Seminal Malondialdehyde Concentration And Superoxide Dismutase, Catalase Activity In Male Infertility

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Citation

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Abstract

Aims & objectives- Reactive oxygen species (ROS) induced lipid peroxidation is associated with sperm function. Malondialdehyde (MDA) concentration, Superoxide dismutase and Catalase (CAT) activity represent the lipid peroxidation and spermicidal antioxidant status respectively. The present study aimed to evaluate the relationship of MDA, Superoxide dismutase and Catalase levels with sperm parameters. Materials & methods-Specimens were divided into two groups: Group I - Normospermia (n=30); Group II - Oligoasthenozoospermia (n=30). Seminal MDA concentration was measured by thiobarbituric acid reaction method. Seminal SOD activity was measured by Marklund and Marklund method. Seminal Catalase activity was measured by potassium dichromate colorimetric assay. Seminal MDA levels, SOD and Catalase activities in both groups were compared. Significance of the data is analyzed using unpaired t-test. Results - MDA concentrations in both groups were significantly different (12.65 ± 1.68 nmole/m vs 21.58 ± 1.54 nmole/ml). SOD & Catalase activities in both groups were also significantly different. SOD levels in oligoasthenozoosperia were 27.99 ± 4.36 Units/ml and in normospermia 12.96 ± 3.35 U/ml whereas levels of catalase in oligiasthenozoosperia were 1.36 ± 0.44 Units/mg of protein and normospermia were 5.12 ± 1.03 Units/mg of protein. SOD & Catalase activities were positively and significantly correlated with the sperm concentration and sperm motility.
Conclusion : Measurement of seminal MDA concentrations, antioxidant enzymes SOD & CAT can be used as a additional, simple and useful tool in predicting quality of sperm parameters.

INTRODUCTION

Infertility has been a major medical problem as well as social stigma. Despite of enormous progress in research, most of the blame for infertility is placed on the female. Advances in understanding of gonadal/sperm physiology have increased our knowledge of male infertility. Defective sperm function is the most prevalent cause of male infertility. [1]. Many environmental, physiological, and genetic factors have been implicated in infertility. Hence, it is very important to identify factors, which affect normal sperm function. Free radical-induced oxidative damage to spermatozoa is a condition, which is gaining considerable attention for its role in inducing poor sperm function [2]. Understanding of such conditions will help in designing new and effective treatment strategies.

Until recently, Reactive oxygen species (ROS), were considered toxic to the human spermatozoa. However a strong evidence suggests that small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities [3], [4],[5].

Theoretically, cellular damage in the semen is due to improper balance between ROS generation and scavenging activities which causes ‘Oxidative stress’.

The increased ROS formation leads to lipid peroxidation. This is correlated with reduced sperm motility [6], [7], due to decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with a reduction in membrane fluidity [8]. Another hypothesis is that H_2O_2 can diffuse across the cell membranes and inhibit the activity of enzyme, glucose-6-phosphate dehydrogenase (G6PD). Inhibition of G6PD decreases the availability of NADPH and results in accumulation of oxidized glutathione, which reduces the antioxidant defense of the spermatozoa [9].

The seminal plasma is well endowed with an array of antioxidants such as Superoxide dismutase (SOD), Catalase. These antioxidants protect spermatozoa against oxidative stress [10],[11],[12]. SOD scavenges superoxide anion and

converts it into less potent hydrogen peroxide. Catalase detoxifies H_2O_2 to water and oxygen [13]

This study was undertaken to correlate detrimental effect of free radicals on sperm physiology in infertile males. The prime aim of the study was to estimate the lipid peroxidation product; MDA & some antioxidants in men with oligoasthenozoospermia compared to normospermia & their correlation with seminal parameters.

