Original Article

# THE EFFECT OF SEMINAL OXIDATIVE STRESS ON MALE FERTILITY

**Tamilselvan K.[*1], SheelaRavinder S.[2], Thangavel G.[3], Subhashini A.S.[2], Padmavathi R.[2]**

[1]Department of Physiology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Puducherry-605 102, India.

[2]Department of Physiology, Sri Ramachandra University, Chennai 600 116, India.

[3]Department of Environmental Health Engineering, Sri Ramachandra University, Chennai 600 116, India

## ABSTRACT

Background: Male infertility occurs as a result of many pathological conditions. The role of oxidative stress is implicated in its cause. This study has been planned to evaluate the effect of oxidative stress on male fertility.

Aim and objective: To estimate the level of seminal plasma malondialdehyde(MDA) in normospermic and oligozoospermic men and to assess its effectson seminal parameters that determines the fertility.

Methodology: It is a hospital based cross-sectional study with the study population comprising of 10 normospermic men for the contol group and 10 oligospermic volunteers for the case group who were recruited based on their seminal parameters of sperm count, motility, morphology and viability .The marker of oxidative stress, malondialdehyde(MDA) was estimated in their seminal samples according to the method of Okhawa et al. to reflect the degree of oxidative stress.

Results: The level of MDA was 10.94 ± 4.03$\mu$g/ml in oligospermic when compared to normospermic men who had 6.37 ± 2.90 $\mu$g/ml and the increase in oligospermic is statistically significant ($p<0.05$) which correlates inversely with the sperm count, motility and viability.

Conclusion: The elevated levels of MDA in the seminal samples of oligospermic men reflects the occurrence of oxidative stress and this is obvious by the reduction in the sperm parameters of count, motility and viability in the oligospermic group,thus leading to infertility.

Keywords: Malondialdehyde, Oxidative stress, Sperm count

## INTRODUCTION

Amongst many an affliction that mankind suffers from, infertility affects a person as a whole in both personal and social spectrums. According to WHO (World Health Organization) statistics nearly 60 to 80 million couples suffer from this condition globally[1]. Of the infertile couples majority suffer from primary infertility worldwide[2].The burden of infertility is borne by 15-20% of the general population and male factor is involved in 20-40% of the condition, according to global survey[3].In India, male factor contributes to 23% and 45% of the infertile men are either oligozoospermic or azoospermic[4].

Male infertility results as a resultof defective sperm parameters that determine the fertilizing capacity of the gamates[5].The clinical criteria of classifying a person into infertile group is based on WHO cut-off values for the seminal parameters[6].

The etiology of male infertility is many and the cause could be either pretesticular, testicular, post-testicular, hormonal factors, environmental influence, personal habits, infections or a composite of the above conditions[7]. Though the causatives are many, ultimately they all impair the quality of semen thus leading to oligozoospermia or azoospermia in severe conditions. Of the cases of infertility, more than 50% remain a mystery with no established cause thus acquiring the term idiopathic

infertility[7]. Attempts to analyse this issue with the target of administering treatment, made the observation that oxidative stress markers were elevated in the seminal plasma of men with idiopathic infertility thus implying the occurrence of oxidative stress (OS) in them[8].

Oxidative stress and diseases

Oxidative stress is implicated in the development of chronic and degenerative conditions such as cancer, arthritis, aging, autoimmune disorders, cardiovascular, neurodegenerative diseases and not sparing, some of the reproductive pathologies[9]. In case of female infertility the role of oxidative stress is implicated in tubal factor infertility, endometriosis, peritoneal factor infertility and in idiopathic infertility as well[10]. Similarly, in men oxidative stress is implicated in many pathophysiological conditions that lead to infertility including the idiopathic infertility.

Oxidative stress and male infertility

In human spermatozoa, like in any other cell of the body, the reactive oxygen species (ROS) produced within physiologic limits assist in sperm function like capacitation, acrosome reaction, and oocyte fusion. However when their production goes uncontrolled overwhelming the limited antioxidant defenses in semen, they damage the sperms leading to oxidative stress[11]. Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants[12].

