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Research Article

Evaluation of total antioxidant capacity in human seminal plasma

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Abstract

Objectives: Male factor infertility accounts for up to half of all cases of infertility. Oxidative stress (OS) is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants, commonly known as reactive oxygen species (ROS).

Methods: Semen samples were obtained from 65 males aged 23-36 years who were a partner in an infertile couple and presented at the urology clinic of Antalya Medicalpark hospital. Routine spermiogram parameters were analyzed according to World Health Organization guidelines. The study was conducted using a normozoospermic group (n=18) with normal semen parameters, and 47 men with abnormal semen parameters; azoospermic (n=16), teratozoospermic (n=16) and oligoasthenoteratozoospermic (n=15). The total oxidant status (TOS), total antioxidant capacity (TAC), activity, and advanced oxidation protein products (AOPPs) levels in semen were determined using spectrophotometric method. The oxidative stress index (OSI) value of the seminal plasma was calculated. The results were analyzed statistically.

Results: The AOPPs level, TOS activity and OSI index value in seminal plasma was significantly higher in the oligoasthenozoospermic, teratozoospermic and azospermic groups compared with the normozoospermic group. In contrast, the TAC activity was higher in normozoospermic group than infertile group.

Conclusion: This study investigated whether the antioxidant status and the extent of protein oxidation in seminal plasma could be a valuable predictor of sperm function. The results suggest that TAC assay and OSI index may be useful markers for male infertility.

Keywords: Advanced oxidation protein products, male infertility, oxidative stress, seminal plasma, total antioxidant capacity

In approximately 30% of male infertility cases, the underlying causes are unknown (idiopathic infertility) [1]. One factor that has been associated with idiopathic male infertility is oxidative stress (OS), which is thought to be responsible for some 30% to 80% of male subfertility [2].

OS, which is related to the production of reactive oxygen species (ROS), has negative effects on the quality of sperm. OS occurs when there is an imbalance; overproduction of ROS leads to an excess of oxidants that exceeds antioxidant capability. The human sperm is highly susceptible to OS; which is associated with an increased rate of cellular damage [3]. Excessive exposure to ROS damages spermatozoa and affects fertilization capacity [4].

ROS are highly reactive oxidizing agents that have one or more unpaired electrons; they are free radicals [5]. ROS serve a valuable purpose and are required to maintain normal cell function, however, an excess can lead to OS [6].

An elevated ROS level may be a result of exogenous or endogenous factors that may contribute to a deficient antioxidant defense. Exogenous factors can include environmental factors such as high temperature, pesticides, and pollution. Lifestyle characteristics, such as alcohol consumption, malnu-

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trition, and obesity, can also be influential. Immature spermatozoa, varicocele, infection in the male reproduction system, some chronic diseases, and autoimmune diseases are examples of endogenous factors [7].

The significant ROS created by the human sperm are hydrogen peroxide, superoxide anion, and hydroxyl radicals. Human seminal plasma as well as sperm have an antioxidant system that will normally regulate ROS production and prevent cellular damage [8]. Evaluation of seminal plasma oxidative profiles may be a significant instrument for the evaluation of sperm reproduction capacity and functional sufficiency [9].

Spermatozoa are protected by various antioxidants and antioxidant enzymes in the seminal plasma; therefore, an antioxidant that reduces OS and improves sperm motility could be useful in the management of male infertility [10]. Antioxidants reduce OS by inhibiting the oxidative chain reaction [11]. The precise role of OS in the etiology of defective sperm function is still not clearly defined. Oxidant/antioxidant results can vary widely. Laboratory tests that can measure the balance in seminal plasma could be a more objective means to determine OS. The oxidative stress index (OSI) is a quick, easy, and inexpensive technique to accurately determine the oxidant/antioxidant ratio in biological samples. The OSI offers a valuable and objective evaluation of redox status [12].

