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# Oxidative stress induces idiopathic infertility in Egyptian males

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**The most common cause of male infertility is idiopathic. Oxidative stress (OS) would play a vital role in etiology of idiopathic male infertility because of its targeting to spermatozoa plasma membrane rich in polyunsaturated fatty acids. To examine OS effect on Egyptian men fertility, sperm samples were obtained from infertile idiopathic patients (25 to 35 years old). The samples were categorized into 4 groups: fertile group (n = 20); azospermia's patients (n = 20); normospermic patients (n = 20) and oligospermic patients (n = 40). Induced OS was tracked by measuring the alteration in prooxidant level (TBARS) as well as activities of antioxidant enzymes superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxide (GPX) and reduced glutathione (GSH). The TBARS levels were significantly high in infertile patients (within a range of 33.89 to 81.77%) compared to the healthy individuals. GST, SOD and GSH were significantly low in oligospermic patients by 33.33, 39.655 and 53.16%, respectively while GPX was higher by 87.5%. In azospermic patients, GSH and SOD activities were lower by 50% while GPX reached its maximum activity (93.75%). For normospermic patients with high immotile sperm, SOD activity was higher by 62.06% while both GSH and GPX were lower by 36.54 and 70.31%, respectively compared to the healthy individuals. Our results obviously emphasize the association of OS level in seminal plasma with the incidence and progression of the idiopathic infertility in infertile patients. Thus, seminal reactive oxygen species (ROS) would be used as a specific and sensitive biomarker for idiopathic male infertility.**

**Key words:** Idiopathic male infertility, azospermia, oligospermia, normospermia, oxidative stress, antioxidant enzymes, thiobarbituric acid reactive species.

## INTRODUCTION

Infertility affects more than 80 millions people around the globe, with one in 10 couples experiencing primary or secondary infertility. Infertility is more prevalent in those countries defined as the infertility belt, namely the central and the southern African countries, whereas many as one-third of the couples in some populations are unable to conceive (Adonaylo and Oteiza, 1999). Globally, the overall prevalence ranges between 8 to 12%, with a core prevalence of primary infertility of about 5% (Inhorn, 2002; Vayena et al., 2001). The causes of infertility have been attributed to a variety of anatomical, genetic, endocrinological and immunological factors (WHO, 1999). It is clear that it is a common problem affecting young couples, and equally clear that it results in considerable distress for those couples affected. The feelings experienced by infertile couples encompass anger, depression, anguish, denial, guilt, shame, inadequacy, shock, isolation and embarrassment (Irvine, 1998).

Factors causing high rates of infertility in parts of the

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developing world are varied, but tubal infertility due to sexually transmitted, postpartum, post-abortive, and iatrogenic infections is widely regarded as the primary form of preventable infertility in the world today (Sciarra, 1997; Reproductive Health Outlook, 2002). A large proportion of male infertility cases are associated either with systemic defects such as diabetes, obesity, varicocele, cystic fibrosis or with infections for mumps, herpes or else with imbalance in levels of gonadal steroids and trophic hormones [example, testosterone, dihydrotestosterone, follicle stimulating hormone, leutinizing hormone, and androgen receptor]. However, in nearly 25% cases of male infertility no organic cause is identified (idiopathic infertility) (Ambasudhan et al., 2003).

Traditionally, the diagnosis of male infertility is based upon the conventional semen profile, constructed according to recognized guidelines (WHO, 1992; Van den Eede, 1995). Though semen analysis is the first diagnostic step routinely employed in the evaluation of the male infertility, it fails to predict the exact cause behind impaired fertility (Anonymous, 1996). This profile incorporates information on the volume of the ejaculate, the concentration of spermatozoa, their motility and their morphological appearance. However, sperm count and sperm motility are the first and most important predictors of fertility potential rather than sperm morphology. In half of the male infertile patients, the cause is not clear and hence such cases are diagnosed with idiopathic infertility. Moreover, idiopathic infertile cases are blindly treated and selected for assisted reproductive techniques without understanding the basic mechanism behind the fertility impairment (Venkatesh et al., 2009a).

