



(RESEARCH ARTICLE)



PLASMA AND SEMINAL ANTIOXIDANT PROFILE IN INFERTILE MEN

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World Journal of Advanced Research and Reviews, 2026, 32(01), 1667–1673

Publication history: Received on 11 September 2026; revised on 21 October 2026; accepted on 23 October 2026

Article DOI: <https://doi.org/10.30574/wjarr.2026.32.1.2674>

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ABSTRACT

Introduction: Infertility affects millions globally, with male factors contributing nearly 50% of cases. Oxidative stress from an imbalance between reactive oxygen species and non-enzymatic antioxidants is linked to impaired sperm quality, yet data on plasma and seminal zinc, copper, glutathione, and malondialdehyde in Nigerian men remain limited.

Objective: To compare seminal fluid parameters and plasma levels of selected non-enzymatic antioxidants (zinc, copper, reduced glutathione, malondialdehyde) between infertile and fertile men in Sokoto Metropolis.

Methodology: A case-control study of 24 infertile and 16 fertile men aged 30–55 years was conducted. Semen analysis followed WHO guidelines. Plasma and seminal glutathione, zinc, copper, and malondialdehyde were measured spectrophotometrically. Data was analysed by independent t-test ($p < 0.05$ was significant).

Results: Infertile men had significantly lower sperm concentration (12.30 ± 3.50 vs $33.25 \pm 4.22 \times 10^6/\text{ml}$) and morphology (4.92 ± 1.59 vs $9.81 \pm 1.13\%$; $p < 0.05$) compared to fertile men. Plasma zinc and copper were lower, while seminal zinc was higher and seminal copper lower in the infertile group ($p < 0.05$). Glutathione and malondialdehyde showed no significant differences among the two-patient population.

Conclusion: Infertile men exhibited disrupted zinc and copper homeostasis with impaired sperm concentration and morphology. Zinc and copper may serve as useful adjunct markers of sperm quality in male infertility evaluation.

Keywords: Male infertility, Oxidative stress, Zinc, Copper, Glutathione, Malondialdehyde

1 INTRODUCTION

Infertility is a life-altering problem that can have devastating consequences on the affected individuals' mental health, though not life-threatening (1). It is a global health issue, with an estimated 48 million couples and 186 million individuals affected worldwide (2). The World Health Organization (WHO) in 2022 referred to infertility as 'a failure to conceive after regular unprotected sexual intercourse for one year (1, 3). About 3-4 million of the 20-35 million couples that experience infertility in Africa were Nigerian (4). Cultural expectations often disproportionately burden women, who frequently experience stigmatization, discrimination, and social exclusion (5).

Contrary to long-standing belief, research has shown that male factors contribute almost 50% to infertility cases (6). The etiology of male infertility has primarily been associated with obesity, hypogonadotropic hypogonadism, and untreated infections. Smoking, alcohol consumption, and environmental exposures can impair male fertility through oxidative stress, hormonal disruption, testicular damage, and sperm DNA damage, adversely affecting sperm count, motility, and quality (6, 7). Excess production of reactive oxygen species (ROS) is increasingly recognized as a key mechanism underlying oxidative stress-related male infertility (8).

ROS are highly reactive oxygen-derived molecules that have an unpaired electron in their outer valence orbital, and they are also known as free radicals (9, 10). They play a regulatory role in activating transcription factors that drive key intracellular signaling pathways essential for normal sperm function, including spermatogenesis, capacitation, maturation, hyperactivation, chemotaxis, acrosome reaction, and sperm oocyte fusion (9, 11).

However, excessive ROS production leads to sperm injury because sperm plasma membranes are highly susceptible to oxidation due to high levels of polyunsaturated fatty acids, such as docosahexaenoic acid in the membrane, and a deficiency in cytoplasmic antioxidant enzymes (11). Excess ROS can also cause significant damage to spermatozoa mitochondria and nuclear DNA, affecting fertilization, embryonic development, and pregnancy in women (12). Errors during spermatogenesis result in abnormal spermatozoa with high cytoplasmic retention and major sources of endogenous ROS in semen (13).

These high ROS levels are closely associated with defects in spermatozoa function, particularly motility (9). Contaminated leukocytes, principally neutrophils and macrophages, are another significant source of ROS in sperm. In fact, neutrophils, when stimulated, generate toxic reactive oxygen intermediates, such as Superoxide anion (O_2^-) and Hydrogen peroxide (H_2O_2) (14). Exogenous sources of ROS include environmental factors such as high temperature, electromagnetic radiation, pollution, and pesticides.

