Egyptian Journal of Medical Research (EJMR)

Assessment of Seminal Alpha Glucosidase among Infertile Oligoasthenoteratozoospermic Men Post Varicocelectomy

Ahmed Fathy Abo Seif [a], *Hanan Mohamed Farhan* [b] *Ahmed Abdelhady Moussa* [a] ,

Nashaat Nabil [a], *Sally Ibrahime Mohammed* [a] *and Ahmed Adel* [c]

[a] Andrology, Sexology and STIs Department, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

[b] Clinical and Chemical pathology Department, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

[c] Andrology Department, Faculty of Medicine, Cairo University, Egypt

Abstract:

Varicocele is the most common cause of male infertility. Alpha-glucosidase (α-GLUC) is normal constituent of human semen produced mainly in epididymis and is evaluated as a marker for seminal quality. The aim of this study was to assess seminal α-GLUC in infertile men with varicocele and post varicocelectomy. This study was conducted at Beni-Suef University Hospital. Sixty Egyptian male participants were divided into Group I: Fertile normozoospermic (n=30) as healthy control, Group II: Infertile oligoasthenoteratozoospermic (OAT) with varicocele (n=30) who underwent varicocelectomy, follow up for 3 months and were classified as Group III: Infertile OAT post varicocelectomy. Semen analysis was performed manually and seminal α-GLUC was assayed by Enzyme Linked Immunosorbent Assay (ELISA). Males with varicocele showed statistically significant decrease regarding sperm count, percentage of motile sperms and percentage of normal sperm forms when compared to healthy subjects. Semen parameters showed significant improvement after varicocelectomy. Seminal α-GLUC was statistically significantly higher among fertile normozoospermic men and post varicocelectomy when compared to infertile OAT men. Statistically significant positive correlation was found between α-GLUC level and semen parameters. Moreover, α-GLUC showed sensitivity of (100% and 85%) and specificity of (100%) at cut off values (≤2.67 pg/ml and ≤4.3 pg/ml) between infertile OAT varicocele when compared to fertile normozoospermic and post varicocelectomy

men, respectively ($p<0.001$). In conclusion, seminal α-GLUC could be suggested as marker for judging quality of semen in infertile men with varicocele where varicocelectomy was found to improve α-GLUC level suggesting it to have a role in fertility among men.

Keyword: Varicocele, oxidative stress, male infertility, seminal alpha-glucosidase.

1. Introduction:

The World Health Organization (WHO) defines infertility as the inability to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. The incidence of infertility is approximately 15% worldwide with male factor estimated to be 50%, but some data suggest that in Middle East the male factor proportion rises up to 70% [2]. The most important causes of male infertility are hormonal defects, congenital problems, inflammatory causes, environmental, lifestyle, genetic factors and varicocele [3].

Varicocele (Vx) is the most common reason for male infertility which is caused by dilatation of pampiniform venous plexus and internal spermatic vein leading to decreased testicular function [4]. Varicocele affects about 15–20% of general male population and 40% of infertile men [5]. Several theories explain the mechanisms by which Vx affects male fertility including scrotal hyperthermia, dysfunction of Leydig cell, retrograde flow of metabolites, hypoxia and impaired testicular artery perfusion [6]; these mechanisms cause male infertility through affecting semen parameters [7].

Varicocele is the most common treatable cause of male factor infertility [8]. Treatment should be offered for patients with palpable varicocele and abnormal semen parameters; varicocelectomy should only be offered for patients who are actively attempting to conceive with normal or potentially correctable infertile partner [9].

The epididymis is known to be of considerable importance in post-testicular development of fertilizing ability of sperms and is a target for fertility regulation [10]. Alpha-glucosidase (α-GLUC) is a normal constituent of human semen, produced mainly in the epididymis [11]. Three bio-chemical constituents of seminal plasma are secreted by the epidiymis; α-GLUC enzyme, glycerophosphocholine and carnitine; all were suggested as epididymal markers for azoospermia; where α-GLUC appears to be the most specific and sensitive for distinguishing between obstructed and patent ducts [12]. It was reported that seminal α-GLUC ejaculate level is a reliable indicator of congenital bilateral absence of vas deferens [13]. Low levels of α-GLUC in semen can be related to epididymitis and defective sperm [12]. Seminal α-GLUC level can be

suggested as a reliable parameter reflecting the epididymal function [14]. The aim of this study was to assess seminal α-GLUC in infertile men with varicocele and post varicocelectomy.

2. Subjects and Methods:

2.1 Subjects:

This case control study was carried out on 60 Egyptian male participants who presented to Andrology, Sexology & STIs Department, Beni-Suef University Hospital. Participants were divided into: Group I: Fertile normozoospermic men (n=30) as healthy age-matched controls, Group II: Infertile oligoasthenoteratozoospermic (OAT) men with varicocele (n=30) and Group III: Infertile OAT post varicocelectomy patients who were patients of group II followed up for 3 months after varicocelectomy.

