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Seminal plasma TEX101 protein as a spermatogenesis biomarker in male infertility

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Abstract

Male infertility may be results from reduced sperm production, or oligozoospermia and azoospermia. Testis-expressed 101 (TEX101) is a glycoprotein that is associated with male fertility, and the disruption of TEX101 results in abnormal semen parameters and sperm function. A case-control study was done to measure seminal plasma TEX101 in 184 volunteers by enzyme-linked immunosorbent assay (ELISA). Significant differences in the mean of TEX101 values were estimated between fertile men (9.64 ng/ml, N= 40), normozoospermic infertile men (8.57 ng/ml, N= 28), and infertile men with oligospermia (5.35 ng/ml, N= 76), and azoospermia (3.19 ng/ml, N= 40). Significant differences between the mean of TEX101 values were estimated in azoospermic infertile men according to the presence of spermatids as no-spermatid (0.66 ng/ml, N=12), few spermatids (3.05 ng/ml, N=16), and moderate spermatids (5.88 ng/ml, N=12). In addition, a significant correlation in oligozoospermia infertile men was found between TEX101 with seminogram. Clinical assay for TEX101 has the potential to diagnose male infertility. And as a biomarker for the seminal fluid quality and as a differential diagnosis of non-obstructive azoospermia.

1. INTRODUCTION

The TEX101 gene, located on the long arm of chromosome 19 at 19q13.31, encodes a membrane-specific glycosylphosphatidylinositol anchored protein known as TEX101 in humans. The Human Protein Atlas reports that TEX101 is only produced by male germ cells (Schiza et al., 2014). Gametogenesis, signal transmission, and increasing protein tyrosine phosphorylation are all essential mechanisms by which TEX10 contributes to the development of germ cells during its cellular localization in the gonads. Additionally, it is crucial to the acrosome response through fertilization (Mobasheri et al., 2015).

Obstructive and non-obstructive azoospermia (NOA) can be differentiated non-invasively using TEX101 (Drabovich et al., 2013), also Robin and his colleagues, used TEX101 to differentiate subtypes of non-obstructive azoospermia, including hypospermatogenesis, Sertoli-cell-only syndrome (SCOS), and maturation arrest (MA) (Robin et al., 2010).

A previous study, assessing the function of TEX101 in spermatogenesis, reported that the expression of TEX101 in germ cells was substantially correlated with the rate of gametogenesis (Tsukamoto et al., 2006). Angiotensin-converting enzyme (ACE) elimination of the GPI-anchored protein TEX101 is crucial for the development of viable spermatozoa, making TEX101 a possible target for the development of an effective male contraceptive drug (Fujihara et al., 2013). According to other studies, TEX 101 is a favorable biomarker for male infertility, and potentially clinical screening for it may substitute diagnostic testicular biopsies and aid in the prediction of sperm retrieval procedure outcomes, and in increasing the accuracy and effectiveness of assisted reproductive techniques (Jarvi et al., 2021, Bieniek et al., 2016).

Based on this concept, studies of the TEX101 protein could help identify a biomarker for unexplained infertility. In this regard, the concentrations of TEX101 were examined to evaluate their correlations with spermiogram and infertility status.

2. MATERIALS AND METHODS

2.1 Participants

The current study was performed on volunteers who attended Rizgary Teaching Hospital, Runahi IVF Center, and Shayi's private clinical laboratory in Erbil City from September 2021 to September 2022. The study included two groups: Fertile (control) group: Included 40 fertile normozoospermic males (sperm concentration ≥ 15 million/ml), and without any previous history of infertility problems or diseases. Infertile group: Included 144 infertile males, categorized as astheno-terato-zoospermia, (n=28), Oligozoospermia (n=76), and Azoospermia (n=40).

2.2 Exclusion criteria

Obstructive azoospermia, abnormal hormonal levels of (LH, FSH, TEST), urogenital tract infections, cryptorchidism, and other chronic diseases were excluded from the study.

2.3 Seminal fluid analysis

After guiding the volunteers on how to collect the semen sample, the seminal fluid was incubated at 37 °C for 20 min. To categorize the seminal fluid, the liquefied semen was assessed according to World Health Organization's (WHO) guidelines (WHO, 2010).