Lifestyle factors such as advanced age, alcohol consumption, smoking, stress, obesity, and poor diet are other external sources of ROS (15). ROS is neutralized by enzymatic and non-enzymatic antioxidants produced intracellularly (12). Spermatozoa with insufficient cytoplasm (13), lost during spermatogenesis, can only generate a few ROS scavengers, relying on antioxidants contained in seminal fluid such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (produced by the prostate) as well. Many non-enzymatic antioxidants, including reduced glutathione (GSH), zinc, and copper, are usually introduced from the blood (16, 17).

Infertile men are prone to oxidative stress due to an overwhelmed physiological mechanism for managing free radicals, whereas fertile men have lower seminal ROS levels (15). Research has shown a strong link between seminal antioxidants, oxidative damage, and sperm quality, which serve as a key indicator of oxidative stress and antioxidant capacity. However, the relationship between seminal and blood antioxidant profiles and their impact on sperm quality remains insufficiently characterized in many African populations. Specific antioxidants were associated with selected seminal parameters, suggesting their potential utility as biomarkers for assessing sperm quality. This study aimed to determine the levels of malondialdehyde (MDA), reduced glutathione (GSH), zinc (Zn), and copper (Cu) in the blood plasma and seminal fluid of infertile men and to investigate their correlation with semen parameters in plasma and seminal fluid.

2 MATERIALS AND METHODS

2.1 Study design

This was a case-control study. A structured questionnaire was used to obtain the participants' sociodemographic and clinical data. 5 mL of blood and seminal samples were collected for analysis. The study was conducted in a secondary and tertiary health facility in Sokoto, Nigeria. Informed consent was obtained from all the participants.

2.2 Sample size

This study's sample size was calculated using a standard formula for determining the minimum sample size, resulting in a sample size of 24. A total of 16 fertile and 24 infertile adult males were recruited as controls and test subjects, respectively.

2.3 Participant selection

Infertile males aged 30-55 were included as case subjects, while those who did not provide informed consent were excluded.

2.4 Sample collection, processing, and data analysis

Participants were instructed to abstain from sexual activity for 5 days. Semen was collected through masturbation into a 50 mL sterile polystyrene container, and a 5 mL blood sample was obtained aseptically via venipuncture into a heparinized tube. The seminal fluid was processed following WHO guidelines, centrifuged at 1000g for 10 minutes to obtain seminal plasma, and stored at -45 °C. The blood samples were centrifuged at 3000 rpm for 5 minutes to obtain plasma, which was separated and stored at -45 °C.

Both seminal and blood plasma samples were analyzed for malondialdehyde (MDA), reduced glutathione, zinc, and copper levels using a spectrophotometric assay. Data were input into an Excel spreadsheet and analyzed using SPSS version 27.0. Results were presented as mean $\pm$ standard error of the mean (SEM). An independent-samples t-test was used for mean comparisons, with a *p-value* $\leq$ 0.05 considered statistically significant.

3 RESULTS

The sociodemographic characteristics of the study participants, including age group, marital status, tribe, occupation, and educational level, are presented in **Table 1**. Participants were aged 30-35 years with 47.5% in the 30–39-year range. The cohort was predominantly Hausa (57.5%), married (90%), civil servants (57.5%), and tertiary-educated (62.5%)

Table 1: Distribution of study participants

Parameters		Test N= 24	Control N= 16	Total N= 40
AGE GROUP	30-39	13	6	19
	40-49	6	5	11
	50 above	5	5	10
MARITAL STATUS	Single	1	0	1
	Married	21	15	36
	Divorced	1	0	1
	Widower	1	1	2
TRIBE	Hausa	15	8	23
	Yoruba	2	4	6
	Igbo	4	3	7
	Others	3	1	4
	Farming	1	0	1

OCCUPATION	Business	6	10	16
	Civil services	17	6	23
EDUCATION	None	1	2	3
	Primary	1	0	1
	Secondary	6	5	11
	Tertiary	16	9	25

Table 2 showed the result of the Seminal Fluid Analysis with the semen concentration (12.30 ± 3.50) (33.25 ± 4.22) and morphology (4.92 ± 1.59) (9.82 ± 1.13), statistically and significantly lower when the test was compared with the control.

Table 2: Comparison of seminal fluid analysis (SFA) of the study participants.