RESULTS

Figure 1

: Showing sperm count, motility, MDA, SOD and Catalase in control and study group

SEMINAL PARAMETERS	GROUP – I NORMOSPERMIA (N=30)	SEM	GROUP-II OLIGOASTHENOZOOSPERMIA (N=30)	SEM
SPERM COUNT (millions/ml)	69.20 ± 37.23	6.687	13.18 ± 9.95*	1.817
MOTILITY (percent)	70.96 ± 15.24	2.73	40.32 ± 21.59*	3.94
MDA (nmols/ml)	12.65± 1.68	0.302	21.58 ± 1.54*	0.281
SOD (Units/ml)	27.99 ±4.36	0.783	12.96 ± 3.35*	0.601
CATALASE (U/mg protein)	1.360 ± 0.44	0.079	5.12 ± 1.03*	0.188

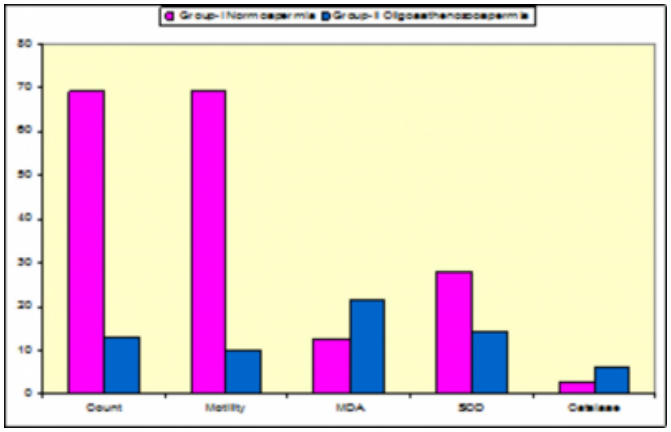
Results are presented as mean ± S.D.

* P < 0.0001

Note: The catalase activity is inversely proportional to the amount of perchromic acid formed by reaction between H_2O_2 and potassium dichromate due to lack of activity of catalase.

Figure 2

Showing comparison of sperm count, motility and levels of seminal MDA in control and study group.



In the above graph sperm count is expressed in millions/ml, motility in percent and MDA in nmols/ml, SOD in units/ml, catalase in U/mgs of protein