Patients with varicocele were classified according to Shridharani et al., 2016 into; Grade I: palpable only during the valsalva maneuver, Grade II: palpable distension on standing upright and Grade III: visible through scrotal skin [8].

The study ethical approval was obtained by the Scientific Research Ethical Committee, Faculty of Medicine, Beni-Suef University (approval number FMBSUREC/10102021/ Mohamed). Data confidentiality was preserved according to the Revised Helsinki Declaration of Bioethics 2008 [15]. Informed consent was obtained from all participants after being provided with adequate information about the study.

2.2 Inclusion Criteria:

a. Normal female factor: Normal uterus and fallopian tubes (no polyps, fibroids, Adhesions, or any other problems); and normal regular ovarian functions.

b. Normal Hormonal factor (FSH, LH, TSH, and prolactin).

c. Suitable Age, weight and environmental factors (no organic solvents or silicones, physical agents, pesticides or chemical dusts).

2.3 Exclusion Criteria:

a. Patients suffering from azoospermia and subclinical Vx.

b. Smoking.

c. Patients with congenital anomalies or leukocytospermia.

d. Blood transfusion, iron therapy and anaemia.

2.4 History taking and examination:

a. Full history taking to exclude the previous exclusion criteria in selected cases.

b. General and clinical examination including assessment of Vx with scrotal Duplex. Clinical examination was carried out in a warm room at the standing position with/without the valsalva manoeuvre. Colour Doppler Ultrasonography was conducted for assurance of Vx and its grade.

2. 5 Sampling:

The ejaculates were obtained after 4-5 days of sexual abstinence into sterile containers. Semen samples were collected into aseptic

tubes and centrifuged for 20 min at 2,000-3,000rpm. The supernatant was collected carefully as seminal plasma sample. Semen analysis and sample extraction were performed as soon as possible after sample collection. The samples for α-GLUC assay were stored at -20℃ until analysis.

2.6 Semen analysis:

Semen analysis was performed and semen was examined manually according to WHO guidelines (2010) [16].

2.7 Surgical technique:

Surgery was performed for group II of infertile OAT men under general anaesthesia using micro-surgical technique sub-inguinal varicocelectomy (MSV) [17], at Beni-Suef University Hospital and Kasr Al-Ainy University Hospital.

2.8 Quantitative detection of seminal α-Glucosidase:

Seminal α-GLUC was assayed by Enzyme Linked Immunosorbent Assay (ELISA) sandwich principle using Human α-glucosidase ELISA Kit supplied by Bio SunLong Biotech Co., LTD, China (Cat no SL3441Hu) according to manufacturer's instructions.

2.9 Statistical analysis:

Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). Qualitative data were described as number and percent. Quantitative data were described as mean ± standard deviation. Receiver operating characteristic (ROC) curve was used for sensitivity, specificity and prediction of cut off values. P-value < 0.05 was considered significant.

a- Student t-test: For normally distributed quantitative variables comparing two studied groups.

b- F-test (ANOVA): For normally distributed quantitative variables comparing more than two groups, and Post Hoc test for pair wise comparisons.

c- Pearson coefficient: For correlation of two normally distributed quantitative variables.

d- Mann Whitney test: For abnormally distributed quantitative variables comparing two studied categories.

e- Kruskal Wallis test: For abnormally distributed quantitative variables comparing more than two studied groups.

3. Results:

Infertile OAT men with varicocele showed statistically significant decrease regarding sperm counts, percentages of motile sperms and normal sperm forms when compared to fertile normozoospermic men; meanwhile significant improvement in semen parameters was found post varicocelectomy when compared to pre-operative corresponding values **(Table 1).**

Table (1): Comparison of different studied parameters among studied groups

Variables	Group I (n = 30)	Group II (n = 30)	Group III (n = 30)	P-value
Age (years) Mean ± SD	23.90 ± 1.25	24.30 ± 1.17	24.30 ± 1.17	0.482
Semen Volume (ml) Mean ± SD	2.69 ± 0.98	2.53 ± 1.51	3.28 ± 0.73	0.009*
p_1		0.318	0.044*	
p_2			0.003*	
Sperm Count (million/mL) Mean ± SD	54.13 ± 22.51	5.29 ± 3.25	30.45 ± 12.62	<0.001*
p_1		<0.001*	0.031*	
p_2			<0.001*	
Total sperm Motility (%) Mean ± SD	55.6 ± 12.1	14.0 ± 6.41	49.50 ± 10.37	<0.001*
p_1		<0.001*	0.594	
p_2			<0.001*	
Sperm normal forms (%) Mean ± SD	6.10 ± 2.64	1.60 ± 0.50	5.20 ± 1.32	<0.001*
p_1		<0.001*	0.092	
p_2			<0.001*	