Advanced oxidation protein products (AOPPs) are created as a result of the reaction between plasma proteins and chlorinated oxidants (e.g., hypochlorous acid catalyzed by the H_2O_2-myeloperoxidase system), and are di-tyrosine-containing cross-linking protein products considered to be novel markers of oxidant-mediated protein damage. Due to the sensitivity, stability, convenience, and low cost of detection, understanding the role of AOPPs in predicting the severity of OS and disease prognosis has become increasingly important [13]. AOPPs have been analyzed in numerous diseases and have been widely accepted as an easily measurable marker of OS [14].

The present study aims to evaluate the seminal plasma total oxidant status (TOS), total antioxidant capacity (TAC), OSI, and AOPP level in infertile men and compared the results with those of healthy controls.

Materials and Methods

This study was approved by the Antalya Training and Research Hospital Non-pharmacological Clinical Research Ethics Committee (no: 34/13). All of the patients provided informed consent.

Study group

A total of 65 males who presented at the Antalya Medicalpark Hospital urology clinic and had semen analysis performed were enrolled in the study.

A detailed medical history was taken and an andrological examination was performed in all cases. Smoking, alcohol use, hypertension, hyperlipidemia, systemic or local infection, leukospermia (leukocyte count $>1\times10^6$/mL), varicocele, current medication use or antioxidant supplementation were exclusion criteria.

The patient group comprised 47 infertile men in couples who had experienced 1 year or more of infertility and for whom female infertility had been ruled out. Eighteen men proven to be healthy and fertile served as the control group.

Semen analysis

Semen samples were collected by masturbation into a clean specimen container after sexual abstinence for 3–5 days and allowed to liquefy at 37°C. The conventional semen parameters of sperm concentration, percentage motility, and normal sperm morphology were assessed according to the criteria provided in the World Health Organization (WHO) laboratory manual for the examination and processing of human semen, 5th edition [15]. The semen analysis was performed in the hospital laboratory using a Makler counting chamber (Sefi Medical Instruments Ltd., Haifa, Israel) with 10-µL aliquot loaded onto a counting chamber for each semen parameter. Morphology smears were scored by using the Kruger criteria [16]. Sperm concentration was expressed as sperm number per milliliter of semen, and motility and morphology were expressed as percentages. The study design used 4 groups based on the ejaculate parameters: Group N (n=18) consisted of samples from males with normal ejaculate (normozoospermia), Group A (n=16) consisted of those lacking spermatozoa in the ejaculate (azoospermia), Group T (n=16) consisted of those with >96% abnormal spermatozoa (teratozoospermia), and Group OAT (n=15) consisted of those with an abnormal motility, count, and morphology (oligoasthenoteratozoospermia).

Seminal plasma was retrieved after centrifugation at 3500 rpm for 15 minutes at room temperature and stored in Eppendorf tubes (3 mL) at -20°C for TAC, TOS, and AOPP measurement.

Evaluation

The seminal plasma TAC was determined using a novel automated colorimetric method developed by Erel [17]. In this assay, a hydroxyl radical, the most potent biological radical, is produced as a result of oxidation of ferrous ion to ferric ion, using ferrous ion solution in Reagent 1 and hydrogen peroxide in Reagent 2. Sequentially-produced radicals, like the brown-colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. The color increases with continued oxidation reactions. Trolox (F. Hoffmann-La Roche Ltd., Basel, Switzerland), a widely used antioxidant in TAC measurement assays, is used to suppress oxidation, which allows for the measurement of the antioxidative capacity of samples against potent free-radical reactions initiated by the hydroxyl radical. The assay has an excellent precision value of >97%. The results are expressed in terms of millimolar Trolox equivalent per liter (mmol Trolox equiv/L).

The TOS value of seminal plasma was also determined using Erel's colorimetric method [17]. Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The

oxidation reaction is enhanced by glycerol molecules, which are abundant in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the number of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromoles of hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv/L).

The OSI value is the TOS to TAC ratio. The TAC units were changed to mmol/L, and the formula OSI (arbitrary unit)=TOS (μmol H_2O_2 Equiv/L)/TAC (mmol Trolox equiv/L) was applied to calculate the OSI [17].

