Levels of Antioxidants and Body Mass Index in Infertile Female Subjects in Sokoto, Nigeria

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ABSTRACT

Infertility is a major reproductive health challenge worldwide and is associated with multiple physiological and biochemical abnormalities, including oxidative stress, nutritional deficiencies, and abnormal body mass index (BMI). Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the antioxidant defense capacity of the body, resulting in cellular and tissue damage. This study investigated the levels of antioxidant enzymes, antioxidant vitamins, and BMI in infertile female subjects attending the Obstetrics and Gynecology (O and G) clinic of Usmanu Danfodio University Teaching Hospital, Sokoto and compared them with fertile controls. Antioxidant enzymes assessed included glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), while antioxidant vitamins A, C, and E were also measured. BMI was calculated using standard anthropometric methods. Results showed that GPx and CAT activities were significantly lower in infertile subjects compared with fertile controls ($p < 0.05$), while SOD showed no statistically significant difference. Antioxidant vitamins A, C, and E were significantly reduced in infertile subjects. In addition, BMI was significantly higher in infertile women compared to fertile controls. These findings suggest that oxidative stress, antioxidant depletion, and elevated BMI may contribute significantly to female infertility.

Key words: Antioxidants, Body mass index (BMI), Enzymes, Infertile Female, Vitamins

INTRODUCTION

Infertility is known to be the inability of a sexually active, non-contraceptive couple to achieve pregnancy after 12 months or more in regular unprotected sexual intercourse. It is a growing global health issue affecting millions of women of reproductive age. WHO (2023) has affirmed that approximately 48 million couples and 186 million individuals worldwide were affected making it a major reproductive and psychosocial challenge. In many African societies, including Nigeria, infertility is associated with emotional trauma, stigma, marital instability, and social discrimination. Agarwal et al. (2017) affirmed that female infertility may result from ovulatory disorders, tubal blockage, uterine abnormalities, hormonal imbalance, endometriosis, and unexplained causes.

Recent studies by Daraghmeh et al. (2025) highlighted oxidative stress as a major factor influencing female reproductive dysfunction. Ojo et al. (2023) has emphasized that oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and antioxidant defense systems, leading to cellular damage. In female reproduction, ROS are highly reactive molecules generated during normal metabolism and are involved in important reproductive processes such as folliculogenesis, ovulation, fertilization, and implantation. However, Daraghmeh et al. (2025) equally opined that excessive ROS production can cause lipid peroxidation, DNA damage, protein oxidation, and apoptosis, which impair ovarian function and reproductive outcomes.

Ghaffari et al. (2023) in their study stated that antioxidant defense mechanisms include enzymatic antioxidants such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), as well as non-enzymatic antioxidants like vitamins A, C, and E. They further stated that GPx detoxifies hydrogen and lipid

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peroxides, SOD converts superoxide radicals to hydrogen peroxide, while CAT further breaks down hydrogen peroxide into water and oxygen. Vitamins A, C, and E act as free radical scavengers and protect reproductive tissues from oxidative injury.

Body mass index (BMI) is another important factor influencing female fertility. Both underweight and overweight women may experience infertility due to hormonal disturbances and ovulatory dysfunction. Shen et al. (2024) observed that obesity has been associated with insulin resistance, chronic inflammation, and increased oxidative stress, all of which can impair ovulation and implantation. Ma et al. (2025) emphasized that excess adipose tissue increases ROS production and alters reproductive hormone metabolism, leading to menstrual irregularities and poor fertility outcomes. Female obesity and underweight are known to adversely affect fertility through alterations of hormone patterns and the menstrual cycle. Elizabeth et al. (2009) in their recent work investigated the effect of increasing maternal BMI on nitrative stress, antioxidant markers of oxidative stress, and protein oxidation in the placenta concluded that placental nitrative stress increased with maternal body weight.