SD: Standard deviation
p: p value for comparing between the studied groups
p_1: p value for comparing between **group I** and each other groups
p_2: p value for comparing between **group II** and **group III**
* P-value <0.05 is statistically significant

Alpha Glycosidase level was statistically significantly higher among fertile normozoospermic control group and infertile OAT men post varicocelectomy when compared to infertile OAT men **(Table 2).**

Table (2): Comparison of Alpha Glycosidase level between the three studied groups

Variable	Group I (n = 30)	Group II (n = 30)	Group III (n = 30)	P-value
Alpha Glycosidase level (pg/ml) Mean ± SD.	8.66 ± 2.05	1.42 ± 0.55	7.18 ± 2.36	<0.001*
p_1		<0.001*	0.036*	
p_2			<0.001*	

SD: Standard deviation
p: p value for comparing between the studied groups
p_1: p value for comparing between **group I** and each other groups
p_2: p value for comparing between **group II** and **group III**
* P-value <0.05 is statistically significant

The seminal studied parameters were compared between grades II and III among infertile OAT with varicocle **(Table 3)** and post varicocelectomy **(Table 4)**; α-GLUC and sperm normal forms were the only variables that showed statistically significant increase in grade III when compared to grade II among post varicocelectomy group.

Table (3): Comparison of different studied parameters among infertile OAT with varicocle according to the grade

Variables	Infertile OAT with varicocle		P-value
	Grade II (n =18)	Grade III (n =12)	
Age (years) Mean ± SD.	24.42 ± 1.24	24.13 ± 1.13	0.600
Semen Volume (ml) Mean ± SD.	2.74 ± 1.72	2.20 ± 1.16	0.427
Sperm Count (million/mL) Mean ± SD.	4.98 ± 3.09	5.74 ± 3.64	0.678
Total sperm Motility (%) Mean ± SD.	13.33 ± 6.85	15.0 ± 5.98	0.521
Alpha Glycosidase level (pg/ml) Mean ± SD.	1.57 ± 0.52	1.21 ± 0.56	0.160
Sperm normal forms (%) Mean ± SD.	1.67 ± 0.49	1.50 ± 0.53	0.571

SD: Standard deviation
P-value>0.05 is not significant

Table (4): Comparison of different studied parameters among infertile OAT post varicocelectomy according to the grade

Variables	Infertile OAT post varicocelectomy		P- value
	Grade II (n =18)	Grade III (n =12)	
Age (years) Mean ± SD.	24.27 ± 1.19	24.33 ± 1.22	0.912
Semen Volume (ml) Mean ± SD.	3.18 ± 0.84	3.39 ± 0.60	0.456
Sperm Count (million/mL) Mean ± SD.	27.73 ± 14.85	33.78 ± 8.93	0.331
Total sperm Motility (%) Mean ± SD.	46.82 ± 11.46	52.78 ± 8.33	0.230
Alpha Glycosidase level (pg/ml) Mean ± SD.	5.58 ± 1.47	9.14 ± 1.62	<0.001*
Sperm normal forms (%) Mean ± SD.	4.27 ± 0.65	6.33 ± 1.0	<0.001*

SD: Standard deviation

* P-value <0.05 is statistically significant

On follow up of infertile OAT group 3 months after varicocelectomy, semen parameters showed significant improvement compared to the corresponding semen parameters pre-operatively among grade II **(Table 5)** and grade III **(Table 6).**

Table (5): Comparison of different studied parameters among grade II Infertile OAT with varicocle and post varicocelectomy

Variables	Grade II infertile OAT		P-value
	With varicocle (n =18)	Post varicocelectomy (n =18)	
Age (years) Mean ± SD.	24.42 ± 1.24	24.27 ± 1.19	0.780
Semen Volume (ml) Mean ± SD.	2.74 ± 1.72	3.18 ± 0.84	0.190
Sperm Count (million/mL) Mean ± SD.	4.98 ± 3.09	27.73 ± 14.85	<0.001*
Total sperm Motility (%) Mean ± SD.	13.33 ± 6.85	46.82 ± 11.46	<0.001*
Alpha Glycosidase level (pg/ml) Mean ± SD.	1.57 ± 0.52	5.58 ± 1.47	<0.001*
Sperm normal forms (%) Mean ± SD.	1.67 ± 0.49	4.27 ± 0.65	<0.001*

SD: Standard deviation

* P-value <0.05 is statistically significant

Table (6): Comparison of different studied parameters among grade III Infertile OAT with varicocle and post varicocelectomy