The concentration of AOPP was measured using a spectrophotometric assay according to the method described by Witko-Sarsat et al. [18]. The technique is based on the color reaction of AOPPs to a potassium iodide solution in an acidic environment. AOPPs concentrations are expressed as micromoles per liter of chloramine-T equivalents (μmol/L).

Statistical analysis

The study data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 software (IBM Corp., Armonk, NY, USA). Normally distributed data were expressed as the mean±SD. The Shapiro-Wilk test was performed to verify the normality of the distribution, and one-way analysis of variance was used to compare the mean age, volume, pH, sperm count, total count, forward motility, immotility, and Kruger morphology of the different groups. Variables with a non-normal distribution were analyzed using the Kruskal-Wallis variance analysis test and the Mann-Whitney U test. Significance was accepted at $p<0.05$ and presented using the median (min-max). The Bonferroni correction was used to test the significance of pairwise differences and a p value of <0.008 was accepted as the level of significance.

Results

The study group was composed of 47 infertile and 18 fertile men (23-38 years old) who presented at a urology clinic. The mean age of the N group was 30.72±5.16 years, while it was 29.80±6.14 in the T group, 30.81±5.35 in the A group, and 32.13±5.57 years in the OAT group. There was no statistically significant difference in age between the groups (p=0.682). The semen analysis results are reported by group in Table 1. The seminal plasma median TOS, OSI, and AOPP values of the A, T, and OAT groups were significantly greater than N group. TAC value of N group was measured higher than infertile groups (Table 2). We also performed a pairwise comparison between all groups to assess the differences. p value ($p<0.008$) was found after Bonferroni correction by using the Mann-Whitney test (Table 3).

The major finding of the present study is the significantly different p values are obtained in the N group compared with the OAT group for TAC, TOS, and OSI parameters. Also when N and A groups are compared TAC (p=0.003) and OSI (p=0.002) parameters are significantly different. All other group comparisons were not statistically significant.

Table 1. Basic parameters of semen analysis results by group

Semen parameters	N (n=18)	T (n=16)	A (n=16)	OAT (n=15)
Semen volume (mL)	4.67±0.56	4.26±0.54	3.55±0.33	3,68±0.31
pH	8.41±0.06	8.40±0.06	8.25±0.06	8.31±0.08
Sperm concentration ($\times10^6 mL^{-1}$)	132.33±19.45	85.95±13.94	0	6.7±1.11
Total sperm number ($\times10^6$ per ejaculate)	589.57±100.41	297.67±40	0	25.33±5.25
Total motility (PR+NP, %)	66.44±2.72	61.14±2.59	0	20.33±4.43
Immotile (IM) (%)	26.61±2.77	32.09±2.42	0	69.2±5.88
Normal morphology (%)	6.72±0.55	1.47±0.27	0	0.93±0.33

Results are presented as mean±SD. Sperm movement definitions according to World Health Organization criteria. N: Normozoospermic; T: Teratozoospermic; A: Azoospermic; OAT: Oligoastenoteratozoospermic; PR: Progressive motility; NP: Non-progressive motility; IM: Immotile spermatozoa.

Table 2. Laboratory characteristics of seminal plasma in the study groups (N=65)

	N group	T group	A group	OAT group	p*<0.05
TAC	1.74 (1.20-2.40)	1.57 (1-2.10)	1.33 (0.90-1.80)	1.19 (0.20-1.60)	<0.001
TOS	18.23 (16.10-19.90)	19.28 (17.70-20.60)	19.98 (17.30-29.10)	20.77 (17.40-36.10)	<0.001
OSI	10.93 (6.79-16.16)	12.58 (9.0-17.3)	15.63 (10.1-18.6)	20.60 (9.3-27.6)	<0.001
AOPP	33.90 (13.70-59.10)	44.38 (23.10-75.90)	54.23 (24.80-83.10)	70.46 (19.70-88.7)	0.004

p*value determined by using the Kruskal-Wallis test. p results which are <0.05 are accepted as stasitically significant. A nominal p value less than 0.05 means that at least one group of infertile groups significantly differs from N group. Results are presented as median (min-max). N: Normozoospermic; T: Teratozoospermic; A: Azoospermic; OAT: Oligoastenoteratozoospermic; TAC: Total antioxidant capacity (mmol Trolox equiv/L); TOS: Total oxidant status (μmol H_2O_2 equiv/L); OSI: Oxidative stress index; AOPP: Advanced oxidation protein product (μmol/L).

