EVALUATION OF OXIDATIVE STRESS IN MALE INFERTILITY

Noor Abdulaala Kadhim Alhafadhi1, Ehab Rasmi Alkhafaji2
1Department of Medical Laboratory Techniques, College of Health and Medical Technology, National University of Science and Technology, Thi Qar, Iraq. E-mail: nooraltaie46@gmail.com
2Dhi Qar Health Department, Iraq.

ABSTRACT:
Immunological infertility cause may contribute to up to 8-19% of the male infertility factors, involved in cryptorchidism, testicular trauma, epididymitis, primary testicular failure, idiopathic infertility, infections. As a result, ASA can be present in infertile men who are either primary or secondary infertile. The aim is to look into the relationship between ASA and trace elements in both primary and secondary infertile men. The research was conducted at the ALBasrah Women and Children's Hospital's Infertility Center. This study was included 96 men, divided into two groups infertile and fertile men. Infertile men (66) were subdivided into a primary (33) and secondary infertile men (33). Other (30) fertile men. After (3-5) days of abstinence, seminal fluids were extracted from both of them, and semen fluid analysis was performed on each specimen. Following liquefaction, the concentration, morphology, and motility grades of the sperm were determined (WHO). The mean values of were significantly increased (p<0.05) (CAT, GPx, and TAC) levels in both primary and secondary infertile men as compared to fertile men, although the gap was non-significant (P>0.05). (GPx, CAT, and TAC) levels in primary infertile men as compared to secondary infertile men (p<0.05), SOD significant increase (P>0.05) in primary infertile men as compared to fertile men, whereas non-significant increase (P>0.05) in both secondary infertile men as compared to fertile men and primary infertile men as compared to secondary infertile men. When comparing fertile men to primary infertile men and secondary infertile men to fertile men, MDA levels were highly significant increase (p<0.001) in both primary infertile men and secondary infertile men as compared with fertile men, while non-significant increase (P>0.05) in primary infertile men as compared to secondary infertile men. The correlation analysis between ASA and enzymatic antioxidant both immunologically infertile parameters t men were; GPx was positive and significantly correlated; CAT was negative and highly significantly correlated; SOD was non-significant and positive; TAOC was negative and highly significantly correlated; and MDA was positive and highly significantly correlated.

Keywords: Male Infertility, Anti Sperm Antibody, Immunological Infertility, GPx, CAT, SOD and TAC.

INTRODUCTION

Infertility remains both prevalent and problematic that affects 13-15% of the couples worldwide medically, psychosocially and socioeconomic status. It isAfter 12 months of unprotected intercourse or therapeutic donor insemination, the inability to achieve a healthy pregnancy is clinically characterized as failure (22). Data from the last 20 years shows that the cause of infertility is found in 30 percent to 50 percent of cases, 50 percent to 70 percent of cases, and 20 percent of cases, the causes are found in both men and women. Human fertility is complicated; it is dependent on a combination of male and female reproductive competence, as well as physiological, genetic, behavioral, and environmental influences, which has restricted our understanding of conception and fertilization mechanisms (29). When the findings of a standard infertility test are common, a diagnosis of unexplained infertility, that the couple is experiencing unexplained infertility) is provided (34). Unidentified male infertility (37-58%) is classified into two categories: unexplained male infertility and idiopathic male infertility; it is a disorder in which fertility deficiency arises naturally or due to an obscure or unknown cause (22). If more than 50% of sperm are linked to IgG or IgA antibodies, immunologic infertility is likely. If more than 10% of spermatozoa are antibody-bound, this can be expected. These immunoglobulins can be present in serum, sperm, and cervical mucus in both men and women. Infertile couples have a 9-36 percent sperm autoimmunity rate, compared to 0.9-4 percent in the fertile population. Infertile couples have a 9-36
percent sperm autoimmunity rate, compared to 0.9-4 percent in the fertile population. (5) Detection of sperm antibodies is seen in 8-21 percent of fertile males. 5-15 percent of male infertility factors could be due to immunological causes (28). The study aimed to determine the correlation of ASA with Enzymatic antioxidant: GPx, CAT, SOD and TAOC.

