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Biochemical Assessments of Seminal Plasma Zinc, Testis-Expressed Sequence 101 and Free Amino Acids and Their Correlations with Reproductive Hormones in Male Infertility

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Abstract

The role of the male factors in the couple's infertility has been significantly increased in recent years due to a sententious assessment of male reproductive functions and enhanced diagnostic tools. We investigated the correlations among the seminal plasma (SP) levels of each of zinc, testis-expressed sequence 101 (TEX101), and free amino acids levels with reproductive hormones in adult fertile and infertile men. The study included 100 infertile men categorized into 50 non-obstructive azoospermic patients and 50 patients with idiopathic oligoasthenoteratozoospermia (iOAT), in addition to 50 fertile controls. Semen analyses, serum ELISA assays for male reproductive hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, and prolactin), colorimetric assays of SP zinc and total proteins, SP free amino acids using high-performance liquid chromatography (HPLC), and ELISA assays of SP TEX101 were performed for all subjects. Infertile men with azoospermia had significantly lower SP median levels of zinc, TEX101, and many SP free amino acids compared to both men with iOAT and fertile controls ($P < 0.05$ for all). There were lower SP levels of zinc and some free amino acids among men with iOAT compared to the fertile controls ($P < 0.05$ for all) with non-significant difference regarding to SP TEX101 ($P > 0.05$). Azoospermic men exhibited negative correlations between FSH, LH, and prolactin with some SP free amino acids ($P < 0.05$ for all), and a positive correlation between glycine with total testosterone ($P < 0.05$). Among iOAT patients, LH and FSH were positively correlated with SP zinc, TEX101, and some measured free amino acids ($P < 0.05$ for all). Total testosterone was positively correlated with some amino acids, while prolactin was negatively correlated with glycine ($P < 0.05$ for all). iOAT and azoospermic men exhibited low SP zinc and some free amino acids levels that were more pronounced in azoospermic men and were significantly associated with the reproductive hormones. TEX101 could be a helpful confirmatory test for azoospermia.

Keywords Seminal plasma · TEX101 · Zinc · Amino acids · Male infertility

Introduction

Male factor infertility is estimated to be present in 50%, accounting for a male in 30% and a contributing female cause in 20% of patients [1]. Adult male infertility can be caused by several factors, were idiopathic male infertility with no identified causative factors diagnosed in 30–40% [2].

Seminal plasma (SP) contains numerous proteins, ribonucleic acids, and many metabolites. These are candidate non-invasive pathogenic and diagnostic biomarkers of male reproductive system, including infertility. Testis-specific biomarkers may not be found in the blood owing to the testicular and epididymal blood barriers [3–6].

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Zinc has a crucial role in the male genital tract development, growth, and maturation necessary for fertility [7, 8]. Reduced zinc levels hinder the activity of various zinc-dependent enzymes required for spermatogenesis, sperm maturation, and activity with subsequent fall in spermatozoa fertilization capacity [9, 10].

SP human testis-expressed sequence 101 (TEX101), also known as testis-expressed sequence 101, is a recently identified protein, notably synthesized by the germ cells of the testis [11, 12]. Bioinformatic analysis shows that TEX101 is a membrane glycosyl-phosphatidyl-inositol (GPI)-anchored protein with a conserved UPAR/Ly6 domain, indicating a similar protein structure as urokinase-type plasminogen activator receptor (uPAR). TEX101 was found to be highly expressed in the testis and is involved in acrosome reaction [11, 13, 14]. Therefore, TEX101 is closely related to the male fertility.

The functions of SP free amino acids are not well-known. They were found to play a vital role as spermatozoa bioenergetics and are possibly involved in a pro-survival detoxification process [15, 16]. SP free amino acids are mainly synthesized in male genital tissues. However, degradation of seminal proteins after ejaculation by semen proteolytic enzymes is another source [17].

Currently, a few studies collectively evaluated SP levels of zinc, TEX101, and free amino acids among infertile men. Therefore, the aim of present study was to investigate the possible role of SP zinc, TEX101, and free amino acid levels and their correlations with reproductive hormones in infertile men compared with healthy fertile men.

Materials and Methods

Study Design and Setting

This cross-sectional study was conducted in the period from January 1st, 2018, to July 1st, 2019. It enrolled100 infertile male patients who were categorized into two groups; A included 50 non-obstructive azoospermic patients, and B included 50 idiopathic oligoasthenoteratozoospermia (iOAT) patients. Additionally, 50 healthy men served as the control fertile group. Healthy controls exhibited normozoospermia and had fathered at least one child with no history of infertility. All patients were selected from the Outpatient Clinics of Dermatology, Venereology and Andrology Departments, Qena and Sohag University Hospitals, Egypt. The study considered the criteria of the Declaration of Helsinki and a written informed consent was obtained from the anonymously enrolled participants after approval of the study by Faculty of Medicine Ethics Committee, South Valley University, Qena, Egypt. Ethical approval code: SVU-MED-MBC004-2017-12.

Patients' Selection Criteria

All included infertile patients with male factor infertility (non-obstructive azoospermia or iOAT) had a history of primary infertility for at least 1 year with normal fertile female partner. Those having history suggestive of epididymo-orchitis, prostatitis, genital trauma, testicular torsion, cryptorchidism, inguinal or genital surgery or undescended testes, obstructive azoospermia, varicoceles, urinary tract infection, chronic medical diseases, or receiving chemotherapy or radiotherapy or hormonal therapy, were excluded from the study. Also, Infertile men with GIT disorders or special diet regimen (e.g., vegetarians) or receiving multivitamins or zinc supplementations in the last 3 months were excluded from the study.

Data Collections

Medical history and demographics recorded included age, residence, smoking status, duration of the marriage, duration of infertility, and type of infertility. A thorough clinical examination and a local genital examination comprised testicular volume measurement by the Prader orchidometer [18]. Presence of varicoceles was excluded by scrotal Doppler US based on published criteria by Dubin & Amelar [19].

Blood Samples

Five milliters of peripheralvenous blood samples was collected in plain collection tubes for serum recovery. Samples were allowed to clot for 30 min at 37 °C before centrifugation, and sera obtained were aliquoted into 1-mL cryotubes and stored at − 20 °C until used. Specific ELISA assays were used according to the manufacturer's instructions, to measure levels of each of follicle-stimulating hormone (FSH) and prolactin (Calbiotech Inc., Spring Valley, CA-USA; Catalog No. FS046F and PR234F, respectively), luteinizing hormone (LH) (Cayman Chemical, Ann Arbor, MI-USA; Catalog No. 500720), and total testosterone (ALPCO, NH-USA; Catalog No. 11-TESHU-E01). Developed color was recorded using EMR-500 microplate ELISA reader.