Oxidative stress (OS), a condition where the production of reactive oxygen species (ROS) overwhelms anti-oxidant levels, has been considered as one of the major factors believed to be involved in idiopathic male infertility. Low levels of ROS are necessary for normal functions of spermatozoa like capacitation, hyperactivation, motility, acrosome reaction, oocyte fusion and fertilization (Agarwal et al., 2004; Venkatesh et al., 2009a). For the past two decades, the pathological role of ROS in the semen has been studied but not well established because of various possible sources associated with excess production of ROS including abnormal sperma-tozoa (Venkatesh et. al., 2009b). It has been postulated that oxidants interfere with normal sperm plasma function via peroxidation of unsaturated fatty acids in the sperm plasma membtrane which results in sperm dysfunction (Barroso et al., 2000). In addition, ROS are known to attack DNA inducing strand breaks and other oxidative-based damage in spermatozoa. High levels of ROS endanger sperm motility, viability and increased midpiece sperm defects that impair sperm capacitation and acrosome reaction. The fertilizing ability of human spermatozoa is inversely related to the sperm ROS production (Gil-Guzman et. al., 2001).

Since the pathophysiology of male infertility is still poorly understood and various diagnostic tests are unable to determine the underlying cause of sperm dys-function, the aim of the present study was to investigate the correlation between OS and incidence of idiopathic infertility and subfertility in Egyptian men. Lipid peroxi-dation, antioxidants enzymes and DNA fragmentation in seminal samples were tested.

## MATERIALS AND METHODS

5,5'-Dithiobis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), tris-HCl, p-nitrobenzyl chloride, trichloroacetic acid (TCA), cumen $H_2O_2$, reduced glutathione (GSH), Foline-Ciocalteau reagent, SOD enzyme, pyrogallol, phenol, chloroform and sodium acetate, were purchased from Sigma Chemical Company (St. Louis, Mo, USA). 1 Kbp ladder was purchased from Bioron (Ludwigshafen, Germany). Kit for total protein determination was bought from Biodiagnostic (Cairo, Egypt). All other chemicals and reagents were of highest quality.

### Experimental design

The study was approved by the ethics committee of Alexandria University, Egypt and has therefore been performed in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki, 1964 and Declaration of Tokyo, 1975). An experienced urologist performed the genital examinations. Male patients with varicocele, hypogonadism, obstructive azoospermia, cytogenentic abnormalities, alcohol, recent drug intake, prolonged illness and exposure to reproductive toxicants were excluded from the study. Patients with normal female partners, that is, normal reproductive history, normal ovulation (by follicular ultrasound scan, luteal phase progesterone levels, and endometrial biopsy), and tubal patency (hysterosalpingogram) were eligible for the study. All participants were gave a written informed consent. A detailed medical history including reproductive history and fertility evaluation of the female partner was documented.

Semen samples were obtained from 80 idiopathic infertile men and 20 fertile donors aged 25 to 35 years old who attended the male infertility clinic, Dr Sherif S. Said, Center for Infertility Research, Alexandria, Egypt. All infertile couples included in this study had a minimum of 1 year of regular unprotected intercourse. The group of healthy male volunteers of proven fertility (initiated a successful pregnancy within the last 12 months before participation in the study) served as the control group.