II. MATERIALS AND METHODS

Determination of Seminal Glutathione Peroxidase (GPx) Activity

Sandwich ELISA as a method using Elabscience kit (E-EL-H5410, USA) GPx was weighed. The antibody specific to GPx has been pre-coated on the microtiter plate included in the package. To combine with the particular antibody, standards or samples were applied to each well of the micro plate. After that, each microplate well was incubated with a biotinylated detection antibody specific for Human GPx and an Avidin-Horseradish Peroxidase (HRP) conjugate. The non-essential part was washed away. Each well was filled with the substrate solution. The enzyme substrate reaction is terminated by adding stop solution to those wells that contain human GPx, biotinylated detection antibody, and avidin HRP conjugate. The color has changed to yellow as a result of the solution.

Reagents

Reference Standard, Concentrated Biotinylated Detection Ab.

Concentrated HRP Conjugate, Reference Standard & Sample Diluent.

Biotinylated Detection Ab Diluent, HRP Conjugate Diluent.

Wash Buffer Concentrate, Substrate Reagent, and Stop Solution.

Procedure

1. Fill each well with 100 mL of normal or sample. At 37°C, incubate for 90 minutes.
2. Mix in 100 mL of biotinylated detection antibody. At 37°C, incubate for 1 hour.
3. Aspirate three times and wash.
4. Pour in 100 liters of HRP Conjugate. At 37°C, incubate for 30 minutes.
5. Aspirate 5 times and wash.
6. Pour in 90 liters of Substrate Reagent. At 37°C, incubate for 15 minutes.
7. Pour in 50 liters of Stop Solution. Immediately determine the OD value at 450 nm.
8. Calculation of the outcomes.

Calculation of the Outcomes

The SOD is proportional to the concentration of Human GPx and is measured spectrophotometrically at wavelength 450 nm. As shown in figure, the concentration of Human GPx in samples was determined by comparing the sample's OD to the standard curve (1).
Seminal Catalase (CAT) activity measurement: The Sandwich ELISA technique is used in this ELISA package (MBS016042, USA). Add the Standard and Sample to the wells that have been pre-coated with objective antibody, then add streptavidin HRP to form an immune complex, incubation, followed by incubation and washing, removal of unbound enzyme, and then add the substrate A and B. The solution will turn blue and then yellow due to the acid effect.

Reagents Include
Wash solution, buffer solution, standard solution, HRP-Conjugate Reagent.
Stop solution, chromogen solution A, chromogen solution B.

Steps to take
1. Fill each well with 50 mL of normal or sample.
2. Pour 10 L of sample into the measuring sample well, followed by 40 L of sample diluent.
3. Fill each well with 100 mL of HRP-conjugated reagent. At 37 degrees Celsius, incubate for 60 minutes.
4. Fill each well with 50 L of chromogen solution A.
5. Pour 50 L of chromogen solution B into each well. At 37°C, incubate for 15 minutes.
6. Pour in 50 liters of Stop Solution. Color shifts from blue to yellow. Immediately determine the OD value at 450 nm.
7. Calculation of the outcomes.

Calculation of the Outcomes
At a wavelength of 450 nm, the color change is measured spectrophotometrically. Figure 2 shows how the concentration of CAT in the sample was determined by comparing the sample’s SOD to the standard curve(2).

Determination of Seminal Superoxide Dismutase (SOD) Activity
The sandwich ELISA kit (E-BC-K020-M, USA) is the form used in this ELISA kit. A SOD-specific antibody has been pre-coated on the microtiter plate included in the package. Using a biotin-conjugated antibody specific to SOD, Standards or samples are attached to microtiter plate wells during the reaction. Then, in each microplate well, Avidin conjugated to Horseradish Peroxidase (HRP) is added and incubated. Only those wells containing SOD, biotin-conjugated antibody, and enzyme-linked immunoglobulin were applied to the TMB substrate solution Avidin that has been enzyme-conjugated can change color. The color shift is calculated spectrophotometrically at a wavelength of 450nm after the enzyme-substrate reaction is terminated by adding sulphuric acid solution. After that, the concentration of SOD in the samples is calculated by comparing the samples’ O.D. to the standard curve.
Reagents

Steps to Take
1. Fill each well with 100 mL of normal or sample. Incubate at 37°C for 2 hours.
2. Aspirate and apply 100L of the Detection Reagent that has been prepared. A. Incubate at 37°C for 1 hour.
3. Aspirate three times and wash.
4. Add 100L of the prepared Detection Reagent B and incubate at 37C for 30 minutes.
5. Aspirate 5 times and wash.
6. Pour in 90 liters of Substrate Solution. At 37°C, incubate for 20 minutes.
7. Pour in 50 liters of stop solution. Immediately determine the OD value at 450nm.
8. Performance calculation.