Semen Samples

Semen samples were obtained by masturbation, after sexual abstinence of 3–5 days. Semen analysis was performed according to WHO criteria [20]. Semen liquefaction time, appearance, viscosity, and volume were assessed macroscopically, while sperm motility, morphology, and concentration were assessed microscopically [21, 22]. Azoospermia was confirmed after centrifugation of semen, while oligozoospermia was diagnosed when sperm concentration is under 20 million/mL [20].

After allowing 30 min for liquefaction, to recover SP, semen samples were centrifuged for 10 min at 4000 rpm and SP

were stored in cryotubes, stored at − 80 °C till used for analysis of the following biochemical biomarkers:

A) SP zinc level was measured colorimetrically (Spectrum Diagnostics, Cairo, Egypt, Catalog No. 330001) [23–26].
B) Chromatographic analysis of SP free amino acids and their derivatives utilized the amino acid S433 automatic analyzer (Sykam GmbH, Germany, Catalog No. 1120001). Using the ninhydrin reagent (Catalog No. S000025) ready to use and citrate buffers at different pH (8.0, 4.2, or 2.9). Eight hundred microliters aliquot of SP poured in a microcentrifuge tube was added to 200 μL 10% sulfosalicylic acid solution and was vortex mixed. The mix was cooled down at 4 °C for 30 min and the supernatant was recovered by centrifugation in a cooling centrifuge at 14000 RPM for 10 min. An aliquot of the supernatant was diluted 1:1 in sample dilution buffer before the chromatographic analysis [27]. The measured amino acids included branched-chain amino acids (valine, leucine, and isoleucine), aromatic amino acids (phenylalanine, tyrosine, and tryptophan), alanine, glycine, aspartic acid, serine, thereonine, histidine, proline, basic amino acids (arginine, lysine, and ornithine), and some amino acid derivatives (aspargine, taurine, and α-amino adipic acid).
C) SP levels of TEX101 were measured by a commercially available specific ELISA kit (Bioassay Technology Laboratory, Shanghai, China, catalog No. E4614Hu).
D) SP total proteins were analyzed colorimetrically using commercially available kit (Spectrum Diagnostics, Cairo, Egypt, catalog No. 310001). The level of each SP biochemical parameter was normalized by dividing by the sample total protein content and then was multiplied by 1000 to get their concentration per mg SP proteins [28].

Statistical Analysis

Kolmogorov-Smirnov test was used to check for data normality. Qualitative data was expressed as frequency: numbers and percentages. Parametric quantitative data was expressed as mean ± standard deviation, while median and inter-quartile range used for non-parametric data. For comparison between two groups, the chi-square test ($\chi 2$) was used for qualitative variables, while the Student's *t* test was used for normally distributed parametric data. For abnormally distributed quantitative variables (non-parametric data), the Mann-Whitney *U* test was used. Correlations were analyzed, using Spearman correlation among quantitative variables in the case of non-parametric data, while Pearson correlation was applied for normally distributed data. *P* value < 0.05 was considered significant. Statistical assessments utilized Statistical Package for Social Sciences (SPSS) for Windows version 20.0 (Armonk, NY: IBM Corp).

Results

Demographic Data of the Study Participants

The current study included 100 infertile men categorized into two groups: 50 azoospermic cases with a mean age of 34.26 ± 4.17 years (mean ± SD), and, 50 iOAT cases with a mean age of 34.92 ± 6.87 years. Fifty fertile control men with a mean age of 32.02 ± 2.80 years were included for comparison. There were insignificant age differences among the three groups. There was a significantly higher frequency of urban residency, heavy smoking, and small-sized testis among azoospermic patients (42%, 18%, and 32%, respectively) vs. both iOAT patients (12%, 8%, and 6%, respectively) and the fertile controls (14%, 6%, and 0%, respectively); $P < 0.05$ for all (Table 1).

Semen Analysis Parameters of the Studied Participants

There were significantly lower mean ± SD values of semen volume of both azoospermic men (2.09 mL ± 0.55) and iOAT patients (2.42 mL ± 0.54) opposed to the fertile controls (2.68 mL ± 0.47), $P < 0.05$ for all, with insignificant difference among the studied participants regarding to the liquefaction time ($P > 0.05$) as presented in (Table 2). There were significantly lower mean values of sperm concentration (million/mL), total sperm motility (%), abnormal sperm morphology (%), and sperm viability (%) among iOAT patients (41.44 ± 31.31, 40.2 ± 21.24, 52.2 ± 20.9, and 45.6 ± 20.94, respectively) compared to the fertile controls (64.2 ± 23.39, 79.2 ± 2.74, 35 ± 5.71, and 62.8 ± 20.46, respectively), $P < 0.05$ for all (Table 2).

Reproductive Hormonal Profiles of the Study Participants

The median serum FSH and LH levels were significantly higher among patients with azoospermia (18.45 μIU/mL and 12.0 mIU/mL, respectively) compared to both iOAT patients (7.4 μIU/mL and 4.95 mIU/mL, respectively) and fertile controls (6.7 μIU/mL and 3.5 mIU/mL, respectively ($P < 0.05$ for all), with insignificant differences in the serum total testosterone and prolactin levels between the studied participants, ($P > 0.05$), (Table 3).

SP Zinc and TEX101 Levels of the Study Participants

The median SP levels of zinc and TEX101 were significantly lower among azoospermia patients (0.283 μg/mg SP protein and 0.08 ng/mg SP proteins, respectively) vs. each of iOAT

Table 1 Demographic, clinical, and semen analysis data of the studied participants

