Iranian Journal of Reproductive Medicine Vol. 2. No.2 pp:58-64, 2004

Correlation between Sperm Quality Parameters and Seminal Plasma Antioxidants Status

Ali Khosrowbeygi, M.S., Nosratollah Zarghami, Ph.D., and Yaghoub Deldar, M.S.

Department of Clinical Biochemistry and RIA, Drug Applied Research Center, Tabriz University of Medical Sciences and Health Services, Tabriz, Iran.

Background: There is growing evidence that damage to spermatozoa by reactive oxygen species (ROS) play a key role in male infertility.
Objective: The aim of the present study was to assess the antioxidant status of seminal plasma by measuring total antioxidant capacity (TAC) and activities of catalase and superoxide dismutase (SOD) in men with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia compared to normozoospermic males, and their correlations with seminal parameters.
Materials and Methods: 46 men with seminal parameters abnormalities divided in three categories: asthenozoospermic (n=15), asthenoteratozoospermic (n=16) and oligoasthenoteratozoospermic (n=15), according WHO criteria, participated in the study. The control group consisted of 25 males with normozoospermia. Catalase activity was measured by Aebi spectrophotometeric method. Commercially available colorimetric assays were used for measuring SOD activity and TAC.
Results: TAC evaluation showed significantly lower values in the total case group (n=46) versus control group (1.05±0.04 mmmol/ml vs. 1.51±0.07 mmol/ml, $p<0.05$). Catalase activity also showed significantly lower values in the total case group (n=46) versus control group (14.40± 0.93 U/ml vs. 21.33±1.50 U/ml). But this difference was not significant for SOD activity (5.31±0.56 U/ml vs. 6.19±0.83 U/ml). Both catalase activity and TAC in asthenozoospermic, asthenoteratozoospermic, oligoasthenoteratozoospermic subjects were significantly lower than normozoospermic males, but SOD activity did not show a significant difference between these groups. Both catalase activity and TAC showed a positively significant correlation with progressively motile sperms and normal sperm morphology, but these correlations with SOD activity were not significant.
Conclusion: Decreasing seminal plasma antioxidant status especially catalase activity and TAC may have significant role in etiology of sperm abnormality.

Keywords: *Seminal plasma, Total antioxidant capacity, Superoxide dismutase, Catalase*

Introduction

In the etiology of male infertility, there is growing evidence that damage to spermatozoa by reactive oxygen species (ROS) play a key role (Alkan *et al*., 1997; Hendin *et al*., 1999; Pasqualotto *et al*., 2000; Taylor CT, 2001; Esfandiari *et al*., 2003). Most studies have implicated oxidative stress as a mediator of sperm dysfunction. The spermatozoa have a high content of polyunsaturated fatty acids (PUFA) within the plasma membrane and a low concentration of scavenging enzymes within the cytoplasm and they are susceptible to the peroxidation in the presence of elevated seminal ROS (Fraczek et al., 2001; Dandekar et al., 2002; Agarwal *et al*., 2002; Agarwal *et al*., 2003; Zarghami and Khosrowbeygi, 2004). Spermatozoa and seminal plasma contain a number of antioxidant systems which counteract the effects of ROS. Spermatozoa possess a low amount of cellular ROS defense system (Fujii *et al*., 2003; Garrido *et al*., 2004). The seminal plasma is well endowed with an array

Corresponding Author:
Dr. Nosratollag Zarghami, Department of Clinical Biochemistry and RIA, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail: nzarghami@hotmail.com

[Downloaded from ijrm.ir on 2024-10-12]

of antioxidants that act as free radical scavengers to protect spermatozoa against oxidative stress (Zini *et al*., 2000; Agarwal *et al*., 2004; Sanocka and Kurpisza, 2004). Seminal plasma contains a number of enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (Peeker *et al*., 1997; Mruk *et al*., 2002; Calamera *et al*., 2003). In addition, it contains a variety of non-enzymatic antioxidants such as vitamin C, vitamin E, pyruvate, glutathione, and carnitine (Meucci et al., 2003).

