ISA Journal of Medical Sciences (ISAJMS)

Homepage: https://isapublisher.com/isajms/

Volume 3, Issue 4, Jul-Aug, 2026 ISSN: 3049-1746

Comparative Serum Vitamin D, Calcium, and Magnesium Levels among Fertile and Infertile Women Attending a Tertiary Hospital in Northwestern Nigeria

Abubakar Mayana Usman[1], Bello Alhaji Mohammed[1], Umar Abdullahi[2], Abidemi Oyaromade[1], Anas Yakubu Ibrahim[3], Mansur Muhammad Birnin Kebbi[4], Muhammad Aliyu Abbas[5]

[1]*Department of Obstetrics and Gynaecology, Federal Medical Center, Gusau, Zamfara State, Nigeria.*
[2]*Department of Internal Medicine, Federal Medical Center / Federal University Gusau, Zamfara State, Nigeria.*
[3]*Department of Psychiatry, Federal Teaching Hospital, Birnin Kebbi, Kebbi State, Nigeria.*
[4]*Department of Obstetrics and Gynaecology, Federal Medical Center, Gusau, Zamfara State, Nigeria.*
[5]*Deparment of Chemical Pathology, Federal Teaching Hospital, Birnin Kebbi, Kebbi State, Nigeria.*

Received: 25.06.2026 | **Accepted:** 23.07.2026 | **Published:** 22.08.2026

***Corresponding Author:** Abubakar Mayana Usman

DOI: *10.5281/zenodo.22055603*

Abstract **Original Research**

Background: Vitamin D, calcium, and magnesium play complementary roles in female reproductive physiology, yet evidence regarding their combined association with infertility remains limited in sub-Saharan Africa. This study compared serum concentrations of these micronutrients between fertile and infertile women attending a tertiary hospital in northwestern Nigeria and evaluated factors associated with their levels.

Methods: A hospital-based comparative cross-sectional study was conducted among 200 women (100 infertile and 100 fertile) attending Usmanu Danfodiyo University Teaching Hospital, Sokoto. Sociodemographic and clinical data were collected using structured questionnaires. Serum 25-hydroxyvitamin D, calcium, and magnesium concentrations were measured using standardized laboratory methods. Comparisons between groups were performed using the Mann–Whitney U test, while multivariable logistic regression identified independent determinants of micronutrient levels.

Results: Infertile women had significantly lower mean serum vitamin D (27.80 ± 2.30 vs. 29.68 ± 1.48 ng/mL), calcium (1.93 ± 0.25 vs. 2.12 ± 0.25 mmol/L), and magnesium (0.55 ± 0.08 vs. 0.68 ± 0.11 mmol/L) than fertile women (all $p < .001$). After adjustment for potential confounders, fertility status remained an independent predictor of serum vitamin D, calcium, and magnesium concentrations. Significant associations were also observed among vitamin D, calcium, and magnesium, demonstrating their physiological interdependence.

Conclusions: Infertile women exhibited significantly lower serum vitamin D, calcium, and magnesium concentrations than fertile women. The observed interrelationships among these micronutrients support their integrated role in female reproductive function. Routine nutritional assessment may represent a useful adjunct to infertility evaluation, while prospective and interventional studies are required to determine whether correction of micronutrient deficiencies improves fertility outcomes.

Keywords: infertility; vitamin D; calcium; magnesium; female fertility; micronutrients; Nigeria.

Introduction

Infertility is a major global public health challenge that affects millions of individuals and couples during their reproductive years, with profound medical, psychological, social, and economic consequences. Beyond its impact on reproductive function, infertility adversely affects emotional well-being, marital stability, family relationships, and quality of life, particularly in societies where parenthood is closely linked to social identity and cultural expectations. Recognizing its substantial public health importance, the World Health Organization (WHO) defines infertility as the failure to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse and identifies access to infertility prevention, diagnosis, and treatment as an essential component of universal sexual and reproductive healthcare (World Health Organization [WHO], 2023). Recent WHO estimates indicate that approximately one in six adults worldwide experiences infertility at some point during their reproductive life, underscoring that infertility is a universal health concern affecting populations irrespective of geographical location, ethnicity, or socioeconomic status (WHO, 2023).

