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Role of SERPIN A5 in Fertilizing Potential of Human Spermatozoa

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ABSTRACT

Background: Infertility affects 12.5% of couples all over the world, with paternal factors accounting for about 50% of infertility cases. It was assumed that SERPIN A5 could be used as a seminal biomarker for evaluation of semen quality in varicocele-related infertility.

Aim: This study aimed to identify the role of SERPIN A5 in fertilizing potential of human spermatozoa.

Methods: This case control study was conducted on 30 cases astheno-teratozoospermia, 30 cases were oligo-astheno-teratozoospermia (OAT) versus 30 age-matched control group. Level of SERPINA5 protein in sperm pellet was measured. Alpha glucosidase was measured. Determination of malondialdehyde (MDA) was conducted.

Results: A significant difference was detected as regards halo, halo percent and acrosin index between control and astheno-teratozoospermia group and between control & oligo-astheno-teratozoospermia and also between astheno-teratozoospermia versus oligo-astheno-teratozoospermia. A significant difference was recorded between studied groups regarding Serpin A5 and DNA fragmentation between control and astheno-teratozoospermia group and between control & oligo-astheno-teratozoospermia and also between astheno-teratozoospermia versus oligo-astheno-teratozoospermia. Higher median serpin A5 was detected among control group followed by astheno-teratozoospermia then oligo-astheno-teratozoospermia. Lower median DNA fragment was detected among control group followed by astheno-teratozoospermia then oligo-astheno-teratozoospermia. Lower mean FSH was detected among control followed by astheno-teratozoospermia and the highest for oligo-astheno-teratozoospermia.

Conclusion: Serpin A5 played an important role in the context of fertilizing potential of human spermatozoa. It significantly increased among healthy subjects compared to oligo-astheno-teratozoospermia.

Keyword: Infertility, SERPIN A5, Oligo-astheno-teratozoospermia, Spermatozoa.

INTRODUCTION

Infertility affects about 12.5% of couples all over the world, with paternal factors representing about fifty percent of infertility cases [1]. Although semen parameters are useful to the physicians for fertility evaluation, detection of novel seminal biomarkers may be helpful [2].

While about 10% of seminal fluid composition is formed in the testes, the remainder is formed by the epididymis, seminal vesicles, and prostate [3]. Proteomics studies could recognize different forms of regulated proteins among experimental groups. This method is utilized to identify biological processes happening in cells and their adjacent microenvironment and could aid in the recognition of new biomarkers. In addition, this approach might be helpful in assessing the alterations happening in biological fluids, which include semen [3]. **Teke *et al*.** [4] identified proteins differentially regulated in spermatozoa specimen obtained from fertile healthy men (FHM) and infertile cases with varicocele (IFPV) pre- and post-varicocelectomy. They suggested that SERPIN A5 can be utilized as a possible seminal marker in terms of semen quality evaluation among IFPV. So, to identify the role of SERPIN A5 in fertilizing potential of human spermatozoa.

PATIENTS AND METHODS

This case-control study enrolled subjects attending the Andrology Outpatient Clinic, Mansoura University Hospital through the period from January 2018 to August 2018. It was carried out at Mansoura University's Faculty of Medicine's Medical Biochemistry Department's Molecular Biology Unit.

The study included control group, which included 30 FHM with normal seminogram and normal sex hormone levels. Varicocele fertile group included 30 subjects diagnosed by Doppler Ultrasound. Varicocele infertile group included 20 cases diagnosed by Doppler Ultrasound and chosen following exclusion of other infertility causes.

Inclusion criteria: subjects whose ages ranged from 20 to 45 years. Varicocele cases were diagnosed with Doppler ultrasound. All cases failed to conceive after at least one year of regular unprotected intercourse. The control group comprised healthy males achieving pregnancy within one year of continuous unprotected intercourse with normal semen analysis.

Exclusion criteria: Cases with systemic diseases, use of medications, or surgeries with potential negative effects on fertility, cases with a history of pelvic/spinal traumas, cases with hypogonadism, hypoandrogenism, and chromosomal aberrations, and cases with azoospermia.