### Standard semen analysis

After a period of 72 h of sexual abstinence, volunteers collected their semen specimens in sterile plastic containers and delivered them to the clinical andrology laboratory in less than 30 min. After liquefaction, semen specimens were evaluated immediately according to the WHO guidelines (volume, sperm concentration, motility and morphology). Morphology smears were scored using the WHO classification and Kruger's strict criteria (Kruger et al., 1986). Sperm concentration was expressed as $10^6$ per 1 ml semen, whereas motility and morphology were expressed as and 14% by Kruger's strict criteria. Seminal leukocytes were percentage. Sperm parameters were considered normal when sperm concentration was $\geq 20 \times 10^6$ per 1 ml semen, motility was $\geq 50\%$, and normal sperm forms were 30% by WHO criteria quantified by a myeloperoxidase staining test, and values were considered to be normal at concentrations of $1 \times 10^6$ peroxidase- positive

leukocytes per 1 ml semen.

According to seminal quality profile, the infertile patients were categorized into three groups as follow; normospermic patients (normal sperm count but motility grade <50%) (n =20), oligospermic patients (sperm concentration ≤20 × $10^6$ sperm per ml) (n = 40) and azospermic patients (sperm count equal zero) (n = 20). Semen samples were centrifuged at 1800 $xg$ for 10 min, then the supernatants and sperm pellets were separately collected and stored at -20°C for further analysis.

## Estimation of seminal plasma lipid peroxidation

Seminal plasma malondialdehyde content, indicator for lipid peroxidation, was assayed in the form of TBA-reactive species (TBARS) according to Tappel and Zalkin (1959). Briefly, 500 µl of seminal plasma was added to 1 ml TCA (15%), mixed well, and then centrifuged at 1800 $xg$ for 10 min. After cooling, 1 ml supernatant was added to 0.5 ml TBA (0.7 g/dl) and boiled for 30 min in boiling water bath then cooled. The absorbance (At) of semen samples was read by a spectrophotometer (Optima, Japan) at 532 nm against reagent blank. The level of seminal TBARS was calculated according to Equation 1:

Seminal TBARS level (nmol/ml) = At / 0.156          (1)

## Determination of seminal plasma glutathione-S- transferase (GST)

100 µl GSH (5 mM), 10 µl p-nitrobenzyl chloride (1 mM in ethanol) and 25 µl seminal plasma were added to 1.365 ml phosphate buffer (0.1 M, pH 6.5), vortexed then incubated for 20 min at room temperature. The absorbance was measured against air at 310 nm. The GST activity was calculated according to Habig et al. (1974) using the following Equation 2:

GST activity (µM/min/mg protein) = At / (1.9 × time × mg protein)  (2)

## Determination of superoxide dismutase (SOD) in seminal plasma

20 µl of seminal plasma (test) or Tris buffer (0.1 M, pH 8.5) (reference) and 10 µl pyrogallol (20 mM in HCl, 10 mM) were added to 1 ml Tris-buffer solution and left at room temperature. The absorbance of test (At) or reference (Ar) was measured at 420 nm against air after 30 and 90 s. The percentage inhibition of pyrogallol auto-oxidation by seminal plasma was calculated according to the following Equation 3:

Inhibition (%) = 100 – [At /min/ml sample) / (Ar/min/ml reference] × 100                                                        (3)

The specific activity of serum SOD was calculated as ng/min/mg protein with dividing the value ng/min/ml by sample protein concentration. One unit of SOD activity is defined as the amount of enzyme which inhibits the rate of auto-oxidation of pyrogallol by 50%. SOD concentration was computed as ng/ml according to a standard curve and was found that one unit equals 153 ng. The enzyme activity was expressed in U/mg protein by dividing value (ng/min/mg protein) by 153 (Marklund and Marklund, 1974).