Calculation of the Outcomes
At a wavelength of 450 nm, the color change is measured spectrophotometrically. As shown in figure, the concentration of CAT in the sample was determined by comparing the sample's SOD to the standard curve 2.

![Figure 2](image_url)
Figure 2. Shows the SOD Standard Curve in an ELISA Package

**Seminal Total Antioxidant Potential (TAOC) Activity Determination**

The basic idea is that This is an ELISA package (Enzyme-Linked Immunosorbent Assay) (MBS164846, USA). Human TAOC antibody has been pre-coated on the counter. The TAOC in the sample was applied, and it binds to the antibodies on the wells. The biotinylated human TAOC Antibody was then applied to the sample, which binds to TAOC. The next step was to add Streptavidin HRP, which binds to the biotinylated TAOC antibody. Unbound Streptavidin-HRP is washed away during a washing phase after incubation.. The color developed in proportion to the amount of human TAOC after the substrate solution was applied. The reaction was stopped by adding an acidic stop solution and measuring the absorbance at 450 nm.

Reagents Include

Procedure
Steps to Take

1. Fill standard wells with 50L of standard.
2. Pour 40 liters of sample into sample wells, followed by 10 liters of anti-TAC antibody.
3. To the sample and norm, add 50 l streptavidin-HRP. At 37°C, incubate for 60 minutes.
4. Fill each well with 50 liters of substrate solution A.
5. Fill each well with 50 liters of substrate solution B. At 37°C, incubate for 10 minutes.
6. Color evolves after adding 50L Stop Solution.
7. Within 10 minutes, determine the OD value at 450nm.
8. Calculation of the outcomes.

Calculation of results: Draw a best-fit curve through the points on the graph by plotting the average OD for each norm on the vertical (Y) axis against the concentration on the horizontal (X) axis. These calculations are best done with computer-based curve-fitting software, and the best-fit line is calculated using regression analysis, standard curve, as shown in figure (3).

![Standard Curve for TAOC in ELISA Kit](image.png)

**Figure 3. Standard Curve for TAOC in ELISA Kit**

**Determination of Seminal Malondialdehyde (MDA) Activity: Principle**

This ELISA kit uses competitive ELISA kit (E-EL-0060, (United States of America) as a tool.

MDA has been pre-coated on the microtiter plate included in the package. During the reaction, MDA in the sample or normal competes for a site on biotinylated detection Ab unique to MDA with a fixed amount of MDA on the solid phase supporter. Excess conjugate and unbound sample or normal are washed from the plate, and each microplate well is incubated with Avidin conjugated to Horseradish Peroxidase (HRP). Then, in each well, a TMB (3, 3, 5, 5-Tetramethylbenzidine) substrate solution was added. Stop solution was used to stop the enzyme-substrate reaction.

**Reagents**

Concentrated Biotinylated Detection Ab, Concentrated HRP conjugate, Reference norm and sample diluent, Biotinylated Detection Ab diluent.

Diluent for HRP conjugate, concentrated wash buffer, substrate reagent, and stop solution.

**Steps to Take**

1. To each well, pour 50 mL of normal or sample.
2 Add 50L Biotinylated Detection Ab to each well right away. At 37°C, incubate for 45 minutes.

3 times aspirate and wash

4 To each well, pour 100L HRP Conjugate. At 37°C, incubate for 30 minutes

5 Aspirate 5 times and wash.

6 Pour in 90 liters of Substrate Reagent. Incubate for 15 minutes at 37°C.

7 Pour in 50 liters of stop solution. Immediately determine the OD value at 450 nm.

8 Calculation of the outcomes.

Calculation of the Outcomes
At a wavelength of 450 nm, the color change was measured spectrophotometrically. As shown in figure, the concentration of MDA in the sample was calculated by comparing the OD of the samples to the standard curve (4).

![Figure 4. Standard Curve for MDA in ELISA Kit](image)

Analytical Statistics
The data were analyzed using SPSS software (Version 23.0), and the values were expressed as the mean values ± SD. P- values <0.05, 0.01 were considered to be statistically significant (Standard deviation), Range: P-value: N.S (P > 0.05), S (P < 0.05), HS (P < 0.01) indicate the level of significance.