Variables	Azoospermic patients ($n = 50$)	iOAT patients ($n = 50$)	Controls ($n = 50$)	P1-value	P2-value	P3-value
Age (mean ± SD)	34.26 ± 4.17	34.92 ± 6.87	33.78 ± 7.12	0.503	0.681	0.417
Residence, no (%)						
• Urban	21 (42%)	6 (12%)	7 (14%)			
• Rural	7 (14%)	10 (20%)	11 (22%)	0.003*	< 0.0001*	0.698
• Suburban	22 (44%)	34 (68%)	32 (64%)			
Duration of marriage (mean ± SD)	6.14 ± 2.18	5.5 ± 2.58	5.4 ± 2.24	0.172	0.097	0.836
Smoking index, no (%)						
• No	30 (60%)	28 (56%)	27 (54%)			
• Mild	2 (4%)	12 (24%)	13 (26%)	0.021*	< 0.0001*	0.757
• Moderate	9 (18%)	6 (12%)	7 (14%)			
• Heavy	9 (18%)	4 (8%)	3 (6%)			
Duration of infertility (mean ± SD)	5.42 ± 1.55	4.86 ± 2.24	5.51 ± 1.23	0.150	0.748	0.075
Local examination, no (%)						
• Testis size						
- Normal sized	34 (68%)	47 (94%)	50 (100%)	0.001*	–	–
- Small sized	16 (32%)	3 (6%)	-			
Semen analysis (mean ± SD)						
• Volume (ml)	2.09 ± 0.55	2.42 ± 0.54	2.68 ± 0.47	0.002*	< 0.0001*	0.011*
• Liquefaction time (min.)	12.4 ± 2.81	12.7 ± 3.23	11.8 ± 3.16	0.626	0.318	0.162
• Sperm concentration (million/ml)	0 ± 0	10.44 ± 31.31	64.2 ± 23.39	< 0.0001*	< 0.0001*	< 0.0001*
• Total sperm motility (%)	0 ± 0	25.27 ± 11.24	79.2 ± 2.74	< 0.0001*	< 0.0001*	< 0.0001*
• Abnormal sperm forms (%)	0 ± 0	98.2 ± 20.9	35 ± 5.71	< 0.0001*	< 0.0001*	< 0.0001*
• Sperm viability (%)	0 ± 0	45.6 ± 20.94	62.8 ± 20.46	< 0.0001*	< 0.0001*	< 0.0001*

*Significant P value < 0.05. Data were expressed as mean ± SD or numbers and percentages. *iOAT* idiopathic oligoasthenoteratozoospermia

N.B: P1 (comparing azoospermia vs. iOAT, P2 comparing azoospermia vs. healthy controls, and P3 comparing iOAT vs. healthy controls)

patients (0.347 μg/mg SP protein and 0.21 ng/mg SP protein, respectively; $P < 0.05$) and the fertile controls (0.451 μg/mg SP protein and 0.22 ng/mg SP protein, respectively; $P < 0.05$ (Table 4). There was significantly lower SP zinc levels among iOAT patients vs. controls ($P < 0.05$), but a non-significant difference between the two groups regarding SP TEX101 levels (Table 4).

There were significantly positive correlations between SP zinc levels and SP TEX-101concentration among both azoospermic men ($r = 0.664$, $P < 0.001$) and iOAT patients ($r = 0.643$, $P < 0.001$), with non-significant correlation could be recorded among fertile controls between the two measured parameters ($r = 0.167$, $P = 0.246$) as presented in (Table 5).

SP Levels of Free Amino Acids Among the Study Participants

Patients with azoospermia exhibited significantly lower median SP contents of several individual free amino acids that included aspartic acid, threonine, serine, asparagine, glycine, alanine, valine, isoleucine, leucine, tyrosine, histidine, tryptophan, ornithine, lysine, arginine, and proline, total amino acids, total branched-chain amino acids, branched-chain amino acids/aromatic amino acid ratio, aromatic amino acid/total amino acids ratio, and branched-chain amino acids/total amino acids ratio compared to men with iOAT. There were significantly lower SP levels of taurine and α-amino adipic acid among the two infertile groups in comparison with the controls, and significantly lower SP levels of taurine, aspartic acid, aspargine, alanine, leucine, histidine, lysine, and proline, and, total amino acids only among men with iOAT compared to the fertile controls, $P < 0.05$ for all (Table 6).

Correlations Between SP Levels of Zinc, TEX101, the Measured Amino Acids and Reproductive Hormones Among the Included Infertile Men

Among patients with azoospermia, SP zinc levels positively correlated with levels of each of aspartic acid ($r = 0.371$, $P = 0.008$), asparagine ($r = 0.314$, $P = 0.026$), glycine ($r = 0.306$, $P = 0.031$), alanine ($r = 0.518$, $P < 0.001$), valine ($r = 0.335$, $P = 0.018$), leucine ($r = 0.455$, $P = 0.001$), proline ($r = 0.416$,

Table 2 Semen analysis parameters of the studied participants

Variables	Azoospermic patients ($n = 50$)	iOAT patients ($n = 50$)	Controls ($n = 50$)	P1-value	P2-value	P3-value
Volume (ml)	2.09 ± 0.55	2.42 ± 0.54	2.68 ± 0.47	0.002*	< 0.001*	0.014*
Liquefaction time (min)	12.4 ± 2.81	12.7 ± 3.23	11.8 ± 3.16	0.626	0.330	0.145
Sperm concentration (million/ml)	0 ± 0	41.44 ± 31.31	64.2 ± 23.39	< 0.001*	< 0.001*	< 0.001*
Total sperm motility (%)	0 ± 0	40.2 ± 21.24	79.2 ± 2.74	< 0.001*	< 0.001*	< 0.001*
Abnormal sperm morphology (%)	0 ± 0	52.2 ± 20.9	35 ± 5.71	< 0.001*	< 0.001*	< 0.001*
Viability (%)	0 ± 0	45.6 ± 20.94	62.8 ± 20.46	< 0.001*	< 0.001*	< 0.001*

*Significant P value < 0.05. Data are expressed as mean ± SD

N.B: P1 (comparing azoospermia vs. iOAT, P2 comparing azoospermia vs. healthy controls, and P3 comparing iOAT vs. healthy controls)

$P = 0.003$), total amino acids ($r = 0.395$, $P = 0.005$), and branched-chain amino acids ($r = 0.428$, $P = 0.002$) as presented in (Table 7). SP levels of TEX101 positively correlated with levels of each of taurine ($r = 0.303$, $P = 0.033$), aspartic acid ($r = 0.450$, $P = 0.001$), glycine ($r = 0.343$, $P = 0.015$), alanine ($r = 0.433$, $P = 0.002$), valine ($r = 0.487$, $P < 0.001$), histidine ($r = 0.280$, $P = 0.049$), total amino acids ($r = 0.386$, $P = 0.006$), and branched-chain amino acids ($r = 0.415$, $P = 0.003$), (Table 7).