Variables	Grade III infertile OAT		P-value
	With varicocle (n =12)	Post varicocelectomy (n =12)	
Age (years) Mean ± SD.	24.13 ± 1.13	24.33 ± 1.22	0.721
Semen Volume (ml) Mean ± SD.	2.20 ± 1.16	3.39 ± 0.60	0.008*
Sperm Count (million/mL) Mean ± SD.	5.74 ± 3.64	33.78 ± 8.93	<0.001*
Total sperm Motility (%) Mean ± SD.	15.0 ± 5.98	52.78 ± 8.33	<0.001*
Alpha Glycosidase level (pg/ml) Mean ± SD.	1.21 ± 0.56	9.14 ± 1.62	<0.001*
Sperm normal forms (%) Mean ± SD.	1.50 ± 0.53	6.33 ± 1.0	<0.001*

SD: Standard deviation

* P-value <0.05 is statistically significant

Statistically significant positive correlation was found between α-GLUC and semen parameters including sperm normal forms, sperm Count and sperm Motility as demonstrated in **(Table 7).**

Table (7): Correlation between different studied parameters

Variables		Age (years)	Semen Volume (ml)	Sperm Count (million/mL)	Total sperm Motility (%)	Alpha Glycosidase (pg/ml)	Sperm normal forms (%)
Age (years)	**R**	1.0	-0.073	-0.047	-0.030	-0.033	-0.125
	P		0.581	0.719	0.822	0.802	0.340
Semen Volume (ml)	**R**		1.0	-0.025	0.220	0.118	0.119
	P			0.847	0.091	0.369	0.365
Sperm Count (million/mL)	**R**			1.0	0.598*	0.724*	0.752*
	P				<0.001*	<0.001*	<0.001*
Total sperm Motility (%)	**R**				1.0	0.811*	0.796*
	P					<0.001*	<0.001*
Alpha Glycosidase (pg/ml)	**R**					1.0	0.910*
	P						<0.001*
Sperm normal forms (%)	**R**						1.0
	P						

r: Pearson coefficient

* P-value <0.05 is statistically significant

Using ROC curve, α-GLUC showed a sensitivity of (100%) and a specificity of (100%) at cut off value (≤2.67 pg/ml) in differentiation between infertile OAT patients with varicocle and control fertile normozoospermic men (p<0.001) **(Table 8) (Figure 1).** Moreover, sensitivity of (85%) and specificity of (100%) at cutoff (≤4.3 pg/ml) was found between infertile OAT patients with varicocele and post varicocelectomy (p<0.001) **(Table 9) (Figure 2).**

Table (8): Prognostic performance of alpha glycosidase level (pg/ml) to discriminate infertile OAT patients with varicocele and fertile normospermic men

Variable	AUC	P	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
Alpha Glycosidase (pg/ml)	1.000	<0.001*	1.000 – 1.000	≤2.67	100.0	100.0	100.0	100.0

AUC: Area Under a Curve

p value: Probability value

CI: Confidence Intervals

PPV: Positive predictive value NPV: Negative predictive value

#Cut off was choose according to Youden index

* P-value <0.05 is statistically significant

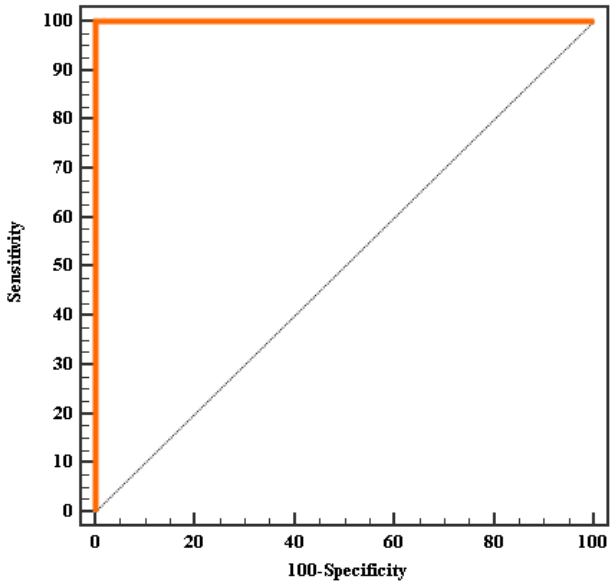

Figure (1): ROC curve for Alpha Glycosidase level (pg/ml) to discriminate infertile OAT patients with varicocle and control fertile normozoospermic men

Table (9): Prognostic performance of alpha glycosidase level (pg/ml) to discriminate infertile OAT patients with varicocele and post varicocelectomy

Variable	AUC	P	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
Alpha Glycosidase (pg/ml)	0.924	<0.001*	0.853 – 0.994	≤4.3	85.00	100.0	100.0	76.9

