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# Relationship between Seminal Malondialdehyde Levels and Sperm Quality in Fertile and Infertile Men

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## ABSTRACT

*The aim of this study was to determine the level of malondialdehyde in seminal plasma of fertile and infertile men and investigate its relationship with sperm quality. Results showed that the mean of ± S.D. MDA concentration in seminal plasma of infertile men (0.94 ± 0.28 nmol/ml) was significantly higher than fertile men (0.65 ± 0.17 nmol/ml) (p value< 0.001), and had negative relationship with sperm count, motility and morphology. Therefore it could be concluded that increase in lipid peroxidation was associated with sperm membrane destructed and high level of MDA.*

**Key words:** Seminal plasma, male infertility, sperm quality, malondialdehyde (MDA), lipid peroxidation

## INTRODUCTION

Reactive oxygen species (ROS), especially superoxide anion ($O_2^{\cdot}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH^{\cdot}$) are highly reactive oxidizing agents that belong to the class of free radicals (Agarwal and Prabakaran, 2005a; Aitken and Fisher, 1994). They have one or more unpaired electron and react with macromolecules for compensation of their deficit electron (Warren et al., 1987). Nevertheless, they have physiological roles, such as acrosome induction and sperm capacitation in low concentration, but they have pathological effects on macromolecules such as polyunsaturated fatty acid, amino acid and sugars in high levels (Agarwal et al., 2003; Agarwal and Prabakaran, 2005b; Sharma and Agarwal, 1996). Therefore, they are like double edged sword. Lipid peroxidation (LPO) is one of the pathological effects from ROS that is associated with oxidation of membrane poly unsaturated fatty acid (PUFA) (Fraczek et al. 2001; Alvarez et al., 1987; Alvarez and Storey, 1995). It can be defined broadly as oxidative deterioration of PUFA (Duru et al. 2000; Agarwal and Saleh, 2002). It attacks the fluidity of sperm plasma membrane, with subsequent loss of the ability for oocyte fusion and fertilization (Mammoto et al., 1996). Human sperm cells in contrast with other cells are particularly susceptible to oxidation of their plasma membranes due to the existence of a high concentration of polyunsaturated fatty acids in the membrane (Aitken et al., 1989a; Jones et al., 1979). PUFA play an important role in ion transport and sperm membrane fluidity, therefore oxidation of sperm membrane PUFA by oxidants (ROS) cause to deficiency in membrane function and sperm death. Malondialdehyde (MDA) is a

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stable peroxidation product of polyunsaturated fatty acids, usually cross linked to proteins. It is a diagnostic tool for lipid peroxidation and the analysis of etiology of male infertility (Aitken et al., 1987b; Laudat et al., 2002). The aim of the present study was to determine the lipid peroxidation levels in fertile and infertile men by MDA measurement and its relation with sperm quality.

## MATERIALS AND METHODS

### Semen populations and collection

Study population included 17 fertile and 23 infertile men. There was no significant difference between age of fertile and infertile men ($p$-value > 0.05). The mean standard ages of fertile and infertile men were 31.29 ± 4.25 and 28.61 ± 4.29 years respectively. All the samples were provided by Fateme Zahra IVF Center, and were evaluated for infertility. Before semen analysis, a questionnaire was distributed to obtain information on age and lifestyle of male including: smoking habits, alcohol use, use or abuse of other substances and drugs and history of orchitis, testicular trauma, sexually transmitted disease, varicocele, inguinal hernia operation and cryptorchidism and none had any of these. Semen samples were collected by intercourse between couples into a sterile container after sexual absence for 2-3 days. A single sample provided by each subject was examined according to the World Health Organization criteria (WHO, 1999) and analyzed for the appearance, volume and consistency. On microscopic examination, sperm concentration, percentage of normal morphology and motile sperm were objectively evaluated. Sperm count and motility were measured according to WHO criteria, whereas percentage of sperm morphology was performed according to Kruger's strict criteria (Kruger et al., 1986).

### Measurement of malondialdehyde

Seminal MDA levels were analyzed according to Rao et al. (1989). MDA was assessed using the thiobarbituric acid method. Briefly, semen samples were centrifuged for 7 min at 2000 g, and then 100 µl of seminal plasma (supernatants) was added in 900 µl of distilled water into glass tube. To each tube, 500 µl of thiobarbituric acid reagent (0.67 g of 2-thiobarbituric acid dissolved in 100 ml of distilled water with 0.5 g NaOH and 100 ml glacial acetic acid added) was added and then heated for 1 h in a boiling water bath (all samples run as duplicates). After cooling temperature, each tube was centrifuged for 10 min at 4,000g and the supernatant absorbance of these was read on a spectrophotometer at 534 nm.