Table 3. Pairwise comparison results among all groups

Groups	TOS	TAC	OSI	AOPP
N vs. A	0.364	**0.003**	**0.002**	0.012
N vs. T	0.082	0.999	0.783	0.377
N vs. OAT	**0.008**	**0.002**	**<0.001**	0.012
A vs. T	0.999	0.059	0.143	0.975
A vs. OAT	0.999	0.999	0.999	0.999
T vs. OAT	0.999	0.039	0.027	0.938

p<0.008 after Bonferroni correction. Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction to adjust or multiple comparisons. TOS: Total oxidant status; TAC: Total antioxidant capacity; OSI: Oxidative stress index; AOPP: Advanced oxidation protein product; N: Normozoospermic; A: Azoospermic; T: Teratozoospermic; OAT: Oligoastenoteratozoospermic.

Discussion

Estimates are that between 10% and 15% of couples around the world are infertile. Semen analysis according to WHO guidelines is widely regarded as the gold standard, and uses sperm parameters such as concentration, motility, and morphology to determine reproduction potential [19]. However, semen analysis remains insufficient to predict male infertility in many cases, and does not include information on sperm function. Studies to determine sperm function and the etiology of this problem suggest that OS has a significant role in the pathogenesis of male infertility [20].

Nonetheless, a precise view of the particular role of the physiological and pathological levels of ROS in male infertility remains to be defined. Though ROS is associated with the pathology of many diseases, including male infertility, a limited quantity of ROS is also necessary for spermatozoa to turn into normal sperm capable of fertilization [21]. Spermatogenesis is a complex process that includes various stages and different cell types; which is why mutations in the mitochondrial genome as a result of excessive ROS may cause infertility by disturbing the formation of mature morphological and functional spermatozoa formation [22].

Infertile male patients frequently have a high level of OS, apoptosis, or sperm DNA damage [23]. Human sperm includes various cell types, including mature and immature spermatozoa, round cells, leukocytes, and epithelial cells. Therefore, most human ejaculate contains potential ROS sources. Leukocytes and immature spermatozoa are the primary sources of ROS [24]. The TAC of the seminal plasma and reproductive fluids is influenced mainly by the antioxidant enzymes-superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and total glutathione (GSH). Reduced glutathione and oxidized glutathione (GSSG) has been shown to protect spermatozoa against lipid peroxidation and OS [25]. A decrease of one antioxidant may be compensated with an increase of another antioxidant; therefore, measurement of TAC provides more valuable information than individual measurements [26].

The TOS value is the measurement of the production of reactive oxygen and/or nitrogen species. Elevated levels of reactive oxygen and nitrogen species can be toxic and damage cellular biomolecules, such as lipids, proteins, or DNA [6].

Agarwal et al. [27] reported that excessive ROS can have a significant effect on sperm motility. An imbalance between ROS and antioxidants causes mitochondrial dysfunction and depletion of adenosine triphosphate, leading to the reduction of cell energy and sperm motility. Also, excessive ROS negatively affects the activity of some enzymes that are necessary for sperm motility, such as glucose-6-phosphate dehydrogenase. Venkatesh et al. [28] researched the relationship between various sperm morphological defects and seminal OS in infertile and fertile males and found that infertile males had a median ROS level 124 times higher than fertile males. Among various morphological defects, the percentage of sperm with cytoplasmic droplets was significantly higher in infertile males, which might have been due to a high level of ROS.

TAC measurement of seminal plasma gives an indirect measurement of OS. Consistent with our work, previous studies have indicated that low seminal TAC values were related to male infertility [29]. TAC values might contribute to the understanding of the pathophysiology of male infertility, irrespective of the clinical diagnosis.