AUC: Area Under a Curve

p value: Probability value

CI: Confidence Intervals

PPV: Positive predictive value NPV: Negative predictive value

#Cut off was choose according to Youden index

* P-value <0.05 is statistically significant

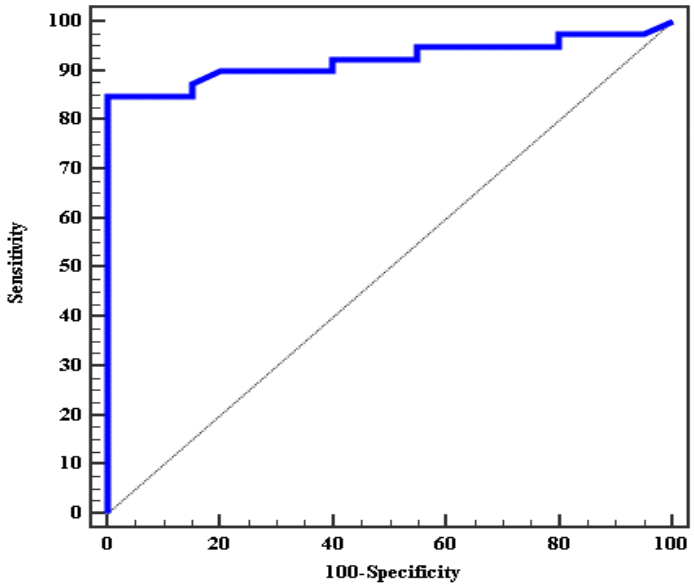

Figure (2): ROC curve for Alpha Glycosidase level (pg/ml) to discriminate infertile OAT patients with varicocele and post varicocelectomy

4. Discussion:

Seminal plasma is the protein rich fluid part of semen which plays a significant role in sperm metabolism and sperm function [18]. Evaluation of seminal plasma composition is important in determination of male infertility, assisted pregnancy outcomes and to find biomarkers able to recognize sperm pathology [19].

The objective of this study was to evaluate the effectiveness of assessment of seminal alpha glycosidase in infertile men post varicocelectomy. Sixty male participants were presented to Andrology, Sexology & STIs department, Beni- Suef University Hospitals. Participants were divided into 30 fertile normozoospermic men as control group, 30 infertile OAT men with varicocele who underwent varicocelectomy and were followed up to 3 months for evaluation before and after surgery. In our study, we found that males with varicocele showed statistically significant decrease regarding sperm count, percentage of normal sperm forms and percentage of motile sperms than healthy subjects who had normal basic semen profiles. These data indicated that varicocele could be a significant factor in spermatogenesis failure. The studied semen parameters showed no statistical difference between grades II and III of varicocele. On follow up of infertile OAT group 3 months after varicocelectomy, semen parameters showed significant improvement compared to the corresponding semen parameters pre-operatively.

Our results came in agreement with Tanaka et al., 2020 [20] who reported that patients with varicocele showed decreased sperm parameters including count, motility and normal morphology. Shaygannia et al., 2021 [21] observed increased loss of spermatic chromatin integrity determined by the increased presence of residual histones; and increased lipid peroxidation and DNA damage among males with varicocele. Similar findings of decreased standard semen characteristics among varicocele patients were also reported by

Ammar et al., 2021; Mostafa et al., 2022 and Pallotti et al. 2022 [22, 7, 18].

Varicoceles are easy to find and fix with surgery which can relieve significant testicular pain, stop testicular shrinking, or determine being the cause of infertility as varicocele can damage the testes over time leading to more shrinking and loss of seminal parameters [23]. Microsurgical varicocelectomy improves testosterone levels in men with varicocele and leads to greater improvement in semen parameters [24]. Significant improvement in sperm motility and sperm quality was observed among patients with clinical varicocele after varicocelectomy [25]. Men with large varicoceles have worse sperm parameters before surgery, but they improve more after surgery than men with small or medium-sized varicoceles [26]. Patients with varicoceles benefit from varicocelectomy because of improvement of classic semen parameters [27]. Morini et al. 2021 [28] suggested detailed morphologic sperm assessment as a crucial parameter expressing the post-surgical semen improvement after varicocelectomy.

In our study, we found that α-GLUC level was statistically significantly higher among fertile normozoospermic control group and infertile OAT men post varicocelectomy when compared to infertile OAT men. Also statistically significant positive correlation was found between α-GLUC level and semen parameters including sperm normal forms, sperm Count and sperm Motility. Moreover, α-GLUC showed sensitivity of (100% and 85%) and specificity of (100%) at cut off values (≤2.67 pg/ml and ≤4.3 pg/ml), respectively in differentiation between infertile OAT patients with varicocle when compared to control fertile normozoospermic men and post varicocelectomy ($p<0.001$).