## Determination of GPx activity in seminal plasma

50 µl seminal plasma were added to 100 µl GSH ( 5 mg in 10 ml Tris-HCL buffer, 50 mM, pH 7.6), 100 µl cummen $H_2O_2$ (50 µl in 10 mL Tris-HCl buffer, 50 mM, pH 7.6) and 750 µl Tris-HCl (50 mM, pH 7.6) and incubated at 37°C for 10 min. One milliliter of TCA (15%) was added to this mixture, centrifuged at 1800 $xg$ for 20 min and then the supernatants were separated off. One milliliter supernatant was added to 2 ml Tris-HCl (0.4 mM, pH 8.9) and 100 µl DTNB (0.0198 g in 5 ml methanol) and incubated for 5 min. The absorbance was measured at 412 nm against distilled $H_2O$. The activity of GPx was calculated according to Paglia and Valentine (1967) with the following Equation 4:

GPx activity (U/g wet tissue) = At × 6.2 × 100 / 13.1 × 0.05 × 10   (4)

## Estimation of reduced glutathione (GSH) in seminal plasma

Briefly, 1 ml seminal plasma (test, t), GSH (1 mg/ml) (standard, st) or $H_2O$ (blank, b) was mixed with 1.0 ml 4% sulphosalicylic acid, incubated at 4°C for 1 h and then centrifuged at 1200 $xg$ for 10 min at 4°C. Afterwards, 0.1 ml supernatant was added to 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml DTNB (0.1 mM, pH 8.0) and the developed yellow color was measured immediately at 412 nm. GSH was assayed according to the following equation of Jollow et al. (1974):

GSH activity (mg/mg protein) = (At – Ab / Ast – Ab) × standard concentration                                                      (5)

## Determination of total protein concentration in seminal plasma

Ten milliliters seminal plasma (test), standard or $H_2O$ (blank) was added to 1 ml Biuret reagent, vortexed for 1 min and allowed to incubate at room temperature for 10 min. The absorbance (A) of samples (t) and standard (st) were read against blank (b) at 546 nm. The total protein concentration in the samples was computed according to Plummer (1978) using the following Equation 6:

Total protein content (g/dl) = (At – Ab / Ast – Ab) × st concentration                                                            (6)

## Detection of seminal DNA fragmentation

DNA fragmentation was detected according to the method of Maniatis and coworkers (1982) with some modification. Seminal pellet (50 µg) was suspended in SET buffer (1 ml sucrose-EDTA-Tris, pH 7.6) by shaking in ice bath. 50 µl of seminal suspension were diluted with 450 µl SET buffer and 15 µl alkaline protease, mixed well and incubated for 30 min at about 50°C. 50 µl 10% SDS were added to the mixture and incubated for 40 to 60 min at 50°C.