### RESULTS

#### III. RESULTS

**Table 1. Antioxidant Activities and Oxidative Stress Index (MDA)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Primary Infertile men (N=33) (Mean ± SD, Range)</th>
<th>Fertile men (N=30) (Mean ± SD, Range)</th>
<th>P Value</th>
<th>Secondary Infertile men (N=33) (Mean ± SD, Range)</th>
<th>Fertile men (N=30) (Mean ± SD, Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminal GPX (ng/ml)</td>
<td>213.06 ±58.88 110-317</td>
<td>275.36±50.62 205-387</td>
<td>0.0001</td>
<td>205.87 ±60.10 106-334</td>
<td>275.36±50.62 205-387</td>
</tr>
<tr>
<td>Seminal CAT (U/ml)</td>
<td>12.21±1.88 5.53-16.43</td>
<td>18.61±2.19 12.41-20.54</td>
<td>0.0001</td>
<td>12.24±1.18 8.65-14.40</td>
<td>18.61±2.19 12.41-20.54</td>
</tr>
<tr>
<td>Seminal SOD (U/ml)</td>
<td>8.43±4.20 2.00-24.00</td>
<td>6.72±2.67 2.50-12.00</td>
<td>0.047</td>
<td>6.79±2.98 2.00-12.00</td>
<td>6.72±2.67 2.50-12.00</td>
</tr>
<tr>
<td>Seminal TAOC (U/ml)</td>
<td>0.47±0.21 0.16-1.00</td>
<td>0.99±0.29 0.50-1.70</td>
<td>0.0001</td>
<td>0.44±0.19 0.12-0.90</td>
<td>0.99±0.29 0.50-1.70</td>
</tr>
<tr>
<td>Seminal MDA</td>
<td>2.46±0.68 1.49±0.31</td>
<td>0.0001</td>
<td>2.39±0.79 1.49±0.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Glutathione Peroxidase (GPx)

Our results in this study as in table (1), showed highly significant decrease inglutathione peroxidase levels of both primary and secondary infertile men (P<0.0001) compared to the fertile control group and a non-significant(increase (P>0.05) in GPx levels of the primary group compared to the secondary group. Our results agree with the results of (19;4;30) But these found differ from the results, who have shown no difference in the GPx levels between infertile and fertile men groups. On the other hand, they discovered that healthy men's seminal GPx activity was ten times higher than infertile men's (20). Increased GPx activity in fertile men's seminal plasma efficiently catalyzes seminal ROS, which can protect sperm from peroxidative damage (17). Seminal plasma GPx reduction may be lead to defective sperm quality and reduced fertilizing capacity (5).

It has been well documented that motility and morphology parameters have a major relationship with GPx behavior. Peroxidative damage to spermatozoa has been suggested to be exacerbated in assisted reproduction techniques that include washing spermatozoa to remove enzymes present in seminal plasma and then exposing them to extended incubation in the absence of these potentially protective enzymes. (46). GPx suppressed GPx activity may enable the development of peroxides from the sperm plasma membrane to the chromatin, where they could induce DNA strand breakage; suppressed GPx activity may allow the production of peroxides from the sperm plasma membrane to the chromatin, where they could induce DNA strand breakage (44).

Catalase (CAT)

(Our results in this study as in table (1), showed a high significant decrease (P<0.0001) in catalase (CAT) levels of both primary and secondary infertile men compared to the fertile control group and an non-significant decrease (P>0.05) in CAT levels of the primary group compared to the secondary group. Our results agree with the results of (5;8;30). On another hand, observed a significant increase of seminal CAT activity in infertile men compared with fertile men. Despite our observations (11) found no difference in CAT-like behaviors between fertile and infertile men (41). There were no major variations in CAT concentrations in seminal plasma between infertile and fertile men, according to the report. (14) The protein expression of CAT in seminal plasma was positively associated with sperm progressive motility and morphology. CAT helps in the decomposition of H2O2 into water and oxygen, preventing LPO and improving motility. Previous research has shown that the mean values of CAT activity in seminal plasma of infertile groups were not substantially different from fertile individuals (26). The current study's findings demonstrate the significance of CAT behavior in seminal plasma as a significant indicator of the ejaculate's antioxidant status. CAT's ability to increase sperm motility suggests that H2O2 is the ROS with the most cytotoxic effect on spermatozoa. (3).