Methods: All cases were subjected to history taking, physical examination, and semen analysis. General examination was conducted to exclude abnormalities related to fertility in the next systems: Endocrine, cardiovascular, respiratory, gastrointestinal and

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neurologic to determine potential abnormalities to be ruled out. Local examination of the testis, epididymis, vas deferens and inguinal region was also conducted to rule out possible abnormalities.

Varicocele diagnosis and grading was done by history taking (aching pain within scrotum, testicular heaviness, testicular atrophy and visible or palpable (could be felt) enlarged vein). Examination and colour Doppler ultrasound grading of reflux were also done.

Semen analysis: After two to five days of abstinence, semen specimens were collected by masturbating and then liquefying for 15 to 30 min at 37 °C. Following full liquefaction, the liquefied semen was separated into two portions. The first was used to perform an acrosin activity test and semen analysis using an Auto Sperm analyzer according to WHO guidelines, while the second was centrifuged at 4000 rpm for 15 min at 4 °C in order to separate the sperm pellet and seminal plasma.

Concentration and sperm motility characteristics were evaluated by Autosperm approach. Acrosin activity was conducted by gelatin-covered microslides and gelatinolysis. Level of SERPIN A5 protein in sperm pellet was measured by ELISA using human SERPIN A5 ELISA kit (SunRed biotechnology China). Measurement of Alpha glucosidase was conducted by using (Episcreen Kit, Belgium). Determination of MDA included Thiobarbituric acid reactive substances.

Ethical Considerations: The study design was approved by Mansoura University Institutional Ethics Committee. An informed written consent was taken from each case. All data were collected by the researcher himself. The Helsinki Declaration was followed throughout the course of the investigation.

Statistical analysis

Data analysis was performed by SPSS (SPSS Inc., PASW statistics for windows version 25, Chicago). Numbers and percentages were used to define qualitative data. Concerning non-normally distributed data, the median was used to define quantitative data after the Kolmogorov-Smirnov test was used to determine normality. The results were deemed significant at the $P \leq 0.05$ level. Concerning non-normally distributed data, the U test was used to compare two researched groups, and the Kruskal Wallis test was used to compare more than two examined groups. The degree and direction of a linear link between two continuous variables that are not normally distributed and/or ordinal variables were assessed using Spearman's correlation. The ROC curve was used to determine the appropriate cutoff point and assess the validity of continuous variables.

RESULTS

Table **(1)** showed that higher median Halo, Halo percent and acrosin index was detected among control group followed by Astheno-teratozoospermia than Oligo-astheno-teratozoospermia (OAT). A significant difference was detected regarding halo, halo percent and acrosin index between control and astheno-teratozoospermia group and between control & Oligo-astheno-teratozoospermia and also between astheno-teratozoospermia versus oligo-astheno-teratozoospermia. There was a significant difference between studied groups regarding serpin A5 and DNA fragmentation between control and astheno-teratozoospermia group and between control & oligo-astheno-teratozoospermia and also between Astheno-teratozoospermia versus Oligo-astheno-teratozoospermia. Higher median serpin A5 was detected among control group followed by astheno-teratozoospermia then oligo-astheno-teratozoospermia. Lower median DNA fragment was detected among control group followed by astheno-teratozoospermia then oligo-astheno-teratozoospermia. There was insignificant difference between studied groups regarding volume. However, a significant difference was detected between studied groups regarding concentration and grades A, B, A/B sperm motility between control versus astheno-teratozoospermia, between control versus oligo-astheno-teratozoospermia and between astheno-teratozoospermia versus oligo-astheno-teratozoospermia.