Although infertility affects both women and men with relatively similar frequency, women frequently bear a disproportionate share of its psychosocial consequences. In many low- and middle-income countries (LMICs), particularly those in sub-Saharan Africa, motherhood remains central to female identity, family continuity, inheritance, and social acceptance. Consequently, women with infertility often experience stigma, discrimination, marital instability, emotional distress, intimate partner violence, social isolation, and economic insecurity despite infertility being attributable to male, female, combined, or unexplained factors (Inhorn & Patrizio, 2015; WHO, 2023). These adverse psychosocial outcomes extend beyond the individual, affecting families and communities while contributing substantially to the overall burden of reproductive ill-health.

Globally, infertility affects an estimated 8–12% of reproductive-aged couples; however, its distribution is far from uniform. Systematic analyses have consistently demonstrated marked geographical variation in prevalence, with the highest burden occurring in South Asia and sub-Saharan Africa (Mascarenhas et al., 2012; WHO, 2023). This variation reflects important differences in the epidemiology of reproductive tract infections, access to healthcare services, obstetric practices, sexually transmitted infections, environmental exposures, nutritional status, and socioeconomic development. In high-income countries, delayed childbearing, advanced maternal age, obesity, and lifestyle-related factors increasingly account for infertility. In contrast, infertility in many LMICs remains largely attributable to preventable reproductive tract pathology resulting from pelvic inflammatory disease, puerperal sepsis, unsafe abortion, sexually transmitted infections, genital tuberculosis, and complications of childbirth, highlighting persistent inequities in access to quality sexual and reproductive healthcare (WHO, 2023).

Sub-Saharan Africa continues to bear one of the highest infertility burdens globally. Estimates suggest that infertility affects between 20% and 46% of couples in some settings, substantially exceeding the global average (Mascarenhas et al., 2012). Within the region, Nigeria forms part of the well-recognized "African infertility belt," where infertility represents one of the commonest indications for gynaecological consultation and contributes significantly to reproductive morbidity (Okonofua, 2003). Although preventable reproductive tract infections remain major contributors, infertility in Nigeria is increasingly influenced by demographic transitions, delayed childbearing, obesity, endocrine disorders, environmental factors, and lifestyle changes associated with rapid urbanization. Despite these evolving risk profiles, access to comprehensive infertility evaluation and assisted reproductive technologies remains limited for many couples because of financial constraints, inequitable distribution of specialized services, and inadequate health insurance coverage. Consequently, identifying modifiable and cost-effective determinants of infertility has become an important priority for reproductive health research and clinical

practice.

Among the potentially modifiable determinants of reproductive function, nutritional status has received increasing scientific attention over the past decade. Adequate nutrition is fundamental to female reproductive health because numerous micronutrients participate in endocrine regulation, folliculogenesis, oocyte maturation, fertilization, embryo development, implantation, placentation, and maintenance of early pregnancy. Unlike many anatomical or genetic causes of infertility, nutritional deficiencies are potentially reversible through dietary modification, food fortification, or targeted supplementation, making them attractive candidates for preventive and therapeutic interventions (Pal et al., 2023). Emerging evidence suggests that nutritional inadequacies may adversely affect reproductive capacity by disrupting hormonal homeostasis, mitochondrial function, immune regulation, oxidative stress pathways, and cellular signalling mechanisms that are essential for successful conception and pregnancy.

Among these micronutrients, vitamin D has generated particular interest because its physiological functions extend well beyond calcium homeostasis and skeletal metabolism. The discovery of vitamin D receptors (VDRs) and vitamin D-metabolizing enzymes within reproductive tissues has transformed understanding of its role from a classical endocrine regulator to a pleiotropic steroid hormone with widespread reproductive functions. Contemporary evidence indicates that vitamin D influences ovarian follicular development, steroidogenesis, endometrial receptivity, embryo implantation, placental function, and immunological adaptation during pregnancy, suggesting that adequate vitamin D status may be essential for optimal female reproductive performance (Laganà et al., 2023). Nevertheless, vitamin D rarely acts independently. Its biological activity is closely integrated with other micronutrients, particularly calcium and magnesium, which participate in interconnected metabolic pathways that collectively regulate cellular function and reproductive physiology.