Seminal plasma as well as the sperms are endowed with an array of antioxidants that scavenge, dispose, and suppress the formation of ROS, or oppose their actions[13]. The antioxidants includes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and non-enzymatic antioxidants like glutathione, vitamin C, vitamin E, selenium, manganese, zinc,flavonoids, omega-3 and omega-6 fatty acids etc[9]. When the scavenging machinery is rendered inefficient or when the production of reactive oxygen species goes unchecked oxidative stress results.

Sources of reactive oxygen species in semen

Morphologically abnormal spermatozoa that retain their cytoplasm in the midpiece due to arrest of spermiogenesis and the polymorphonuclear leukocytes and macrophages that contaminates the seminal plasma are the main sources of high ROS productionin human[13,14]. Apart from endogenous causes local and systemic infective and inflammatory conditions which are grouped as exogenous cause also can lead to infertility[15].

Oxidative stress and sperm damage

1. Lipid peroxidation

The membrane of the human sperm, unlike any other somatic membranes is rich with poly unsaturated fatty acids (PUFAs) that contain unconjugated double bonds separated by methylene groups with weak methyl carbon-hydrogen interaction that makes them vulnerable for oxidative damage[16]. These PUFAs are the targets for ROS produced within the cell, leading to lipid peroxidation. When peroxidative changes occur in membrane lipids, it impairs the membrane fluidity and the spermatogenesis. This autocatalytic self-propagating reaction increases the membrane permeability for ions and inactivates the membrane bound receptors and enzymes[11]. Peroxidation of sperm membrane lipids may disturb regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually in membrane fusion, thus leading to infertility[17].

**2. DNA damage**

The mechanism of DNA damage by free radicals involves modification of bases, production of base free sites, deletions, frame shifts, DNA cross links, chromosomal rearrangements, single and double strand DNA breaks, gene mutations and polymorphism, denaturation and DNA base-pair oxidation. Self -repair mechanism comes into play when the insult to the genetic material is minimal. Oocyte is also capable of repairing the damaged DNA of the sperms. But when the damage is extensive it leads to apoptosis of the sperms and when a gamete with damaged Y chromosome takes part in

fertilization it can result in the infertility of the offspring by gene deletion in Y chromosome[18].

### 3. Apoptosis

Apoptosis, the programmed cell death plays an important role in eliminating abnormal sperms. But in oxidative stress the free radicals disrupt the mitochondrial membranes leading to the release of cytochrome-c which in turn activates caspases leading to apoptosis[17]. Apoptosis may also result when cell surface protein Fas is expressed in high proportion[19].

By all the above mechanisms, oxidative stress impairs the sperm functions and its viability thus leading to infertility. Therefore this study has been designed as a pilot effort to look for the relation between the oxidative stress marker, malondialdehyde, the end-product of lipid peroxidation, and the sperm parameters between normospermicand oligozoospermic men.

## AIM & OBJECTIVES

Aim

To estimate the level of seminal plasma malondialdehyde (MDA) in normospermic and oligozoospermic men and to assess its effects on seminal parameters that determine the fertility.

Objectives

a. To compare the sperm count between the normospermic and oligospermic group.

b. To compare the malondialdehydelevel between the normospermic and oligospermic group.

c. To compare the seminal parameters viz morphology, motility and viability between the two groups to establish the effect of oxidative stress on the seminal parameters.

## MATERIALS AND METHODS

## METHODOLOGY

The study was carried out in Sri Ramachandra University after obtaining Institutional Ethical Clearance. It is a hospital based cross sectional study and men who visited andrology department for evaluation of infertility (10 oligospermic men and10 normospermic men) were selected .The volunteers were selected based on their preliminary seminal analysis report particularly the sperm count and a screening questionnaire was administered to collect information about the exclusion criteria. Men between the age group of 20 and 35 were selected with sperm count of $< 20$ million/ml for oligospermic and $\geqslant 20$ million/ml for normospermic groups. Men on infertility treatment, on antioxidant therapy and those with active infections were excluded.