### Statistical analysis

Mean standard (mean ± S.D.) of sperm parameters quality in the fertile and infertile men were analyzed by descriptive statistic. The relationship of the MDA levels with sperm count, motility, morphology and semen volume were also compared. Samples T-test and linear regression model was applied to the compare seminal MDA and sperm quality in all the samples.

## RESULTS

The mean values of examined sperm parameters in the fertile and infertile men are shown in Table 1. Sperm quality in fertile men was higher than infertile men. The concentrations of seminal MDA in both the groups were significantly different. The mean of MDA concentration in infertile men was significantly higher than the fertile men (Fig. 1, $p$-value< 0.001). The levels of MDA in fertile and infertile men were 0.65 ± 0.17 and 0.94 ± 0.28 nmol/ml respectively. The ratio of seminal MDA from infertile to fertile men was 1.44. Results showed that there was a negative relationship between MDA levels with sperm concentration, motility and normal morphology. This correlation was significant between the fertile and infertile men (Fig. 2), but not significant in only fertile or infertile men (Fig. 3). On the other hand, although there was a negative significant correlation between MDA levels with sperm count counts (Fig. 2A, $p$-value=0.007), motility (Fig. 2B, $p$-value< 0.001) and normal morphology (Fig. 2C, $p$-value< 0.001) between fertile and infertile men but this correlation was not significant in any fertile or infertile men (Fig. 3). On the other hand, that there is a positive correlation between semen volume and MDA levels. This correlation was significant both between in fertile and infertile men (Fig. 2D, $p$-value= 0.003) and in only fertile (Fig. $3D_1$, $p$-value <0.05) or infertile men (Fig. $3D_2$, $p$-value <0.05). Therefore, (I) high level of MDA in seminal plasma of infertile men was a sign of increasing oxidative stress associated with decrease in sperm quality and the risk of idiopathic

male infertility, (II) there was a significant difference between MDA levels and sperm parameters quality between fertile and infertile men, but this correlation was not significant from fertile or infertile men and (III) high semen volume was associated with high levels of MDA.

**Table 1**- Sperm parameters quality in fertile and infertile men.

| Sperm parameters | Fertile men | Infertile men | *p*-value |
|---|---|---|---|
| Sample number (n) | 17 | 23 | |
| Age (Years) | 31.29 ± 4.25 | 28.61 ± 4.29 | >0.05 |
| Semen volume (ml) | 4.4 ± 1.28 | 4.27 ± 1.37 | =0.762 |
| Sperm count ($\times 10^6$/ml) | 102 ± 23.36 | 34.78 ± 26.81 | <0.001 |
| Total sperm ($\times 10^6$) | 450.26 ± 162.70 | 184.98 ± 106.38 | <0.001 |
| Motile sperm (%) | 68.17 ± 8.99 | 39 ± 14.75 | <0.001 |
| Normal morphology (%)* | 16.18 ± 4.31 | 4.21 ± 3.23 | <0.001 |
| MDA concentration (nmol/ml) | 0.65 ± 0.17 | 0.94 ± 0.28 | <0.001 |

Results are presented as mean ± S.D.
*Normal morphology assayed according to Kruger's strict criteria.

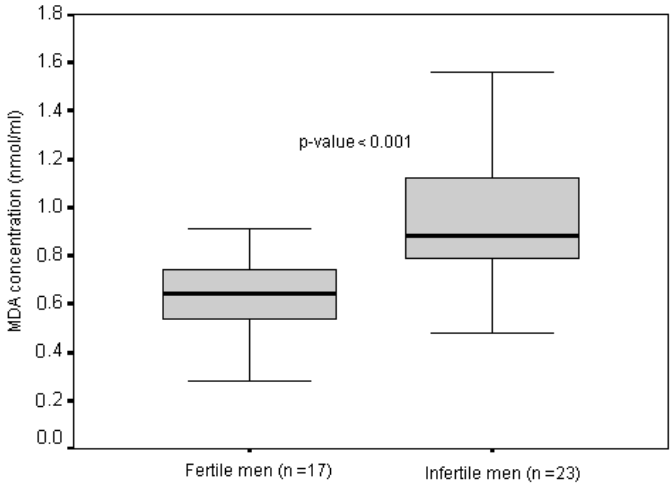
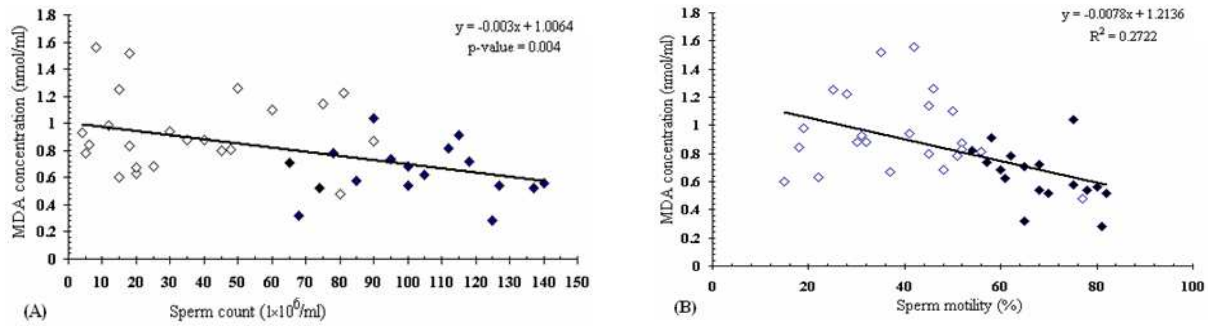



