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# Effects of Seminal Plasma Antioxidant Potential on Semen Quality and Male Fertility

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## ABSTRACT

It is very important to identify the factors which affect normal sperm functions. New Bio-chemical parameters in seminal plasma were analyzed to determine the biochemical factors that affect normal sperm function. The research groups consists of infertile groups Asthenozoospermia (n=31) and Normozoospermia (n=27) with a healthy men as a control (n=24). The patients have been selected and examined according to the World Health Organization (2010). In addition the seminal fluid analysis the biochemical parameters were analyzed in the seminal plasma of each sample including; Total antioxidant capacity, Catalase, Glutathione Reductase , glutathione and Malondialdehyde.Results showed a decrease in seminal plasma total antioxidant capacity of Astheno-zoospermia and Normozoospermia , and a significance decrease in catalase level for both infertile groups, Malondialdehyde level significantly increased in both infertile groups and Glutathione level significantly decreased in Asthenozoospermia group as compared to control group. Antioxidants have an important role in sperm protection via disturbing the balance in ROS production which destroys sperm plasma membrane causing loss sperm activity and it's ability to fertilize the egg.

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*Keywords:* Male infertility, Sperm activity, Semen TAC, Lipid peroxidation

## 1. Introduction

Male infertility problems can occur when sperms are limited in number or function [1]. A large proportion of infertile men fail to fertile the female ovum because of lack of sperm (Azoospermia) or too little sperm (Oligozoospermia); infertility may also be due to abnormal sperm morphology (Teratozoospermia) and insufficient sperm motility (Asthenozoospermia) [2].Many men of apparently normal have problem impregnating their partners even when their fertility status by routine semen analysis is considered normal. These cases are classified as idiopathic infertility [3]. This suggests that the routine semen analysis (measurement of sperm motility, count, viability and morphology) does not necessarily provide complete diagnostic information [4]. Therefore, it is very important to identify the factors/conditions which affect normal sperm functions. Among various causes, oxidative stress (OS) has been attributed as an important factor to affect the fertility status and Sperm function [5]. OS results from an imbalance between production and removal of reactive oxygen species (ROS) [6]. Hence, ROS must be inactivated continuously to maintain only the small amount necessary to maintain normal cell function [7].

Usually, ROS kept in normal levels by the action of enzymatic as well as non-enzymatic antioxidative defense mechanisms [8]. However, excessive production of ROS can damage sperm, and ROS has been extensively studied as one of the major mechanism of infertility, and high level of ROS in the semen leads to decrease of sperm quality and function [9], all cellular components

components including lipids, proteins, nucleic acids, and sugars can be destroyed by a situation of OS [5]. This article studies the effect of OS on the seminal plasma quality and sperm function.

## 2. Materials and methods

The population of this study consists of 68 infertile men of which 31 Asthenozoospermia ASZ and 37 Normozoospermia, with a healthy man as a control group (n=24). All patients were attending the andrology laboratories in Sulaymaniyah governorate. Seminal fluid analyzed according to the World Health Organization **(**2010**)** criteria [10]. After semen analysis

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seminal plasma has been separated by centrifugation at (4000 g) and seminal plasma were preserved at (-45 C°) untill biochemical analysis which included; Total antioxidant capacity TAC, Catalase CAT, Glutathione Reductase GRD, glutathione GSH and Malondialdehyde (MDA).

*2.1. Total Antioxidant Capacity Assay*

Total Antioxidant Capacity (TAC) assayed using (Cell Biolab, USA) kit. The assay based on the reduction of copper (II) to copper (I) by antioxidants. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 490 nm. The concentration of antioxidant was calculated from uric acid standard curve and the results expressed as µM/ml.

*2.2. Catalase Assay*

CAT assayed using (Cell Biolab, USA) kit, based on the decomposition of $H_2O_2$ by catalase, the remaining $H_2O_2$ in the reaction mixture facilitates the coupling reaction of 4-aminoantipyrin in conjunction with an Hourse Radish Peroxidase catalyst forming quinoneimine dye which correlates to the amount of $H_2O_2$ remaining in the reaction mixture. The quinoneimine dye coupling product is measured at 520nm, which correlates to the amount of hydrogen peroxide remaining in the reaction mixture.

*2.3. Glutathione Reductase Assay*

GRD assayed using (Oxford, UK) assay kit. The assay is based on the oxidation of NADPH to $NADP^+$ catalyzed by GRD. One GRD activity unit is defined as the amount of enzyme catalyzing the reduction of one micromole of GSSG per minute at pH 7.6 and 25°C. The reduction of GSSG is determined indirectly by the measurement of the consumption of NADPH, as demonstrated by a decrease in absorbance at 340 nm as a function of time. The enzyme activity determined and samples GRD activity is calculated by plotting the absorbance of samples on the standard curve, results were expressed as mU/mL.

*2.4. Lipid Peroxidation Assay*

Lipid peroxidation (LPO) is measured by reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) according to a method by Rao *et al.* [11]. In the presence of heat and acid, TBA reacts with MDA under heat and low pH in a ratio 1:2 to form a pink color $[TBA]_2$-malondialdehyde. The intensity of the color at 532 nm corresponds to the level of lipid peroxidation.