2.4 Seminal plasma TEX101 ELISA measurement

The lasting semen fluids were used for TEX101 analysis. Samples of semen were centrifuged for 5 minutes at 3,000 rpm. The supernatants were taken out and put into Eppendorf tubes for storage at −20 °C until analysis. TEX101 analysis was performed by the commercial ELISA kit (Sunlong Biotech Co., China, Lot no:20220215, REF: SL2833Hu). Seminal plasma TEX101 concentration was calculated as ng/ml. TEX101 ELISA kit analysis was performed at the Biotechnology Laboratory, College of Education, Salahadden University.

2.5 Testicular biopsy

A testicular biopsy is a surgical procedure performed by the urologist to collect a small tissue sample from the testicles for diagnostic

purposes. It was typically done to investigate the cause of infertility or abnormal testicular function. The procedure was performed under general anesthesia. After scrotal disinfection, a teeny incision was made in the scrotum to access the testicle, a teeny piece of tissue was then extracted for examination by a biologist under a microscope to assess the presence of sperm, the health of the testicular tissue, and any abnormalities (Girsh, 2021).

2.6 Statistical analysis: The statistical analysis was achieved using the Statistical Package for the Social Sciences, version 16.0 (SPSS). The data were presented as the mean and standard deviation, and differences between means were determined using ANOVA. followed by least significant difference (Tukey test), and the associations between values were investigated using the Spearman correlation test for p-values ≤ 0.05.

3. RESULTS AND DISCUSSION

Outcome of the seminogram of our study that measures various parameters showed significant differences between fertile and infertile males (Table 1).

Table 1: Seminogram characteristics between fertile and infertile categories.

Parameters		Healthy participants (N=40)	Infertile participants			P. value
			Normozoospermia (N=28)	Oligozoospermia (N=76)	Azoospermia (N=40)	
Volume (ml)		3.89+0.99 ^{abc}	3.25+1.23 ^{bcd}	3.56+1.60 ^{cd}	3.09+1.87 ^{d}	0.094
Liqufication		30.00+0.00	30.74+9.17	30.00+0.00	28.65+5.96	0.257
Concentration x10^6/ml		81.58+16.19 ^{a}	34.82+22.76 ^{b}	3.17+3.10 ^{c}	0.00+0.00 ^{d}	0.000
Total Count x10^6/ejaculate		318.09+99.38 ^{a}	105.23+69.38 ^{b}	11.55+12.64 ^{c}	0.00+0.00 ^{d}	0.000
Motility %	Rapid progression	50.50+8.07 ^{a}	5.71+9.59 ^{bc}	3.11+5.33 ^{c}	0.00+0.00 ^{d}	0.000
	Slow progression	17.50+3.92 ^{a}	12.54+9.72 ^{b}	7.23+7.52 ^{c}	0.00+0.00 ^{d}	0.000
	Non- progression	6.00+2.82 ^{a}	3.93+2.84 ^{bc}	4.14+4.40 ^{c}	0.00+0.00 ^{d}	0.000
	Immotile	26.00+6.81 ^{a}	77.82+20.12 ^{b}	85.53+12.15 ^{c}	0.00+0.00 ^{d}	0.000
Morphology %	Normal morphology	61.38+11.77 ^{a}	4.54+8.23 ^{bc}	2.49+6.46 ^{c}	0.00+0.00 ^{d}	0.000
	Abnormal morphology	38.63+11.77 ^{a}	95.46+8.23 ^{bc}	97.51+6.46 ^{c}	0.00+0.00 ^{d}	0.000

-The mean difference is significant at the 0.05 level (2-tailed)
- Combined different letters mean no significant differences between groups

Statistical analysis results revealed a significant decline of TEX101 protein concentration in infertile men azoospermia (3.19±2.69) and oligospermia (5.35±2.93) compared with infertile normozoospermia men (8.57±2.2) and healthy participants (9.64±1.44) (Figure 1). Furthermore, in the seminal plasma of infertile men with azoospermia, there was a significant decrease in TEX101 protein level according to the quantities of spermatids (Figure 2).

Correlation values (*r*) in addition to p-values of infertile participants have been presented in Table 2. TEX101 levels in oligozoospermic participants were correlated positively and significantly with concentration (*r*=0.727), total count (*r*=0.671), slow motility (*r*=0.487), non-progressive motility (*r*=0.256), and normal morphology (*r*=0.270), while correlated negatively and significantly with immotility (*r*=-0.490), and abnormal morphology (*r*=-0.270).