Table (1): Comparison of HALO, HALO percent, acrosin index, SerpinA5, DNA fragment and semen analysis between studied groups

		Control N=30	**Astheno-teratozoospermia N=30**	**Oligo-atheno-teratozoospermia N=30**	**P value**
Halo		17.4 (12.8-23.7)	13.5 (9.4-16.8)	10.55 (9-14.6)	P1=0.001* P2=0.001* P3=0.001*
Halo percent		74 (63-90)	39 (8-82)	21(6-61)	P1=0.001* P2=0.001* P3=0.001*
Acrosin index		12.87 (8.98-19.49)	5.24 (0.94-11.07)	2.13 (0.62-7.56)	P1=0.001* P2=0.001* P3=0.001*
SerpinA5		37.5 (31.66-45.45)	26.97 (21.76-38.51)	20.66 (10.13-31.34)	P1=0.001* P2=0.001* P3=0.001*
DNA fragment		19 (13-28)	39 (25-55)	66.5 (45-90)	P1=0.001* P2=0.001* P3=0.001*
Semen Analysis	**Volume**	3.65 (2-7)	3.45 (1-8.2)	4 (1.5-8.4)	P1=0.721 P2=0.282 P3=0.471
	Conc. (mill /ml)	71.45 (44.8-96)	29.5 (20.8-53.33)	5.45 (0.46-18.67)	P1=0.001* P2=0.001* P3=0.001*
	Grade A motility	53 (41-60)	16 (0-36)	3 (0-20)	P1=0.001* P2=0.001* P3=0.001*
	Grade A+B motility	58.5(44-67)	32(4-46)	10.5(0-41)	P1=0.001* P2=0.001* P3=0.001*

Used test Kruskal Wallis test, p1: difference between control & Astheno-teratozoospermia, p2: difference between control & Oligo-astheno-teratozoospermia, p3: difference between Astheno-teratozoospermia & Oligo-astheno-teratozoospermia. *significant.

Table **(2)** illustrated a significant difference between studied groups (between control versus astheno-teratozoospermia, between control versus oligo-astheno-teratozoospermia and between astheno-teratozoospermia versus oligo-astheno-teratozoospermia). Higher median velocity, linear velocity, linearity index and morphology were detected among control groups followed by astheno-teratozoospermia then oligo-astheno-teratozoospermia. There was a significant difference between control versus astheno-teratozoospermia regarding WBCS, round cells, malondialdehyde, total antioxidant capacity and alpha glucosidase. Regarding comparison between control versus oligo-astheno-teratozoospermia, significant difference was detected regarding WBCS, malondialdehyde, total antioxidant capacity and Alpha glucosidase. Regarding comparison between astheno-teratozoospermia versus Oligo-astheno teratozoospermia and significant difference was detected regarding round cells, malondialdehyde and alpha glucosidase.

Table (2): Comparison of velocity, linearity, morphology and laboratory findings between studied groups.

		Control N=30	Astheno-teratozoospermia (N=30)	Oligo-atheno-teratozoospermia (N=30)	P value
Velocity		79.65 (63.4-95.4)	35.95(17.6-39.9)	23.2(5.4-74.5)	P1=0.001* P2=0.001* P3=0.001*
Linear velocity		62.7 (42.9-73.4)	21.7(11.1-49.4)	11.2(1.2-42.6)	P1=0.001* P2=0.001* P3=0.001*
Linearity index		78.65 (58.72-92.56)	60.92(48.7-87.79)	50.53(14.77-97.4)	P1=0.001* P2=0.001* P3=0.001*
Morphology		51.5 (40-66)	12(2-22)	2(0-10)	P1=0.001* P2=0.001* P3=0.001*
Laboratory findings	**WBCs**	0.6 (0.4-0.8)	0.6(0.4-4.4)	0.6(0.3-5.4)	P1=0.047* P2=0.01* P3=0.535
	Round cells	2.05 (0.4-3.4)	2.55(1.4-5.6)	2.4(0.4-4.7)	P1=0.001* P2=0.053 P3=0.014*
	Malondialdehyde	1.76 (1.13-3.44)	2.84(1.16-8.1)	3.5(0.78-11.9)	P1=0.017* P2=0.001* P3=0.008*
	Total antioxidant capacity	2.17 (1.24-3.75)	1.17(0.6-1.7)	0.96(0.56-1.41)	P1=0.001* P2=0.001* P3=0.052
	Alpha glucosidase	62.7 (35.7-90.7)	33.9(22.7-54.8)	23.9(18.4-44.6)	P1=0.001* P2=0.001* P3=0.001*

Used test Kruskal Wallis test, p1: difference between control & Astheno-teratozoospermia, p2: difference between control & Oligo-astheno-teratozoospermia, p3: difference between Astheno-teratozoospermia & Oligo-astheno-teratozoospermia. *significant.