The findings on the seminal plasma catalase and SOD activities and TAC are controversial. The study conducted by Siciliano *et al.* (2001) showed seminal plasma enzymatic (catalase and SOD) and nonenzymatic (TAC) antioxidant capacity do not alter in the asthenozoospermic specimens, whereas SOD activity is lower in oligoasthenozoospermic samples than normozoospermic males. Hsieh *et al*. (2002) investigation showed that there is not a significant difference in seminal plasma or sperm SOD activity between normozoospermic and oligo- or asthenozoospermic males. This group also observed that seminal plasma SOD activity does not correlate significantly with sperm motility and concentration. Tkaczuk-Wlach *et al*. (2002) observed that whole semen SOD activity is higher in men with oligoszoospermia than those with normozoospermia. Koca *et al*. (2003) showed that seminal plasma TAC in infertile asthenozoospermic and asthenoteratozoospermic males is lower than fertile men. They also observed a positive correlation between seminal plasma TAC and sperm motility.

Therefore, we sought to assess the antioxidant status of seminal plasma by measuring TAC and activities of catalase and SOD in men with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia compared to normozoospermic males, and their correlations with seminal parameters.

Materials and Methods

Semen Samples

A case-control study with a simple random sampling was designed. Following Institutional Review Board approval, semen samples were collected from case and control groups. All specimens were collected into sterile plastic containers by masturbation at the clinical andrology laboratory at University Teaching Women Hospital after an abstinence period of 48-72 hrs and analyzed within 1h of collection. After allowing at least 30 min for liquefaction to occur, semen analysis was performed to measure sperm concentration, percentage progressively motile sperms normal sperm morphology in accordance with the recommendations of the World Health Organization (WHO 1999) using Sperm Quality Analyzer IIC (SQA IIC, United Medical Systems Inc, Santa Ana, CA, USA) (Suzuki *et al*., 2002; Shibahara *et al*., 2002). The WHO criteria for sperm normality were as follows: sperm concentration $\geq 20\times10^6$/ml of ejaculate, progressively motile sperms $\geq$ 50% and normal sperm morphology $\geq$ 30%. The case group consisted of men with asthenozoospermia (n=15), asthenoteratozoospermia (n=16) and oligoasthenoteratozoospermia (n=15). The control group consisted of 25 males with normozoospermia. Samples with a leukocyte concentration $>1\times10^6$ /ml of ejaculate were excluded from the study. Liquefied semen samples were centrifuged at 15000g for 15 minutes (Conquer *et al*., 1999). The supernatant seminal plasma was then carefully removed and transferred to Eppendrof tubes. The seminal plasma was frozen at -20ºC until examination.

Assays

a) Total antioxidant capacity. Total antioxidant capacity (TAC) was measured with the colorimetric method using Randox total antioxidant status kit (Randox Laboratories Ltd, UK) (Said *et al*., 2003; Meucci *et al*., 2003). The frozen seminal plasma was thawed by placing the vials in a water bath at 37°C for 20 minutes and immediately assessed for its antioxidant capacity. Twenty microliters of seminal plasma was added to 1 mL of the reconstituted chromogen, ABTS-metmyoglobin (10 mL vial with 10 mL of phosphate-buffered saline buffer). Twenty microliters of Trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) at a concentration of 1.71 mmol/L was used as the standard. Whereas 20 µl of deionized water was used as a blank. One milliliter of chromogen was added to the standard, and blank samples. With spectrophotometer adjusted at a wavelength of 600 nm, the initial absorbance (A_1) was read. Two hundred microliters of H_2O_2 (250µmol/L) was then added to all tubes, and absorbance (A_2) was read exactly after 3 minutes. The difference between A_2 and A_1 (ΔA) was calculated. The TAC of the sample was then calculated by the following formula: TAC= Concentration of the Standard × (ΔA Blank – ΔA Sample)/(ΔA Blank – ΔA Standard). The results were expressed as mmol/l.

[Downloaded from ijrm.ir on 2024-10-12]

Table I. Seminal parameters in normozoospermic, asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic males.

Variables	Concentration (10^6/ml)	Progressively Motile (%)	Normal Morphology (%)
Normozoospermic (n=25)	100.28±4.87	58.60±1.30	39.96±0.96
Asthenozoospermic (n=15)	62.40±1.09	46.07±0.30	31.07±0.25
Asthenoteratozoospermic (n=16)	39.00±2.75	36.75±1.38	23.56±0.89
Oligoasthenoteratozoospermic (n=15)	13.73±1.19	18.53±1.28	16.80±0.33

Variables are reported as mean±SEM.