Figure 3

Showing correlation of sperm count and seminal MDA concentration

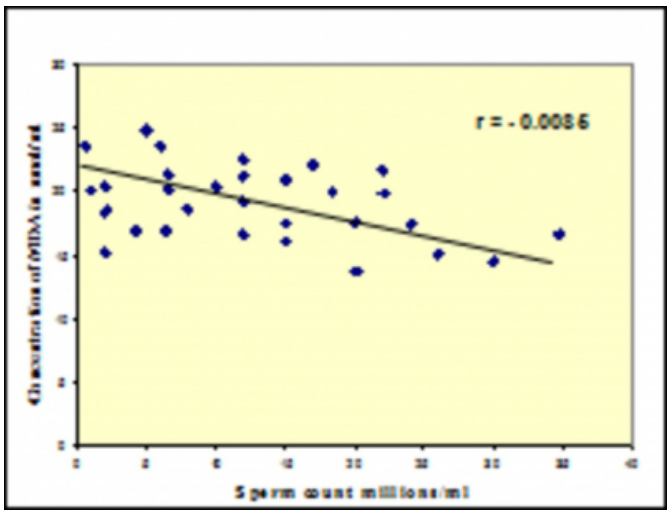


Figure 4

: Showing correlation of seminal SOD activity with Sperm Count

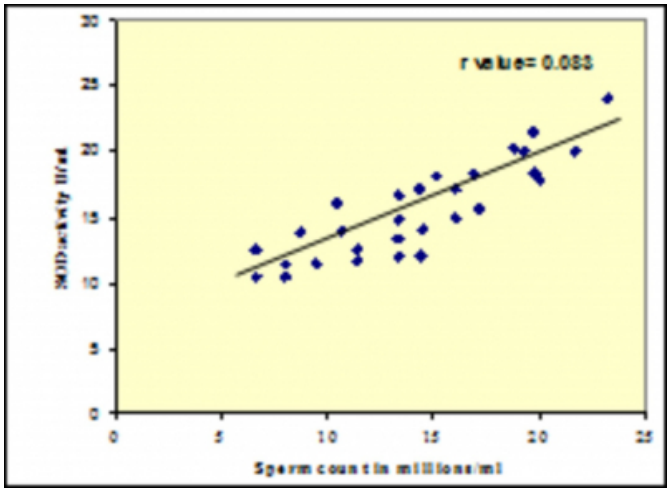
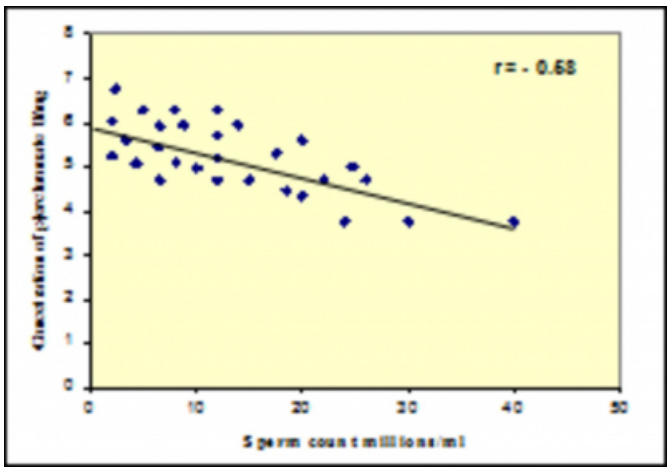


Figure 5

: Showing correlation of seminal Catalase activitiy with Sperm Count



The catalase activity is inversely proportional to the amount of perchromic acid formed by reaction between H_2O_2 and potassium dichromate due to lack of activity of catalase

Figure 6

: Showing correlation of seminal SOD activity with seminal MDA concentration

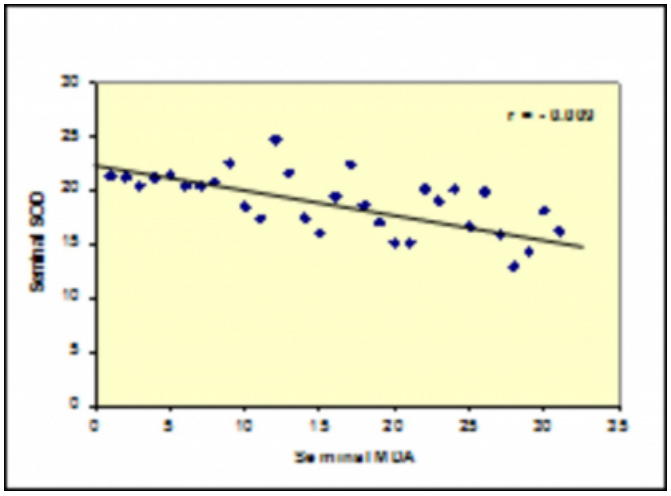
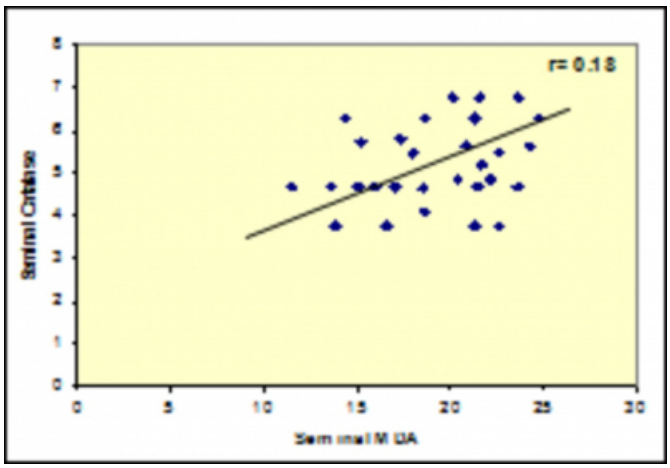


Figure 7

: Showing correlation of seminal Catalase activitiy with seminal MDA



RESULT

The concentration of seminal MDA in both groups were significantly different. Individuals with oligoasthenozoospermia are related with the elevated MDA concentrations. The levels of MDA in Group I & Group II were 12.65 ± 1.68 and 21.58 ± 1.54 nmols/ml respectively (p< 0.0001). The activities of seminal plasma SOD in Group I & Group II were 27.99 ± 4.36 & 12.96 ± 3.35 unit/ml respectively (p< 0.0001). The values of catalase activities in Group I & Group II were 1.360 ± 0.44 & 5.12 ± 1.03 U/mg of protein respectively (p< 0.0001). The Sperm concentration is negatively correlated seminal MDA concentration (Graph 2) (r = -0.0086) whereas seminal SOD & CAT activities (measured in terms of perchromic acid which is inversely proportional) positively correlated with sperm concentration (Graph 3 & 4) (r = 0.083, r = -0.58) resp. We also correlated seminal MDA concentration with levels of seminal SOD & CAT where we noted negative correlation of these antioxidant with seminal MDA (Graph 5 & 6) resp.