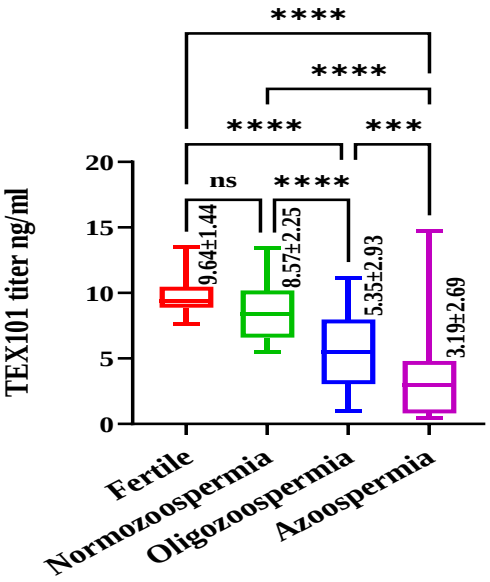

Figure 1: Seminal plasma TEX101 levels between healthy and infertile categories.

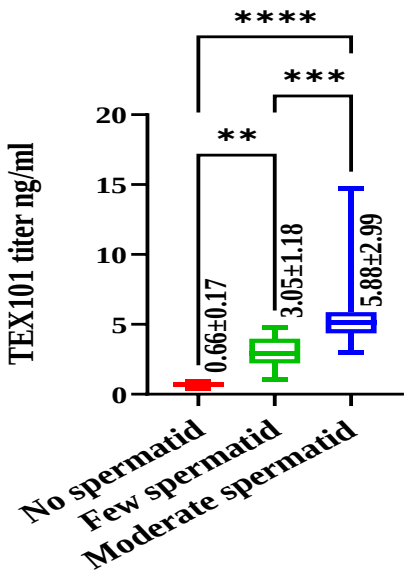

Figure 2: Seminal plasma TEX101 levels in sub grouped azoospermic infertile participants.

Table 2: Correlation of seminal plasma TEX101 with seminogram among infertile categories.

Parameters		Infertile men categories					
		Normozoospermia		Oligozoospermia		Azoospermia	
		Pearson correlation	p. value	Pearson correlation	p. value	Pearson correlation	p. value
Volume ml		-0.399	0.135	0.051	0.659	-0.003	0.986
Concentration x10^6/ml		0.332	0.084	0.727**	0.000	NA	NA
Total Count x10^6/ ejaculate		0.170	0.386	0.671**	0.000	NA	NA
Motility %	Rapid progression	0.226	0.247	0.218	0.059	NA	NA
	Slow progression	0.374	0.050	0.487**	0.000	NA	NA
	Non- progression	0.037	0.852	0.256*	0.025	NA	NA
	Immotile	-0.294	0.129	-0.490**	0.000	NA	NA
Morphology %	Normal	0.270	0.165	0.270*	0.018	NA	NA
	abnormal	-0.270	0.165	-0.270*	0.018	NA	NA

**: Correlation is significant at the 0.01 level (2-tailed).* : Correlation is significant at the 0.05 level (2-tailed).
NA: Not applicable

4. DISCUSSION

In this study TEX101 protein, which is known to help in resolving unexplained male infertility, was examined. This protein might represent a completely novel possible factor in male infertility (Burton et al., 2022, Erbayram et al., 2021) and others figure out the crucial roles of TEX101 in spermatogenesis (Masutani et al., 2020).

A seminogram is a medical test performed to significantly in infertile men compared to fertile men (Farman et al., 2019). We found significant dimensions of seminal TEX101 concentration in infertile men compared to fertile men (Figure 1). Only testicular biopsy provides a conclusive diagnosis for obstructive (OA) and nonobstructive (NOA) azoospermia, as well as for the NOA subtypes of hypospermatogenesis, Sertoli cell-only syndrome and maturation arrest

(Witherspoon and Flannigan, 2019). Studies have identified TEX101 as a biomarker for OA and NOA differential diagnosis as well as for NOA subtype classification. Clinical screening for TEX101 have the possibility to replace most diagnostic testicular biopsies and make the sperm retrieval process more predictable (Attayia et al., 2021). Others have shown how useful TEX101 is as a test for stratifying azoospermia types, assessing the effectiveness of vasectomy procedures, and deciding which patients to better choose for sperm retrieval (Korbakis et al., 2017). Taking into consideration the outcomes of previous studies and our findings, the TEX101 amount is close to zero in cases that have no spermatids generated from testicular germ cells, thus the amount of TEX101 is a remarkable signal for the existence of an abundance of spermatids (Figure 2).