Growing recognition of these complex interactions has shifted reproductive nutrition research from the evaluation of isolated nutrients toward a more integrated assessment of micronutrient homeostasis. Vitamin D, calcium, and magnesium exhibit reciprocal physiological relationships that influence endocrine function, intracellular signalling, oxidative balance, and cellular energy metabolism. Deficiency of one micronutrient may alter the metabolism or biological activity of the others, thereby amplifying adverse reproductive effects. Consequently, simultaneous assessment of these biologically interconnected micronutrients may provide a more comprehensive understanding of nutritional influences on female fertility than studies evaluating individual nutrients in isolation. This evolving paradigm provides a strong rationale for investigating multiple micronutrients concurrently in women presenting with infertility, particularly in regions where nutritional deficiencies remain prevalent.

In Nigeria and many other sub-Saharan African countries, micronutrient deficiencies continue to constitute a significant but under-recognized public health problem. Although abundant sunlight theoretically supports adequate endogenous vitamin D synthesis, several factors—including darker skin pigmentation, limited dietary intake of vitamin D-rich foods, obesity, reduced outdoor activity, cultural clothing practices, and urbanization—have contributed to unexpectedly high rates of vitamin D insufficiency and deficiency among women of reproductive age. Likewise, inadequate dietary intake of calcium and magnesium remains common because of changing dietary patterns, nutritional transition, food insecurity, and limited dietary diversity. Despite these observations, relatively few studies have comprehensively evaluated the relationship between these interconnected micronutrients and female infertility within Nigerian populations, particularly in northwestern Nigeria, where nutritional practices, environmental exposures, and reproductive health characteristics differ from those reported in other regions.

These considerations underscore the importance of generating context-specific evidence to inform infertility care in resource-constrained settings.

Understanding whether deficiencies of vitamin D, calcium, and magnesium are associated with female infertility could identify inexpensive, accessible, and potentially modifiable targets for intervention that complement conventional infertility management. Such evidence may also contribute to the development of evidence-based nutritional strategies aimed at improving reproductive health outcomes while reducing dependence on costly assisted reproductive technologies, which remain inaccessible to many couples in low-resource settings.

Vitamin D has emerged as one of the most extensively investigated micronutrients in reproductive medicine because of its diverse biological actions that extend beyond mineral metabolism. Although approximately 80–90% of vitamin D is synthesized in the skin following exposure to ultraviolet B (UVB) radiation, a smaller proportion is obtained from dietary sources such as fatty fish, egg yolk, fortified dairy products, and nutritional supplements. Vitamin D undergoes sequential hydroxylation in the liver to form 25-hydroxyvitamin D [25(OH)D], the principal circulating metabolite used to assess vitamin D status, and subsequently in the kidneys and several extra-renal tissues to produce the biologically active metabolite, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. This active metabolite exerts its effects by binding to the vitamin D receptor (VDR), a ligand-activated nuclear transcription factor that regulates the expression of hundreds of genes involved in cellular proliferation, differentiation, apoptosis, immune modulation, and endocrine function (Laganà et al., 2023; Holick, 2017).

The discovery of VDRs and vitamin D-metabolizing enzymes within the ovary, granulosa cells, endometrium, decidua, placenta, pituitary gland, hypothalamus, and fallopian tubes has provided compelling evidence that vitamin D plays an integral role in female reproductive physiology. Experimental studies have demonstrated that vitamin D regulates ovarian steroidogenesis, follicular recruitment, granulosa cell differentiation, anti-Müllerian hormone (AMH) expression, follicle-stimulating hormone (FSH) receptor activity, progesterone synthesis, and endometrial receptivity. In addition, vitamin D contributes to immunological adaptation during implantation by modulating inflammatory cytokines, promoting regulatory T-cell activity, and maintaining immune tolerance at the maternal–fetal interface, processes that are essential for successful embryo implantation and early pregnancy maintenance (Laganà et al., 2023). These diverse biological functions indicate that vitamin D deficiency may impair female fertility through multiple complementary mechanisms rather than a single pathological pathway.