Table II. Seminal plasma total antioxidant capacity (TAC) and catalase and superoxide dismutase (SOD) activities in normozoospermic, asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic males.

Variables	TAC (mmol/l)	Catalase (U/ml)	SOD (U/ml)
Normozoospermic (n=25)	1.51±0.07	21.33±1.50	6.19±0.83
Asthenozoospermic (n=15)	1.12±0.06*	13.76±1.63*	4.82±0.54
Asthenoteratozoospermic (n=16)	1.02±0.08*	16.66±5.84*	6.14±1.50
Oligoasthenoteratozoospermic (n=15)	1.01±0.09*	12.61±1.65*	4.82±0.39

Variables are reported as mean±SEM.

**Significantly lower than normozoospermic group (p<0.05).*

b) SOD activity. SOD activity was measured by a kit from Randox (Ransod) (Randox Laboratories Ltd, UK). This method employs xanthine and xanthine oxidase to generate superoxide radicals which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD inhibits reduction of INT by 50% under the conditions of the assay. After thawing, the seminal plasma was diluted 30-fold with 10 mM phosphate buffer, pH 7.0. Assay was performed at 37°C. Phosphate buffer was used as blank. Mixed substrate and xanthine oxidase were added into standards and sample tubes and mixed well. With spectrophotometer adjusted at a wavelength of 505 nm, the initial absorbance (A_1) was read. Final absorbance (A_2) was read exactly after 3 minutes. Percentages of inhibition of standards and samples were calculated. The SOD activity was measured using calibration curve of percentage inhibition for each standard against Log_{10} of standards SOD activity and expressed as U/ml.

c) Catalase activity. Catalase activity was estimated by the method of Aebi (Aebi, 1984). It can degrade hydrogen peroxide which can be measured directly by the decrease in the absorbance at 240 nm. The hydrogen peroxide was diluted with phosphate buffer pH 7.0 and its initial absorbance was adjusted between 0.5 to 0.6 absorbance unit at 240 nm. The decrease in the absorbance was measured. One unit of catalase activity was defined as the amount of catalase which absorbed in 30 sec at 25°C. The catalase activity was then calculated from the change in absorbance and finally expressed as U/ml.

Statistical analysis

Based on a pilot study, using an α value of 0.05 and a β value of 0.2 (80% power), the minimum sample size required was 15 samples per group. The Mann-Whitney U test was used to compare total case group with control group. Comparison between each group was carried out using Kruskal-Wallis test. Coefficients of correlation were calculated using Spearman's correlation analysis. All hypothesis tests were two-tailed with statistical significance assessed at the p value <0.05 level with 95% confidence intervals (CI). The data were expressed as the mean ± SEM. Statistical computations were calculated using SPSS 11.5 for windows software (SPSS Inc, Chicago, IL, USA).

Results

Seminal parameters of the control (normozoospermic subjects) and the case (asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic subjects) groups are reported in Table I. Seminal plasma antioxidant status of the specimens is shown in Table II. TAC evaluation was significantly lower in the total case group (n=46) than in the control group (1.05±0.04 mmmol/ml vs. 1.51±0.07 mmol/ml, p<0.05). Catalase activity was also significantly lower in the total case group (n=46) than the control group (14.40± 0.93 U/ml vs. 21.33±1.50 U/ml, p<0.05). But this difference was not significant for SOD activity (5.31±0.56 U/ml vs. 6.19±0.83 U/ml). Both catalase activity and TAC in asthenozoospermic, asthenoteratozoospermic,

[Downloaded from ijrm.ir on 2024-10-12]

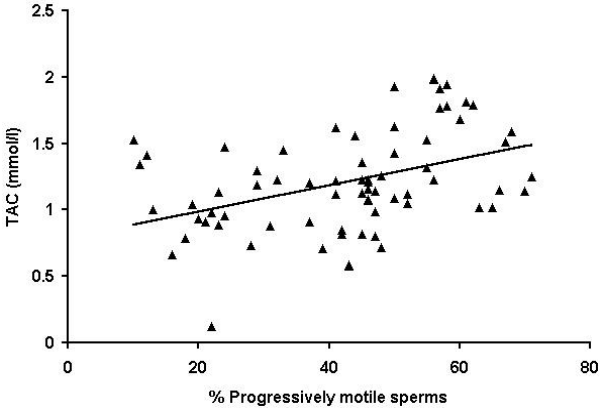

Figure 1. Correlation between percentage progressively motile sperms and seminal plasma total antioxidant capacity (TAC). Regression analysis is for total of case and control groups (r=0.45, p<0.05).