Previous research has reported that the molecular properties of TEX101 may change based on its sub-cellular localization within the testis. TEX101's molecular variant may be associated with its function(s) (Yoshitake et al., 2008). Others have shown that altering the structure of the TEX101 gene or removing it harms the functioning of germ cells by reducing TEX101 expression (Jin et al., 2006). Endo et al. (2016) reported that normal TEX101 expression is linked to motility and the acrosome reaction, otherwise un-usual expression may contribute to the causes of azoospermia, immobile sperm cell, and failed progressive motility in unexplained infertility and asthenospermia (Endo et al., 2016). Others demonstrated a positive association of TEX101 protein expression with semen and sperm parameters, which included sperm concentration, sperm progressive motility, and normal morphology (Hammood et al., 2019). Based on the findings and interpretations of previous studies, we found strong correlations between most of the seminogram parameters and seminal plasma TEX101. As shown in Table 2, we evaluated the correlation of TEX101 concentrations with seminogram of infertile groups, in the oligozoospermic group, we realized statistically positive correlations with sperm concentration, total number, slow and non-progressive motility, normal morphology, and negative correlations with sperm immotility, abnormal morphology. These results emphasize that TEX101 levels are important in the evaluation of male infertility.

5. CONCLUSIONS

The present study postulated that the TEX101 level has a detrimental impact on germ cell production and spermatogenesis in the testis by altering the sperm concentration of infertile (oligospermia) men and the spermatid concentration of infertile (azoospermic) men. Also, pre-operative seminal TEX-101 can be employed as a predictor biomarker of sperm retrieval in NOA.

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Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

Al-Barzinji, R. M. & Al-Naqshbandi, A. A. 2017. Estimation Of Leptin And Leptin Receptor Concentrations In Seminal Plasma Of Primary Infertile Men. *Journal Of The Faculty Of Medicine Baghdad,* 59**,** 160-164.

Attayia, A. K., Jewad, A. K. M. J. & Ali, N. H. 2021. Differential Diagnosis Of Obstructive-From Non-Obstructive-Azoospermia By Quantification Of Seminal Fluid Biomarkers In Infertile Men. *Innovative Journal Of Medical And Health Science,* 11**,** 1897−1903.

Bieniek, J. M., Drabovich, A. P. & Lo, K. C. 2016. Seminal Biomarkers For The Evaluation Of Male Infertility. *Asian Journal Of Andrology,* 18**,** 426-433.

Burton, J., Wojewodzic, M. W., Rounge, T. B. & Haugen, T. B. 2022. A Role Of The Tex101 Interactome In The Common Aetiology Behind Male Subfertility And Testicular Germ Cell Tumor. *Frontiers In Oncology,* 12, 1-13.

Drabovich, A. P., Dimitromanolakis, A., Saraon, P., Soosaipillai, A., Batruch, I., Mullen, B., Jarvi, K. & Diamandis, E. P. 2013. Differential Diagnosis Of Azoospermia With Proteomic Biomarkers Ecm1 And Tex101 Quantified In Seminal Plasma. *Science Translational Medicine,* 5**,** 1-11.

Endo, S., Yoshitake, H., Tsukamoto, H., Matsuura, H., Kato, K., Sakuraba, M., Takamori, K., Fujiwara, H.,

Takeda, S. & Araki, Y. 2016. Tex101, A Glycoprotein Essential For Sperm Fertility, Is Required For Stable Expression Of Ly6k On Testicular Germ Cells. *Scientific Reports,* 6**,** 1-11.

Erbayram, F. Z., Menevse, E. & Dursunoglu, D. 2021. Semen Testis Expressed Protein 101 And Spermatid-Specific Thioredoxin Reductase 3 Levels May Be Biomarkers In Infertile Male. *Turkish Journal Of Biochemistry,* 46**,** 581-586.

Farman, H. A., Assi, M. A. & Salih, M. B. M. 2019. Effect Of Tex101 Protein, Vitamin D And Zinc On Men Fertility. *Biochemical And Cellular Archives,* 19**,** 4315-4318.

Fujihara, Y., Tokuhiro, K., Muro, Y., Kondoh, G., Araki, Y., Ikawa, M. & Okabe, M. 2013. Expression Of Tex101, Regulated By Ace, Is Essential For The Production Of Fertile Mouse Spermatozoa. *Proceedings Of The National Academy Of Sciences,* 110**,** 8111-8116.