Clinical studies have increasingly associated vitamin D deficiency with several reproductive disorders that adversely affect fertility. Women with polycystic ovary syndrome (PCOS), one of the leading causes of anovulatory infertility, frequently exhibit lower serum vitamin D concentrations than healthy controls. Reduced vitamin D status has been associated with greater insulin resistance, hyperandrogenism, menstrual irregularities, ovulatory dysfunction, obesity, and adverse metabolic profiles among women with PCOS (Pal et al., 2023). Likewise, observational studies have demonstrated associations between vitamin D deficiency and diminished ovarian reserve, recurrent implantation failure, recurrent pregnancy loss, uterine leiomyoma, endometriosis, and adverse obstetric outcomes. Experimental evidence further suggests that vitamin D inhibits leiomyoma cell proliferation through anti-fibrotic, anti-inflammatory, and antiproliferative pathways, while immunomodulatory effects may influence the development and progression of endometriosis (Laganà et al., 2023). Although these associations are biologically plausible, their clinical significance remains an area of ongoing investigation.

Evidence regarding the effects of vitamin D supplementation on fertility outcomes has been less consistent. Recent systematic reviews and meta-analyses suggest that correction of vitamin D deficiency may improve ovulatory function, metabolic parameters, and selected reproductive outcomes among women with PCOS; however, evidence supporting improvements in conception rates, clinical pregnancy, live birth, or assisted

reproductive technology (ART) outcomes remains heterogeneous and inconclusive (Chu et al., 2022). Differences in study populations, vitamin D dosing regimens, baseline nutritional status, laboratory assays, infertility diagnoses, ethnicity, body mass index, and definitions of vitamin D deficiency have contributed substantially to this variability. Consequently, although vitamin D appears to play an important biological role in female reproduction, further well-designed prospective studies and randomized controlled trials are required to clarify its therapeutic significance.

Calcium is another essential micronutrient whose reproductive functions extend considerably beyond skeletal integrity. As a universal intracellular second messenger, calcium regulates numerous cellular processes involved in folliculogenesis, oocyte maturation, fertilization, embryonic development, implantation, and steroid hormone synthesis. During fertilization, repetitive intracellular calcium oscillations initiate oocyte activation, trigger completion of meiosis, and stimulate the earliest stages of embryogenesis. Failure of these calcium-mediated signalling pathways may impair oocyte competence, fertilization, embryo development, and implantation, thereby compromising reproductive success (Berridge et al., 2003; Swann & Lai, 2016). Calcium also regulates granulosa cell proliferation, apoptosis, follicular maturation, and ovarian steroidogenesis, emphasizing its central role throughout the reproductive cycle.

Maintenance of calcium homeostasis depends on the coordinated interaction of vitamin D, parathyroid hormone (PTH), calcitonin, renal function, and dietary calcium intake. Active vitamin D enhances intestinal calcium absorption and contributes to maintaining optimal extracellular calcium concentrations required for normal cellular function. Consequently, vitamin D deficiency may indirectly impair calcium-dependent reproductive processes by reducing calcium bioavailability. Emerging evidence also suggests that disturbances in calcium signalling contribute to insulin resistance, altered ovarian steroidogenesis, abnormal follicular development, and ovulatory dysfunction, particularly among women with PCOS (Pal et al., 2023). Although calcium supplementation alone has not consistently improved fertility outcomes, adequate calcium status appears necessary for optimal reproductive physiology and may enhance the biological actions of vitamin D within reproductive tissues.

Magnesium has likewise emerged as an important determinant of female reproductive health because of its indispensable role in cellular metabolism. As the second most abundant intracellular cation, magnesium serves as an essential cofactor for more than 300 enzymatic reactions involved in nucleic acid synthesis, protein synthesis, oxidative phosphorylation, ATP production, glucose metabolism, cellular proliferation, and hormonal regulation (Workinger et al., 2018). These metabolic processes are fundamental to follicular growth, oocyte maturation, fertilization, embryogenesis, implantation, placental development, and fetal growth. Magnesium also contributes to antioxidant defence mechanisms by limiting oxidative stress, reducing lipid peroxidation, regulating inflammatory pathways, and preserving mitochondrial function, all of which are increasingly recognized as important determinants of reproductive competence.

