# OXIDATIVE STRESS BIOMARKERS IN INFERTILE MEN: A COMPARATIVE STUDY IN KANO, NORTHWESTERN NIGERIA

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## ABSTRACT

**Background**: Male infertility affects millions of couples worldwide, with oxidative stress implicated as a potential contributor. Male infertility is a complex condition with multiple genetic and hormonal factors contributing to its etiology.

**Aim**: This research aimed to estimate serum level oxidative stress biomarkers (Malondialdehyde (MDA), Super Oxide Dismutase (SOD) and Glutathione Peroxidase (GPx)) among infertile men in kano.

**Method:** A cross-sectional comparative study was conducted; one hundred and twenty (120) participants were recruited into the study. The study participants were divided into two groups. Sixty (60) infertile men and sixty (60) fertile control men. Oxidative stress biomarkers were assessed using ELISA technique from both infertile men and fertile control men.

**Result:** From these findings; 80% infertile men attending Abubakar Imam Urology Center, Kano have primary infertility, 20% have erectile dysfunction, 18% have history of infertility, 8.3% are cigarette smokers, 70% have oligospermia. There was significant oxidative stress with increased levels of malondialdehyde and decrease levels of superoxide dismutase and glutathione peroxidase among infertile men (p-value <0.0001).

**Conclusion:** These findings suggest oxidative stress may contribute to the pathophysiology of male infertility. These results have important implications for the diagnosis and treatment of male infertility. There is need to develop oxidative stress biomarkers testing profile for infertile patients, this will enable better diagnosis and management of infertility and potentially improving treatment outcomes.

**Keywords**: Male Infertility, Oxidative Stress, Reactive Oxygen Species, Antioxidants,

## INTRODUCTION

Male infertility is defined as the inability of a male to accomplish pregnancy with a fertile female after 12 months of regular unprotected sexual intercourse (Younes *et al.*, 2016). Previously the problem of infertility in couples was ascribed to female alone and hence bears the majority of the psychosocial effects especially in Africa. This in most cases is due to cultural believe and insufficient knowledge and misunderstanding. Male infertility is well-thought-out when identifiable female causes of infertility are excluded and semen quantity and quality fail to fulfill WHO criteria (Cao *et al.*, 2017).

Researchers had revealed that male factors account for 40-50% of infertility in human. Male infertility is commonly due to deficiencies in the semen and semen quality is used as a surrogate measure of male fertility.

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It is estimated that 60% of married couples having regular unprotected sexual intercourse, achieve pregnancy after 6 months of co-habitation, 90% achieve pregnancy by 12 months and 95% between 18-24 months (Ahmed *et al*., 2010). In more than 50% of male infertility cases, the causes remain unknown, and the infertility is thus classified as idiopathic. Idiopathic infertility affects a high percentage of infertile men who cannot be effectively treated by the treatment modalities available (Olooto, 2012).

Male-factor infertility is a notable health issue all over the world including Africa and other developing nations. The prevalent rate differs between and within countries. For instance, in the United Kingdom and the United States of America, it is estimated to be 6% and 10% respectively. In Denmark, it is estimated to be in the region of 15.7% (Abarikwu, 2018).

The prevalence of infertility in Sub-Sahara Africa ranges from 20% to 40%. Although, in Africa due to its unique socio-cultural setting the focused has been on the female but fertility problems are shared by both male and female sexes. Male factor represents 40–50% of all infertility in Nigeria although it varies from one region to another, and the causes also vary from place to place (Ahmed et al., 2010).

A study in the mid-western Nigeria showed that about 50% of the 780 couples evaluated were observed to have varied causes of infertility (Uadia and Emokpae, 2015). In the southwest, male factor was reported to be responsible for 42.4% infertility cases, while in Maiduguri, North-Eastern Nigeria, infertility is the reason for about 40% of all gynecological consultations. In Kano, 40.8% prevalence was reported, 46% in Ile-Ife and 55–93% was observed in Enugu, Eastern Nigeria for male factor infertility (Uadia and Emokpae, 2015).