Among patients with iOAT, SP zinc significant positively correlated with levels of each of leucine ($r = 0.345$, $P = 0.014$), tryptophan ($r = 0.983$, $P < 0.001$), total amino acids ($r = 0.475$, $P < 0.001$), aromatic amino acids ($r = 0.852$, $P < 0.001$), branched-chain amino acids/aromatic amino acid ratio ($r = 0.484$, $P < 0.001$), and aromatic amino acid/total amino acids ratio ($r = 0.750$, $P < 0.001$). There were no significant correlations between SP TEX101 with the measured SP free amino acids (Table 8). Additionally, SP zinc positively correlated with seminal volume ($r = 0.373$, $P = 0.008$). Also, there were positive correlations between seminal volume and levels of each of leucine ($r = 0.318$, $P = 0.024$), tyrosine ($r = 0.429$, $P = 0.002$), and proline ($r = 0.401$, $P = 0.004$). Additionally, there was significant positive correlation between liquefaction time and glycine content ($r = 0.421$, $P = 0.001$). There were significant correlations between total sperm motility and levels of each of glycine ($r = 0.327$, $P = 0.022$) and arginine ($r = 0.435$, $P = 0.002$). There were significant correlations between sperm viability and levels of each of glycine ($r = 0.336$, $P = 0.018$), tyrosine ($r = 0.451$, $P = 0.012$), and arginine ($r = 0.324$, $P = 0.022$).

As regards the correlations of the measured SP biochemical parameters with the serum reproductive hormones among azoospermic patients, FSH level negatively correlated with levels of each of threonine, serine, and arginine ($r = -0.947$, $P = 0.000$; $r = -0.732$, $P = 0.016$; and $r = -0.680$, $P = 0.030$, respectively). LH level negatively correlated with levels of each of threonine and serine ($r = -0.914$, $P = 0.000$; and r = −0.793, $P = 0.006$, respectively). In contrast, total testosterone level positively correlated with glycine ($r = 0.763$, $P = 0.010$). Prolactin level negatively correlated with serine content ($r = -0.688$, $P = 0.028$), (Table 9).

Among iOAT patients, FSH level positively correlated with levels of each of aspartic acid, valine, ornithine, proline, zinc, and TEX101 ($r = 0.658$, $P = 0.038$); $r = 0.669$, $P = 0.035$; $r = 0.645$, $P = 0.044$; $r = 0.653$, $P = 0.040$; $r = 0.908$, $P = 0.000$, and $r = 0.909$, $P = 0.000$, respectively). LH level positively correlated with levels of each of aspartic acid, alanine, valine, histidine, ornithine, proline, total branched-chain amino acids, zinc, and TEX101 ($r = 0.714$, $P = 0.020$; $r = 0.638$, $P = 0.047$; $r = 0.718$; $P = 0.019$; $r = 0.699$, $P = 0.024$;

Table 3 The reproductive hormonal profiles of the studied participants

Variables	Azoospermic patients ($n = 50$)	iOAT patients ($n = 50$)	Controls ($n = 50$)	P1-value	P2-value	P3-value
FSH (μIU/ml)	18.45 (9.63–28.03)	7.4 (5.88–10.4)	6.7(6.25–8.8)	0.009*	0.019*	0.679
LH (mIU/ml)	12 (4.93–16.88)	4.95 (2.48–8.33)	3.5(1.8–6.4)	0.023*	0.028*	0.440
Total testosterone (ng/ml)	2.55 (1.05–4.28)	3.2 (2.18–4.05)	3.7(2.95–4.7)	0.684	0.254	0.371
Prolactin (ng/ml)	9.5 (6.88–13.55)	6.9 (5.13–8.23)	6.7(5.35–7.3)	0.063	0.055	0.679

*Significant P value < 0.05. Data were expressed median and inter-quartile range (IQR). *iOAT* idiopathic oligoasthenoteratozoospermia, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone

N.B: P1 (comparing azoospermia vs. iOAT, P2 comparingazoospermia vs. healthy controls, and P3 comparing iOAT vs. healthy controls)

Table 4 Seminal plasma zinc and TEX-101 levels of the investigated infertile men compared to healthy controls

Variables	Azoospermic patients ($n = 50$)	iOAT patients ($n = 50$)	Controls ($n = 50$)	P1-value	P2-value	P3-value
Zinc (μg/mg SP proteins)	0.283 (0.218–0.386)	0.347 (0.227–0.740)	0.451 (0.238–0.745)	0.024*	0.01*	0.027*
TEX-101 (ng/mg SP proteins)	0.08 (0.06–0.13)	0.21 (0.07–0.23)	0.22 (0.08–0.25)	0.024*	0.005*	0.411

*Significant P value < 0.05. Data are expressed as median and inter-quartile range (IQR). *SP* seminal plasma, *TEX-101* testis-expressed sequence 101
N.B: P1 (comparing azoospermia vs. iOAT, P2 comparing azoospermia vs. healthy controls, and P3 comparing iOAT vs. healthy controls)

$r = 0.692$, $P = 0.027$; $r = 0.766$, $P = 0.010$; $r = 0.720$, $P = 0.019$; $r = 0.898$, $P = 0.000$, and $r = 0.842$, $P = 0.0002$, respectively). Total testosterone level positively correlated with contents of each tyrosine and total aromatic amino acids/total amino acids ratio ($r = 0.717$, $P = 0.020$, and $r = 0.676$, $P = 0.032$, respectively. Prolactin level negatively correlated with glycine content ($r = -0.682$, $P = 0.030$ respectively), (Table 10).

Discussion

Male factor infertility has an increasing role in reproductive infertility of the couples as assessment of male reproductive functions became a mandatory item in the reproductive profiling of any couple. However, azoospermia and iOAT are still challenging topics in infertility management [29].

Normal testicular measures of the adults have been well-known to be at least 4.6 cm in length and 2.6 cm in width with a volume varying from 18 to 20 cm^3. Since 85% of the volume of the testis is linked with the production of sperms, the reduced size of the testis denotes a deteriorated spermatogenesis potentiality [29, 30]. In the current study, azoospermic patients displayed smaller testicular size than those with iOAT. In agreement, Han et al. [31] and Kitilla [32] both showed that azoospermic patients with small testicular sizes were likely to complain of deteriorated spermatogenesis.

Despite of the already-established damaging effects of smoking on general health, and men reproductive system, smoking is still a universally common habit [33, 34]. In the present study, nearly 40% of infertile men were smokers with a significantly higher frequency of heavy smokers among azoospermia patients. The impact of smoking established in different semen biological parameters as smoking enhances the reactive oxygen species, and so leading to oxidative stress [34].

The correlation of the rate of fertility with residency location is pivotal to characterize effect of demographic variables [35]. In the present study, urban residencies among azoospermic patients were higher than patients with iOAT. This was in line with Kulu [36], who reported higher fertility in rural areas in well-developed countries.