Among Chinese infertile men, the level of α-GLUC in normozoospermia was significantly higher than that in subfertile and infertile men, while the level of α-GLUC in subfertile men was significantly

higher than that in infertile men; the authors concluded that α-GLUC is a key marker for judging the quality of seminal plasma in Chinese men [29]. Fraczek et al. 2020 [30] found that the amount of seminal α-GLUC activity was related to the amount of sperm, total sperm count, and semen volume. Moronkeji and Emokpae 2020 [31] reported that α-GLUC activity decreased with decreasing sperm count showing lowest levels among azoospermic versus highest among normozoospermic males while it correlated positively with percentage of sperm motility, concluding that α-GLUC can be used for the evaluation of semen quality as an adjunct to traditional semen analysis.

The ejaculated semen has both cellular spermatozoa and non-cellular parts where the non-cellular part gives spermatozoa energy, protection, acrosome reaction, and help capacitation; which are all crucial for the ability of reproduction [32]. Oxidative stress (OS) is a crucial pathway in pathogenesis of testicular damage in males with varicocele [33]. Physiologic antioxidants are present in the seminal plasma being classified as enzymatic and non-enzymatic antioxidants [34]. Alpha glucosidase enzyme activity is positively correlated with conventional sperm parameters, seminal adenosine tri phosphate level and fertilisation capacity of spermatozoa but negatively correlated with reactive oxygen species (ROS) levels and concentration of peroxidase-positive white blood cell, both of which have adverse effects on fertilisation [35]; suggesting α-GLUC to have a role in male fertility; since OS and elevated levels of seminal and sperm ROS may participate to up to 80% of male infertility diagnosis [36]. Another theory explaining the role of α-GLUC in fertility among men; is the strong correlation between the activity of α-GLUC and seminal 5α-dihydrotestosterone (5α-DHT) in the ejaculate which suggests that measurement of α-GLUC can be indicative of androgens effect on epididymal function which depends mainly on 5α-DHT

concentration at epithelial level [35]. Increased α-GLUC in the postoperative semen predicts improved patency, considering that postoperative low α-GLUC level may reflect residual obstruction; moreover increased seminal postoperative α-GLUC can predict improved pregnancy outcomes and successful intrauterine insemination [37].

5. Conclusion and Recommendations:

In conclusion, seminal α-GLUC could be suggested as a marker for judging quality of semen in infertile men with varicocele where varicocelectomy was found to improve α-GLUC level. Alpha glucosidase may be suggested to play a role in fertility among men. The fact that only a small number of people took part in this study is still a limitation. More researches need to be done especially with larger sample populations.

Declaration of Conflicting Interests

The authors declared no potential conflict of interests.

Funding

This research received no fund.

Ethical approval

The study was approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University (approval number FMBSUREC/10102021/ Mohamed).

Informed consent

A signed consent was obtained from each participant.

6. References:

1. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018.
2. Agarwal, A., Finelli, R., Durairajanayagam, D., Leisegang, K., Henkel, R., Salvio, G., Aghamajidi, A., Sengupta, P., Crisóstomo, L., Tsioulou, P.A., Roychoudhury, S., Finocchi, F., Darbandi, M., Mottola, F., Darbandi, S., Iovine, C., Santonastaso, M., Zaker, H., Kesari, K.K., Nomanzadeh, A., Gugnani, N., Rambhatla, A., Duran, M.B., Ceyhan, E., Kandil, H., Arafa, M., Saleh, R.,

Shah, R., Ko, E., & Boitrelle, F. (2022). Comprehensive Analysis of Global Research on Human Varicocele: A Scientometric Approach. World J Mens Health, 40(4):636-652. doi: 10.5534/wjmh.210202.

3. Vander Borght, M., & Wyns, C. (2018). Fertility and infertility: Definition and epidemiology. Clin Biochem, 62:2-10. doi: 10.1016/j.clinbiochem.2018.03.012.
4. Lomboy, J.R., & Coward, R.M. (2016). The Varicocele: Clinical Presentation, Evaluation, and Surgical Management. Semin Intervent Radiol; 33(3):163-9. doi: 10.1055/s-0036-1586143.
5. Mongioì, L. M., Mammino, L., Compagnone, M., Condorelli, R. A., Basile, A., Alamo, A., La Vignera S, Morgia G, Russo GI, Calogero, & A. E. (2019). Effects of Varicocele Treatment on Sperm Conventional Parameters: Surgical Varicocelectomy Versus Sclerotherapy. Cardiovascular and Interventional Radiology, 42(3):396-404. DOI: 10.1007/s00270-018-2136-4.
6. Mostafa, T., Rashed, L., & Taymour, M. (2016). Seminal cyclooxygenase relationship with oxidative stress in infertile oligoasthenoteratozoospermic men with varicocele. Andrologia. 48:137-142.
7. Mostafa, T., El Taweel, A. E. A. I., Rashed, L. A., Mohammed, N. A., & Akl, E. M. (2022). Assessment of seminal cystatin C levels in infertile men with varicocele: A preliminary study. Andrologia, 54(1), e14278.
8. Shridharani, A., Owen, R. C., Elkelany, O. O., & Kim, E. D. (2016). The significance of clinical practice guidelines on adult varicocele detection and management. Asian journal of andrology, 18(2), 269–275. https://doi.org/10.4103/1008-682X.172641
9. Lundy, S. D., & Sabanegh, E. S. (2018). Varicocele management for infertility and pain: a systematic review. Arab journal of urology, 16(1), 157-170.
10. James, E. R., Carrell, D. T., Aston, K. I., Jenkins, T. G., Yeste, M., & Salas-