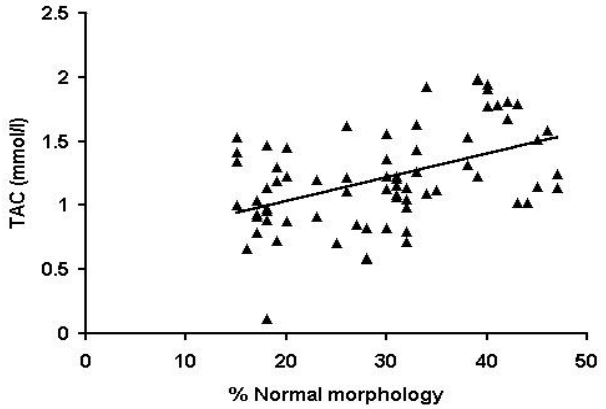

Figure 2. Correlation between percentage normal sperm morphology and seminal plasma total antioxidant capacity (TAC). Regression analysis is for total of case and control groups (r=0.45, p<0.05).

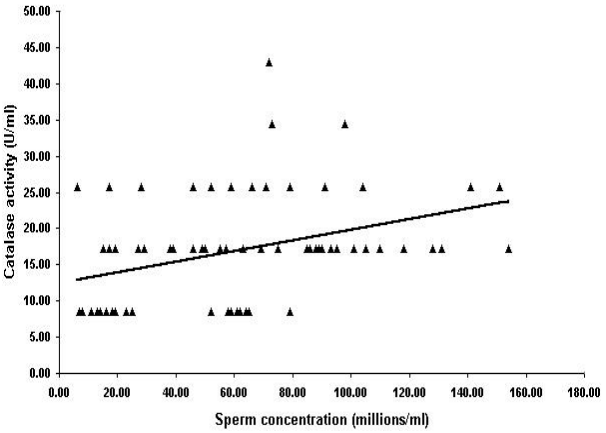

Figure 3. Correlation between sperm concentration and seminal plasma catalase activity. Regression analysis is for total of case and control groups (r=0.41, p<0.05).

oligoasthenoteratozoospermic subjects were significantly lower than normozoospermic males (p<0.05), but SOD activity did not show any significant differences (p>0.05) (Table II).

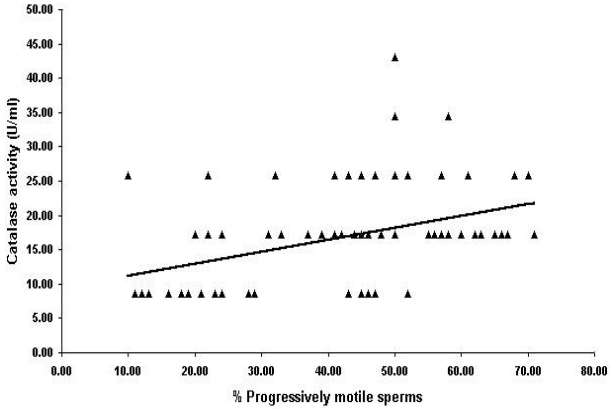

Figure 4. Correlation between percentage progressively motile sperms and seminal plasma catalase activity. Regression analysis is for total of case and control groups (r=0.41, p<0.05).