Testosterone, FSH, and LH assessments are essential in the management plan of male infertility [37]. FSH binds to receptors on the Sertoli cells and activates spermatogenesis. LH enhances testosterone synthesis in Leydig cells that in turn affects the peritubular cells of the seminiferous tubules and Sertoli cells to enhance spermatogenesis [38]. FSH is considered as a biomarker of spermatogenesis and Sertoli cell functions and the risk of abnormal semen parameters is negatively linked with its blood concentrations. In the same way, the testicular volume is considered as an essential clinical marker of spermatogenesis that is negatively correlated with testicular functions [39, 40]. In the infertile men, an elevated FSH level is a consistent indicator of germinal epithelial damage and related to azoospermia and severe oligozoospermia [41]. In this study, there were significantly higher LH and FSH serum levels among azoospermic patients than those with iOAT. These findings are in accordance with previous reports [37, 42–44]. Additionally, in the present study, there was no significant difference in the median serum total testosterone

Table 5 Correlation between SP zinc with TEX-101 among the study participants

Variables/study participants	Zinc (μg/mg SP proteins)					
	Azoospermic patients ($n = 50$)		iOAT patients ($n = 50$)		Controls ($n = 50$)	
	r	P	r	P	r	P
TEX-101 (ng/mg SP proteins)	0.664	< 0.001*	0.643	< 0.001*	0.167	0.246

*Significant P value < 0.05. *SP* seminal plasma, *TEX-101* testis-expressed sequence 101

Table 6 Seminal plasma levels of free amino acids levels of the studied participants

Variables	Azoospermic patients ($n = 50$)	iOAT patients ($n = 50$)	Controls ($n = 50$)	P1-value	P2-value	P3-value
Taurine	0.22 (0.21–0.33)	0.27 (0.18–0.38)	0.44 (0.42–0.46)	0.285	< 0.001*	< 0.001*
Aspartic acid	0.66 (0.32–0.90)	0.89 (0.70–1.2)	1.2 (1–1.5)	< 0.001*	< 0.001*	< 0.001*
Threonine	1 (0.9–1.4)	1.6 (1.3–1.8)	1.7 (1.4–1.9)	< 0.001*	< 0.001*	0.843
Serine	3.2 (2.9–3.4)	3.62 (3.51–3.75)	3.972 (3.65–3.87	< 0.001*	< 0.001*	0.346
Asparagine	3.72 (2.8–4.1)	3.99 (3.88–4.20)	4.35 ± 0.25 4.3 (4.18–4.60)	0.001*	< 0.001*	< 0.001*
α–Amino adipic acid	0.36 (0.15–0.45)	0.4 (0.22–0.66)	1.75 (1.6–1.8)	0.651	< 0.001*	< 0.001*
Glycine	1.64 (1.2–1.89)	1.98 (1.9–2.1)	1.86 (1.49–1.93)	< 0.001*	< 0.001*	0.651
Alanine	0.39 (0.28–0.59)	0.5 (0.44–0.53)	1.4 (1.3–1.4)	0.029*	< 0.001*	< 0.001*
Valine	1.3 (0.97–1.9)	1.86 (1.37–2.21)	1.91 (1.47–2.71)	< 0.001*	< 0.001*	0.365
Isoleucine	0.93 (0.37–0.99)	1.31 (1.22–1.39)	1.29 (1.22–1.31)	< 0.001*	< 0.001*	0.331
Leucine	1.28 (1.1–1.45)	1.62 (1.56–1.69)	1.78 (1.68–1.87)	< 0.001*	< 0.001*	< 0.001*
Tyrosine	0.84 (0.78–1)	1.16 (0.91–1.28)	1.14 (1.11–1.22)	< 0.001*	< 0.001*	0.748
Phenylalanine	0.63 (0.54–0.69)	0.72 (0.29–1.2)	0.73 (0.39–1.89)	0.801	0.365	0.802
Histidine	0.88 (0.59–0.98)	1.28 (1.22–1.33)	1.5 (1.28–1.51)	< 0.001*	< 0.001*	< 0.001*
Tryptophan	0.35 (0.28–0.43)	0.47 (0.33–0.7)	0.44 (0.30–0.5)	0.006*	0.01*	0.843
Ornithine	1.16 (0.88–1.35)	2 (1.5–2.4)	2 (1.7–2.3)	< 0.001*	< 0.001*	0.604
Lysine	1 (0.87–1.33)	1.8 (0.94–2.8)	2.6 (2.1–2.8)	< 0.001*	< 0.001*	< 0.001*
Arginine	1.3 (0.95–1.4)	1.67 (1.52–1.85)	1.61 (1.42–1.95)	0.001*	0.001*	0.41
Proline	0.19 (0.15–0.26)	0.25 (0.22–0.27)	1.5 (1.49–1.1.73)	< 0.001*	< 0.001*	< 0.001*
Total amino acids	22.55 (19.23–24.24)	28.45 (26.81–29.31)	30.43 (29.88–31.69)	< 0.001*	< 0.001*	< 0.001*
Aromatic amino acids	2.14 (1.97–2.27)	2.24 (1.71–2.93)	2.36 (2.13–2.39)	0.404	< 0.001*	0.704
Branched-chain amino acids	3.48 (3.18–3.85)	4.92 (4.29–5.32)	4.12 (4.10–5.12)	< 0.001*	0.001*	0.517
Branched-chain amino acids/aromatic amino acids ratio	1.48 (1.12–1.75)	2.3 (1.99–2.55)	2.4 (2.01–2.75)	<0.001*	<0.001*	0.176
Aromatic amino acids/total amino acids ratio	0.076 (0.07–0.081)	0.11 (0.09–0.133)	0.12 (0.08–0.141)	< 0.001*	< 0.001*	0.418
Branched-chain amino acids/total amino acids ratio	0.159 (0.15–0.171)	0.169 (0.152–0.191)	0.171 (0.172–0.187)	0.002*	< 0.001*	0.204

*Significant P value < 0.05. Data are expressed as median and inter-quartile range (IQR). *SP* seminal plasma

N.B: P1 (comparing azoospermia vs. iOAT, P2 comparing azoospermia vs. healthy controls, and P3 comparing iOAT vs. healthy controls)

levels comparing azoospermic and iOAT men. This was in line with many researchers [42, 43, 45].

Presence of a large number of tissue-specific proteins and other metabolites in SP provides a high source of biomarkers for the assessment of male fertility [4]. Each of the glands of the male reproductive system is isolated from the systemic circulation by a strong tissue-blood barrier. So, reproductive gland-specific proteins are exemplary not presented in blood serum. Identification of both testis-specific and germ cell type-specific proteins secreted into semen notably by spermatocytes, spermatids, or spermatozoa should supply markers that meticulously pinpoint the stages of spermatogenesis [3, 6].