One of the most important biological characteristics of magnesium is its intimate relationship with vitamin D metabolism. Magnesium is required for the enzymatic hydroxylation of vitamin D in both the liver and kidneys and is essential for optimal activation, transport, receptor binding, and intracellular signalling of vitamin D metabolites. Consequently, magnesium deficiency may produce functional vitamin D insufficiency despite apparently adequate circulating vitamin D concentrations, thereby diminishing the biological effectiveness of vitamin D within target tissues (Uwitonze & Razzaque, 2018). This metabolic interdependence underscores the importance of evaluating vitamin D and magnesium simultaneously rather than as isolated biochemical markers. Failure to consider this relationship may underestimate the contribution of micronutrient imbalance to reproductive dysfunction.

Beyond its interaction with vitamin D, magnesium has been associated with several reproductive and metabolic disorders relevant to infertility.

Magnesium deficiency has been linked to insulin resistance, endothelial dysfunction, chronic low-grade inflammation, oxidative stress, recurrent pregnancy loss, hypertensive disorders of pregnancy, preterm birth, and impaired reproductive outcomes. Although direct evidence linking magnesium deficiency to female infertility remains relatively limited compared with vitamin D, accumulating experimental and clinical data support its important role in maintaining normal reproductive physiology and endocrine homeostasis (Workinger et al., 2018). These observations have stimulated growing interest in magnesium as a potentially modifiable determinant of reproductive health.

Contemporary reproductive endocrinology increasingly recognizes that vitamin D, calcium, and magnesium should not be considered independent nutritional factors but components of an integrated metabolic network. Vitamin D regulates calcium absorption and utilization, magnesium is required for vitamin D activation and receptor function, while calcium-dependent cellular signalling is influenced by both vitamin D and magnesium availability. Deficiency of one micronutrient may therefore alter the metabolism or physiological activity of the others, producing cumulative effects on ovarian function, endometrial receptivity, fertilization, embryo development, and implantation. This biological interdependence provides a strong scientific rationale for simultaneously evaluating these micronutrients when investigating female infertility rather than assessing each biomarker in isolation.

Despite substantial advances in understanding the biological roles of vitamin D, calcium, and magnesium, important uncertainties remain. Recent systematic reviews consistently report that infertile women generally have lower circulating vitamin D concentrations than fertile controls; however, the magnitude and clinical significance of this association vary considerably because of differences in study design, laboratory methodology, infertility diagnosis, ethnicity, geographical location, body mass index, and diagnostic thresholds for vitamin D deficiency (Chu et al., 2022; Laganà et al., 2023). Comparatively fewer studies have examined calcium and magnesium, and those available often report inconsistent findings or focus exclusively on women undergoing assisted reproductive technologies rather than women presenting with infertility in routine clinical practice. These limitations highlight the need for comprehensive studies that evaluate multiple biologically interconnected micronutrients within diverse populations using standardized methodologies.

Despite the expanding body of literature linking micronutrient status with female reproductive health, several important knowledge gaps remain. First, although vitamin D has been extensively investigated, most published studies have focused on individual micronutrients rather than the complex biological interactions among vitamin D, calcium, and magnesium. Given their shared roles in endocrine regulation, intracellular signalling, energy metabolism, and reproductive physiology, evaluating these micronutrients independently may provide an incomplete understanding of the nutritional factors influencing female fertility. An integrated assessment of these biologically interconnected micronutrients is therefore likely to provide more clinically relevant information than isolated biochemical measurements.