After that, 565 µl phenol/chloroform mixture (1:1) were added, mixed gently, centrifuged at 8200 $xg$ for 5 min, and then the upper aqueous layer was separated. The re-extraction of the aqueous layer including DNA fibers with phenol/chloroform mixture was repeated for further purification. To the recovered aqueous phase, sodium acetate (3 M) was added to a final concentration of 0.25 M, then 2 volumes of cold absolute ethanol were mixed well and incubated on ice for about 20 min. Afterwards, the mixture was centrifuged at 8200 $xg$ for 2 min. The supernatant was decanted and the pellet was washed with 70% ethanol then centrifuged for 5 min at 8200 $xg$. Ethanolic supernatant was decanted and the pellet (DNA fiber) was dried in an oven at 65°C and then re-suspended in 250 µl TE buffer (Tris-EDTA buffer, pH 8.0). DNA was quantified spectrophotometrically at 260 nm. DNA was then loaded onto agarose gel (5 ug/ lane). DNA was resolved by constant voltage mode electrophoresis (mini submarine, 80v, 45 min) on a 1.2% agarose gel containing 0.5 ug/ml ethidium bromide. A ladder (1K bp) served as a DNA base pair marker. Gels were illuminated with 300 nm UV light and a photographic record was taken.
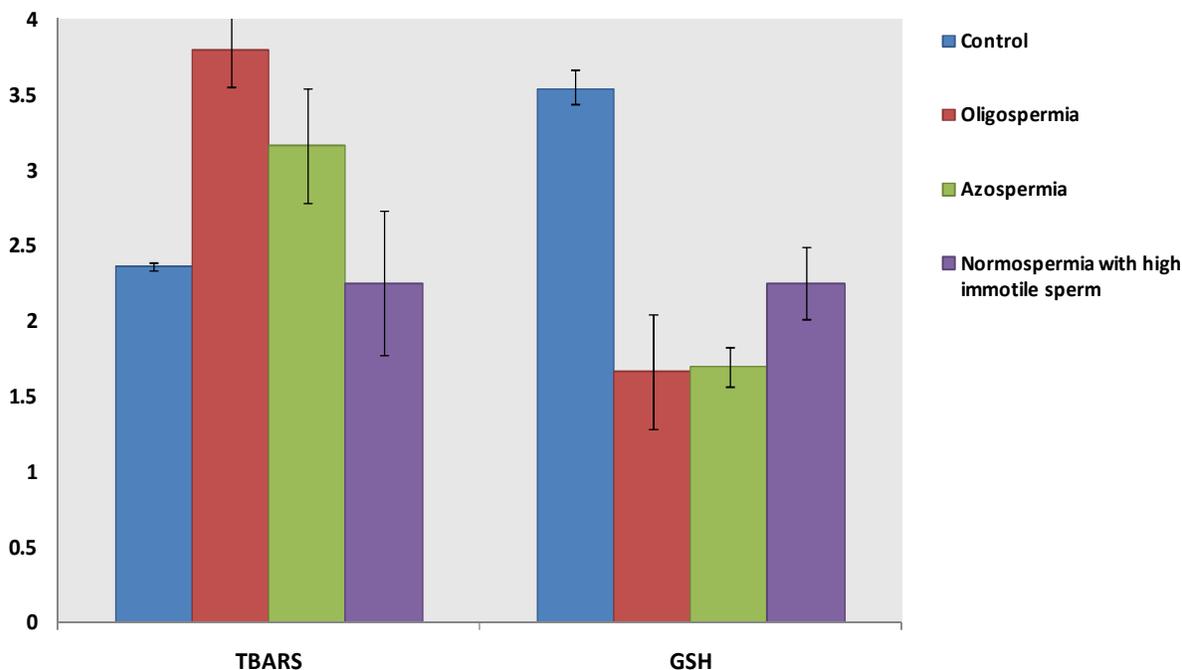
**Figure 1:** Prooxidants (TBARS) and nonenzymatic antioxidants (GSH) seminal plasma concentration of idiopathic infertile men and fertile donors.

### Statistical analysis

All data are expressed as the mean ± standard error of means (S.E.M). The difference between infertile groups and the fertile one was statistically compared at $P<0.05$. Seminal characteristics, enzymes activities, and lipid peroxidation were compared among the groups tested using the unpaired Student's t-test. The obtained data were analyzed by one-way analysis of variance (ANOVA) using Primer of Biostatistics (V5) software program.

### RESULTS

Seminal volume, sperm concentration, motility, morphology and leukocytes count are detailed in Table 1. There was no significant difference between the fertile donors and idiopathic men in terms of semen volume (ml) and leukocytes count ($1 \times 10^6$ peroxidase-positive leukocytes per ml semen) at $P< 0.05$. However, leukocytes count in azoospermia patients was significantly increased to $27.5 \times 10^6$/ml. Sperm motility (%) was significantly dropped from 60.6% in fertile group to 30.0 and 22.76% in normospermia and oligospermia patients, respectively. Furthermore, percentages of normal sperm were significantly decreased in normo-spermia and oligospermia groups compared to fertile one. As earlier proved in previous studies, we found that semen samples of azoospermia patients were completely free of motile sperm and have zero normal ones.

As shown in Figure 1, seminal TBARS levels were significantly increased in oligospermic, azoospermic and normospermic patients by 61.0, 33.9 and 81.8%, respectively compared to the fertile group at $P < 0.05$. On the other hand, GSH (seminal nonenzymatic antioxidant) was significantly decreased in all idiopathic groups compared to fertile one at $P < 0.05$. Activities of antioxidant enzymes are shown in Table 2. No significant differences were observed in GST activities in azoospermic and normospermic patients compared to fertile males while GST activity was significantly decreased in oligospermic patients at $P < 0.05$.