Huetos, A. (2020). The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. International Journal of Molecular Sciences, 21(15), 5377.

11. Peña, P., Risopatrón, J., Villegas, J., Miska, W., Schill, W.B., & Sánchez, R. (2004). Alpha-glucosidase in the human epididymis: topographic distribution and clinical application. Andrologia, 36(5):315-20. doi: 10.1111/j.1439-0272.2004.00625.x.
12. Eertmans, F., Bogaert, V., Poecke, T.V., & Puype, B. (2014). An Improved Neutral a-Glucosidase Assay for Assessment of Epididymal Function—Validation and Comparison to the WHO Method. Diagnostics , 4(1), 11; https://doi.org/10.3390/diagnostics4010001.
13. Olesen, I.A., Andersson, A.M., Aksglaede, L., Skakkebaek, N.E., Rajpert-de Meyts, E., Joergensen, N., & Juul, A. (2017). Clinical, genetic,biochemical, and testicular biopsy findings among 1213 men evaluated for infertility. Fertil. Steril, 107, 74–82.e7.
14. Andrade, D. L., Viana, M. C., & Esteves, S. C. (2021). Differential Diagnosis of Azoospermia in Men with Infertility. Journal of clinical medicine, 10(14), 3144. https://doi.org/10.3390/jcm10143144.
15. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects, The 59th WMA General Assembly, Seoul, South Korea. 2008
16. World Health Organization (WHO). WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. WHO Press; Geneva, Switzerland: 2010.
17. Lee, J. Y., Yu, H. S., Ham, W. S., Kang, D. H., Kim, K. H., Chung, D. Y., & Cho, K. S. (2014). Microsurgical intermediate subinguinal varicocelectomy. International Surgery, 99(4), 398-403.
18. Pallotti, F., Barbonetti, A., Rastrelli, G., Santi, D., Corona, G., & Lombardo, F. (2022). The impact of male factors and

their correct and early diagnosis in the infertile couple's pathway: 2021 perspectives. J Endocrinol Invest, 45, 1807–1822. https://doi.org/10.1007/s40618-022-01778-7.

19. Lazzarino, G., Listorti, I., Muzii, L., Amorini, A. M., Longo, S., Di Stasio, E., & Bilotta, P. (2018). Low-molecular weight compounds in human seminal plasma as potential biomarkers of male infertility. Human Reproduction, 33(10), 1817-1828.
20. Tanaka, T., Kobori, Y., Terai, K., Inoue, Y., Osaka, A., Yoshikawa, N., Shimomura, Y., Suzuki, K., Minami, T., Iwahata, T., Onota, S., Yamamoto, A., Sugimoto, K., & Okada, H. (2022). Seminal oxidation-reduction potential and sperm DNA fragmentation index increase among infertile men with varicocele. Hum Fertil, 25(1):142-146. doi: 10.1080/14647273.2020.1712747.
21. Shaygannia, E., Nasr-Esfahani, M. H., Sotoodehnejadnematalahi, F., & Parivar, K. (2021). Is ferroptosis involved in ROS-induced testicular lesions in a varicocele rat model? Basic and Clinical Andrology, 31(1), 1-13.
22. Ammar, O., Tekeya, O., Hannachi, I., Sallem, A., Haouas, Z., & Mehdi, M. (2021). Increased Sperm DNA Fragmentation in Infertile Men with Varicocele: Relationship with Apoptosis, Seminal Oxidative Stress, and Spermatic Parameters. Reprod Sci, 28(3):909-919. doi: 10.1007/s43032-020-00311-6.
23. Teixeira, T.A., Pariz, J.R., Dutra, R.T., Saldiva, P.H., Costa, E., & Hallak, J. (2019). Cut-off values of the Johnsen score and Copenhagen index as histopathological prognostic factors for postoperative semen quality in selected infertile patients undergoing microsurgical correction of bilateral subclinical varicocele. Transl Androl Urol, 8(4):346-355. doi: 10.21037/tau.2019.06.23.
24. Dabaja, A. A., & Goldstein, M. (2016). When is a varicocele repair indicated: the dilemma of hypogonadism and erectile dysfunction? Asian journal of