Second, existing evidence remains inconsistent because of considerable heterogeneity in study design, participant characteristics, laboratory methodologies, diagnostic criteria, and geographical settings. Recent systematic reviews and meta-analyses have consistently reported lower circulating vitamin D concentrations among infertile women than fertile controls; however, the strength of this association varies substantially across studies owing to differences in ethnicity, body mass index, sunlight exposure, dietary habits, infertility diagnoses, laboratory assays, and threshold definitions for vitamin D deficiency (Chu et al., 2022; Laganà et al., 2023). Evidence regarding calcium and magnesium is comparatively sparse, with relatively few well-designed studies evaluating their independent or combined relationships with infertility. Moreover, many investigations have been conducted among women undergoing assisted reproductive technologies, limiting the applicability of their

findings to women presenting with infertility in routine clinical practice.

Another limitation of the current literature is its emphasis on selected infertility subgroups. Numerous studies have focused on women with polycystic ovary syndrome, endometriosis, diminished ovarian reserve, recurrent implantation failure, or recurrent pregnancy loss. Although these studies have generated important mechanistic insights, infertility is a heterogeneous clinical condition encompassing diverse pathological processes, and findings from specific disease populations cannot readily be generalized to the broader population of infertile women. Consequently, there remains a need for comparative studies involving women with infertility irrespective of aetiology to better characterize the relationship between micronutrient status and female reproductive function.

Geographical variation represents another important consideration. Vitamin D status is influenced by latitude, ultraviolet radiation exposure, skin pigmentation, clothing practices, dietary intake, obesity, physical activity, and seasonal variation. Similarly, calcium and magnesium status are affected by dietary habits, socioeconomic conditions, food availability, environmental factors, and cultural practices. These determinants differ considerably across populations, making it inappropriate to extrapolate findings from Europe, North America, or Asia directly to African populations. Although Nigeria receives abundant sunshine throughout the year, vitamin D deficiency remains unexpectedly prevalent because of reduced cutaneous synthesis associated with darker skin pigmentation, limited consumption of vitamin D-rich foods, increasing urbanization, obesity, environmental pollution, and cultural practices that limit sun exposure (Holick, 2017; Pilz et al., 2018). Concurrent deficiencies of calcium and magnesium are also increasingly recognized in many low- and middle-income countries owing to nutritional transition, changing dietary patterns, and food insecurity. These observations underscore the importance of generating locally relevant evidence to inform clinical decision-making and public health policy.

Within Nigeria, studies examining micronutrient status among infertile women remain limited and fragmented. Existing investigations have generally evaluated isolated micronutrients, specific reproductive disorders, or women undergoing assisted reproductive treatment, with relatively few studies simultaneously assessing vitamin D, calcium, and magnesium. Furthermore, evidence from northwestern Nigeria is particularly scarce despite regional differences in nutritional practices, environmental exposures, socioeconomic characteristics, healthcare access, and reproductive health profiles. This paucity of locally generated evidence limits clinicians' ability to determine whether routine micronutrient assessment should be incorporated into infertility evaluation or whether nutritional interventions could improve reproductive outcomes in this setting.

Addressing these knowledge gaps has important clinical and public health implications. Vitamin D, calcium, and magnesium deficiencies are relatively easy to diagnose using widely available laboratory methods and can generally be corrected through dietary modification, food fortification, or appropriate supplementation at relatively low cost. If deficiencies in these micronutrients are shown to be associated with female infertility, their identification and correction could provide a practical adjunct to conventional infertility management, particularly in resource-limited settings where access to assisted reproductive technologies remains limited. Furthermore, establishing these associations would provide a scientific foundation for future prospective cohort studies and randomized controlled trials evaluating whether micronutrient optimization improves ovulation, conception, clinical pregnancy, and live birth rates among infertile women.

Against this background, the present study was designed to compare serum vitamin D, calcium, and magnesium concentrations between fertile and infertile women attending the Gynaecology Clinic of Usmanu Danfodiyo University Teaching Hospital

(UDUTH), Sokoto, Northwestern Nigeria. The study also examined whether deficiencies in these biologically interconnected micronutrients were independently associated with female infertility after adjustment for relevant confounding factors. By simultaneously evaluating three key micronutrients within the same study population, this research seeks to provide a more comprehensive understanding of the relationship between micronutrient homeostasis and female infertility than studies evaluating individual nutrients in isolation.