The GPx activity increased up to two folds in both oligospermic and azoospermic patients while decreased dramatically (three folds) in normospermic patients compared to GPx activity in fertile subjects, at $P < 0.05$. Semen samples of oligospermic and azoospermic patients showed a significant decrease in their SOD activities by 39.66 and 50%, respectively than those of fertile subjects, at $P < 0.05$. On the other hand, normospermic patients showed a significant increase in SOD activity by 61.1% than that of fertile group. The seminal protein contents significantly increased in oligospermic patients but decreased in normospermic patients compared to the fertile group, at $P < 0.05$ (Table 2). No significant difference in protein content was noticed between azoospermia samples and fertile ones.

Figure 2 shows the qualitative changes in the integrity of the genomic DNA extracted from sperm palette of the different infertile groups. The electrogram generated from the gel electrophoresis showed that all infertile semen DNA had a dramatic oligonucleosome-length degradation

**Table 1.** Semen characteristics of fertile group and idiopathic infertile patients.

| Semen characteristic | Fertile group ( n = 20) | Idiopathic infertile patients | | |
| --- | --- | --- | --- | --- |
| | | Normosepermia ( n = 20) | Oligospermia ( n = 20) | Azoospermia ( n = 40) |
| Volume (ml) | 3.45 ± 0.26 | 3.2 ± 0.302 | 3.55 ± 0.26 | 3.88 ± 0.97 |
| Concentration [1] | 92.5 ± 13.7 | 47.83 ± 16.62* | 5.14± 0.56* | - |
| Motility [2] | 60.5 ± 1.5 | 30 ± 3.5* | 22.76 ± 5.5* | - |
| Morphology [3] | 66.5 ± 1.8 | 42.2 ± 3.42* | 31.5 ± 2.66* | - |
| Leukocytes [4] | 1.5 ± 0.76 | 2.8 ± 1.1 | 2.7 ± 1.57 | 27.5 ± 2.9* |

Results are the mean ± SEM. *significant difference between fertile and infertile groups was detected at $P < 0.05$, by using one way. The obtained data were analyzed by one-way analysis of variance (ANOVA) using Primer of Biostatistics (Version 5) software program.[1] Sperm count ($10^6$/ml); [2] motility (% motile sperm); [3] sperm morphology (% normal).

**Table 2.** Activities of antioxidants enzymes and protein content of seminal plasma of fertile group and idiopathic infertile patients

| Antioxidants enzymes and protein content | Fertile group ( n = 20) | Idiopathic infertile patients | | |
| --- | --- | --- | --- | --- |
| | | Normosepermia ( n = 20) | Oligospermia ( n = 20) | Azoospermia ( n = 40) |
| GPx (IU) | 6.4 ±0.92 | 1.9 ± 0.3* | 12 ± 0.93* | 12.4 ± 2.9* |
| GST (uM/min × $10^{-3}$) | 4.2 ± 0.2 | 4.3 ± 0.3 | 2.8 ± 0.2* | 3.5 ± 0.9 |
| SOD  (U/mg) | 0.58±0.01 | 0.94±0.04* | 0.35±0.02* | 0.29±0.01* |
| Seminal protein content (g/dl) | 7.23 ± 0.38 | 4.21 ± 0.4* | 9.6 ± 0.27* | 5.9 ± 0.9 |

