst formation rate, available embryo rate, and quality embryo rate between the two groups. Correlation analysis was conducted using the Pearson product-moment correlation coefficient. Furthermore, logistic regression analysis was executed to ascertain whether the TEX101 protein in seminal plasma constituted a risk factor affecting IVF fertilization rate. To evaluate the predictive value of the TEX101 protein concentration in seminal plasma for IVF fertilization rate, a Receiver Operating Characteristic (ROC) curve was constructed. A significance level of $P < 0.05$ was considered as indicating statistical significance.

3. Results

Comparison of general semen parameters and IVF treatment outcomes between the two groups (refer to Table 1).

Table 1. Comparison of general semen parameters and IVF treatment outcomes between the two groups

Group	High DFI (≥30%)	Normal DFI (< 30%)	*P*
n	36	35	
Semen volume (ml)	3.87±1.95	3.99±1.46	0.767
Sperm concentration ($\times 10^6$ /ml)	56.07±35.61	121.07±68.72	<0.001
Total sperm count ($\times 10^6$)	219.86±172.13	443.88±251.59	<0.001
Sperm motility rate (%)	31.39 ± 24.50	64.66 ± 17.50	<0.001
PR (%)	23.56 ± 17.78	50.57 ± 15.07	<0.001
Sperm malformation rate (%)	98.56 ± 1.45	91.97 ± 4.16	<0.001
IVF fertilization rate	57.1% (264/462)	82.3% (455/553)	<0.001
Blastocyst formation rate	75% (114/152)	83.1% (242/291)	0.040
Available embryo rate	40.4% (101/250)	50.8% (192/378)	0.035
Quality embryo rate	21.6% (54/250)	30.2% (114/378)	0.046
TEX101 protein concentration in seminal plasma(ng/ml)	1346.38±1011.63	3215.57±2540.99	<0.001

3.1 Correlation between TEX101 protein concentration in seminal plasma and various semen parameters

Correlation analysis was performed using the Pearson product-moment correlation coefficient. The results indicated no correlation between TEX101 protein concentration in seminal plasma and semen volume ($r=-0.048$) ($P > 0.05$). However, it showed a positive correlation with sperm concentration ($r=0.276$), total sperm count ($r=0.432$), sperm motility rate ($r=0.280$), and Progressive Motility Rate (PR) ($r=0.288$) ($P < 0.05$). Furthermore, it displayed a negative correlation with sperm malformation rate ($r=-0.238$) and sperm DNA fragmentation rate ($r=-0.423$) ($P < 0.05$). (Refer to Table 2, Figure 2, and Figure 3.)

Table 2. Correlation of TEX101 Protein Concentration in Seminal Plasma with Semen Parameters and IVF Fertilization Rate

	r	*P*
Semen volume	-0.048	0.692
Sperm concentration	0.276	0.020
Total sperm count	0.432	0.000
Sperm motility rate	0.280	0.018
PR	0.288	0.015
Sperm malformation rate	-0.238	0.046
DFI	-0.423	0.000
IVF fertilization rate	0.269	0.023

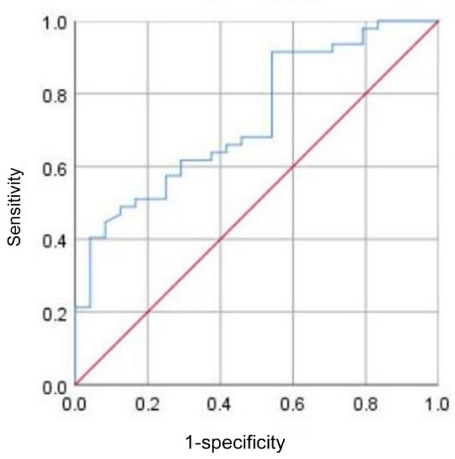

Figure 1. ROC curve of plasma TEX101 protein concentration predicting IVF fertilization rate.