There is no consensus on a TAC reference range for seminal plasma in healthy, fertile males. Researchers working with an Italian group used a total antioxidant status assay and reported a mean seminal plasma result of 1.21±0.16 mmol/L in a control group of 25 and a median of 1.19 with a range of 1.0 to 1.4 mmol/L in another control group of 18 [30]. The median seminal plasma TAC value of the healthy men in our study was 1.74 (1.20-2.40) mmol Trolox equiv/L.

Sharma et al. [31] and others have observed that the ROS-TAC scores of seminal plasma can be used as OS indicators and may be correlated with male infertility. Antioxidants may be a means to reduce ROS in cases of low TAC. In our study, TAC levels are significantly lower in the infertile groups (A, T and OAT groups) compared to that N group (p<0.001). On the other-hand the OSI levels of the A, T and OAT groups are significantly higher than N group (p<0.001). Our results are consistent with the results of Khosrowbeygi et al. [32] and Koca et al. [33].

Khosrowbeygi et al. [32] noted that the TAC level was significantly lower in asthenospermic, astenoteratozoospermic, and oligoasthenoteratozoospermic groups. Koca et. Al. [33] also found that the seminal plasma TAC was lower in males with astenoteratozoospermia, asthenospermia, and oligoasthenoteratozoospermia when compared with fertile males. A low TAC in seminal plasma may contribute to reduced fertilization capacity and defective sperm quality.

Anomalies to the head and neck of the sperm are characteristic of teratozoospermia, which can seriously affect fertility (Fig. 1). Sperm head abnormalities are often caused by chromatin and acrosome anomalies [34]. In our study, although no significant difference was established between normozoospermia and teratozoospermia groups in terms of median TAC, TOS,

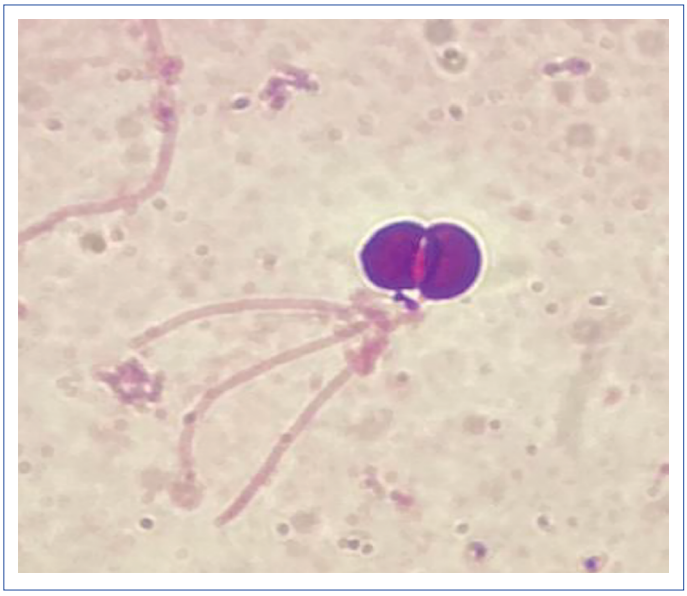

Figure 1. Teratozoospermic "kissing" sperm. Specimen stained using Diff-Quick (Dade Behring Inc., Newark, NJ, USA) and viewed at ×1000 magnification with a Nikon E-50i microscope (Nikon Corp., Tokyo, Japan) using an immersion oil objective lens.

AOPP and OSI values, it has been recognized that the TAC, TOS, AOPP and OSI can be affected by numerous factors. There is a need for more extensive studies of this topic.

Our literature review revealed only a limited number of studies using the OSI in human seminal plasma. A study performed by Barik et al. [35] found a lower OSI value in men with normal semen parameters than in men with abnormal semen parameters, but the finding was not statistically significant. Conflicting data have been reported about the relationship between OS and sperm DNA damage. Verit et al. [36] did not find any relationship between sperm DNA damage and OS in normozoospermic infertile men They suggested that the pathophysiology of idiopathic infertility cannot be explained by sperm DNA damage or seminal OS.