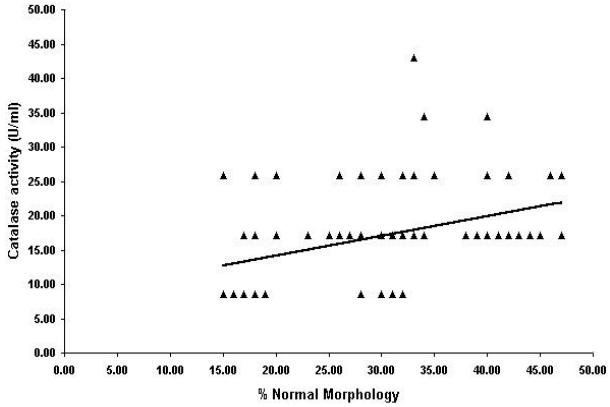

Figure 5. Correlation between percentage normal sperm morphology and seminal plasma catalase activity. Regression analysis is for total of case and control groups (r=0.42, p<0.05).

Then, we examined the correlation between seminal parameters and seminal plasma TAC and activities of SOD and catalase in each group and in overall. TAC showed a positively significant correlation with percentages progressively motile sperms (r=0.45, p<0.05) (Fig. 1) and normal sperm morphology (r=0.45, p<0.05) (Fig. 2) in overall, but these correlations were not significant in each group. We also observed a direct significant correlation between catalase activity and sperm concentration (r=0.41, p<0.05) (Fig. 3), percentage progressively motile sperms (r=0.41, p<0.05) (Fig 4), and percentage normal sperm morphology (r=0.42, p<0.05) (Fig 5) in overall. These correlations were not significant in each group. There was not a significant correlation between SOD activities and seminal parameters in general and also in each group.

[Downloaded from ijrm.ir on 2024-10-12]

Discussion

The most relevant findings of this study were (i) both catalase activity and TAC in asthenozoospermic, asthenoteratozoospermic, oligoasthenoteratozoospermic subjects were significantly lower than normozoospermic males, but SOD activity did not show any significant differences, and (ii) both catalase activity and TAC showed a positively significant correlation with progressively motile sperms and normal sperm morphology, but these correlations were not significant with SOD activity.

Siciliano *et al*. (2001) evaluated antioxidant capacity of seminal plasma in asthenozoospermic and oligoasthenozoospermic specimens with normal viscosity and hyperviscosity. They observed that in semen with normal viscosity catalase and SOD activities and TAC do not alter in asthenozoospermic specimens compared with normozoospermic men, whereas SOD activity declines in oligoasthenozoospermic males. In contrast to Siciliano *et al*. (2001), we observed a significant decrease in catalase activity and TAC in men with asthenozoospermia compared to normozoospermic men. Our finding about SOD activity in asthenozoospermic men was similar to Siciliano *et al*. (2001). The results obtained in our study were in accordance with the study of Hsieh *et al*. (2002) and Koca *et al*. (2003) and contradicted the study of Tkaczuk-Wlach *et al*. (2002).

Immature spermatozoa with abnormal morphology and cytoplasmic retention are the most source of ROS production in semen (Pasqualotto *et al*., 2000). This has been confirmed by Gil-Guzman et al. (2001) study. They showed there is a direct significant correlation between the average ROS level and the rate of abnormal forms in semen. Gil-Guzman et al. (2001) also observed that there is an indirect significant correlation between seminal plasma TAC and ROS levels. They suggested that this indirect correlation between TAC and ROS might be associated with an increase in the consumption of soluble, non-enzymatic antioxidants in seminal plasma which is resulted from over production of ROS. In our study the correlation between TAC and the level of normal morphology was direct. This finding could indirectly confirm the Gil-Guzman et al. (2001) suggestion. The indirect correlation between lipid peroxidation and sperm motility has been shown by Keskes-Ammar *et al*. (2003). In our study both TAC and catalase, two defenses against ROS, showed direct correlation with sperm motility. Keskes-Ammar *et al*. (2003) and our study might suggest that the higher level of antioxidant status prevents lipid peroxidation in spermatozoa and therefore results in higher sperm motility.

Hsieh *et al*. (2002) observed a slightly positive correlation between seminal plasma SOD activity and sperm concentration. Their interpretation was that higher concentrations of spermatozoa might produce higher levels of SOD. The positive significant correlation between seminal plasma catalase activity and sperm concentration that observed in our study may be interpreted similar to Hsieh *et al*. (2002).