**Figure 1** - Comparison of MDA levels in seminal plasma of fertile and infertile men.



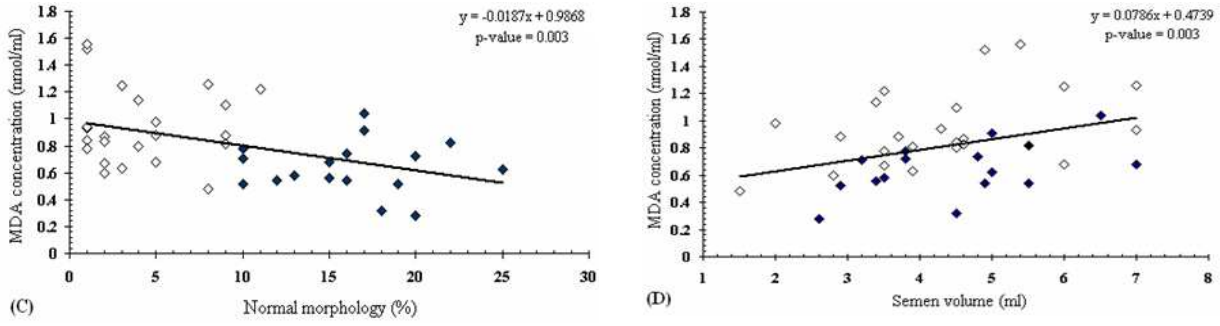



**Figure 2** - Correlation of MDA concentration with sperm count (A), sperm motility (B), normal sperm morphology (C) and semen volume (D) between fertile (■) and infertile (□) men.