After the recruitment,informed written consent was obtained from the volunteers. The participants were requested to give a fresh seminal sample for the study, after a period of 48 hours of abstinence. Samples were collected in sterile containers under aseptic precautions.ID coding was done for the groups accordingly. The samples collected were transferred to the lab immediately and centrifuged for 10 min at a rate of 3500 rpm at 4°C. 2 ml of the supernatant of the centrifuged samples was transferred to an eppendorf and stored in deep freezer until analysis.

LABORATORY METHOD FOR THE ASSESSMENT OF LIPID PEROXIDATION:

Estimation of Malondialdehyde (also known as TBARS):

The method involved heating of 0.2ml of seminal fluid with 0.8 ml saline (0.9%), 0.5ml of Butylated Hydroxyl Toluene (0.05%) and 3.5 ThioBarbituricacid (0.8%) reagent for 90 min in a boiling water bath. After cooling, the solution was centrifuged at 2000 rpm for 10 min, the absorbance of the supernatant was determined at 532 nm using spectrophotometer against a blank that contained all the reagents minus the biological sample. The values were expressed in mcg/ml seminal fluid. (Ohkawa H. et al 1979)[20].

## STATISTICAL ANALYSIS

The data collected was analysed using SPSS 11.4 software. The two groups were compared using Student's t-test. The level of significance was taken at 5%.

## RESULTS

This cross sectional study was conducted among 10 oligospermic men with a mean age of 30.1 ± 2.56 years and 10 normospermic men with a mean age of 30.2 ± 2.74 years.

Comparison of sperm count levels between Normospermic and Oligospermic groups

Fig 1 shows the variation in the levels of sperm count between the normospermic and oligospermic groups. The sperm count of the normospermic group is 49.40 ± 20.88 million/ml while that of the oligospermic group is 7.80 ± 7.31 million/ml. The count is reduced in the oligospermic men with ap value <0.001 which is statistically significant.

Comparison of TBARS levels between Normospermic and Oligospermic groups

The bar plot (Fig 2) shows the elevated levels of malondialdehyde in the oligospermic samples.

The mean levels of MDA (TBARS) in normospermic men is 6.37 ± 2.90 μg/ml while in oligospermic it is 10.94 ± 4.03 μg/ml . The levels are found to be elevated in the oligospermic group with a p value 0.009 which is statistically significant.

Comparison of seminal parameters between normal &oligospermic subjects

From the Table 1 it could be observed that the percentage of motile sperms and sperms with normal morphology varied significantly between the groups with P value < 0.001 and vitality is reduced significantly in oligospermic group with p value 0.015.

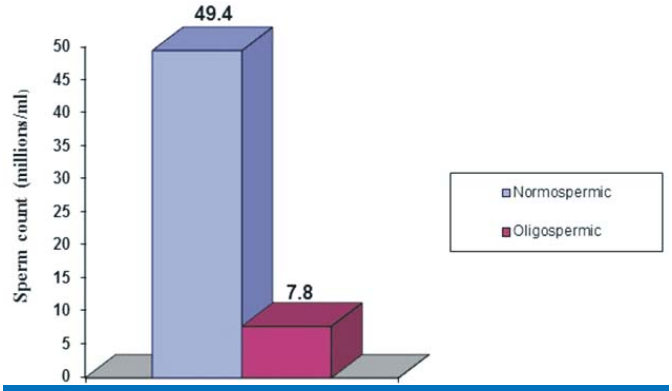


Fig1: Comparison of sperm count levels between Normospermic and Oligospermic groups