Results are the mean ± SEM. *significant difference between fertile and infertile groups was detected at $P < 0.05$ by using one way. The obtained data were analyzed by one-way analysis of variance (ANOVA) using Primer of Biostatistics (Version 5) software program.
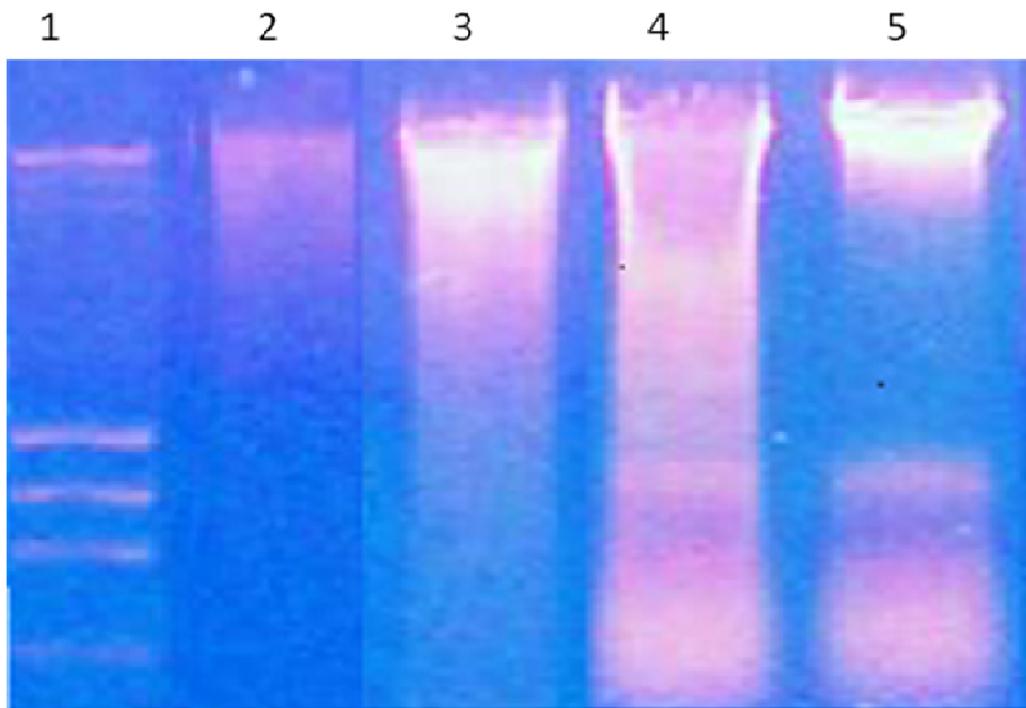


**Figure 2:** DNA agarose gel electrophoresis of semen samples: Lane 1, base pair marker; lane 2, fertile DNA; lane 3, oligospermic DNA; lane 4, azoospermic DNA and lane 5, Normosepermic DNA

of DNA, characterized by mixed smearing and laddering or only smearing of DNA fragments (lanes 3, 4 and 5) (Figure 2). Whereas, DNA isolated from control samples (lane 2) showed total ladder and smear negativity.

## DISCUSSION

Male infertility is one of the most prevalence disorder worldwide and accounts as serious social problem. Above 25% of infertile men are diagnosed as idiopathic (Sigman et al., 1998) which worsen the situation. Infertility cases diagnosed as idiopathic maybe due to instrumental limitations used in andrology laboratories and/or physicians assessment which varied according to physician accumulative experience. On the other hand, there is a near complete overlap in the semen characteristics between fertile and infertile men, barring those with absolute azoospermia. Men with sperm concentrations below 12.5 million/ml have up to a 25% pregnancy rate whereas those with counts between 12.5 and 25 million have up to a 44% spontaneous pregnancy rate. These would otherwise be considered as abnormal semen parameters (Smith et al., 1977; Gupta and Kumar, 2002).

Though the sperm count, percent sperm motility and morphology are the most accessed sperm parameters during the infertility evaluation, the link between less motility and sperm abnormalities and ROS production is rarely considered. There are many contaminants in semen like leukocytes, bacteria and immature germ cells produce high ROS levels (Ollero et al., 2001). This conforming the presence of high lipid peroxidation level in azoospermic patients because their semen had high level of leukocytes (Table 1).