andrology, 18(2), 213–216. https://doi.org/10.4103/1008-682X.169560

25. Hammadi, I. J., Saeed, G. T., & Tawney, R. H. A. (2015). Evaluation of the Effects of Surgical Varicocelectomy on the Seminal Fluid Parameters in Patients with Clinical and Subclinical Varicocele. Journal of the Faculty of Medicine Baghdad, 57(1), 40-44.
26. Pasqualotto, F.F., Lucon, A.M., Sobreiro, B.P., Pasqualotto, E.B. & Arap, S. (2004). Effects of Medical Therapy, Alcohol, Smoking, and Endocrine Disruptors on Male Infertility. Revista do Hospital das Clínicas, 59, 375-382. http://dx.doi.org/10.1590/s0041-87812004000600011
27. Miyaoka, R., & Esteves, S. C. (2012). A critical appraisal on the role of varicocele in male infertility. Advances in urology, 597495. https://doi.org/10.1155/2012/597495
28. Morini, D., Spaggiari, G., Daolio, J., Melli, B., Nicoli, A., De Feo, G., Valli, B., Viola, D., Garganigo, S., Magnani, E., Pilia, A., Polese, A., Colla, R., Simoni, M., Aguzzoli, L., Villani, M. T., & Santi, D. (2021). Improvement of sperm morphology after surgical varicocele repair. Andrology, 9(4), 1176–1184. https://doi.org/10.1111/andr.13012
29. Qiu, Z., Chu, Q., Zhang, W., Luo, C., & Quan, S (2018). Level of neutral alpha-1,4-glucosidase in seminal plasma of Chinese men. Andrologia, 50(3). doi: 10.1111/and.12948.
30. Fraczek, M., Wojnar, L., Kamieniczna, M., Piasecka, M., Gill, K., Kups, M., Chopyak, V., Havrylyuk, A., Nakonechnyy, J., Nakonechnyy, A., Wozniak, T., & Kurpisz, M. (2020). Seminal Plasma Analysis of Oxidative Stress in Different Genitourinary Topographical Regions Involved in Reproductive Tract Disorders Associated with Genital Heat Stress. International journal of molecular sciences, 21(17), 6427. https://doi.org/10.3390/ijms21176427
31. Moronkeji, M.A. & Emokpae, M.A. (2020). Alteration of neutral alpha

glucosidase in seminal plasma and correlation with sperm motility among men investigated for infertility Nigeria: a cross-sectional study. Fertil Sci Res, 7:111-6. DOI:10.4103/2394-4285.288724.

32. Vickram, S., Rohini, K., Srinivasan, S., Nancy Veenakumari, D., Archana, K., Anbarasu, K., Jeyanthi, P., Thanigaivel, S., Gulothungan, G., Rajendiran, N., & Srikumar, P. S. (2021). Role of Zinc (Zn) in Human Reproduction: A Journey from Initial Spermatogenesis to Childbirth. International journal of molecular sciences, 22(4), 2188. https://doi.org/10.3390/ijms22042188
33. Cho, C.L., Esteves, S.C., & Agarwal, A (2016). Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl, 18(2):186-93. doi: 10.4103/1008-682X.170441.
34. Kowalczyk, A. (2022). The Role of the Natural Antioxidant Mechanism in Sperm Cells. Reproductive sciences, 29(5), 1387–1394. https://doi.org/10.1007/s43032-021-00795-w
35. Li, J.P., Zhang, X.Z., Wu, J.G., Liang, Z.Y., Tian, Y.H., Chen, C., Tang, Y.G. & Zhang, F.B. (2019).Seminal plasma neutral alpha-glucosidase activity as an early predictor of patency and natural pregnancy after microsurgical vasoepididymostomy. Andrologia, 51, (5)https://doi.org/10.1111/and.13235
36. Castleton, P. E., Deluao, J. C., Sharkey, D. J., & McPherson, N. O. (2022). Measuring Reactive Oxygen Species in Semen for Male Preconception Care: A Scientist Perspective. Antioxidants, 11(2), 264. https://doi.org/10.3390/antiox11020264
37. Namekawa, T., Imamoto, T., Kato, M., Komiya, A., & Ichikawa, T. (2018). Vasovasostomy and vasoepididymostomy: Review of the procedures, outcomes, and predictors of patency and pregnancy over the last decade. Reproductive medicine and

biology, 17(4), 343–355. https://doi.org/10.1002/rmb2.12207.