Vitamin E is a fat-soluble vitamin found in the ovary especially in follicular fluid. Many studies including Chitrah et al. (2008) have shown the role of vitamin E as an antioxidant within the body. In cells and organelles, vitamin E is the first line of defense against lipid peroxidation. Findings from Rigotti et al. (2007) have shown the roles of vitamin E in RBC flexibility and longevity in immune function, and its positive effects on fertility. Savita et al. (2008) have also shown that increased OS is associated with decrease in antioxidants and fertility. Vitamin E directly neutralizes superoxide anion, hydrogen peroxide, and hydroxyl radical. It is called a chain breaking antioxidant because of its ability to terminate a free radical chain reaction. Ruder et al. (2009) have shown that Vitamin E increases the number of embryos developing into the expanded blastocysts and increases the viability of embryos exposed to heat shock.

Vitamin C, also known by the terms ‘ascorbic acid’, ‘L-ascorbate’ or ‘ascorbate’, is one of the essential nutrients to be provided through the diet as stated by Paszkowszki et al. (1999). Mezatti et al. (1996) observed that vitamin C levels in the plasma are inversely proportional to age, that is, there is a 20% decrease in plasma concentration with an increase in age. In addition to its antioxidant property, Kazmierzczak et al. (2020) had found that vitamin C has a therapeutic effect in treating cancer. Luck et al. (2020) also reported that vitamin C in reproductive health is primarily due to its three primary functions, collagen biosynthesis, its role in the synthesis of steroid and peptide hormones, and its antioxidant property. Frei et al. (2019) also reiterated the role of vitamin C in scavenging free radicals and protects cells from the deleterious effect of ROS and metal-oxygen complexes on the gametes during gametogenesis and fertilization. Vitamin C has a biological role in the menstrual cycle and helps in various reproductive processes like follicle development, production of the hormones by the ovary, tissue remodeling, repair of the follicle after ovulation, and protection of the oocyte from oxidative damage.

According to Abdollahifar et al. (2019) the ovary is considered a pool of L-ascorbate, and its turnover forms evidence for the influence of vitamin C in reproduction. Fatemi et al. (2009) observed that large measures of vitamin C are present in theca interna, granulosa cells of the mature graafian follicle, and luteinizing granulosa cells, due to the high reducing potential of the ascorbate to protect the oocyte from damage caused by ROS. Graafian follicle and corpus luteum are actively involved in synthesis of steroid hormones, which include: estrogen and progesterone. Hence, Moser et al. (1992) concluded that the accumulation of *L*-ascorbate in these two sites is helpful since the hydroxylation requires ascorbate in the steroidogenesis pathway. Few studies including Padoyatti et al. (2016) have found that collagen is necessary for developing ovarian follicles; it is also essential to repair the ruptured Graafian follicle after the ovulation and the luteinization of the ovulated follicle. Hence, the role of *L*-ascorbate in the synthesis of collagen in the ovary is observed.

According to Fieder et al. (2014) the antioxidant effects of carotenoid, fat-soluble vitamin A, include the absorption of peroxyl radicals and singlet oxygen. Vitamin A was shown by Vaskova et al. (2014) to be essential in several physiological processes, especially in reproduction, the immune system via an inflammatory response as it is related to the expression of cyclooxygenases, the generation of nitric oxide, prostaglandins, cell differentiation, vision, and bone metabolism. Vitamin A participates in signaling during

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embryogenesis as reported by Batiogly et al. (2012) via the induction of activation of retinoic acid receptor (RAR) and retinoid X receptor (RXR) transcription factors. The binding of RAR and RXR to RARE sites in DNA as reported by Metzler and Sandell, (2016) activates the expression of developmental genes. It is this positive effect on embryo development as confirmed by a study conducted by Wdowiak and Fillip (2020) that reflected the addition of retinol to the blastocyst's culture media which improved the development of rabbit embryos.

Despite the increased evidence on the consequences of oxidative stress and obesity in infertility, there is limited information on the combined assessment of the antioxidant enzymes, antioxidant vitamins, and BMI among infertile female subjects attending Obstetrics and Gynaecology (O and G) clinics, especially in developing countries. Therefore, this study was undertaken to evaluate the levels of antioxidant enzymes, antioxidant vitamins and body mass index in infertile female subjects compared with the fertile controls, in order to provide useful information for improved infertility management.

Objectives of the Study

1. Determine the levels of antioxidant enzymes (SOD, CAT and GPx) in infertile female subjects and fertile controls
2. Assess the levels of antioxidant vitamins A, C and E in infertile female subjects compared with fertile controls
3. Determine the body mass index (BMI) of infertile female subjects and fertile controls
4. Compare antioxidant enzymes and vitamins between infertile female subjects and fertile female controls
5. Determine the possible role of oxidative stress and antioxidant depletion in female infertility.