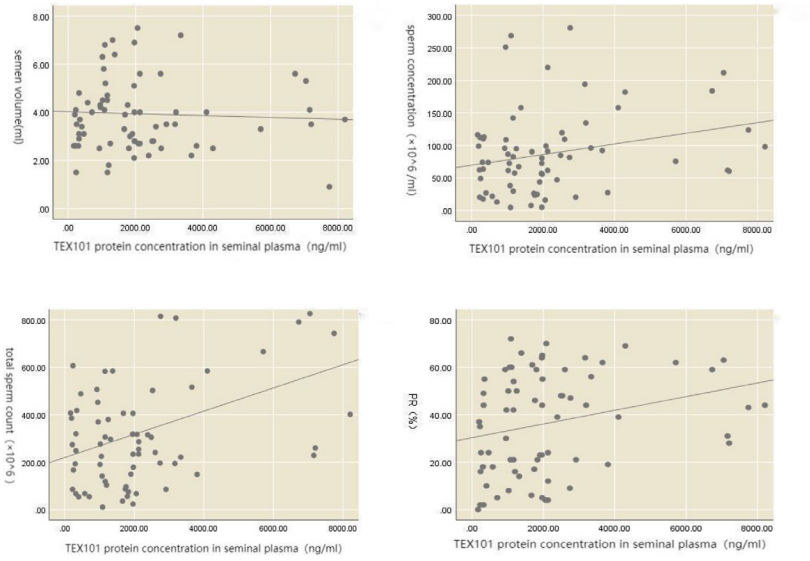

Figure 2. Correlation between TEX101 Protein Concentration in Seminal Plasma and Semen Volume. Correlation between TEX101 Protein Concentration in Seminal Plasma and Sperm Concentration. Correlation between TEX101 Protein Concentration in Seminal Plasma and Total Sperm Count. Correlation between TEX101 Protein Concentration in Seminal Plasma and PR.

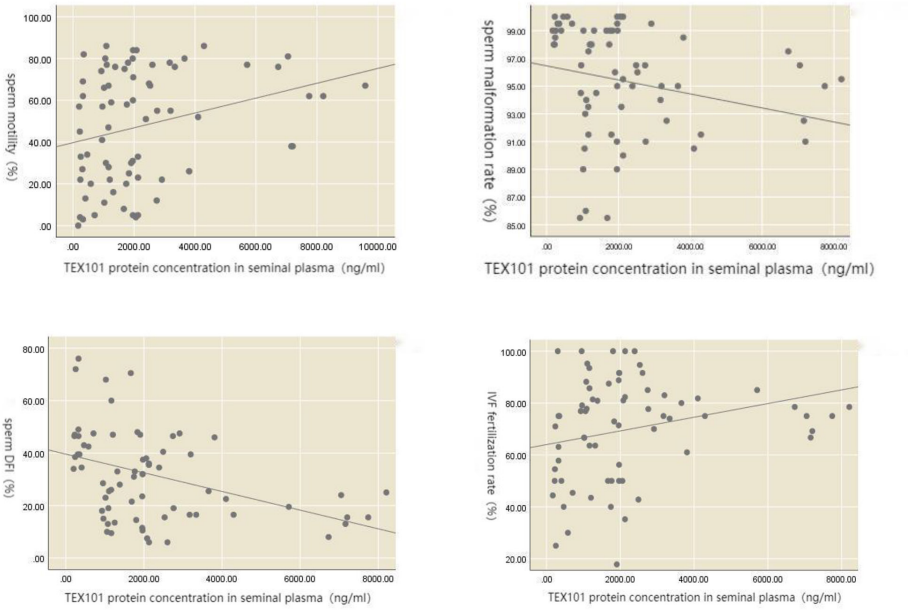

Figure 3. Correlation between TEX101 Protein Concentration in Seminal Plasma and Sperm Motility. Correlation between TEX101 Protein Concentration in Seminal Plasma and Sperm Malformation Rate. Correlation between TEX101 Protein Concentration in Seminal Plasma and Sperm DFI. Correlation between TEX101 Protein Concentration in Seminal Plasma and IVF Fertilization Rate.