Superoxide Dismutase (SOD)

Our results in this study as in (table (1), showed an significant increase (P<0.05) in superoxide dismutase (SOD) levels of primary infertile group compared to the fertile control group and a non-significant increase (P>0.05) in secondary infertile men compared to the fertile control group and a non-significant increase (P>0.05) in primary infertile group compared to the secondary infertile group. Our results agree with the results of (4;7;30). And disagreement the study of significant higher infertile men as compared to fertile men (42). On another hand Significant, lower infertile men as compared to fertile men (5).

Immature spermatozoa generate primary O₂ (1). This anion is disputed to H₂O₂ by SOD an operation For human spermatozoa, H₂O₂ is the most toxic ROS, causing LPO and cell death at high concentrations. As a result, maintaining sperm motility requires a combination of SOD and H₂O₂ activities in the sperm (24). This finding backs up the findings of other researchers who had previously reported their findings (45). It should be noted that in a fertile sample, SOD seminal plasma protects against lipid peroxidation of phospholipid and phospholipid-bound fatty acids (41).

Total Antioxidant Capacity (TAOC)

Our results in this study as in (table (1), showed a high significant decrease (P<0.0001) in (TAOC) levels of both primary and secondary infertile men compared to the fertile control group and a non-significant increase (P>0.05) in TAOC levels of the primary group compared to the secondary group. Our results agree with the results (36;35). Semen TAOC Nutrition, vitamin availability, age, illness, and other factors may all have an impact on ability.
Malnutrition is the cause of low semen TAOC in the infertile population, according to our findings. It is possible that low semen TAOC in the infertile community is due to a lack of health culture, as they eat little food rich in antioxidants.

The discovery of particular genes and pathways impacted by oxidants led to the theory that ROS function as subcellular messengers in gene regulatory and signal transduction pathways. Additionally, antioxidants can activate numerous genes and pathways (6), also several groups have now shown that using a variety of molecular genetic techniques, the expression of a wide range of genes is regulated by H$_2$O$_2$ (23). It has recently been discovered that AOs, oxidants, and factors influencing the state of cellular redox potential control gene expression of many enzymes and proteins. It has also been shown that free radicals directly or indirectly oxidize redox-sensitive proteins. (37). Many enzymes, as well as transcription factors, have been proposed as potential targets for altered redox status (12). Demonstrate that induced OS is the source of altered steroid hormone receptor expression and distribution, as well as altered gene expression of certain AO enzymes. (12).

They came to the conclusion that, regardless of the clinical diagnosis, total antioxidant ability can play a role in the pathophysiology of male infertility.

Furthermore, we discovered that lower levels of total antioxidant ability were linked to sperm concentration, motility, and morphology., suggested that ROS synthesis and TAOC in the seminal fluid could be used as markers of OS and that they were linked to male infertility. As a result, low TAOC levels mean that antioxidants are being used to detoxify an abundance of reactive oxygen) organisms. (39)

**Malondialdehyde (MDA)**

Our results in this study as in table (1), showed highly significant increase (P<0.0001) in MDA levels of both primary and secondary infertile men as compared to the fertile control group and a non-significant increase (P>0.05) in MDA levels of the primary group compared to the secondary group. Our results agree with the results of (19;32;8).

Malondialdehyde may be used as a marker of oxidative stress in males; a rise in seminal MDA levels may be attributed to an increase in ROS generation due to the excessive oxidative damage caused by these infertile persons. Many other essential biomolecules, including membrane lipids, can be oxidized by these oxygen species. (Peroxidation of polyunsaturated phospholipids) and disturbance of the functions carried out by the sperm membrane and impairs the fertilizing capacity of spermatozoa (24).

OS are often linked to low sperm quality and male infertility. It has been discovered that seminal ROS levels are high in 40%–88% of non-selected infertile patients. Recent studies, however, have discovered that normospermic men are more likely to have normal sperm counts. normal semen analysis) infertile men have ROS higher levels and reduced the total antioxidant capacity (TAOC) levels in comparison with The exact prevalence of OS in normospermic infertile men is unknown; the exact prevalence of OS in normospermic fertile men is unknown. Despite this, a randomized study involving a small number of patients discovered that 11% of normospermic infertile men have OS. (10). Surprisingly, ROS can significantly reduce male reproductive capacity without causing significant changes in sperm parameters. The negative effects of ROS on genetic material, sperm capacitation, and acrosome reaction may explain this disability. ROS are thought to be stand-alone indicators of male factor infertility (22). According to studies, detecting MDA concentrations in seminal plasma can help with the diagnosis of male infertility caused by an excess of (ROS) in the male reproductive system.The presence of reactive oxygen species (ROS) in infertile sperm makes these sperm more vulnerable to peroxidative damage. (31).