In our findings, the median value of OSI in seminal plasma of the A group p=0.002 and the OAT group (p<0.001) was significantly higher than that of the control group. Our results suggested that an elevated OSI was associated with impaired sperm quality, which could lead to infertility.

AOPPs might be formed during the oxidative stress reaction of plasma proteins with chlorinated oxidants and have been considered potential novel markers of oxidant-mediated protein damage [18]. AOPPs could be considered to be true mediators of the proinflammatory effect of OS in seminal plasma. Therefore, the AOPP level may serve as a useful additional diagnostic measure of semen quality and male reproductive potential [14].

Kratz et al. [37] found a higher level of AOPP concentration in the seminal plasma of an azoospermia group compared with a teratozoospermia group. Similarly, Jianwei et al. [38] found that the seminal plasma level of AOPPs and ROS was significantly elevated in patients with teratozoospermia and that the seminal plasma AOPP level was significantly higher in a severe teratozoospermia group.

Against Kratz et al. study, we observed no statistically significant difference in paired group comparisons of AOPP level. This may be due to the small size of the study groups.

Conclusion

From the present study, it may be concluded that oxidative stress is increased in men with abnormal semen parameters. The increase in oxidants may eventually lead to defects in sperm morphology and cause male infertility. In our present study, the OAT group demonstrated increased seminal plasma oxidative stress pattern against normozoospermic individuals. The presence of lower levels of TAC and higher levels of OSI in infertile groups (A, T and OAT groups) compared to N group shows the presence of oxidative stress in seminal plasma.

Therefore, seminal plasma oxidative profiles may be an important tool for better assessment of sperm reproductive capacity. Clearly, more multicenter studies are needed to further examine and understand the assessment of male infertility.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by the Antalya Training and Research Hospital Non-pharmacological Clinical Research Ethics Committee (No: 34/13, Date: 06/02/2014).

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References

1. Tadros NN, Sabanegh ES. Empiric medical therapy with hormonal agents for idiopathic male infertility. Indian J Urol 2017;33(3):194–8. [CrossRef]
2. Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, Sadeghi MR. Reactive oxygen species and male reproductive hormones. Reprod Biol Endocrinol 2018;16(1):87. [CrossRef]
3. Sikka SC, Rajasekaran M, Hellstrom WJ. Role of oxidative stress and antioxidants in male infertility. J Androl 1995;16(6):464–8.
4. Maneesh M, Jayalekshmi H. Role of reactive oxygen species and antioxidants on pathophysiology of male reproduction. Indian J Clin Biochem 2006;21(2):80–9. [CrossRef]
5. Sikka SC. Oxidative stress and role of antioxidants in normal