In the current study, SP zinc, TEX101, and free amino acid levels were measured and compared among healthy controls, azoospermic patients, and iOAT men. We found significantly lower SP zinc levels among azoospermic men compared with iOAT patients. Zinc is a vital trace mineral for normal male reproductive functions and SP zinc concentrations represent the prostatic secretory functions, and have antioxidant activity [46]. Zinc particularly affects conversion of testosterone into dihydrotestesterone as the converting enzyme (5α-reducatse) is zinc dependent [47]. Our findings are in line with previous investigators [48–51]. The reduction in the SP zinc level may result from insufficient intake, decreased absorption, enhanced losses, or enhanced demands [52]. SP zinc levels were previously linked with men sperm quality [53].

Proteins with testis-specific expression, such as TEX101, have emanated as essential biomarkers for testicular sperm extraction outcomes and for differentiation between hypospermatogenesis, maturation arrest, and non-obstructive subtypes of Sertoli cell-only syndrome [4]. Studies comparing SP levels of TEX101 among infertile men are scarce. The

Table 7 Correlations between specific SP free amino acids with both SP zinc and SP TEX-101 concentrations among azoospermic patients

SP amino acids (μmol/mg SP proteins)	Zinc (μg/mg SP proteins)	TEX-101 (ng/mg SP proteins)
Taurine	r = 0.157	r = 0.303
	P = 0.278	*P* = 0.033*
Aspartic acid	r = 0.371	r = 0.450
	P = 0.008*	*P* = 0.001*
Threonine	r = 0.216	r = 0.255
	P = 0.132	*P* = 0.074
Serine	r = −0.016	r = 0.058
	P = 0.913	*P* = 0.687
Aspargine	r = 0.314	r = 0.221
	P = 0.026*	*P* = 0.122
α-amino adipic acid	r = 0.063	r = − 0.068
	P = 0.662	*P* = 0.639
Glycine	r = 0.306	r = 0.343
	P = 0.031*	*P* = 0.015*
Alanine	r = 0.518	r = 0.433
	P = < 0.001**	*P* = 0.002*
Valine	r = 0.335	r = 0.487
	P = 0.018*	*P* = < 0.001*
Isoleucine	r = − 0.118	r = − 0.199
	P = 0.414	*P* = 0.166
Leucine	r = 0.455	r = 0.163
	P = 0.001**	*P* = 0.259
Tyrosine	r = 0.078	r = − 0.174
	P = 0.588	*P* = 0.226
Phenylalanine	r = 0.183	r = 0.095
	P = 0.205	*P* = 0.514
Histidine	r = 0.139	r = 0.280
	P = 0.335	*P* = 0.049*
Tryptophan	r = −0.173	r = −0.08
	P = 0.23	*P* = 0.581
Ornithine	r = 0.263	r = 0.272
	P = 0.065	*P* = 0.056
Lysine	r = −0.105	r = 0.002
	P = 0.47	*P* = 0.991
Arginine	r = 0.257	r = 0.262
	P = 0.072	*P* = 0.066
Proline	r = 0.416	r = 0.204
	P = 0.003*	*P* = 0.155
Total amino acids	r = 0.395	r = 0.386
	P = 0.005*	*P* = 0.006*
Aromatic amino acid	r = 0.039	r = − 0.072
	P = 0.786	*P* = 0.619
Branched-chain amino acids	r = 0.428	r = 0.415
	P = 0.002*	*P* = 0.003**
Branched-chain amino acids/ Aromatic amino acid ratio	r = 0.181	r = 0.108
	P = 0.209	*P* = 0. 30
Aromatic amino acid/ Total amino acids ratio	r = − 0.125	r = − 0.222
	P = 0.389	*P* = 0.121
Branched-chain amino acids/ Total amino acids ratio	r = 0.224	r = 0.208
	P = 0.118	*P* = 0.148

Table 8 Correlations between specific SP free amino acids with both SP zinc and SP TEX-101 concentrations among iOAT patients

SP amino acids (μmol/mg SP proteins)	Zinc (μg/mg SP proteins)	TEX-101 (ng/mg SP proteins)
Taurine	r = 0.243 *P* = 0.089	r = − 0.066 *P* = 0.648
Aspartic acid	r = 0.07 *P* = 0.627	r = − 0.068 *P* = 0.637
Threonine	r = 0.085 *P* = 0.559	r = − 0.005 *P* = 0.975
Serine	r = − 0.224 *P* = 0.118	r = − 0.191 *P* = 0.183
Aspargine	r = − 0.02 *P* = 0.89	r = 0.058 *P* = 0.687
α-Amino adipic acid	r = − 0.177 *P* = 0.218	r = 0.054 *P* = 0.709
Glycine	r = 0.182 *P* = 0.21	r = − 0.163 *P* = 0.264
Alanine	r = − 0.25 *P* = 0.08	r = − 0.082 *P* = 0.571
Valine	r = 0.067 *P* = 0.644	r = − 0.217 *P* = 0.129
Isoleucine	r = 0.096 *P* = 0.507	r = − 0.09 *P* = 0.535
Leucine	r = 0.345 *P* = 0.014*	r = 0.066 *P* = 0.648
Tyrosine	r = 0.029 *P* = 0.844	r = − 0.076 *P* = 0.602
Phenylalanine	r = − 0.035 *P* = 0.81	r = 0.009 *P* = 0.952
Histidine	r = 0.147 *P* = 0.309	r = − 0.181 *P* = 0.208
Tryptophan	r = 0.983 *P* = < 0.001**	r = − 0.074 *P* = 0.608
Ornithine	r = 0.204 *P* = 0.156	r = − 0.034 *P* = 0.815
Lysine	r = 0.201 *P* = 0.162	r = 0.105 *P* = 0.468
Arginine	r = − 0.247 *P* = 0.084	r = − 0.061 *P* = 0.676
Proline	r = 0.064 *P* = 0.661	r = 0.121 *P* = 0.403
Total amino acids	r = 0.475 *P* = < 0.001*	r = − 0.037 *P* = 0.799
Aromatic amino acid	r = 0.852 *P* = < 0.001*	r = − 0.051 *P* = 0.728
Branched-chain amino acids	r = 0.147 *P* = 0.309	r = − 0.193 *P* = 0.179
Branched-chain amino acids/aromatic amino acid ratio	r = 0.484 *P* = < 0.001*	r = − 0.014 *P* = 0.923
Aromatic amino acid/total amino acids ratio	r = 0.750 *P* = < 0.001*	r = − 0.054 *P* = 0.709
Branched-chain amino acids/total amino acids ratio	r = − 0.136 *P* = 0.346	r = − 0.139 *P* = 0.336

present study observed significantly lower SP TEX101 level among azoospermic men in comparison with iOAT patients. This was in accordance with Korbakis et al. [12].