DISCUSSION

The most relevant findings of this study are

i) There is significant increase in concentration of MDA in oligoasthenozoospermia on comparison with normospermia and negative correlation was observed between the MDA concentration and sperm count. MDA production, which reflects the peroxidation of

polyunsaturated phospholipids, the major components of sperm membrane[18]. MDA

measurements are physiological and relevant because major loss of sperm function may occur with minimal damage to the membranes that envelop the sperm and/or divide key intracellular sperm compartments Thus, increased MDA activity represents lipid peroxidation of sperm membrane and inhibits sperm motility and viability.

Our results of MDA are concurrent with Kobayashi et al. and Hiesh et al. Kobayashi et al. [19] Kobayashi et al demonstrated elevated seminal MDA concentration in patients with oligoasthenoteratozoospermia. While our results are in contrast with Suleiman et al. and Heidar et al. Suleiman et al. (2001) [20] demonstrated that MDA concentration in the seminal plasma was not related with the sperm concentration and motility.

ii) We also measured antioxidant enzymes superoxide dismutase and Catalase. The SOD & CAT activities showed positive correlation with sperm concentration. We found negative correlation between SOD, CAT and MDA in seminal plasma. Immature spermatozoa generate primary superoxide anion. This anion is dismutated to hydrogen peroxide by SOD. Detoxification of hydrogen peroxide is carried out by Catalase. Hydrogen peroxide is the primary toxic ROS for human spermatozoa, as 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. Our studies of SOD are at par with Marek et al. and Siciliano et al. Marek et al.(2007)[21] reported significantly lower semen SOD activity in oligoasthenozoospermic patients, comparing to the normospermic men. Their study showed a positive correlation between seminal SOD activity and semen parameters - concentration and overall motility, where as Siciliano et al. (2001)[22] observed decline in SOD activity in oligoasthenozoospermia. Our findings of Catalase are in accordance with Khosrowbeygi et al.and Siciliano et al. Khosrowbeygi et al (2007)[23] observed decrease in seminal Catalase in infertile group compared to fertile group. Siciliano et al observed significant decrease in catalase activity and total antioxidant capacity (TAC) in men with asthenozoospermia compared to normozoospermia. In contrast Heidar et al.(2008) [24] found a positive correlation between total activity of CAT with total content of MDA in seminal plasma in normozoospermic samples.

Human spermatozoa exhibit a capacity to generate ROS. Production of very low amounts of ROS in semen appears to play a physiological role in regulating normal sperm functions, whereas high levels of ROS endanger sperm function and viability. Oxidative stress due to excessive production of ROS, impairs the antioxidant defense mechanisms, or it precipitates a range of pathologies that are believed to affect the male reproductive function [25]. Abnormal ROS production is associated with defective sperm function [26],[27]. A fine balance between ROS production and recycling is essential for spermatogenesis. Excessive generation of seminal ROS, mainly by neutrophils but also by immobile sperm, morphologically abnormal sperm, or morphologically normal but functionally abnormal sperm, can be a cause of male infertility [28]. Exposure of spermatozoa to ROS has been associated with cellular injury, which includes DNA damage and lipid peroxidation[29]. High lipid peroxidation may reduce the capacity of the sperm to undergo acrosomal reaction and fertilization [30] along with it's capability for oocyte fusion.

Seminal Antioxidant enzymes, such as SOD, Catalase could scavenge and detoxify the free radicals. A balance is maintained between the amount of ROS and antioxidants. Cellular damage arises on disturbed equilibrium, especially when the cellular scavenging systems cannot eliminate the increased ROS. Lipid peroxidation damages the cell plasma membrane, which leads to loss of cytosolic components and cell death.