Table **(3)** showed a significant difference between each of studied pairs regarding testosterone and FSH with higher mean testosterone among control followed by astheno-teratozoospermia and the least for oligo-astheno-teratozoospermia. Lower mean FSH was detected among control followed by Astheno-teratozoospermia and the highest for oligo-astheno-teratozoospermia. Insignificant difference was detected between studied groups regarding estradiol, prolactin and LH.

Table (3): Comparison of hormonal assay between studied groups.

	Control N=30	Astheno-teratozoospermia (N=30)	Oligo-atheno-teratozoospermia (N=30)	P value
Testosterone (ng /ml)	859 (94-1106)	667.5(312-1077)	428(288-1200)	P1=0.025* P2=0.001* P3=0.001*
Estradiol (E2) (pg/ml)	33 (23.9-44.7)	35.3(22-88)	35.4(20-123)	P1=0.292 P2=0.072 P3=0.448
Prolactin (ng /ml)	6.4 (3.5-12.7)	7.5(2.3-12.4)	7.55(3.9-14.9)	P1=0.322 P2=0.065 P3=0.385
LH (mIU /ml)	6.4 (3.4-9.5)	6.8(3.4-9.7)	8.05(3.0-10.7)	P1=0.604 P2=0.08 P3=0.208
FSH (mIU /ml)	6.1 (3.8-7.7)	7.4(5.3-9.4)	10.25(6.4-18.4)	P1=0.001* P2=0.001* P3=0.001*

Used test Kruskal Wallis test, p1: difference between control & Astheno-teratozoospermia, p2: difference between control & Oligo-astheno-teratozoospermia, p3: difference between Astheno-teratozoospermia & Oligo-astheno-teratozoospermia,*significant

Table **(4)** illustrated significant lower median halo, halo percent and acrosin index among cases than control group (p<0.001 each). There was significant lower median Serpin A5 among cases than control group with median value in control group 37.5 ranging from 31.66 to 45.45 versus 24.96 ranging from 10.13 to 38.51 among cases group. Median DNA fragmentation was higher among cases than control group with significant difference between them. There was insignificant difference between studied groups regarding volume (P=0.532). However, a significant difference was detected between studied groups regarding concentration and grades A, A+B sperm mobility. Higher median semen concentration, grade A and grade A+B sperm motility among control than cases group, while grade B motility was more frequent among cases than control group. Significant increases in median velocity, linear velocity, linearity index and morphology were detected among control group than cases group.

Table (4): Comparison of HALO, HALO percent, acrosin index, SerpinA5, DNA fragment, semen analysis, velocity, linearity and morphology between cases and control groups

	Control N=30	**Cases N=60**	**P value**
Halo	17.4 (12.8-23.7)	12(9-16.8)	<0.001*
Halo percent	74(63-90)	25(6-82)	<0.001*
Acrosin index	12.87(8.98-19.49)	3.01(0.62-11.07)	<0.001*
SerpinA5	37.5(31.66-45.45)	24.96(10.13-38.51)	<0.001*
DNA fragment	19(13-28)	50(25-90)	<0.001*
Semen Analysis			
Volume	3.65(2-7)	3.6(1-8)	0.532
conc. (mill /ml)	71.45(44.8-96)	19.74(0.46-53.33)	<0.001*
Grade A motility	53(41-60)	6(0-36)	<0.001*
Grade A+B motility	58.5(44-67)	25(0-46)	<0.001*
Velocity	79.65(63.4-95.4)	33.45(5.4-74.5)	<0.001*
Linear velocity	62.7(42.9-73.4)	16.3(1.2-49.4)	<0.001*
Linearity index	78.65(58.72-92.56)	56.89(14.77-97.4)	<0.001*
Morphology	51.5(40-66)	5(0-22)	<0.001*