High concentrations of H$_2$O$_2$ induce LPO and result in cell death (38).In sperm cell membrane PUFAs are essential to produce plasma membrane,Fluidity that is required to participate in the membrane fusion events associated with fertilization (41).

induce ROS LPO reduces membrane fluidity and impairs the structure and function of spermatozoa by targeting PUFA, and several studies have shown that the cellular changes caused by LPO have a significant impact on various parameters of semen and spermatozoa function.. (16).
Due to the high content of polyunsaturated fatty acids in their plasma membrane, spermatozoa are highly susceptible to harm from excessive levels of ROS, even though the oxidative state of semen has little effect on basic semen characteristics other than motility. (40).

The excessive production of ROS (O2) and H2O2 by the sperm themselves may be result in peroxidation of (PUFAs) of the plasma membrane. As a result, the fluidity of the Spermatozoa membrane assured by then complex network of PUFAs is compromised by the (ROS) and inhibits the proper membrane fusion, with the oocytes (13). The results of the LPO assay exhibit the excellent correlation with the degree to which function of sperm is impaired in the terms of motility and the capacity for spermatoocyte fusion (18). LPO of sperm membrane it is considered to be the key mechanism of this (ROS) induced sperm damage leading to infertility (9). In addition, the breakdown products resulting from LPO, such as (MDA), can combine with several functional groups on molecules, including lipoproteins, proteins, DNA and RNA. Therefore, (MDA) can have the cytotoxic effects of Spermatozoa (16).

Table (2) the correlation analysis between ASA and enzymatic antioxidant parameters in all immunological infertility studied. In this current study was found of results for GPx was positive and significantly correlated with ASA (P=0.013, r = 0.254). CAT was negative and highly significantly correlated with ASA (P <0.0001, r = -0.570). SOD was positive and non-significantly correlated with ASA (P=0.881, r=0.016). TAC was negative and highly significantly correlated with ASA (P<0.0001, r = -0.356). However, less or not found of references about correlated ASA with enzymatic antioxidant (GPx, CAT, SOD and TAOC). But MDA was positive and highly the significantly correlated (ASA) (P<0.0001, r = 0.412). Lipid peroxidation may be responsible for some cases of male infertility by disrupting sperm functions and quality, especially sperm motility and morphology (15). One of the underlying etiologies in the process leading to sperm dysfunction has been identified as oxidative stress (OS) (33), as well as DNA damage (25;43). Overproduction of reactive oxygen species (ROS) beyond cellular antioxidant scavenging ability causes oxidative stress. Human ejaculate has been found to be polluted with possible ROS sources. In any ejaculate, this causes oxidative damage and the loss of function of some spermatozoa. The key origins of ROS in semen have been described as immature spermatozoa and seminal leukocytes (21). Increased ROS levels cause lipid peroxidation, which affects the sperm cell membrane and causes harm to intracellular proteins, leading to infertility indirectly. Despite mounting evidence that OS plays a role in male infertility, the effectiveness of antioxidant therapy is also up for debate (3). As ASA with a strong degree of correlation (P0.0001, r = 0.412). Lipid peroxidation may be responsible for some cases of male infertility by disrupting sperm functions and quality, especially sperm motility and morphology (15). One of the underlying etiologies in the process leading to sperm dysfunction has been identified as oxidative stress (OS) (33), as well as DNA damage (25;43). Overproduction of reactive oxygen species (ROS) beyond cellular antioxidant scavenging ability causes oxidative stress. Human ejaculate has been found to be polluted with possible ROS sources. In any ejaculate, this causes oxidative damage and the loss of function of some spermatozoa. The key origins of ROS in semen have been described as immature spermatozoa and seminal leukocytes (21). Increased ROS levels cause lipid peroxidation, which affects the sperm cell membrane and causes harm to intracellular proteins, leading to infertility indirectly. Despite mounting evidence that OS plays a role in male infertility, the effectiveness of antioxidant therapy is still up for debate. (2).

REFERENCES

454-460.