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the scavenging capacity of available antioxidants resulting in redox paradox. Sperm cells are vulnerable to ROS because of the abundance of polyunsaturated fatty acids in their plasma membrane and cytoplasm and limited antioxidant capacity and DNA repair system. Significant levels of ROS are required for maturation of spermatozoa, acrosome reaction, capacitation, hyperactivation, and sperm-oocyte fusion. Excessive ROS production, however, overwhelms the neutralizing capability of antioxidants (enzymatic and nonenzymatic) in the seminal plasma. ROS are formed as natural byproducts of oxygen during metabolism and have important roles in cell signaling and homeostasis. Sources of ROS can be endogenous or exogenous and body antioxidant defense mechanism aims to neutralize the harmful effects of these pro-oxidants molecules (Huang *et al.*, 2018).

Increased ROS along with decreased antioxidant defense result in redox imbalance, reduced sperm motility and sperm DNA damage. Spermatozoa are extremely prone to the deleterious effects of ROS due to the large amounts of unsaturated fatty acids found in their cell membranes. Reactive oxygen species promote peroxidation of lipids, resulting in intracellular oxidative burden. The sequence of events involves lipid peroxidation, loss of membrane integrity with increased permeability, reduced sperm motility, structural DNA damage, and apoptosis. Several intrinsic and extrinsic factors have been associated with increased OS in the male reproductive system (Huang *et al*., 2018).

Accordingly, the aim of this study was to evaluate the level of oxidative stress biomarkers among infertile men in kano.

## MATERIALS AND METHODS

### Study Design and Subjects

This was a cross-sectional comparative study design hospital-based study conducted at Abubakar Imam Urology Centre, Kano. Kano State is located in Northern Nigeria.

The study subjects were recruited from infertile male subjects attending Abubakar Imam Urology Centre, Kano. Age matched fertile men used as controls were recruited around Kano metropolis. Sixty (60) participants from the case group and sixty (60) from the control group were recruited for the detection of oxidative stress biomarkers.
Purposive sampling technique was applied for selection of the participant visiting the infertility and urology clinic weekly spanning for 12 months.
Ethical approval was obtained from the Ethics Committee of kano State Ministry of Health, Kano. (NHREC/17/03/2018).

**Data Collection**

Well-structured questionnaire was administered to the participants, Socio demographics such as age, History of infertility, occupation, Western education status, ethnicity, smoking status were collected. Weight and height (anthropometric) were collected by trained nurses following standard protocols. BMI was calculated by dividing the weight (kg) by the height (meters) squared

**Specimen Collection and Processing**

Four milliliters (4mls) of whole blood were collected from the median cubital vein using standard blood collection kits. The whole blood was collected in plain container, it was left to clot and centrifuged. The serum was separated from the plain container and transferred in to sample bottles for analysis of oxidative stress biomarkers (Malondialdehyde (MDA), Super Oxide Dismutase (SOD) and Glutathione Peroxidase (GPx).

**Analytical methods:** SOD and GPx were analyzed using enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc, (2019) for quantitative determination of SOD and GPx levels in human serum while Serum MDA was analyzed TBA chemical method. All chemicals and reagents were of analytical grade from Sigma Aldrich Chemical Company, USA.

**Statistical analysis**

The data was edited and summarized in Microsoft Excel spreadsheet. Missing values and outliers were identified and recorded appropriately. Normality test and tests of significance were conducted using GraphPad Prism version 8. Categorical variables from the participants' clinical attributes were summarized using frequency and percentage. Continuous variables were summarized as mean ± standard deviation. Two independent group comparison was made using independent sample t-test. Significance level was set at p value≤0.05 at 95% confidence interval.

## RESULTS

This study comprised of two (2) groups. Group one (1) were infertile men and group two (2) were fertile men as controls. Each group comprise of sixty (60) participants. Table 1 shows the Socio-demographic characteristics of the study participants. A total of 120 participants recruited for this study comprised sixty (60) infertile subjects and sixty (60) fertile as controls. The mean age of the patients was 36.68±5.3 and that of control was 36.47±6.64. There was significant increase in the BMI of the infertile group when compared with control (p=0.0287) as showed in table 1.