SFA	Test (n = 24)	Control (n = 16)	p- value
SV (ml)	2.59 ± 0.30	2.93 ± 0.25	$P > 0.05$
SC ($\times 10^6$ ml)	12.30 ± 3.50	33.25 ± 4.22	$P < 0.05$
SMT (%)	22.50 ± 4.43	31.25 ± 4.30	$P > 0.05$
SMP (%)	4.92 ± 1.59	9.81 ± 1.13	$P < 0.05$

Key: SV = semen volume; SC = semen concentration; SMT = semen motility; SMP = semen morphology; n = number of participants; p-values ≤ 0.05 were considered statistically significant.

Table 3 showed the results of the biochemical analysis. The mean values for each marker in Test Plasma and Semen were compared with their respective controls. The plasma values of zinc (62.22 ± 7.06) (96.83 ± 7.6) and copper (292.73 ± 23.85) (394.4 ± 37.9) were statistically and significantly higher in the control group. The semen values of zinc (606.03 ± 70.40) (380.24 ± 57.51) and copper (131.41 ± 15.46) (279.33 ± 6.60) in the control group were also found to be statistically significant.

Table 3: Comparison of blood plasma test and control; seminal test and control of selected biochemical parameters of the study participants.

Parameters	Test (n = 24)	Control (n = 16)	p- value
Plasma			
GSH ($\mu\text{mol/L}$)	26.02 ± 1.98	28.65 ± 1.94	$P > 0.05$
Zn($\mu\text{g/dl}$)	62.22 ± 7.06	96.84 ± 7.64	$P < 0.05$
Cu($\mu\text{g/dl}$)	292.74 ± 23.85	394.43 ± 37.91	$P < 0.05$
MDA (nmol/ml)	9.07 ± 0.30	9.30 ± 0.39	$P > 0.05$
Semen			
GSH ($\mu\text{mol/L}$)	355.29 ± 6.82	367.08 ± 12.60	$P > 0.05$
Zn($\mu\text{g/dl}$)	606.03 ± 70.40	380.24 ± 57.51	$P < 0.05$
Cu ($\mu\text{g/dl}$)	131.41 ± 15.46	279.33 ± 60.59	$P < 0.05$
MDA (nmol/ml)	8.43 ± 0.45	8.01 ± 0.54	$P > 0.05$

Key: Values are mean $\pm$ Standard error of mean; GSH = reduced glutathione; Zn = zinc; Cu = copper; MDA = malondialdehyde; n = number of participants; p-values ≤ 0.05 were considered statistically significant.

Table 4 showed the comparison between the seminal and plasma of the test group. The mean value of GSH (355.29 ± 6.82) (26.02 ± 1.98) and Zinc (606.03 ± 70.40) (62.22 ± 7.07) were statistically significantly higher in semen, while copper (131.41 ± 15.46) (292.73 ± 23.85) was statistically significantly higher in plasma.

Table 4: Comparison of the seminal and plasma tests for selected biochemical markers

Parameter	Test semen (n = 24)	Test plasma (n = 24)	p- value
GSH (μmol/L)	355.29± 6.82	26.02± 1.98	P < 0.05
Zn(μg/dl)	606.03± 70.40	62.22± 7.07	P < 0.05
Cu(μg/dl)	131.41± 15.46	292.73± 23.85	P < 0.05
MDA (nmol/ml)	8.43± 0.45	9.07± 0.30	P > 0.05

Key: Values are mean ± Standard error of mean; GSH = reduced glutathione; Zn = zinc; Cu = copper; MDA = malondialdehyde; n = number of participants; p-values ≤ were considered statistically significant.

4 DISCUSSION

Infertility affects approximately 15–20% of couples worldwide, with male factors contributing to about 50% of cases. Reactive oxygen species (ROS) play a central role in the pathophysiology of male infertility, with elevated seminal ROS levels reported in approximately 30–80% of subfertile men (18). Antioxidant defence systems maintain ROS homeostasis; however, excessive ROS production can overwhelm these defences, triggering lipid peroxidation, sperm DNA damage, and impaired sperm function and fertilising capacity (19). Semen analysis remains the primary diagnostic test for male infertility; however, it may not fully capture functional sperm abnormalities (18). Therefore, complementary biochemical assessments are needed to provide a more comprehensive evaluation of male reproductive function.