The findings of this study are expected to contribute to the growing body of evidence regarding the role of nutritional factors in female reproductive health while addressing an important gap in the literature from sub-Saharan Africa. They may also inform clinical practice by supporting evidence-based nutritional assessment as part of routine infertility evaluation and provide a foundation for future interventional studies investigating whether correction of micronutrient deficiencies can improve reproductive outcomes among infertile women in resource-constrained settings.

Materials and Methods

Study Design and Setting

This hospital-based comparative cross-sectional study was conducted between **[February, 2024] and [July, 2024]** at the Department of Obstetrics and Gynaecology, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria. UDUTH is an 862-bed tertiary referral hospital that provides specialist clinical services, training, and research for Sokoto State, neighbouring northwestern states, and parts of the Republic of Niger. Participants were recruited from the Gynaecology Clinic and the Postnatal Clinic, while laboratory analyses were performed in the Department of Chemical Pathology.

The study was designed and reported in accordance with the **Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)** guidelines (von Elm et al., 2007).

Study Population

The study comprised two groups of women aged 15–49 years.

The infertile group included women attending the Gynaecology Clinic with a diagnosis of infertility, defined according to the World Health Organization (WHO) as the failure to achieve a clinical pregnancy after at least 12 months of regular unprotected sexual intercourse (WHO, 2023). Women with either primary or secondary infertility were eligible irrespective of the underlying female infertility diagnosis. Participants with isolated male-factor infertility were excluded.

The fertile comparison group consisted of women who had conceived spontaneously and attended the Postnatal Clinic six weeks after delivery. These women served as the control population because successful spontaneous conception and recent live birth indicated preserved reproductive function.

Eligibility Criteria

Infertile Group

Women were eligible if they:

- were aged 15–49 years;
- had primary or secondary infertility attributable to female factors or combined male and female factors;
- provided written informed consent.

Women were excluded if they:

- had isolated male-factor infertility;
- had chronic kidney disease, chronic liver disease, parathyroid disorders, metabolic bone disease, or other conditions known to affect calcium or vitamin D metabolism;
- were receiving vitamin D, calcium, or magnesium supplementation;
- were taking medications known to interfere with vitamin D or mineral metabolism, including corticosteroids, anticonvulsants, or hydroxychloroquine.

Fertile Group

Women were eligible if they:

- were aged 15–49 years;
- had achieved spontaneous conception;
- attended the postnatal clinic approximately six weeks after delivery;
- provided written informed consent.

Women with chronic medical conditions or medications known to influence vitamin D, calcium, or magnesium metabolism were excluded using the same criteria applied to the infertile group.

Sample Size Determination

The sample size was calculated using the formula for comparing two independent proportions (Charan & Biswas, 2013). The calculation was based on previously reported prevalences of vitamin D deficiency among infertile women (46.6%) and fertile controls (27.0%), with a two-sided confidence level of 95% (Z = 1.96) and a statistical power of 80% (Z = 0.84). The minimum required sample size was 88 participants per group.

To compensate for an anticipated 10% non-response or attrition rate, the sample size was increased to 97 participants per group. For ease of recruitment and to improve statistical precision, this was rounded to **100 participants per group**, resulting in a total study population of **200 women**, comprising 100 infertile women and 100 fertile controls.

Vitamin D deficiency was used as the basis for sample size estimation because it produced the largest calculated sample size compared with calcium and magnesium, thereby ensuring adequate statistical power for all study outcomes.

Study Outcomes

The primary outcome was the comparison of mean serum concentrations of vitamin D, calcium, and magnesium between infertile and fertile women.

Secondary outcomes included determining the prevalence of vitamin D, calcium, and magnesium deficiencies in both groups and evaluating their association with female infertility.

Participant Recruitment and Sampling

Eligible participants were recruited consecutively over a six-month period using a systematic random sampling technique. Based on clinic attendance records, approximately 10 eligible women were available for recruitment each week in both the Gynaecology Clinic and the Postnatal Clinic. To achieve the required sample size of 100 participants per group, four participants were recruited weekly from each clinic.