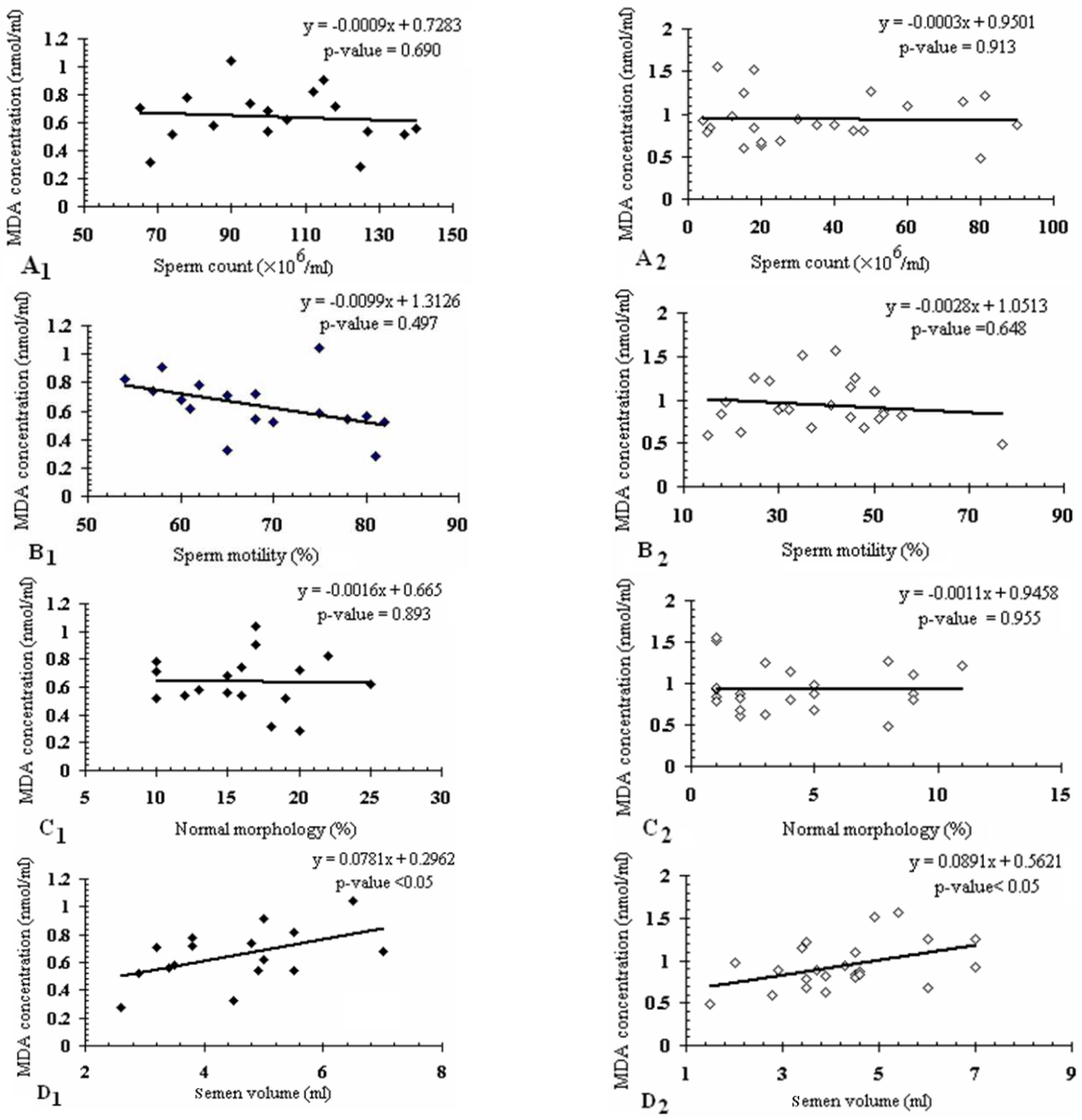



**Figure 3** - Comparison of MDA concentration with sperm count (A), sperm motility (B), normal sperm morphology (C) and semen volume (D) in the fertile (■) and infertile (□) men.

## DISCUSSION

In fact, all cellular compounds including lipids, proteins, nucleic acid and sugars are potential targets for ROS (Zalata et al., 2004). ROS induces the oxidative stress (OS) which decreases the membrane fluidity and impairs its function (Saleh and Agarwal, 2002). Indeed, this decrease in fluidity could affect the membrane transport activity and thereby affect on the surviving of sperm. A number of studies have shown that lipid peroxidation affects the sperm concentration, motility, morphology and related with poor sperm quality (Huang et al. 2000; Hsieh et al, 2006; Gomez et al., 1998). Kobayashi et al. (1991) demonstrated that MDA level in spermatozoa was significantly related to the number of immotile sperm. Suleiman et al. (1996) demonstrated that the MDA concentration in the seminal plasma was not correlated with the sperm concentration and motility. In this work the negative significant correlation was observed between lipid peroxidation with sperm concentration, motility and normal morphology between fertile and infertile men which was compatible with the findings of Kobayashi et al (1991), Huang et al. (2000), Hsieh et al. (2006), Zalata et al. (2004), Suleiman et al. (1996) and Gomez et al. (1998). But this correlation was not significant in the fertile or infertile men. MDA level was not significant variable in fertile or infertile men. It appeared that oxidative stress induced with ROS was one reason for low sperm quality in infertile men. Some studies have suggested that ROS attack the integrity of DNA in sperm nucleus by causing base modification, DNA strand breaks and chromatin cross-linking (Duru et al., 2000; Aitken and Baker, 2006). On the other hand, DNA damage included by excessive levels of ROS could accelerate the process of germ cell apoptosis, leading to decline in sperm counts associated with male infertility (Agarwal and Allamaneni; 2004; Aitken and Krausz, 2001). Motility is indispensable for the spermatozoa, as it has to travel the female reproductive tract to reach the site of fertilization. Studies have found that the levels of ROS correlate with the motility of spermatozoa (Aitken et al., 1989a; Iwasaki and Gagnon, 1992; Agarwal et al., 1994; Armstrong et al., 1999). Peroxidative damage to the sperm membrane and axonemal proteins appears to be the cause of permanent impairment in sperm motility (Agarwal and Allamaneni, 2004). Excessive ROS causes ATP to deplete rapidly resulting in decreased phosphorylation of axonemal proteins and cause transient impairment of motility (De Lamirande and Cagon, 1992). Lipid peroxidation has also a deleterious effect on the ultramorphological status of the sperm cells and thereby on the male fertilization potential (Zabludovsky et al., 1999; Kessopoulou et al., 1992). This study showed that there was a positive correlation between semen volume and MDA levels. This finding was consistent with the results of some other studies (Aleksandra et al., 2004). It appeared that increase in semen volume was associated with high levels of abnormal sperm and leukocytes which were major sours for ROS production (Aleksandra et al., 2004). These finding suggested that oxidative stress was involved in low sperm quality and the etiology of male infertility. The measure of MDA could be useful diagnostic tool for estimation of oxidative stress.

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