and abnormal sperm function. Front Biosci 1996;1:e78–86.
6. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ Jr, Agarwal A. The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 1999;14(11):2801–7. [CrossRef]
7. Tremellen K. Oxidative stress and male infertility--a clinical perspective. Hum Reprod Update 2008;14(3):243–58.
8. de Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J Androl 1992;13(5):379–86.
9. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. Am J Reprod Immunol 2008;59(1):2–11. [CrossRef]
10. Kim JG, Parthasarathy S. Oxidation and the spermatozoa. Semin Reprod Endocrinol 1998;16(4):235–9. [CrossRef]
11. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4(8):118–26. [CrossRef]
12. Abuelo A, Hernández J, Benedito JL, Castillo C. Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. Animal 2013;7(8):1374–8.
13. Hazen SL, Hsu FF, Heinecke JW. p-Hydroxyphenylacetaldehyde is the major product of L-tyrosine oxidation by activated human phagocytes. A chloride-dependent mechanism for the conversion of free amino acids into reactive aldehydes by myeloperoxidase. J Biol Chem 1996;271(4):1861–7. [CrossRef]
14. Heinecke JW, Li W, Daehnke HL 3rd, Goldstein JA. Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages. J Biol Chem 1993;268(6):4069–77. [CrossRef]
15. WHO. WHO Laboratory manual for the examination and processing of human semen. 5th ed. Geneva, Switzerland: WHO Press; 2010.
16. Kruger TF, Ackerman SB, Simmons KF, Swanson RJ, Brugo SS, Acosta AA. A quick, reliable staining technique for human sperm morphology. Arch Androl 1987;18(3):275–7. [CrossRef]
17. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38(12):1103–11.
18. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, et al, Descamps-Latscha B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996;49(5):1304–13. [CrossRef]
19. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci 2015;8(4):191–6. [CrossRef]
20. Agarwal A, Allamaneni SS. Sperm DNA damage assessment: a test whose time has come. Fertil Steril 2005;84(4):850–3.
21. Chen SJ, Allam JP, Duan YG, Haidl G. Influence of reactive oxygen species on human sperm functions and fertilizing capacity including therapeutical approaches. Arch Gynecol Obstet 2013;288(1):191–9. [CrossRef]
22. Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P. Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev 2016;28(1–2):1–10.
23. Garrido N, Meseguer M, Simon C, Pellicer A, Remohi J. Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility. Asian J Androl 2004;6(1):59–65.
24. Henkel RR. Leukocytes and oxidative stress: dilemma for sperm function and male fertility. Asian J Androl 2011;13(1):43–52.
25. Pahune PP, Choudhari AR, Muley PA. The total antioxidant power of semen and its correlation with the fertility potential of human male subjects. J Clin Diagn Res 2013;7(6):991–5.
26. Chen SS, Chang LS, Wei YH. Oxidative damage to proteins and decrease of antioxidant capacity in patients with varicocele. Free Radic Biol Med 2001;30(11):1328–34. [CrossRef]
27. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health 2014;32(1):1–17. [CrossRef]
28. Venkatesh S, Singh G, Gupta NP, Kumar R, Deecaraman M, Dada R. Correlation of sperm morphology and oxidative stress in infertile men. Iran J Reprod Med 2009;7:29–34.
29. Pasqualotto FF, Sharma RK, Kobayashi H, Nelson DR, Thomas AJ Jr, Agarwal A. Oxidative stress in normospermic men undergoing infertility evaluation. J Androl 2001;22(2):316–22.
30. Fingerova H, Novotny J, Barborik J, Brezinova J, Svobodova M, Krskova M, et al. Antioxidant capacity of seminal plasma measured by TAS Randox. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2007;151(1):37–40. [CrossRef]
31. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ Jr, Agarwal A. The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 1999;14(11):2801–7. [CrossRef]
32. Khosrowbeygi A, Zarghami N, Deldar Y. Correlation between sperm quality parameters and seminal plasma antioxidants status. Iran J Reprod Med 2004;2(2):58–64.
33. Koca Y, Ozdal OL, Celik M, Unal S, Balaban N. Antioxidant activity of seminal plasma in fertile and infertile men. Arch Androl 2003;49(5):355–9. [CrossRef]
34. Chemes HE, Alvarez Sedo C. Tales of the tail and sperm head aches: changing concepts on the prognostic significance of sperm pathologies affecting the head, neck and tail. Asian J Androl 2012;14(1):14–23. [CrossRef]
35. Barik G, Chaturvedula L, Bobby Z. Role of oxidative stress and antioxidants in male infertility: an interventional study. J Hum Reprod Sci 2019;12(3):204–9. [CrossRef]
36. Verit FF, Verit A, Kocyigit A, Ciftci H, Celik H, Koksal M. No increase in sperm DNA damage and seminal oxidative stress in patients with idiopathic infertility. Arch Gynecol Obstet 2006;274(6):339–44. [CrossRef]
37. Kratz EM, Piwowar A, Zeman M, Stebelová K, Thalhammer T. Decreased melatonin levels and increased levels of advanced oxidation protein products in the seminal plasma are related to male infertility. Reprod Fertil Dev 2016;28(4):507–15.
38. Jianwei T, Tingting X, Zhuolin Q, Jing L, Wenting YE, Song Yali S. Association of advanced oxidation protein products in seminal plasma with teratospermia and outcome parameters of *in vitro* fertilization. J South Med Univ 2019;39(8):937–43.