MATERIALS AND METHODS

Thirty normal & thirty infertile males between age group 20-45 years were included in the study on voluntary basis. Exclusion criteria included positive HIV status, history of tuberculosis, endocrinological disorders. Semen was obtained by masturbation after at least 72 hours of sexual abstinence. Semen samples were collected into sterile containers. After liquefaction, samples were centrifuged to separate seminal plasma which was analyzed for volume and sperm count, sperm motility and morphology microscopically according to WHO guidelines [14]. All semen samples were divided into two groups: Group I - Normal healthy controls i.e. Normospermia (n=30) with sperm count $> 20 \times 10^6$ per ml & motility > 50%; Group II – Infertile males i.e. Oligoasthenozoospermia (n=30) with sperm count $< 20 \times 10^6$ per ml & motility <50%.

Then the semen samples were analyzed for MDA concentration and SOD, Catalase activities. MDA levels were analyzed according to methods described by Rao et al [15] MDA was assessed using the thiobarbituric acid method. Briefly, 800 µl of seminal plasma was added into glass tube.

To each tube, 1.2 ml of thiobarbituric acid was added and then heated for ten minutes in a boiling water bath. After cooling, to each tube 2 ml of freshly prepared 1 N NaOH was added and absorbance was read on a spectrophotometer at 534 nm. The activity of the superoxide dismutase was estimated by method described by Marklund & Marklund [16] bases on autoxidation of pyrogallol. Catalase activity has been determined in human seminal plasma of normal and infertile groups by potassium dichromate method described by Sinha [17] based on reduction of dichromate acid to perchromic acid in presence of hydrogen peroxide. The concentration of perchromic acid is inversely proportional to activity of catalase. To calculate the activity of catalase the values of seminal proteins required are calculated by lowry' method.

CONCLUSION

There has been a phenomenal growth in our knowledge of male reproduction, sperm function and development of diagnostic tools and treatment modalities. Evaluation of oxidative stress status and use of antioxidants is not routine in clinical practice.

MDA measurements are physiological and relevant because major loss of sperm function may occur with minimal damage to the membranes that envelop the sperm and/or divide key intracellular sperm compartments. It has been studied that the reduction of MDA by using antioxidant therapy was correlated with the improvement of fertilization rates[4]. Increased MDA concentrations has shown a significant increase in the number of immotile spermatozoa whereas the reduction in MDA level by using antioxidants therapy correlates with improvement in fertilization rate[22], [23].

Our study shows, high ROS levels in study group due to lipid peroxidation represented by MDA in semen, should be used as an additional marker in diagnosis of male infertility. It is also important to determine the antioxidant status of semen as it is a major defense mechanism.

These tests have significant bearing on the fertility of semen sample. Our study found that these tests are highly reliable, reproducible & simple to perform.

Thus to conclude - these tests should be used as an adjunct to routine analysis of semen which are carried out in the laboratories in order to find out root cause of male infertility.

They are an established index for the analysis of sperm quality.