Used test: U test
*significant

Table **(5)** illustrated a significant difference between control versus cases regarding WBCs, round cells, malondialdehyde, total antioxidant capacity and alpha glucosidase. Higher WBCS count, round cells, malondialdehyde was detected among cases than control group. However, higher total antioxidant capacity and alpha glucosidase were detected among control than cases group. There was a significant difference between studied groups regarding testosterone and FSH. On the other hand, there was insignificant difference between studied groups regarding estradiol, prolactin and LH.

Table (5): Comparison of laboratory findings & hormonal assay between studied groups

	Control N=30	**Cases N=60**	**P value**
WBCs	0.6(0.4-0.8)	0.6(0.3-5.4)	<0.001*
Round cells	2.05(0.4-3.4)	2.4(0.4-5.6)	<0.001*
Malondialdehyde	1.76(1.13-3.44)	3.1(0.78-11.9)	<0.001*
Total antioxidant capacity	2.17(1.24-3.75)	1.04(0.56-1.7)	<0.001*
Alpha glucosidase	62.7(35.7-90.7)	29.65(18.4-54.8)	<0.001*
Hormonal Assay			
Testosterone(ng/ml)	859(94-1106)	566.5(288-1200)	<0.001*
Estradiol (pg/ml)	33(23.9-44.7)	35.3(20-123)	0.096
Prolactin(ng/ml)	6.4(3.5-12.7)	7.5(2.3-14.9)	0.091
LH(mIU/ml)	6.4(3.4-9.5)	7.4(3-10.7)	0.095
FSH(mIU/ml)	6.1(3.8-7.7)	8.7(5.3-18.4)	0.001*

Used test: U test *significant

Table **(6)** illustrated significant positive correlation between serpin A5 and halo, halo percent and acrosin index among studied sample (p<0.001 each). There was significant negative correlation between serpin A5 and DNA fragment (r=-0.769), positive with concentration, grade A, grade A+B (r=0.805, 0.741, & 0.766 respectively). There was significant positive correlation was detected between serpin A5 and velocity, linear velocity, linearity index and morphology (p<0.001 each). There was a significant positive correlation between serpin A5 and total antioxidant capacity (r=0.699) and between serpin A5 and alpha glucosidase (r=0.666). A significant negative correlation was detected between serpin A5 and WBCS count (r=-0.270) and between serpin A5 and malondialdehyde (r=-0.600). There was significant positive correlation between serpin A5 and testosterone (r=0.458). A significant negative correlation was recorded between serpin A5 and FSH (r=-0.614).

Table (6): Correlation between serpin A5 and halo, halo percent, acrosin index, DNA fragmentation, volume, conc. (mill /ml), grade A, A+B motility, serpin A5 and velocity, linear velocity, linearity index, morphology, inflammatory, antioxidant markers and hormonal assessment among studied sample (n=90)

	SerpinA5		
	r	95%CI	P
Halo	0.679	0.546-0.783	0.001*
Halo percent	0.674	0.537-0.770	0.001*
Acrosin index	0.698	0.568-0.791	0.001*
DNA fragment	-0.769	-0.838, -0.670	0.001*
Volume	-0.03	-0.226, 0.156	0.716
conc. (mill /ml)	0.805	0.706-0.868	0.001*
Grade A motility	0.741	0.646-0.800	0.001*
Grade A+B motility	0.766	0.678-0.828	0.001*
Velocity	0.719	0.596-0.796	0.001*
Linear velocity	0.744	0.626-0.818	0.001*
Linearity index	0.618	0.476-0.727	0.001*
Morphology	0.775	0.680-0.834	0.001*
WBCs	-0.270	-0.461, -0.057	0.01*
Round cells	-0.175	-0.392, 0.054	0.09
malondialdehyde	-0.600	-0.725, -0.442	0.001*
Total antioxidant capacity	0.699	0.567, 0.798	0.001*
Alpha glucosidase	0.666	0.529, 0.757	0.001*
Testosterone (ng /ml)	0.458	0.247-0.642	0.001*
Estradiol (E2) (pg/ml)	-0.052	-0.277, 0.164	0.630
Prolactin (ng /ml)	-0.182	-0.390, 0.035	0.086
LH (mIU /ml)	-0.179	-0.374, 0.026	0.091
FSH (mIU /ml)	-0.614	-0.735, -0.458	0.001*