We observed a significantly positive correlation between SP zinc levels and SP TEX-101 content among both infertile groups included in the current study. We could not trace such correlation in the literature. This could add additional role of zinc in enhancing male fertility and improving the spermatozoa fertility potential both in vivo and in vitro, which require further researches to confirm this finding.

Amino acids are the building units of the spermatozoal protein and have other distinctive functions such as maintain the spermatozoa viability by binding toxic heavy metals, reduction of free radicals, protecting cells against denaturation, provide oxidizable substrate to spermatozoa, and have a buffering action in the SP [54, 55]. Studies that tackled SP amino acids profiling and their derivatives in infertile men are lacking. Since metabolomics remains under-utilized in male infertility, it would be interesting to show how the metabolome levels of different amino acids and their derivatives can be

Table 9 Correlations of serum reproductive hormones with the measured SP biochemical parameters (zinc, TEX-101, and specific free amino acids) among azoospermic patients

Variables	FSH (μIU/ml)		LH (mIU/ml)		Total testosterone (ng/ml)		Prolactin (ng/ml)	
	r	*P*	*R*	*P*	*R*	*P*	*r*	*P*
Taurine	0.159	0.660	0.326	0.358	− 0.213	0.554	0.464	0.177
Aspartic acid	− 0.312	0.380	− 0.075	0.836	0.006	0.988	− 0.058	0.874
Therionine	− .947*	0.000	− .914*	0.000	− 0.391	0.264	− 0.370	0.292
Serine	− .732*	0.016	− .793*	0.006	− 0.413	0.235	− .688*	0.028
Aspargine	− 0.032	0.929	− 0.134	0.712	− 0.007	0.986	0.484	0.156
A-amino adibic acid	0.322	0.364	0.210	0.560	0.177	0.625	− 0.576	0.081
Glycine	− 0.023	0.951	− 0.121	0.738	.763*	0.010	0.143	0.693
Alanine	− 0.551	0.099	− 0.449	0.193	− 0.436	0.208	0.032	0.930
Valine	− 0.178	0.623	− 0.368	0.295	− 0.171	0.636	0.034	0.926
Isoleucine	− 0.438	0.206	− 0.474	0.166	− 0.202	0.576	0.338	0.339
Leucine	0.320	0.368	0.232	0.520	− 0.353	0.317	0.484	0.156
Tyrosine	− 0.348	0.324	− 0.252	0.483	− 0.329	0.354	− 0.006	0.987
Phenyl alanine	− 0.415	0.234	− 0.220	0.541	− 0.014	0.970	0.399	0.254
Histidine	− 0.180	0.619	− 0.385	0.273	0.059	0.871	− 0.118	0.746
Tryptophan	− 0.153	0.672	− 0.164	0.650	− 0.034	0.925	0.588	0.074
Ornithine	− 0.590	0.073	− 0.464	0.177	− 0.439	0.205	− 0.094	0.797
Lysine	− 0.062	0.866	0.148	0.683	− 0.174	0.631	0.532	0.113
Amonia	− 0.503	0.139	− 0.303	0.394	− 0.333	0.347	− 0.416	0.232
Arginine	− .680*	0.030	− 0.558	0.093	− 0.347	0.326	− 0.116	0.750
Proline	0.388	0.269	0.363	0.303	0.173	0.633	0.402	0.249
Total (mmol/L)	− 0.569	0.086	− 0.454	0.188	− 0.379	0.279	0.106	0.770
Aromatic amino acids	− 0.424	0.222	− 0.297	0.405	− 0.239	0.506	0.312	0.380
Branched-chain amino acids	0.181	0.617	− 0.007	0.984	− 0.128	0.724	0.253	0.481
Branched-chain:aromatic ratio	0.491	0.150	0.309	0.385	0.144	0.692	− 0.091	0.803
Aromatic: total ratio	− 0.216	0.548	− 0.133	0.714	− 0.021	0.953	0.387	0.269
Branched: total ratio	0.488	0.153	0.284	0.427	0.139	0.702	0.133	0.714
Zinc (μg/mg SP proteins)	0.357	0.312	0.337	0.341	0.068	0.852	− 0.105	0.773
TEX-101 (ng/ mg SP proteins)	0.391	0.264	0.414	0.234	0.180	0.618	− 0.038	0.918

altered in the human SP of infertile men. A study in Human Metabolome Database (Version 3.6) reveals that SP is not a common source of metabolomics studies for investigations of male infertility. It can be reported that there are only 9 human SP metabolites identified [56].

The current study revealed significantly lower many SP amino acids that included aspartic acid and its amide asparagine, valine, leucine, isoleucine, threonine, serine, glycine, alanine, tyrosine, histidine, tryptophan, ornithine, lysine, arginine, proline, total amino acids, branched-chain amino acids/aromatic amino acid ratio, aromatic amino acid/total amino acids ratio, and branched-chain amino acids/total amino acids ratio among patients with azoospermia in comparison to men with iOAT. Papp et al. [57] established the importance of basic amino acids in male reproduction. They investigated the alterations in SP levels of arginine, ornithine, and total amino acid levels in different forms of pathospermia. Arginine and ornithine were the most overly decreased amino acids, and if corrected, they may assist in enhancing the fertility potential of the spermatozoa. Furthermore, Nissen et al. [58] reported significantly lower SP levels of each of aspartic acid, glutamic acid, threonine, glycine, alanine, and isoleucine among infertile men.