References

1. Hull, M., Glazener, C., Kelly, N., Conway, D., Foster, P., Hunton, R., Coulson, C.,Lambert, P., Watt, E. and Desai. K. (1985) Population study of causes, treatment andoutcome of infertility.Br Med J 291, 1693- 1697
2. Sikka, S. C., Rajasekaran, M. and Hellstrom, H. J. (1995) Role of oxidative stress and antioxidants in male infertility. J. Androl. 16, 464-468.
3. Kovalski, N. N., de Lamirande, E. and Gagnon, C. Reactive oxygen species generated by Human neutrophils inhibit sperm motility:protective effects of seminal plasma and scavengers.Fertil. Steril.1992; 58, 809–816.
4. Saleh, R. A., Agarwal, A., Kandirali, E., Sharma, R. K.,Thomas and Nada. Leukocytospermia is associated with increased reactive oxygen species production by Human spermatozoa. Fertil. Steril. 2002;78, 1215–1224.
5. Jannsen, Y. M., Van-Houton, B., Borm, P. J. and Mossuran, B. T. (1993) Cell and tissue responses to oxidative damage.Lab. Invest.1993; 69, 261– 265.
6. Aitken, R. J. and Fisher, H. Reactive oxygen species generation and human spermatozoa:The balance of benefit and risk. Bioassays.1994; 16, 259–267.
7. de Lamirande, E. and Gagnon, C. Impact of reactive oxygen species on spermatozoa: a balancing act between Beneficial and detrimental effects.Hum. Reprod.1995; 10, 15– 21.
8. Aitken, R. J.Molecular mechanisms regulating human sperm functions. Mol. Hum. Reprod.1997; 3, 169-173.
9. Twigg, J., Irvine, D. S. and Aitken, R. J.Oxidative damage to DNA in human spermatozoa does not preclude Pronucleous formation at intracytoplamic sperm injection. Hum. Reprod1995;13, 1864-1871.
10. Zini A, Garrels K, Phang D: Antioxidant activity in the semen of fertile and infertile men.Urology 2000, 55:922-926.
11. Agarwal A, Nallella KP, Allamaneni SS, Said TM: Role of antioxidants in treatment of male infertility: an overview of the literature. Reprod Biomed Online 2004, 8:616-627.
12. Sanocka D, Kurpisz M: Reactive oxygen species and sperm cells.Reprod Biol Endocrinol 2004, 2:12. 6.
13. Sikka, S. C., Rajasekaran, M. and Hellstrom, W. J. G.Role of oxidative stress and antioxidants in male infertility.J. Androl. 1995;16, 464–468.
14. World Health Organization. World Health Organization Laboratory Manual for the Examination of Human Semen and Semen- Cervical Mucus Interaction, 3rd ed. Cambridge: Cambridge University Press, 1992.
15. Rao B, Souflir JC, Martin M, David G. Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility. Gamete Res 1989;24:127-34.
16. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem.1974; 47: 469-74
17. Sinha Ak. Colorimetric assay of catalase. Anal.Biochem.1972, 47:389
18. Cummins JM, Jequier AM, Kan R. Molecular biology of human male infertility: links with aging, mitochondrial genetics and oxidative stress. Mol Reprod Dev 1994;37:345-62.
19. Kobayashi T, Miyazaki T, Natori M, Nozawa S. Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod 1991;6:987-91

20. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. J. Androl 1996;17:530-7
21. Gil-Guzman E, Ollero M, Lopez MC, Sharma RK, Alvarez JG, Thomasv AJ Jr, Agarwal A:Differential production of reactive oxygen species by subsets of human Spermatozoa at different stages of maturation. Hum. Reprod. 2001, 16:1922-1930.
22. Siciliano L, Tarantino P, Longobardi F, Rago V, De Stefano C, Carpino A: Impaired seminal antioxidant capacity in human semen with hyperviscosity or oligoasthenozoospermia. J. Androl 2001,22:798-803.
23. Ali Khosrowbeygi 1 and Nosratollah Zarghami. Levels of oxidative stress biomarkers in seminal plasma and their relationship with seminal parameters.BMC Clinical Pathology 2007, 7:6
24. Heidar Tavilani, Mohamad T. Goodarzi, Asad Vaisi-raygani, Saeedeh Salimi, Taghi Hassanzadeh Activity of antioxidant enzymes in seminal plasma and their elationship with lipid peroxidation of spermatozoa.International Braz J urol;34:485-491.
25. Ramadan A. Saleh and Ashok Agrawal. Oxidative Stress and Male Infertility: From Research Bench to Clinical Practice.Journal of Andrology, 2002;23:737- 752
26. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients.Fertil Steril. 1992;57:409-16.
27. Mazzilli F, Rossi T, Marchesini M, Ronconi C, Dondero F. Superoxide anion in human Semen related to seminal parameters and clinical aspects. Fertil Steril 1994;62:862-8.
28. Plante M, de Lamirande E, Gagnon C.Reactive oxygen species released by activated neutrophils, but not by Deficient spermatozoa, are sufficient to affect normal sperm motility.Fertil Steril 1994;62:387-93.
29. Potts RJ, Notarianni LJ, Jefferies TM. Seminal plasma reduces exogenous oxidative damage to human sperm,determined By the measurement of DNA strand breaks and lipid peroxidation.Mutat Res 2000;447:249-56.
30. Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod.1989;41:183-97

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