According to WHO guidelines (1992), our results showed that semen of oilgospermic and normospermic patients were likely normal in terms of normal morphological sperm. However, both infertile groups were lower in normo-morphological sperms than that of fertile group (Table 1). Furthermore, these two infertile groups showed a vigorous decrease in sperm motility. This increase in sperm deformities and decrease in motility were associated with high level of lipid peroxidation and low content of GSH (non-enzymatic antioxidant) (Figure 2). The previous observations would be due to occurrence of OS (Adonaylo and Oteiza, 1999) and/or the production of ROS (Said et al., 2005) in idiopathic infertile men. On the other hand, TBARS elevation which is due to ROS production decreases the membrane fluidity and thus impairs linear progression motility (Kumer et. al., 2009). Furthermore, in oligospermic men, the spermatozoa produce higher levels of ROS compared to fertile men (Sharma and Agarwal, 1996). Lewis et al. (1994) stated that the total antioxidant capacity of the seminal plasma in infertile men is lower than that in fertile men.

It well known that superoxide anion is believed to be the primary free radical produced by the immature spermatozoa (Venkatesh et al., 2009b). Superoxide anion is eliminated by the action of superoxide dismutase (SOD), a metallic antioxidant enzyme use iron as cofactor plays an active role during appearance of stress as a result of free radical generation (Sridevi et al., 1998), so it is logically that SOD activity increased in the patients of normospermic as body adaptation phenomenon in order to prevent the incidence of OS. A decrease in SOD means there is an imbalance between prooxidant and oxidants scavenger system and this occurs when lipid peroxidation overload take places (Wu and Cederbaum, 2003).

In our study, an unexpected increase in GPx activity took place in both oilgospermic and azoospermic patients. This might be due to GPx overexpression as a result of OS. Wu and Cederbaum (2003) stated that GPx helps to remove hydrogen peroxideby using GSH as cofactors to remove hydrogen peroxide, the increase in GPx activities could be combat free radical generation during OS.

Sperm DNA is organized in a specific manner that keeps the nuclear chromatin compact and stable (Agarwal and Allamaneni, 2005). Sperm with stable DNA has the ability to decompensate and transmit the DNA correctly during fertilization process at appropriate time (Verit et al., 2006). However, several alterations in DNA structure have been reported in infertile men such as chromatin micro-deletions, aneuploidy and DNA strand breaks (Aitken 1999; Verti et al., 2006). There are several factors lead to DNA damage such as poor sperm morphology (Venkatesh et al., 2009c), ROS formation (Rajesh et al., 2002) and high level seminal leukocytes (Agarwal and Said, 2005). We noticed a complete DNA fragmentation in semen specimens of infertile patients while DNA bands of fertile males were native and compact. This correlation between seminal ROS and such DNA damage is in agreement with that reported by Agarwal and Said (2005). The association between sperm DNA damage and idiopathic infertility was studied before but the results were conflicting. Saleh et al. (2003) demonstrated that sperm DNA damage was increased in idiopathic infertile patients compared with controls ($P < 0.05$). Another study did not find any difference in DNA damage in idiopathic infertile men (Hughes et al., 1996). However, Verit et al. (2006) did not find any change or relation between sperm DNA damage and total oxidative status in normozoospermic infertile men.

Agarwal and Said (2005) postulated that the presence of leukocytes in semen, indicator for inflammation, produces cytokines and ROS which can potentially alter spermatogenesis and cause DNA aberrations

In conclusion, our results are emphasizing the association of OS occurrence in seminal plasma with the incidence and progression of idiopathic male infertility in Egyptian patients. In addition, DNA damage is significantly increased in those infertile patients. Thus, seminal ROS levels would    used primary biomarkers for

idiopathic infertility diagnosis. However, the impact of the clinical significance and management options has always been a subject of controversy among the scientific community specialized in this field.

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