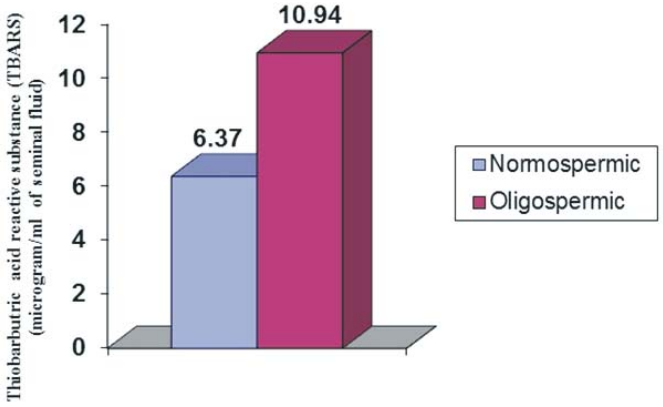


Fig 2: Comparison of TBARS levels between Normospermic and Oligospermic groups

| Parameters | Type | Mean | Std. Deviati | p value |
|---|---|---|---|---|
| Spermcount (millions/ml) | Normal | 49.40 | 20.88 | < 0.001** |
| | Oligospermia | 7.80 | 7.31 | |
| Motility (%) | Normal | 55.00 | 10.80 | <0.001** |
| | Oligospermia | 16.50 | 14.92 | |
| Morphology (%) | Normal | 5.60 | 2.55 | <0.001** |
| | Oligospermia | 1.60 | 1.07 | |
| Vitality (%) | Normal | 78.40 | 10.54 | 0.015* |
| | Oligospermia | 47.60 | 34.63 | |

Table 1: Comparison of seminal parameters between normal &oligospermic subjects

**very significant

*significant

## DISCUSSION

Mammalian spermatozoa are very susceptible to oxidative stress which impairs sperm motility, viability and fertilizing potentials[21].The occurrence of oxidative stress in seminal plasma can be reflected by elevated levels of malondialdehyde (MDA), an end product of lipid peroxidation in the semen.

MDA estimation in our study showed significant elevated levels in the oligospermic samples which correlates positively with sperm immotility[22,23]. The decreased sperm count reflecting compromised sperm survival is due to the lipid peroxidation of sperm membrane, represented by the high MDA levels. Similar observations were reported in

oligoasthenozoospermic subjects by *Hsieh et al. in 2006*[24],Kobayashi et al. in 1991[22]and Fraczeket al.in 2001[23].

It is thus inferred from our study that the concentration of malondialdehyde is significantly elevated in the seminal plasma of oligospermic men when compared to normospermic men. This significant elevation in their concentration reflects theoccurrence of oxidative stress in the seminal plasma leading to decreased sperm count, impaired motility &viability by the pathologic mechanism of peroxidative damage to the sperm cells which is marked by the elevated levels of MDA in the samples of the same population. Studies have made inferences that show an inverse correlation between seminal plasma antioxidant concentration and oxidative stress in idiopathic as well as in many other pathologic conditions that impair fertility. This close relation between antioxidant capacity and fertility status has become the rationale in antioxidant therapy in infertility. Therefore measures to maintain the local antioxidant machinery can help in the improvement of the condition.

## CONCLUSION

This pilot study assessing the relation between oxidative stress and male infertility, makes an observation of elevated level of malondialdehyde (MDA), the marker of oxidative stress, in the oligozoospermic population thus referring to the oxidative stress that had occurred in them. The supposition of oxidative stress occurrence is supported by the significant reduction in the semen quality reflected by the decrease in the sperm count, motility and viability in the same category of people.

It could be thus inferred that the oxidative stress that occurred in the seminal plasma of the oligozoospermic men could be the reason for the decline in the sperm count, viability of the surviving sperms and motility of those scanty survivors.

## LIMITATIONS

- Sample size of this study is less, nevertheless this pilot effort serves to evaluate the methods of assessing oxidative stress status which can help in the future large scale studies.
- As oxidative stress results as an imbalance between free radical production and its scavenging,the assessment of antioxidants in the seminal plasma could have reflected the conditionbetter.

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**Conflict of Interest: NIL**

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