In the current study, correlation analysis among parameters measured for azoospermic men revealed negative correlations between some SP amino acids with FSH, LH, and prolactin, and positive correlation between glycine and total testosterone, which could be explained by spermatogenic failure among these patients. Among iOAT patients, LH and FSH positively correlated with SP zinc, TEX101, and specific

Table 10 Correlations of serum reproductive hormones with the measured SP biochemical parameters (zinc, TEX-101, and specific free amino acids) among iOAT patients

Variables	FSH (μIU/ml)		LH (mIU/ml)		Total testosterone (ng/ml)		Prolactin (ng/ml)	
	r	*P*	*r*	*P*	r	*P*	r	*P*
Taurine	0.141	0.698	0.131	0.719	− 0.116	0.751	0.529	0.116
Aspartic acid	0.658*	0.038	0.714*	0.020	− 0.146	0.687	− 0.246	0.494
Therionine	0.202	0.576	0.286	0.424	0.419	0.228	− 0.031	0.931
Serine	− 0.033	0.927	− 0.016	0.964	0.237	0.509	− 0.551	0.099
Aspargine	0.405	0.246	0.524	0.120	0.035	0.924	− 0.260	0.468
α-amino adipic acid	0.120	0.741	0.222	0.537	− 0.326	0.358	− 0.599	0.068
Glycine	0.182	0.615	0.241	0.503	0.086	0.814	− 0.682*	0.030
Alanine	0.623	0.054	0.638*	0.047	0.038	0.917	− 0.200	0.580
Valine	0.669*	0.035	0.718*	0.019	− 0.086	0.814	− 0.153	0.672
Isoleucine	− 0.395	0.258	− 0.413	0.236	0.105	0.772	− 0.084	0.817
Leucine	0.479	0.162	0.594	0.070	− 0.163	0.653	− 0.479	0.162
Tyrosine	− 0.306	0.390	− 0.298	0.403	0.717*	0.020	0.270	0.450
Phenyl alanine	0.006	0.987	0.046	0.899	0.603	0.065	0.346	0.327
Histidine	0.623	0.054	0.699*	0.024	− 0.455	0.186	− 0.496	0.145
Tryptophan	0.135	0.711	0.215	0.551	− 0.294	0.409	0.044	0.904
Ornithine	0.645*	0.044	0.692*	0.027	− 0.275	0.442	− 0.100	0.784
Lysine	0.397	0.256	0.418	0.229	− 0.432	0.213	− 0.207	0.565
Amonia	0.138	0.705	0.211	0.559	− 0.112	0.759	− 0.094	0.796
Arginine	0.300	0.400	0.383	0.275	0.376	0.284	0.017	0.962
Proline	0.653*	0.040	0.766*	0.010	− 0.111	0.759	− 0.470	0.171
Total (mmol/L)	0.469	0.171	0.578	0.080	0.084	0.818	− 0.300	0.400
Aromatic amino acids	− 0.070	0.849	− 0.011	0.975	0.627	0.053	0.373	0.288
Branched-chain amino acids	0.622	0.055	0.720*	0.019	− 0.070	0.848	− 0.506	0.135
Branched-chain: aromatic ratio	0.268	0.454	0.213	0.554	− 0.593	0.071	− 0.594	0.070
Aromatic: total ratio	− 0.279	0.435	− 0.260	0.469	0.676*	0.032	0.603	0.065
Branched: total ratio	0.051	0.888	0.016	0.965	− 0.204	0.573	− 0.168	0.642
Zinc (μg/mg SP proteins)	0.908*	0.000	0.898*	0.000	− 0.228	0.526	− 0.150	0.680
TEX-101 (ng/mg SP proteins)	0.909*	0.000	0.842*	0.002	− 0.372	0.289	0.080	0.827

amino acids. Additionally, total testosterone serum levels positively correlated with some amino acids, while prolactin negatively correlated with glycine. The marked positive association between SP zinc and serum LH and FSH indicates that zinc could be directly related to the pituitary-gonadal axis [49]. Akinloye et al. [49] found significant positive correlations between SP zinc concentrations with both serum FSH and LH among infertile men. Ozturk et al. [59] have reported that zinc deficiency in male rats negatively affects not only testosterone production but also FSH and LH secretions.

Among our iOAT patients, there was a significantly positive correlation between SP zinc and seminal volume. Also, the seminal volumes positively correlated with SP levels of leucine, tyrosine, and proline. The semen liquefaction time, total sperm viability, and motility positively correlated with glycine and arginine. Likewise, Qiaoet al. [60] found that sperm concentrations positively correlated with levels of some amino acids detected by GC-MS, among iOAT patients. Engel et al. [61] described a positive association between sperm concentration and SP glutamine level with no significant correlations between the morphology of sperms with neither the amino acids nor the biogenic amine sperm contents.

To the best of our knowledge, this is the first study that reports the possible correlations between SP levels of several amino acids, TEX101, and zinc among infertile men. Among patients with azoospermia, there were significant positive correlations between SP zinc level with SP levels of aspartic acid, asparagine, glycine, alanine, leucine, proline, total amino

acids, and branched-chain amino acids, and, significant positive correlations between SP TEX101 with SP levels of taurine, aspartic acid, glycine, alanine, valine, histidine, total amino acids, and branched-chain amino acids. Among the included patients with iOAT, there were significant positive correlations between SP zinc with SP levels of leucine, tryptophan, total amino acids, aromatic amino acids, branched-chain amino acids/ aromatic amino acid ratio, and aromatic amino acid/ total amino acids ratio.

Conclusion

Assessments of SP zinc, TEX101, and amino acids are essential in the evaluations of infertile men beside the routine reproductive hormones and semen analysis as those could be useful in the management plan of such patients. Reproductive hormones were significantly correlated with SP zinc, TEX101, and some free amino acids levels that reflect the essential role of the measured biochemical parameters in assessing the male reproductive capacity. The positive correlation between SP zinc and SP TEX101 among infertile men with the significant reduction of SP TEX-101 levels in azoospermic men confirms the fertility potential effects of zinc in male infertility and the possible use of SP TEX101 assay as a helpful test for azoospermia. Trials on the improving effects of supplementing amino acids to patients with iOAT, especially, taurine, leucine, histidine, and lysine (which are essential amino acids that could not be synthesized by the human body), could be investigated.

Study Limitations We could not include azoospermic patients with obstructive azoospermia so as to be able to investigate the possible utility of SP TEX101 for differentiation of obstructive from non-obstructive forms. We could not include OAT with varicoceles to be able to compare those with vs. those without varicoceles and assess the effect of varicoceles on the measured biomarkers.

Authors' Contributions Study concept and design: MHH, THS, and HMI; Patients' selection, clinical evaluation, and routine semen analyses: HMI, MAE-H, MHH, and HMF; Blood and semen sampling and biochemical assays: MHH; Statistical analysis: MO, HMI, HMF, MAE-H, and MHH; Literature research: MHH, HMI, MAE-H, MO, and HMF; First manuscript drafting: MHH; The authors approved the final version of the manuscript.

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Compliance with Ethical Standards

Ethics Approval and Consent to Participate The study was approved by the local Ethics Committee of Medical Research of the Faculty of Medicine, South Valley University, Qena, Egypt (Ethical approval code: SVU-MED-MBC004-2017-12.), and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from every participant who was anonymously enrolled.

Competing Interests The authors declare that they have no conflicts of interest.

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