Semen analysis revealed significantly lower sperm concentration (12.30 ± 3.50) and normal sperm morphology (4.92 ± 1.59) in the test group compared with controls. However, semen volume and motility did not differ significantly between groups ($p > 0.05$). These findings are consistent with reports by Umar *et al.* and Igibah *et al.* (20, 21) suggesting impaired sperm concentration and morphology among affected participants. Similar to our findings, Umar *et al.* and Igibah *et al.* reported oligozoospermia and teratozoospermia, with sperm morphology below 4% associated with reduced fertility potential. Both studies also identified asthenozoospermia, whereas motility reduction in our study was not statistically significant. These similarities may reflect shared regional environmental exposures, including heat and pollution, while differences in motility findings may be attributable to variations in sample size, participant characteristics, and laboratory procedures.

In this study, plasma non-enzymatic antioxidant levels, Zn, and Cu were significantly lower in the test group. Seminal copper was also significantly reduced, whereas seminal zinc was significantly elevated. Despite these changes, GSH and MDA levels did not differ significantly between groups in either plasma or semen, suggesting a complex relationship between antioxidant depletion and oxidative stress.

Our findings are consistent with those of Emokpae and Bamidele (22), Akinloye *et al.*, and Abiodun and Moronkeji (22–24) who reported significantly lower zinc and copper levels in infertile males than in controls. However, our study found significantly higher seminal zinc levels in the infertile group, contrary to the findings of Emokpae and Bamidele. This discrepancy may reflect differences in sample size, participant characteristics, or zinc supplementation among infertile participants, although these explanations remain speculative. Blood and semen contain essential trace elements, including Zn and Cu, which play vital roles in normal physiological functions and male reproductive health (24). Zinc is particularly important for testicular development, spermatogenesis, sperm integrity, and fertility. Evidence suggests that zinc deficiency may impair spermatogenesis and contribute to male infertility, while excessive zinc levels may disrupt oxidative balance and induce adverse effects on sperm function (25).

Cu is important for several metalloenzymes of the antioxidant system. The ionic form can rapidly form free radicals and induce oxidation, which could be harmful to many body cells at high levels, including human spermatozoa (24). Even though GSH and MDA have been associated with increased ROS generation, Palani and Alahmar (26) also reported no statistical intergroup differences in GSH and MDA levels between infertile and fertile men. The absence of MDA elevation despite antioxidant depletion may be because samples were processed immediately as they were received, preventing cold shock and oxygen-induced lipid peroxidation.

Comparison of plasma and seminal biochemical parameters among infertile participants revealed significantly higher seminal GSH and zinc levels, whereas copper levels were significantly higher in plasma. No statistically significant difference was observed in MDA levels between the two compartments. Akinloye *et al.* (23) reported significantly higher Cu levels in plasma than in semen, consistent with our findings, but observed no significant difference in Zn levels between the two biological fluids.

A major limitation of this study was its small sample size, largely attributable to the limited number of infertile men seeking medical evaluation, which prolonged participant recruitment. Community sensitisation and awareness campaigns may help address this challenge by encouraging early consultation and participation in infertility research. Despite this limitation, the findings highlight the potential value of seminal biochemical parameters as complementary markers to conventional seminal fluid analysis in evaluating male infertility.

5 CONCLUSION

In conclusion, infertile men in Sokoto Metropolis demonstrated significantly reduced sperm concentration and morphology, alongside altered zinc and copper levels in plasma and seminal fluid. These findings suggest that disturbances in trace element homeostasis may be associated with impaired sperm quality and male infertility. Although glutathione and malondialdehyde levels did not differ significantly between infertile and fertile men, the observed alterations in zinc and copper highlight the potential relevance of biochemical assessment in infertility evaluation. Therefore, measuring selected trace elements alongside routine semen analysis may provide complementary information for assessing male reproductive health. However, further studies involving larger populations are warranted to clarify the relationship between antioxidant status, trace element balance, and male infertility.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The technical assistance of the scientists in the Department of Chemical Pathology, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto is well appreciated

Disclosure of conflict of interest

The authors declare that there is no known competing financial interest or personal relationship that could have appeared to influence this work.

Statement of ethical approval

We followed the ethical standards of the institutional research ethics committee and the 1964 Helsinki Declaration. The Institution's Research Ethics Committee approved the study under approval numbers UDUTH/HREC/2019/No.310 and SHS/SUB/VOL.1.

Statement of informed consent

Subjects recruited into the study gave a written informed consent prior to the sample collection by filling in a standard informed consent form.

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