Immature spermatozoa generate primary superoxide anion (Agarwal *et al*., 2002). This anion is dismuted to hydrogen peroxide by SOD activity. Detoxification of hydrogen peroxide is carried out by catalase activity. Hydrogen peroxide is the primary toxic ROS for human spermatozoa that its high concentration induces lipid peroxidation and results in cell death. Therefore, the balance of the SOD and catalase activities in semen is important for maintaining sperm motility (Hsieh *et al*., 2002). In Hsieh *et al*. (2002) study they observed that there is a positive but nonsignificantly correlation between SOD activity and sperm motility. They interpreted that higher SOD activity may scavenge the generation of ROS, which may lower the cytotoxicity to spermatozoa. In present study, we also measured catalase activity that showed a significant difference between case and control groups and correlated with sperm motility. According to these results, we suggest further investigation on detoxifying of hydrogen peroxide by catalse.

One advantage of our study was centrifugation of semen samples at high speed to separate seminal plasma from spermatozoa and other cells and particles. The membrane-bound oxidases or antioxidants associated with cellular debris and/or organellescan influence seminal plasma SOD and catalase activity (Zini *et al*., 2000; Zini *et al*., 2002). A limitation of our study was the small sample size. For this reason, interpretation of the results should be carried out with caution.

In summary, both catalase activity and TAC were significantly correlated with sperm motility and morphology. But, SOD activity in seminal plasma was not correlated with seminal qualities. Therefore, it is concluded that decreasing seminal plasma antioxidant status, especially catalase activity and TAC, may have significant role in the etiology of sperm abnormality. However, the results of this study could provide a database for further research especially about catalase, because

[Downloaded from ijrm.ir on 2024-10-12]

characteristics of catalase in ejaculate have not yet been studied in depth. Further study with higher sample size for investigating the association between catalase activity and seminal parameters is warranted.

Acknowledgements

This research was granted by Drug Applied Research Center of Tabriz University of Medical Sciences and Health Services, No 5 79 401. The authors thank Mr. Amir-Mansour Vatankhah for his laboratory assistance, and IVF center of Alzahra hospital for their cooperation in this research.

References

Aebi H. Catalase in vitro. (1984) *Methods Enzymol* **105**:*121-126.*

Agarwal A., Saleh R.A. (2002) Role of oxidants in male infertility: rationale, significance, and treatment. *Urol Clin North Am* **29**: *817-827.*

Agarwal A., Saleh R.A., Bedaiwy M.A. (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* **79**: *829-843.*

Agarwal A., Nallella K.P., Allamaneni S.S., Said T.M. (2004) Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod Biomed Online* **8**: *616-627.*

Alkan I., Simsek F., Haklar G., Kervancioglu E., Ozveri H., Yalcin S., Akdas A. (1997) Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants. *J Urol* **157**:*140-3.*

Calamera J., Buffone M., Ollero M., Alvarez J., Doncel G.F. (2003) Superoxide dismutase content and fatty acid composition in subsets of human spermatozoa from normozoospermic, asthenozoospermic, and polyzoospermic semen samples. *Mol Reprod Dev* **66***: 422-430.*

Conquer J.A., Martin J.B., Tummon I., Watson L., Tekpetey F. (1999) Fatty acid analysis of blood serum, seminal plasma, and spermatozoa of normozoospermic vs. asthenozoospermic males. *Lipids* **34**: *793-799.*

Dandekar S.P., Nadkarni G.D., Kulkarni V.S., Punekar S. (2002) Lipid peroxidation and antioxidant enzymes in male infertility. *J Postgrad Med* **48**:*186-189.*

Esfandiari N., Sharma R.K., Saleh R.A., Thomas A.J. Jr., Agarwal A. (2003) Utility of the nitroblue tetrazolium reduction test for assessment of reactive oxygen species production by seminal leukocytes and spermatozoa. *J Androl* **24**: *862-870.*

Fraczek M., Szkutnik D., Sanocka D., Kurpisz M. (2001) Peroxidation components of sperm lipid membranes in male infertility. *Ginekologia Polska* **72**: *73-79.*

Fujii J., Iuchi Y., Matsuki S., Ishii T. (2003) Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues. *Asian J Androl* **5**:*231-242.*

Garrido N., Meseguer M., Simon C., Pellicer A., Remohi J. (2004) Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility. *Asian J Androl* **6**: *59-65.*