Table 2 shows clinical characteristics/risk factors of the infertile group. The duration of infertility among the infertile group are; less than 5 years 45(75%), those between 6-10 years are 14(23.3%) and those above 10 years 1(1.7%). On the nature of infertility, 48(80%) had primary infertility while 12(20%) had secondary infertility. Those with erectile dysfunction were 12(20%) while 48(80%) had no erectile dysfunction. From table 2 below, 11(18.3%) had family history of infertility while 49(81.7%) had no family history of infertility. The nature of the semen analysis are as follows; those with azoospermia 16(26.7%), those with oligospermia 42(70%) and those with oligo-teratospermia 2(3.3%). Among these infertile group 5(8.3%) are smokers while the remaining 55(91.7%) are non-smokers.

Serum MDA level was significantly higher in infertile group when compared with control ($p<0.0001$), while SOD and GPx showed significantly lower level in the infertile group when compared with control ($p=0.0389$ and $p<0.0001$ respectively) as shown in table 3

Table 1: Socio Demographics Characteristics of Study Participants

| **Parameter** | **Infertile (n=60)** | **Controls (n=60)** | **p-value** |
|---|---|---|---|
| Age (yrs) | 36.68±5.31 | 36.47±6.64 | p=0.8439* |
| BMI (kg/m$^2$) | 25.35(23.80-27.00) | 24.25(21.73-25.98) | p=0.0287$^{\#}$ |

n (%) = number of participants (percentage)
Age presented as mean ± SD.*Determined by independent sample t-test,
BMI presented as median and interquartile range. #Determined by Mann Whitney U test

Table 2: Clinical Characteristics/Risk Factors of the Study Participants

| **Parameter** | **Frequency** | **%** |
|---|---|---|
| Duration of infertility (years) | | |
| < 5 | 45 | 75.0 |
| 6-10 | 14 | 23.3 |
| >10 | 1 | 1.7 |
| Nature of infertility | | |
| Primary | 48 | 80.0 |
| Secondary | 12 | 20.0 |
| Erectile dysfunction | | |
| Yes | 12 | 20.0 |
| No | 48 | 80.0 |
| Family history | | |
| Yes | 11 | 18.3 |
| No | 49 | 81.7 |
| Semen analysis | | |
| Azoospermia | 16 | 26.7 |
| Oligospermia | 42 | 70.0 |
| Oligo-teratospermia | 2 | 3.3 |
| Smoking | | |
| Yes | 5 | 8.3 |
| No | 55 | 91.7 |

Table 3: Mean Comparison of Oxidative Stress Biomarkers (MDA, SOD and GPx) Among Infertile Men and the Control Group

| **Parameter** | **Infertile men (n=60)** | **Control group (n=60)** | **t-value** | **p-value*** |
|---|---|---|---|---|
| MDA (nmol/ml) | 1.71±0.39 | 0.95±0.14 | 14.18 | <0.0001 |
| SOD (IU/L) | 1.67±0.40 | 1.84±0.52 | 1.99 | 0.0389 |
| GPx (IU/L) | 54.26±13.79 | 72.03±15.29 | 6.65 | <0.0001 |

MDA: Malondialdehyde; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; Presented as mean ± SD.*Determined by independent sample t-test.

## DISCUSSION

In this study, the mean age of patients was 36.68±5.3 years, while the control group had a mean age of 36.47±6.64 years. This finding aligns with a similar study from Ilorin, North Central Nigeria (Jimoh et al., 2012). Notably, 48(80%) of the patients in this study had primary infertility, which is consistent with the findings of Geidam & Idrisa (2014) from Maiduguri, Northeastern Nigeria.

Our results showed a significant increase in serum Malondialdehyde (MDA) levels among infertile men compared to controls ($p<0.0001$). In contrast, antioxidant markers such as Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) were significantly lower in the infertile group ($p=0.0389$ and $p<0.0001$, respectively). These findings are consistent with the work of Olatunbosun et al. (2018), who also observed marked oxidative stress in infertile men in Ilorin, Nigeria. The increased MDA and decreased antioxidant levels in our study point to the presence of oxidative stress (OS) in the infertile group.