*2.5. Glutathione Assay*

Reduced glutathione is measured according to the method of Moron et *al.* [13], based on the reaction of the aliphatic thiol compounds with 5,5-dithiobis (2-nitro-benzoic acid) [DTNB] at pH 8.0. The optical density of yellow color complex developed by the reaction of GSH is measured at 412nm. result is expressed as µmol glutathione/ mL.

## 3. Results and Discussions

The mean±SD of sperm parameters of different causes infertile men and fertile groups shown in the table 1, data showed a significant decrease in sperm activity in ASZ group as compared to fertile (43.66±16.58 and 57.44±11.11 %) and progressive motility (17.97±8.06 and 43.88±14.96 %) respectively.

Table 2 represents a significant difference in ASZ biochemical parameters as compared to fertile group, we found a decrease in TAC level (439.1±207.9 and 834.5±253.7 µM) and a decrease in CAT activity (39.24 ± 32.24 and 76.98±15.32 U/ml). Also we found a decrease in GSH level (127.77±59.08 µmol/mL and 174.12±61.25 µmol/mL) and an increase in the LPO Level (131.45±119.61 nmol/mL and 44.13±13.07 nmol/mL) in ASZ group as compared to fertile group, while no significance seen in GRD and total protein levels.These results agree with Tarish [14] , Atig et al. [15], Mahfoz et al. [16], Mahfoz et al reported a decrease in TAC level for infertile (1310 µM) as compared with fertile (1700 µM), and Khosrowbeygi and Zarghami [17], they have reportedTAC (1.05 ± 0.04 and 1.63 ± 0.08 mM).

**Table 1:** mean±sD of sperm parameters of infertiles and fertile groups

| Parameters | ASZ (n=31) | NOZ (n=27) | Fertile (n=24) |
|---|---|---|---|
| Count millions/mL | 78.04±46.81 | 75.91±43.79 | 58.31±30.70 |
| Live % | 69.76±13.84 | 78.40±10.06 | 74.50±10.01 |
| Active % | 43.66±16.58 | 57.31±8.48 | 57.44±11.11 |
| Progressive % | 17.97±8.06 | 39.39±6.09 | 43.88±14.96 |
| Non progres. % | 25.68±14.65 | 17.91±8.91 | 13.56±11.92 |
| sluggish % | 26.10±12.70 | 21.11±5.09 | 17.06±7.84 |
| Normal morphology % | 50.95±14.48 | 52.98±17.21 | 61.04±11.79 |

**Table 2:** Mean±SD of semen biochemical parameters of infertiles and fertile group

| Parameters | ASZ (n=31) | NOZ (n=27) | Fertile (n=24) |
|---|---|---|---|
| TAC µM | 439.1±207.9 | 454.5±184.9 | 834.5±253.7 |
| CAT U/mL | 44.70±32.49 | 39.24±32.24 | 76.98±15.36 |
| GRD mU/mL | 24.00±17.69 | 26.27±13.35 | 16.02±9.66 |
| T.protein g/dL | 4.48±1.14 | 4.20±0.88 | 4.17±1.60 |
| Gluthatione µmol/mL | 127.77±59.08 | 166.04±66.29 | 174.12±61.25 |
| MDA nmol/mL | 131.45±119.6 | 113.82±21.90 | 44.13±13.07 |
| TAC µM | 439.1±207.9 | 454.5±184.9 | 834.5±253.7 |

One possible mechanism by which motility is decrease due to OS is the negative effect of ROS on ATP production [18]. Uncontrolled production of ROS that exceeds the Antioxidants capacity of the seminal plasma leads to OS which is harmful to spermatozoa (SPZ) [5]. Unfortunately, sperm are unable to repair the damage induced by excessive ROS because they lack the cytoplasmic enzyme systems that are required to accomplish this repair [19], [20], this make the seminal plasma antioxidants as the most important role in the defense against ROS attack and it is one of the features that make SPZ unique in their susceptibility to oxidative damage [19].

Low level of Antioxidants may cause alternation in gene expression of CAT as mentioned by Bilińska *et al.* who conclude that induced OS is the cause of altered expression and altered gene expression of some Antioxidants enzymes [21]. Decrease in CAT activity is the cause of accumulation of $H_2O_2$, and it is likely that $H_2O_2$ is responsible for more damage to cell membrane, structure and function [18]. $H_2O_2$ has the ability diffuse across the membranes into the cells and inhibit the activity of enzymes such as G6PDH, leading to a decrease in the availability of NADPH. These changes can cause a decrease in the Antioxidant defenses of the SPZ, which ultimately leads to the peroxidation of membrane phospholipids [7].

Sperm are highly susceptible to damage by excess concentrations of ROS due to the high content of PUFAs within their plasma membrane [22], LPO of ASZ (131.45±119.61 nmol/mL) and (44.13±13.07 nmol/mL) for fertile group. ($NADPH+H^+$) is coenzyme of glutathione reductase, it formally donates a hydrogen molecule, thereby re-reducing GSSG to GSH [23]. Therefore, the elevation of $H_2O_2$ impaired the re-reducing of GSSG, this is the cause of decrease GSH level in this ASZ group.