MATERIALS AND METHODS

Study Design

The study was a case control study conducted at the gynaecological clinic of Usmanu Danfodiyo University Teaching Hospital, (UDUTH) Sokoto.

Classification of Infertility in the Study

Primary Infertility were females who had never conceived despite regular unprotected sexual intercourse for at least one year.

Secondary Infertility were females who had conceived previously but were unable to achieve another pregnancy after at least one year of regular unprotected intercourse.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

Test Group;

Females diagnosed with infertility by a gynecologist

Females who consented to participate

Controls Group;

Apparently healthy fertile women with at least one successful pregnancy

Females within the same reproductive age range as test group

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Females who consented to participate

Exclusion Criteria

Test Group;

Menopausal stage individuals

Male factor infertility

Control Group;

Pregnancy

Females who did not consent to participate

Sample Size

The ante-natal and post-natal clinic delivered 3,000 births per year with 12,000 to 15,000 visits annually. Family planning units received 30 patients daily with 7,500 to 7,800 annually (info@uduth.org.ng). The sample size was determined using Cochran formula for cross-sectional studies. Margin of error was set at 5% (0.05). Standard normal deviation was set at 95% confidence interval (1.96). Thus, the minimum required sample size was calculated approximately 50 for each group.

The study included fifty infertile (test group) females and fifty fertile (control) females all of reproductive age. The subjects were selected under defined criteria (inclusion criteria stated below). The test group were recruited with newly diagnosed and follow-up patients who had general infertility problem either primary or secondary. New infertility cases were recruited and followed up to known cause of infertility by tagging their files. 50 healthy fertile female volunteers matched by age with the study group and a history of regular menstrual cycles lasting from 28 – 30 days, recruited from the family planning unit at presentation before the commencement of family planning constituted the control group.

These subjects were referred to the laboratory for pregnancy test. Patients with negative pregnancy test were recruited with their consent by filling in questionnaire which was interviewer-administered and venous blood taken. All the subjects (both test and control) had their history taken and physical examination conducted. After obtaining their consent, the relevant clinical findings and results of investigations were documented using a structured questionnaire, which was interviewer-administered. Weight and height of subjects were taken to determine their BMI. Patients gave full consent and the interview was conducted in the native language (Hausa) or English.

Matching Procedures for Controls

Fertile controls were age-matched with infertile subjects to minimize confounding variables associated with age-related oxidative stress and reproductive function.

Participants were classified according to the World Health Organization BMI classification:

Underweight: <18.5 kg/m²

Normal weight: 18.5–24.9 kg/m²

Overweight: 25.0–29.9 kg/m²

Obese: ≥30 kg/m²

Sample Collection and Analysis

About 5 mL of venous blood was collected aseptically from each participant into plain sample bottles. Blood samples were allowed to stand undisturbed to clot and then centrifuged at 4000 rpm for 10 minutes to obtain a

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clear serum. The serum samples were stored at −20°C until analysis. The serum samples were analyzed for antioxidant enzymes (GPx, SOD, CAT) and antioxidant vitamins (A, C, and E). GPx activity was determined according to the method described by Paglia and Valentine (1967), using hydroperoxide as substrate. SOD activity was determined according to the method reported by Marklund (1980). Catalase activity was determined according to Johansson and Borg (1998) cayman catalase assay kit. Vitamin A was determined using Rutkowski et al. (2006). Vitamin C was assayed by method of Natelson (1971) while vitamin E was assayed using Hashim and Schuttrnger (1966).

Laboratory Quality Assurance Protocols

To ensure reliability and accuracy of results, all reagents were prepared according to manufacturer specifications. Analytical instruments were calibrated before analysis and Standard operating procedures (SOPs) were strictly followed. Quality control sera and standards were analyzed alongside samples. All assays were performed in duplicates to ensure reproducibility. Samples were analyzed within recommended storage periods to prevent degradation. Laboratory personnel adhered to standard biosafety and quality control guidelines.