Oxidative stress (OS) is considered a leading cause of male infertility (Takeshima et al., 2021). While reactive oxygen species (ROS) are essential for the physiological function of sperm, excessive ROS levels can disrupt sperm function and lead to infertility. This disruption occurs through mechanisms such as lipid peroxidation and DNA damage (Huang et al., 2018).

In a similar study which focused on effects of ROS on sperm characteristics, Alahmar (2019) found that sperm characteristics were significantly impaired in infertile men with high ROS levels in their semen, as assessed by chemiluminescence. ROS can damage sperm DNA, causing strand breakage, base modifications, and chromatin cross-linking, which affect sperm quality and fertility. This finding further emphasized the implication of ROS and oxidative stress in male infertility.

Similarly, Fainberg & Kashanian (2019) reported that antioxidants in the seminal plasma of infertile men were significantly lower than in fertile controls. They also found that ROS levels in spermatozoa were negatively correlated with sperm quality. The pathophysiology of OS in male infertility involves increased ROS production coupled with a compromised antioxidant defense, leading to sperm DNA damage. Even though this finding focused on seminal plasma unlike in our study that focus on antioxidant levels in the blood, the two findings explained decreased antioxidant defense and development infertility in men.

ROS-induced sperm DNA damage is a critical factor in male infertility. This damage can have long-term consequences, including increased risks for childhood diseases such as neuropsychiatric disorders (e.g., autism and schizophrenia), dominant gene mutations (e.g., Apert syndrome), and childhood cancers like retinoblastoma (Takeshima et al., 2021). Moreover, oxidative stress is known to affect telomere integrity, which is essential for genome stability. Telomeres erode more rapidly under oxidative stress, contributing to chromosome instability, apoptosis, and cellular aging, all of which are linked to carcinogenesis (Barati et al., 2020).

Lipid peroxidation, a key consequence of oxidative stress, contributes to the generation of free radicals and lipid aldehydes, such as acrolein, 4-hydroxy-2-nonenal (4-HNE), and MDA. These substances are associated with DNA damage in both nuclear and mitochondrial genomes, resulting in shorter telomeres, the formation of 8-hydroxy-deoxyguanine (8-OHdG), and mitochondrial DNA fragmentation. Such damage can impair sperm motility, disrupt the sperm membrane, and hinder fertilization capacity. Furthermore, the accumulation of 8-OHdG can limit the DNA repair ability of sperm, leading to genomic instability and infertility. This genomic instability has been associated with an increased incidence of genetic aberrations in embryos, which can result in childhood cancers, neurodevelopmental disorders, and genetic mutations.

## CONCLUSION

In conclusion, oxidative stress plays a pivotal role in male infertility, contributing significantly to sperm dysfunction, DNA damage, and impaired spermatogenesis. The balance between reactive oxygen species (ROS) and antioxidants is crucial for maintaining normal sperm function, and an imbalance resulting from either excessive ROS production or insufficient antioxidant defense may lead to infertility. Studies have shown that oxidative stress is closely linked with various male reproductive pathologies, including varicocele, low semen quality, and poor sperm motility.

Addressing lifestyle factors that contribute to oxidative stress, such as smoking, poor diet, and environmental pollutants, may help reduce the incidence of male infertility linked to oxidative damage. Overall, oxidative stress remains a critical target for improving male fertility outcomes, and a deeper understanding of its mechanisms, along with genetic and environmental factors, will likely lead to more effective management strategies for male infertility.

## RECOMMENDATIONS

1. In the diagnosis of male infertility, it is recommended that oxidative stress biomarkers level should be evaluated to assess the oxidative stress.
2. Antioxidant's vitamins and mineral should be included as part of treatment regimen in order to prevent oxidative DNA damage.
3. Healthy lifestyle is recommended. Individuals should avoid tobacco smoking, marijuana and alcohol use as it damages sperm DNA.

**Conflict of Interest:** The authors have no conflict of interest to declare.

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