3.2 Analysis of TEX101 protein concentration in seminal plasma and fertilization rate in IVF

Correlation analysis, conducted using the Pearson product-moment correlation coefficient, revealed a positive correlation between TEX101 protein concentration in seminal plasma and IVF fertilization rate ($r=0.269$) ($P < 0.05$), as indicated in Table 2. To further ascertain the predictive value of TEX101 protein concentration for IVF fertilization rate, TEX101 protein concentration was considered the independent variable, and whether the fertilization rate was below 65% served as the dependent variable [11]. Binary logistic regression analysis was executed, and it was found that TEX101 protein concentration was a significant factor favoring fertilization ($P < 0.05$). (Refer to Table 3.) An ROC curve was generated, with a predicted critical value of 817.40 ng/ml. The area under the ROC curve was 0.732, sensitivity was 91.5%, and specificity was 45.8% (See Figure 1).

Table 3. Binary Logistic Regression Analysis of Fertilization Rate

Investigation parameter	β	OR	95% *CI*	*P*
TEX101 protein concentration in seminal plasma	1.007	2.737	(1.491, 5.023)	0.001

4. Discussion

In recent years, the incidence of infertility has been on the rise, making the diagnosis and treatment of male infertility a growing societal concern. The gold standard for assessing male fertility is whether a partner can achieve pregnancy. Additional indicators include Time to Pregnancy (TTP) and semen quality. Computer-Assisted Semen Analysis (CASA) is a vital method for fertility assessment, encompassing parameters like semen volume, sperm concentration, and motility. Furthermore, other factors such as sperm DFI and Statin B have been considered, but they possess certain limitations. Hence, the quest for novel indicators to evaluate male fertility holds great significance.

This study revealed that there is no connection between sperm DFI and semen volume. However, it exhibits a negative relationship with sperm concentration, total number, PR, IVF fertilization rate, blastocyst formation rate, available embryo rate, and high-quality embryo rate. Sperm DNA fragments can arise at any stage of spermatogenesis. Unhealthy habits, environmental pollution, and exposure to chemicals can increase sperm DFI. The mechanisms of sperm DNA damage encompass oxidative stress within the body, irregular sperm apoptosis, and improper sperm

chromatin assembly [12]. Under normal conditions, the level of reactive oxygen species (ROS) in semen maintains a balance with the antioxidant defense system. Nevertheless, fragmented sperm DNA is more susceptible to ROS attack and destruction. Oxidative stress disrupts the structural integrity of the sperm membrane, impacting sperm survival. Alterations in sperm dynamic, morphological, and functional parameters ultimately affect the sperm's ability to fuse with oocytes [13]. Sperm DNA damage disrupts paternal genetic material, affecting protein transcription and acrosomal enzyme synthesis, consequently diminishing fertilization capacity. The initial expression of sperm DNA occurs during embryo culture, and when influenced by sperm DNA damage, it may impede embryo development, resulting in fetal abnormalities, decreased implantation rates, and increased miscarriage rates [14-16]. Some researchers have observed that when sperm DFI exceeds 25%, there are lower concentrations, viability, and normal form rates. Moreover, the rates of D3 quality embryos, clinical pregnancy, and live births are reduced [17]. In a study in China, Zhang Kun et al. [18] found that when sperm DFI is equal to or greater than 30%, PR, normal form rate, and fertilization rate are significantly reduced, while the rate of spousal abortions is higher.