r: Spearman correlation coefficient, *significant

Table **(7)** showed that area under curve (AUC) for serpin A5 in the differentiation between cases and control was excellent (AUC=0.969) with the best detected cutoff point from the curve was ≥ 32.0 yielding sensitivity 90% and specifically 96.7%. The AUC for serpin A5 in differentiating between astheno-teratozoospermia versus control was excellent (AUC=0.939) with the best detected cutoff point from the curve was ≤ 32.0 yielding sensitivity 80% and specificity 96.7%. The AUC for serpin A5 in differentiating between oligo-astheno-teratozoospermia versus astheno-teratozoospermia was good (AUC=0.787) (best cutoff point ≥ 26.08) yielding sensitivity 76.7% and specificity 66.7%.

Table (7): ROC curve of SerpinA5 in differentiating between cases versus control, between Astheno-teratozoospermia versus control and between oligo-astheno-teratozoospermia versus astheno-teratozoospermia

Area	Std. Error	P value	Asymptotic 95% Confidence Interval		**Cutoff point**	**Sensitivity**	**Specificity**
			Lower Bound	Upper Bound			
SerpinA5 in differentiating between cases versus control							
.969	.015	.001*	.940	.999	≥32.0	90.0%	96.7%
SerpinA5 in differentiating between Astheno-teratozoospermia versus control							
.939	.029	.001*	.882	.995	≤32.0	80.0%	96.7%
SerpinA5 in differentiating between oligo-astheno-teratozoospermia versus astheno-teratozoospermia							
.787	.059	.001*	.670	.903	≥26.08	76.7%	66.7%

DISCUSSION

One-third of males with normal semen parameters complain of infertility of unknown cause. In this context, alteration in sperm protein expression might be a potential cause of infertility [5].

SERPINE2 is a member of the serpin family. In spite of numerous recorded roles of SERPINE2 in tumour development, the histologic distribution of SERPINE2 and its expression levels in several tumours remain not clear. It has been displayed that SERPINE2 expression differed based on growth stages and tissue types. SERPINE2 is differentially expressed in several tumours and their normal tissue counterparts. SERPINE2 serves diverse roles in several tumours and as a result could serve as a talented biomarker for tumour diagnosis and prognosis [6].

We aimed to identify the role of SERPIN A5 in fertilizing potential of human spermatozoa. This was a case-control study that conducted on 30 cases astheno-teratozoospermia, 30 cases are oligo-astheno-teratozoospermia versus 30 age-matched control group to assess relationship between seminal serpin A5 and male infertility. Of note, there are a limited number of studies that discussed the role of serpin A5 in humans, most of the previous studies were mainly emphasized on mice [7], murine [8] and bulls [9].

The present study demonstrated that higher median Halo, Halo percent and acrosin index were detected among control group followed by astheno-teratozoospermia than oligo-astheno-teratozoospermia. A significant difference was detected regarding halo, halo percent and acrosin index between control and astheno-teratozoospermia group and between control & oligo-astheno-teratozoospermia and also between astheno-teratozoospermia versus oligo-astheno-teratozoospermia. Also, there were significant reduction in median halo, halo percent and acrosin index among cases than among control group ($p<0.001$ each). Likewise, **Zalata *et al.*** [10] displayed that the existence of oxidative stress (OS) in a subject with leukocytospermia and/or oligo-astheno-teratozoospermia is accompanied by an impairment of sperm function (as revealed by acrosin activity).