Chemicals and Reagents

Analytical graded chemicals and reagents were used for this research. Catalase assay kit (item number: 707002), superoxide dismutase kit (item number: 706002), and Glutathione peroxidase kit (item number: 703102) were all products of Cayman Chemical Company, USA. Vitamin A, C and E were all product of Lab Tech Chemicals, India.

Equipment

Micropipette reader RT 2100C Rayfo Life and Analytical Science Company Limited, China was used to take absorbance of analysis involving kits. Analysis of minerals was carried out using Atomic absorption spectrophotometer (AAS) AA240FSAAS (Varian Medical Systems Inc. USA), while vitamins analysis was carried out using Spectrophotometer (Optima sp-300), United States of America.

Ethical Considerations and Informed Consent Procedures

Ethical approval for the study was obtained from the Ethical Committee of Usmanu Danfodiyo University Teaching Hospital. All participants were adequately informed about the purpose and procedures for the study. Consent was obtained from each participant before sample collection. Participants were assured of confidentiality of their data.

Determination of BMI

The height and weight of each participant were measured using a stadiometer and weighing scale, respectively. BMI was calculated using the formula:

$BMI = weight(kg)/height(m^2)$

Statistical Analysis

Results were expressed as mean $\pm$ SEM by using instat software (version 3.0) San Diego, USA. Differences between group means were determined using independent sample t-test. One-way ANOVA, Tukey-Kramer multiple comparison test was used to check for significant difference in biochemical parameters among factors identified in female infertility. The significance level was set at $P < 0.05$.

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RESULTS

Table 1: Antioxidant enzyme activities and BMI in fertile and infertile female subjects

Parameters	Infertile subjects	fertile (control) subjects	P-value
GPx (nmol/min/ml)	7.66 ± 0.13*	25.24 ± 0.55	0.0001
SOD (U/ml)	0.51 ± 0.03	0.65 ± 0.05	0.0657
CAT (nmol/min/ml)	24.65 ± 2.20*	104.14 ± 2.24	0.0001
BMI (kg/m^2)	29.87 ± 0.79*	24.74 ± 0.41	0.0010

*Values differ from the control significantly at P<0.05. values expressed as mean ± SEM, GPx-gluthatione peroxidase, SOD-superoxide dismutase, CAT-catalase, BMI- body mass index

Table 2: Levels of antioxidant vitamins (A, C and E) and BMI in fertile and infertile female subjects

Parameters	Infertile subjects	fertile (control) subjects	P-value
Vitamin A (mg/dl)	0.61 ± 0.05*	0.79 ± 0.06	0.0235
Vitamin C (mg/dl)	1.67 ± 0.11*	2.15 ± 0.07	0.0005
Vitamin E (mg/dl)	0.23 ± 0.03*	0.66 ± 0.04	0.0001
BMI (kg/m^2)	29.87 ± 0.79*	24.74 ± 0.41	0.0010

*Values differ from the control significantly at P<0.05. values are expressed as mean ± SEM.

DISCUSSION

The present study demonstrated significantly lower levels of antioxidant enzymes (GPx and CAT), antioxidant vitamins (A, C, and E), and significantly higher BMI among infertile female subjects compared with fertile controls. These findings strongly suggest the involvement of oxidative stress and abnormal body weight in the pathogenesis of female infertility. The significantly reduced GPx and CAT activities observed in table 1 among infertile women indicate impairment in enzymatic antioxidant defense mechanisms. GPx and CAT are essential antioxidant enzymes responsible for detoxifying hydrogen peroxide and lipid peroxides generated during oxidative metabolism. Reduction in these enzymes may lead to excessive accumulation of reactive oxygen species (ROS), resulting in oxidative damage to ovarian tissues, oocytes, and reproductive cells. Similar findings were reported in a recent review by Ilesanmi and Ilesanmi (2025), in Akure, Nigeria which highlighted reduced antioxidant enzyme activity as a major contributor to infertility.

The findings of this study also agree with reports from Tanzania, where infertile women demonstrated altered oxidative stress biomarkers and increased obesity compared with fertile controls (Kasililika et al., 2021). Likewise, a recent study by Olszak-Wąsik et al. (2026) in Poland identified BMI as one of the strongest determinants of oxidative stress imbalance among women undergoing infertility treatment. The study showed that increased BMI was associated with reduced superoxide dismutase activity, increased lipid peroxidation, and decreased antioxidant capacity.