The TEX101 protein, expressed in the testes, is a glycoprotein-encoding gene crucial for spermatogenesis and sperm function. It is situated on the surface of germ cells [19]. Following the initiation of spermatogenesis, the TEX101 protein vanishes from spermatogonia but reappears in spermatocytes, sperm cells, and testicular sperm. Ultimately, it is shed from the surface of sperm cells stored in the epididymal tube [20]. In this study, the concentration of TEX101 protein in the spermatoplasm of infertile patients displayed a negative correlation with sperm DFI. This correlation may be attributed to oxidative stress and abnormal sperm apoptosis. ROS represent intermediate products of spermatogenesis oxidation reactions, including oxygen free radicals and hydroxyl free radicals. The semen's antioxidant defense system safeguards the integrity of sperm DNA. However, reactive oxygen species are generated during the oxidation reactions of immature sperm during their transit and development in the epididymis. When the damage inflicted by reactive oxygen on sperm DNA surpasses the oxidative defense system's capacity, it results in an imbalance known as oxidative stress [21]. Studies have demonstrated that human sperm are highly sensitive to oxidative stress [22]. Apoptosis plays a vital role in maintaining the balance of spermatogenic cells, supporting cells, and sperm numbers during sperm development and maturation. Furhchi, utilizing mouse models, illustrated that pro-apoptotic and anti-apoptotic factors are pivotal in spermatogenesis and the preservation of a substantial number of male germ cells [23]. Normal spontaneous apoptosis of spermatogenic cells not only regulates the number of spermatogenic cells in the testes but also eliminates abnormal or damaged cells. However, abnormal apoptosis can lead to an increase in DFI, a decrease in sperm quantity and motility, and reduced TEX101 protein expression.

Study demonstrated that TEX101 protein concentration is a favorable factor influencing IVF fertilization rates, and binary logistic regression analysis established its statistical significance ($P < 0.05$). The ROC curve yielded a cut-off value of 0.732, with a predictive sensitivity of 91.5% and specificity of 45.8%. These findings suggest that TEX101 protein concentration holds predictive value for IVF fertilization rates below 65%. Previous research has shown that TEX-101 serves as a potential biomarker to distinguish men with abnormal semen parameters from those with normal semen. With a cut-off value of 1.84 ng/mL, seminal TEX-101 demonstrates 100% sensitivity, specificity, and negative and positive predictive values in predicting male infertility [24]. As a specific marker protein of germ cells, the TEX101 protein remains on the sperm cell surface after synthesis and modifies sperm cell surface molecules through interactions in the epididymis, significantly contributing to sperm capacitation and fertilization20. Studies have revealed that post-sperm-egg binding, the TEX101 protein binds to cumulus cells, elevating Ca2+ levels within cumulus cells and cumulus-oocyte complexes. This leads to the release of intracellular progesterone, ultimately inducing the acrosome reaction [25]. The key acrosomal enzyme involved in these reactions is a serine proteolytic enzyme, which exists in the acrosomal and equatorial membranes as an inactive proenzyme. Its activity is associated with the IVF fertilization rate [26].

This study highlights that TEX101 seminal plasma protein has predictive value for IVF fertilization rates. Future investigations can explore the relationship between the TEX101 seminal plasma protein, embryo implantation rates, and clinical pregnancy rates. This protein's value in assessing assisted reproductive pregnancy outcomes and evaluating the efficacy of male infertility and fertility treatments by measuring its concentration merits further research. Some international scholars have detected TEX101 and ECM1 proteins in seminal plasma and found that their combined detection provides higher sensitivity and specificity for distinguishing OA from NOA. Similarly, the combination of TEX101 and SPAG1 also yields similar results [27].

TEX101 protein is highly expressed in the seminal plasma of patients with normal DFI, but its levels significantly decrease in patients with high DFI. This protein serves as a reflection of the sperm quality of infertility patients and exhibits predictive value for IVF fertilization rates. It holds promise as a novel indicator for assessing male fertility and IVF fertilization rates.

Funding

This study was supported by the Guangdong Medical Science and Technology Research Fund Project (B2023457); Foshan City "14th Five-Year Plan" Medical High-Level Key Specialty Construction Project (FSGSP145020).

Author Contributions

LLL and CB carried out the studies, participated in collecting data, and drafted the manuscript. PT and HDX performed the statistical analysis and participated in its design. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The Author(s) declare(s) that there is no conflict of interest.

Acknowledgments

Not applicable.

Data Availability Statement

All data generated or analysed during this study are included in this published article and its supplementary information file.

Research Ethics and Patient Consent

This study was approved by the Ethics Committee of The First People's Hospital of Foshan. Written informed consent was obtained from all patients.

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