GSH protect sperm plasma membrane [24], [25], especially the Mid-piece region which is important in SPZ motility [15], [26]. Low GSH level reduced Antioxidant activity resulting in disruption in the SPZ membrane [27], and eventually LPO of sperm plasma membrane. LPO of mid-piece cause ATP to deplete rapidly resulting in decreased phosphorylation of axonemal proteins and cause transient impairment of motility [28]. This approved experimentally by Wang, artificially induce OS by incubation with $H_2O_2$ which has been shown to inhibit sperm motility, decrease ATP, and dissipate the mitochondrial membrane potential [29]. The phosphor-rylation of flagellar proteins is required to the initiation and maintenance of sperm motility. Dyneins located on the arms of the outer microtubular doublets of SPZ flagella needs activation of ATPase to maintain flagellar bending of ATPase to maintain flagellar bending [30].

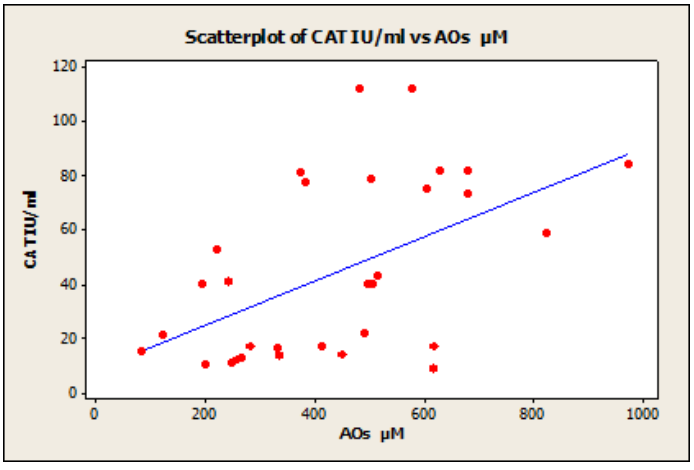


**Figure 1**: The Correlation between TA and CAT Activity in the ASZ group

In the considering of NOZ group results represent a significant difference in ASZ biochemical parameters as compared to fertile group, is a decrease in TAC level (454.5±184.9 and 834.5±253.7 µM) and a decrease in CAT activity (39.24 ± 32.24 and 76.98±15.32 U/ml). Also we found an increase in the LPO Level (113.82±21.90 nmol/mL and 44.13±13.07 nmol/mL) in ASZ group as compared to fertile group, while no significance seen in GRD, total protein and GSH levels.

Acrosomal dysfunction can be considered as one of the important causes of infertility [32]. $H_2O_2$ needed for stimulation and the ability of the SPZ to undergo then acrosome reaction and oocyte fusion [31], but high level of $H_2O_2$ is detrimental to the SPZ, inhibiting fusion with the oocytes [33], this is Because of High ROS ($H_2O_2$) levels may lead to alteration of sperm membrane permeability and fluidity possibly via LPO of sperm plasma membrane [34]. LPO may damage membrane integrity with increased cell membrane permeability, thus leading to enzyme inactivation in sperm acrosome and eventually loss of the ability to penetrate the female ovum [35].

A positive correlation seen between TAC and CAT ($r$= 0.804, $p$ =0.001), as shown in the figure (3.2), this can be explained as mentioned earlier by the gene expression theory by Bilińska *et al.* [21].

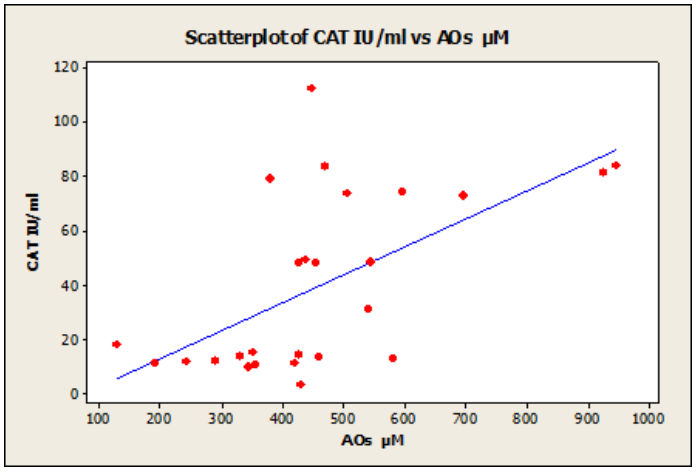


**Figure 2:** The Correlation between SP AOs and CAT Activity in the NOZ group

## 4. Conclusions

We concluded that antioxidants has an important role in sperm function male infertility via disturbing the balance in ROS production which influences the LPO process in sperm plasma membrane in difference places (mid-piece and acrosome) causing loss sperm activity and ability to penetrate the ovum.

## Conflict of interest

None.

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