Regarding laboratory parameters, the current study illustrated a significant difference between control versus astheno-teratozoospermia regarding WBCS, round cells, malondialdehyde, total antioxidant capacity and alpha glucosidase. Regarding comparison between control versus oligo-astheno teratozoospermia; significant difference was detected regarding; WBCS, malondialdehyde, total antioxidant capacity and alpha glucosidase. Regarding comparison between astheno teratozoospermia versus oligo-astheno teratozoospermia. Significant difference was detected regarding round cells, malondialdehyde and alpha glucosidase. This comes in the same line with **Dutta *et al.*** [11] who conducted a meta-analysis assessing OS role on sperm functions and demonstrated that in general, ROS are important for sperm maturation, hyperactivation, capacitation, and fertilization. On the other hand, numerous factors could interfere with ROS levels, which ultimately result in lipid peroxidation, and sperm DNA fragmentation, and therefore infertility. A lot of laboratory tests could be utilized in infertile males to diagnose OS. A therapeutic plan typically includes antioxidants and avoidance of the causative factor. Likewise, **Aitken *et al.*** [12] revealed that OS has an adverse effect on sperm function by disturbing DNA integrity due to concomitant damage to proteins and lipids present in the sperm plasma membrane, with subsequent affection of cellular permeability.

Concerning hormonal assay, our study demonstrated a significant difference between each of studied pairs regarding testosterone and FSH with higher mean testosterone among control followed by astheno-teratozoospermia and the least for oligo-astheno-teratozoospermia. Lower mean FSH was detected among control followed by astheno-teratozoospermia and the highest for oligo-astheno-teratozoospermia. Insignificant difference was detected between studied groups regarding estradiol, prolactin and LH. It has been demonstrated that FHM have a testosterone/oestradiol ratio more than 15, and males with a ratio below 10 usually have a defect in sperm formation [13]. Regarding the comparison of serpin A5, the current study displayed a significant difference between the studied groups regarding serpin A5 and DNA fragmentation between the control and astheno-teratozoospermia groups and between the control and OAT groups and also between the astheno-teratozoospermia and OAT groups. Higher median serpin A5 was detected among the control group, followed by astheno-teratozoospermia and then OAT (37.5, 26.9 & 20.66, respectively). In agreement, **Teke *et al.*** [4] conducted their study on seminal specimens, which were obtained from twenty IFPV pre- and post-varicocelectomy and from fourteen FHM as controls samples. They demonstrated that the increase in serpin A5 after varicocele surgery could be due to improved semen quality, proposing that serpin A5 is a possible seminal marker for evaluation of semen quality in the context of varicocele-related infertility. Likewise, **Panner *et al.*** [5] displayed the overexpression of serpin A5 ($P=0.0073$) in males with unexplained male infertility (UMI). The global proteomic profile of normozoospermic infertile males varies from that of normozoospermic FHM. Based on their results they concluded that serpin A5 could potentially act as noninvasive protein markers accompanied by the fertilization of the spermatozoa in UMI.

This cames in the same line with experimental studies as **Cao *et al.*** [14] conducted their study on 8-week-old ICR mouse COCs added to HTF medium and crushed to get the post-modified HTF medium. Compared to HTF medium use, the fertilization rate and number of sperm combined with the zona pellucida (ZP) were associated with a significant increase following in vitro capacitation (IVC) using the post-

modified HTF medium ($P < 0.01$). Proteomic and Western blotting analyses displayed that serpin A5 level in sperm was associated with a significant increase following IVC with the post-modified HTF medium ($P < 0.05$).