Girsh, E. 2021. *A Textbook Of Clinical Embryology*, Cambridge University Press.

Hammood, S. A., Al-Sallami, A. S. & Al-Khafaji, S. M. 2019. Study The Relationship Between Tex101 Protein, Inhibin B And Testosterone Hormone In Azoospermia And Severe Oligospermia In Infertile Patients. *Indian Journal Of Public Health,* 10**,** 1275-1279.

Jarvi, K. A., Schlegel, P., Schiza, C., Drabovich, A., Lau, S., Soosaipillai, A., Korbakis, D., Brinc, D., Mullen, B. & Diamandis, E. 2021. Semen Biomarker Tex101 Predicts Sperm Retrieval Success For Men With Testicular Failure. *F1000research,* 10**,** 1-13.

Jin, H., Yoshitake, H., Tsukamoto, H., Takahashi, M., Mori, M., Takizawa, T., Takamori, K., Ogawa, H., Kinoshita, K. & Araki, Y. 2006. Molecular Characterization Of A Germ-Cell-Specific Antigen, Tex101, From Mouse Testis. *Zygote,* 14**,** 201-208.

Korbakis, D., Schiza, C., Brinc, D., Soosaipillai, A., Karakosta, T. D., Légaré, C., Sullivan, R., Mullen, B., Jarvi, K. & Diamandis, E. P. 2017. Preclinical Evaluation Of A Tex101 Protein Elisa Test For The Differential Diagnosis Of Male Infertility. *Bmc Medicine,* 15**,** 1-16.

Masutani, M., Sakurai, S., Shimizu, T. & Ohto, U. 2020. Crystal Structure Of Tex101, A Glycoprotein Essential For Male Fertility, Reveals The Presence Of Tandemly Arranged Ly6/Upar Domains. *Febs Letters,* 594**,** 3020-3031.

Mobasheri, M. B., Shirkoohi, R., Zendehdel, K., Jahanzad, I., Talebi, S., Afsharpad, M. & Modarressi, M. H. 2015. Transcriptome Analysis Of The Cancer/Testis Genes, Daz1, Aurkc, And Tex101, In Breast Tumors And Six Breast Cancer Cell Lines. *Tumor Biology,* 36**,** 8201-8206.

Robin, G., Boitrelle, F., Leroy, X., Peers, M.-C., Marcelli, F., Rigot, J.-M. & Mitchell, V. Assessment Of Azoospermia And Histological Evaluation Of Spermatogenesis. *Annales De Pathologie*, 2010. 182-195.

Schiza, C. G., Jarv, K., Diamandis, E. P. & Drabovich, A. P. 2014. An Emerging Role Of Tex101 Protein As A Male Infertility Biomarker. *Journal Of The International Federation Of Clinical Chemistry,* 25**,** 9-26.

Tsukamoto, H., Yoshitake, H., Mori, M., Yanagida, M., Takamori, K., Ogawa, H., Takizawa, T. & Araki, Y. 2006. Testicular Proteins Associated With The Germ Cell-Marker, Tex101: Involvement Of Cellubrevin In Tex101-Trafficking To The Cell Surface During Spermatogenesis. *Biochemical And Biophysical Research Communications,* 345**,** 229-238.

Vander Borght, M. & Wyns, C. 2018. Fertility And Infertility: Definition And Epidemiology. *Clinical Biochemistry,* 62**,** 2-10.

Who 2010. Examination And Processing Of Human Semen. *Geneva: World Health,* Fifth.

Witherspoon, L. & Flannigan, R. 2019. Meiotic Arrest In Azoospermic Men: Current Implications Of A Recently Reported Novel Technique For Classification Of Arrest Stage. *Fertility And Sterility,* 112**,** 1044-1045.

Yoshitake, H. & Araki, Y. 2020. Role Of The Glycosylphosphatidylinositol-Anchored Protein Tex101 And Its Related Molecules In Spermatogenesis. *International Journal Of Molecular Sciences,* 21**,** 1-17.

Yoshitake, H., Shirai, Y., Mochizuki, Y., Iwanari, H., Tsubamoto, H., Koyama, K., Takamori, K., Ogawa, H., Hasegawa, A. & Kodama, T. 2008. Molecular Diversity Of Tex101, A Marker Glycoprotein For Germ Cells Monitored With Monoclonal Antibodies: Variety Of The Molecular Characteristics According To Subcellular Localization Within The Mouse Testis. *Journal Of Reproductive Immunology,* 79**,** 1-11.