Gil-Guzman E., Ollero M., Lopez M.C., Sharma R.K., Alvarez J.G., Thmas A.J. J.r., Agarval A. (2001) Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. *Hum Reprod* **16**:*1922-1930.*

Hendin B.N., Kolettis P.N., Sharma R.K., Thomas A.J. Jr., Agarwal A. (1999) Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J Urol* **161**: *1831-1834.*

Hsieh Y.Y., Sun Y.L., Chang C.C., Lee Y.S., Tsai H.D., Lin C.S. (2002) Superoxide dismutase activities of spermatozoa and seminal plasma are not correlated with male infertility. *J Clin Lab Anal* **16**:*127-131.*

Keskes-Ammar L., Feki-Chakroun N., Rebai T., Sahnoun Z., Ghozzi H., Hammami, S. et al. (2003) Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* **49***:83-94.*

Koca Y., Ozdal O.L., Celik M., Unal S., Balaban N. (2003) Antioxidant activity of seminal plasma in fertile and infertile men. *Arch Androl* **49**: *355-359.*

Meucci E., Milardi D., Mordente A., Martorana G.E., Giacchi E., De Marinis L., Mancini A. (2003) Total antioxidant capacity in patients with varicoceles. *Fertil Steril* **3**:*1577-1583.*

Mruk D.D., Silvestrini B., Mo M.Y., Cheng C.Y. (2002) Antioxidant superoxide dismutase - a review: its function, regulation in the testis, and role in male fertility. *Contraception* **65**: *305-311.*

Pasqualotto F.F., Sharma R.K., Nelson D.R., Thomas A.J. Jr., Agarwal A. (2000) Relationship between oidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril* **73**: *459-64.*

[Downloaded from ijrm.ir on 2024-10-12]

Peeker R., Abramsson L., Marklund S.L. (1997) Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa. *Mol Hum Reprod* **3**:*1061-1066.*

Said T.M., Kattal N., Sharma R.K., Sikka S.C., Thomas A.J. Jr., Mascha E., Agarwal A. (2003) Enhanced chemiluminescence assay vs colorimetric assay for measurement of the total antioxidant capacity of human seminal plasma. *J Androl* **24**: *676-680.*

Sanocka D., Kurpisz M. (2004) Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol* **2**:*12.*

Shibahara H., Suzuki T., Obara H., Hirano Y., Onagawa T., Taneichi A., Takamizawa S., Sato I. (2002) Accuracy of the normal sperm morphology value by Sperm Quality Analyzer IIC: comparison with the strict criteria. *Int J Androl* **25**: *45-48.*

Siciliano L., Tarantino P., Longobardi F., Rago V., De Stefano C., Carpino A. (2001) Impaired seminal antioxidant capacity in human semen with hyperviscosity or oligoasthenozoospermia. *J Androl* **22**: *798-803.*

Suzuki T., Shibahara H., Tsunoda H., Hirano Y., Taneichi A., Obara H., Takamizawa S., Sato, I. (2002) Comparison of the Sperm Quality Analyzer IIC variables with the computer-aided sperm analysis estimates. *Int J Androl* **25**: *49-54.*

Taylor C.T. (2001) Antioxidants and reactive oxygen soecies in human fertility. *Environmental Toxicology and Pharmacology* **10**: *189-198.*

Tkaczuk-Wlach J., Kankofer M., Jakiel G. (2002) Activity of superoxide dismutase and glutathione peroxidase in human semen in normozoospermia and spermatopathy. *Ann Univ Mariae Curie Sklodowska* **57**: *369-375.*

World Health Organization. (1999) WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interactin. *3erd edn Cambridge University Press, Cambridge, UK.*

Zarghami N., Khosrowbeygi A. (2004) Evaluation of lipid peroxidation as an indirect measure of oxidative stress in seminal plasma. *Iranian Journal of Reproductive Medicine* **2**: *34-39.*

Zini A., Garrels K., Phang D. (2000) Antioxidant activity in the semen of fertile and infertile men. *Urology* **55**: *922-926.*

Zini A, Fischer MA, Mak V, Phang D, Jarvi K. (2002) Catalase-like and superoxide dismutase-like activities in human seminal plasma. *Urol Res* **30**: *321-323.*

[Downloaded from ijrm.ir on 2024-10-12]