To blockade serpin A5 effect following IVC, a serpin A5 antibody was introduced to the post-modified HTF medium. This resulted in a significant drop in both the quantity of sperm combined with the ZP and the fertilization rate ($P < 0.05$). When recombinant mouse serpin A5 protein (one–two μg/ml) was introduced to HTF medium, both the number of sperm and the rate of fertilization increased significantly ($P < 0.01$) when combined with ZP. Additionally, prior to the freezing of human semen, 5 μg/ml of recombinant human serpin A5 protein was introduced. The percentage of intact acrosomes and normal sperm morphology rose dramatically ($P < 0.05$) when serpin A5 protein was added. **Cao *et al.*** [14] provided a reference approach for optimizing sperm quality in terms of IVC process. **Teke *et al.*** [4] revealed that PSA ratios didn't change significantly following varicocelectomy in infertile cases, whereas serpin A5 levels was associated with a significant increase. Similarly, **Camargo *et al.*** [15] recorded increased serpin A5 levels after varicocelectomy. **Gaffney *et al.*** [16] displayed that the main action of varicocelectomy was to enhance sperm capacitation. The proof recommends that serpin A5 could be used as a seminal biomarker, which has to be assessed pre- and post-varicocelectomy, when taking into consideration the outcomes of **Gaffney *et al.*** [16] and our study, where serpin A5 was associated with a significant upregulation following varicocele management, and given that serpin A5 is identified to have a role in sperm capacitation.

In terms of humans, the sperm's ability to bind to the ZP was assessed in the existence of various levels of anti-serpin A5 or serpin A5 in the media. In addition, a lower anti-serpin A5 concentration raised the sperm ability to bind to the ZP. In addition, serpine 2 has been recorded to be a decapacitating factor in rodents [17]. Processing sperm for in vitro fertilization could have increased sperm damage and facilitated sperm capacitation [18]. As a result, the authors concluded that an increase in serpin A5 concentration may have offered adequate protection to the sperms and bulls with higher concentrations of serpin A5 on the sperm head, and tail labeled percentage. Increased CL may be resulted from resistance to sperm processing. Further investigations are essential to identify whether serpin A5 or DAG1 can be utilized as a fertility biomarker particularly in humans [9].

Regarding DNA fragments, the current study reported that lower median DNA fragments were detected among control group followed by astheno-teratozoospermia then oligo-astheno-teratozoospermia (19, 39 & 66.5 respectively). Likewise, **Avendaño *et al.*** [19] revealed that there was a highly significant negative relationship between the percentage of fragmented DNA and embryo quality, which was established for the transferred embryos. The ROC curve analysis displayed that the DNA-FI and embryo quality were significant predictors of pregnancy. They demonstrated that the likelihood of becoming pregnant increased by 3.5 times when the percentage of fragmented DNA was less than or equal to 17.6%. Likewise**, Liu *et al.*** [20] displayed that sperm DNA fragmentation index (DNA-FI) has a correlation with age, sperm concentration, PR, and deformity rate. Sperm DNA-FI could be utilized to assess male fertility efficiently. On the other hand, sperm DNA-FI has established to be essential in the male fertility assessment, however its importance as an indicator of pregnancy outcomes after ART, which needs more investigations.

Our study revealed that insignificant correlation was detected between serpin A5 and velocity, linear velocity, linearity index and morphology ($p > 0.05$ each). On the contrary, **Uhrin *et al.*** [7] demonstrated that disruption of the serpin A5 gene in mice was associated with male infertility due to abnormalities in abnormal sperm morphology and decreased motility.

Regarding serpin A5 sensitivity, the current study showed that AUC for serpin A5 in differentiating between cases versus control was excellent (AUC=0.969) with the best detected cutoff point from the curve was ≥ 32.0 yielding sensitivity of 90% and specificity of 96.7%. It showed that AUC for serpin A5 in differentiating between astheno-teratozoospermia versus control was excellent (AUC=0.939) with the best detected cutoff point from the curve was ≤ 32.0 yielding sensitivity of 80% and specificity of 96.7%.

CONCLUSION

Serpin A5 played an essential role in the context of fertilizing potential of human spermatozoa. It significantly increased among healthy subjects compared to oligo-astheno-teratozoospermia. In addition, it seems to have a significant correlation with total antioxidant capacity & prolactin level.

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