Although SOD activity was lower in infertile women in the present study, the reduction was not statistically significant. This may indicate partial preservation of SOD activity or the presence of compensatory antioxidant mechanisms. Similar findings have been reported in previous studies by Ghaffari et al. (2023) on unexplained infertility and oxidative stress, where some antioxidant enzymes showed variable responses depending on disease severity, nutritional status, and obesity levels.

The significantly lower levels of vitamins A, C, and E among infertile women as shown in table 2 suggest depletion of non-enzymatic antioxidant reserves due to excessive ROS generation. Vitamin E protects

membrane lipids against peroxidation, vitamin C neutralizes aqueous free radicals and regenerates vitamin E, while vitamin A supports epithelial and reproductive tissue integrity. Reduced levels of these vitamins may impair follicular maturation, fertilization, and embryo implantation. Similar reductions in antioxidant vitamins have been reported in studies among infertile women in Nigeria and other developing countries. Daraghmeh et al. (2025) reported that vitamin E protects cell membranes against lipid peroxidation, vitamin C scavenges aqueous free radicals and regenerates oxidized vitamin E, while vitamin A maintains reproductive tissue integrity and cellular differentiation.

The significantly elevated BMI observed among infertile female subjects indicated overweight or obesity, which may have contributed to infertility through endocrine disruption, insulin resistance, chronic inflammation, and oxidative stress. In addition, Shen et al. (2024) observed that obesity increases systemic inflammation and oxidative stress, thereby worsening antioxidant depletion and reproductive dysfunction. Obesity has been associated with menstrual irregularities, anovulation, altered estrogen metabolism, and poor reproductive outcomes. Recent international evidence has shown that increasing BMI shifted the body toward a pro-oxidative state characterized by impaired antioxidant enzyme activity and increased oxidative damage.

Furthermore, oxidative stress and BMI appear to interact synergistically in female infertility. Ma et al. (2025) stated that excess adipose tissue produces inflammatory cytokines and ROS, which may damage reproductive tissues and alter endometrial receptivity. This relationship supported previous findings that obesity-related oxidative stress negatively affects ovarian reserve, oocyte quality, and implantation success. Therefore, routine evaluation of oxidative stress markers and BMI may be beneficial in infertility management. These findings agree with recent studies by Saleh et al. (2024) suggesting that antioxidant supplementation and weight management can improve reproductive outcomes in infertile women.

CONCLUSION AND RECOMMENDATION

This study has demonstrated that infertile female subjects attending the Obstetrics and Gynecology clinic exhibited significantly reduced levels of key antioxidant enzymes, including glutathione peroxidase and catalase, as well as markedly lower concentrations of antioxidant vitamins A, C, and E when compared with fertile controls. These findings indicated a compromised antioxidant defense system and increased oxidative stress, which may adversely affect ovarian function, oocyte quality, fertilization, and implantation. Furthermore, the significantly elevated body mass index observed among infertile female subjects suggests that overweight and obesity may exacerbate infertility through hormonal imbalance, chronic inflammation, insulin resistance, and increased generation of reactive oxygen species. Therefore, oxidative stress, antioxidant depletion, and elevated BMI appear to be important interrelated factors in the pathogenesis of female infertility. Early evaluation of these parameters, coupled with antioxidant supplementation, dietary improvement, and effective weight management strategies should be considered as they may play a collective vital role in improving fertility outcomes and enhancing reproductive health among infertile women.

Study Limitations and Potential Confounding Factors

The important findings of this study notwithstanding, some of the acknowledged limitations include: lifestyle-related confounding factors such as dietary habits, alcohol consumption, physical activity, psychological stress, environmental exposure, and socioeconomic status that may have influenced antioxidant levels and oxidative stress markers. Additionally, hormonal profiles, insulin resistance, and polycystic ovarian syndrome (PCOS) were not extensively evaluated in this study and may have influenced the observed biochemical parameters. BMI also has limitations as a measure of adiposity because it does not distinguish between visceral fat and lean body mass.

Future studies involving multicenter recruitment, detailed hormonal evaluation, dietary assessment, and longitudinal follow-up are recommended to better clarify the relationship between oxidative stress, BMI, and female infertility.

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