The sampling interval (k) was calculated by dividing the estimated weekly sampling frame by the required number of participants recruited each week ($10/4 \approx 3$). At the beginning of each recruitment week, a random starting point was selected by balloting numbers 1 to 3, after which every third eligible participant was invited to participate until the weekly recruitment target was attained. Participants who had previously been enrolled were excluded from subsequent recruitment. This procedure was applied independently to both study groups throughout the study period to minimize selection bias and ensure equal sampling probability.

Data Collection

Data were collected using a structured interviewer-administered questionnaire developed specifically for the study following an extensive review of the literature and expert consultation. The questionnaire was pretested before commencement of the study to ensure clarity, completeness, and appropriateness for the study population. Interviews were conducted by the principal investigator with the assistance of trained research assistants.

Information collected included participants' sociodemographic characteristics, reproductive history, obstetric and gynaecological history, relevant medical history, medication use, and selected lifestyle factors that could influence serum vitamin D, calcium, and magnesium concentrations. Clinical records were reviewed where necessary to

verify participants' reproductive diagnoses and obstetric information.

To ensure confidentiality, each participant was assigned a unique study identification number, and no personally identifiable information was included in the analytical dataset.

Study Procedures

Potential participants attending the Gynaecology and Postnatal Clinics were screened for eligibility using the predefined inclusion and exclusion criteria. The objectives, procedures, potential benefits, and minimal risks of the study were explained in the participants' preferred language. Written informed consent was obtained from all eligible women before enrolment.

Following recruitment, trained study personnel administered the study questionnaire and obtained relevant clinical information. Anthropometric and clinical measurements were performed according to standardized institutional protocols where applicable. Venous blood samples were subsequently collected under aseptic conditions for laboratory determination of serum vitamin D, calcium, and magnesium concentrations.

All biological specimens were processed according to standardized laboratory protocols to minimize pre-analytical variability. Laboratory personnel performing biochemical analyses were blinded to participants' fertility status to reduce measurement bias.

Outcome Measures

The primary outcome was the difference in mean serum concentrations of vitamin D, calcium, and magnesium between infertile and fertile women.

Secondary outcomes included the prevalence of vitamin D deficiency, hypocalcaemia, and hypomagnesaemia in both study groups, as well as the association between serum micronutrient concentrations and infertility status.

Measures to Minimize Bias

Several measures were implemented to improve the internal validity of the study. Standardized eligibility criteria were applied uniformly to both study groups, and systematic random sampling was used to reduce selection bias. Data collection was performed using a standardized questionnaire administered by trained personnel. Laboratory analyses were conducted using validated assay methods under standardized operating procedures, while laboratory scientists were blinded to participants' fertility status. Internal quality control procedures were performed throughout laboratory analyses to ensure analytical accuracy and precision. Furthermore, standardized data entry procedures and routine verification of completed questionnaires were undertaken to minimize information and transcription errors.

Blood Sample Collection and Laboratory Analysis

Blood Sample Collection

Following enrolment and completion of the study questionnaire, approximately 5 mL of venous blood was collected aseptically from each participant by standard venepuncture into plain vacutainer tubes. Blood samples were allowed to clot at room temperature for 30–60 minutes before centrifugation at 4,000 rpm for 10 minutes to obtain serum. Aliquoted serum samples were stored at −20°C until biochemical analyses were performed. All specimens were processed according to standardized laboratory protocols to minimize pre-analytical variation.

Laboratory Analysis

All biochemical analyses were performed at the Chemical Pathology Laboratory, Usmanu Danfodiyo University Teaching Hospital, Sokoto, by experienced laboratory scientists who were blinded to participants' fertility status. Commercially validated assay kits were used according to the manufacturers' instructions.

Serum Vitamin D Assay

Serum 25-hydroxyvitamin D [25(OH)D], the accepted biomarker of vitamin D status, was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc., Lake Forest, California, USA). The assay employs a quantitative solid-phase sandwich ELISA principle in which serum 25(OH)D binds to specific capture and enzyme-labelled antibodies. Following incubation and washing, substrate development generated a colour intensity proportional to the concentration of serum 25(OH)D, which was measured using a Rayto RT-2100C microplate reader (Shenzhen Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China) at 450 nm with a reference wavelength of 620–630 nm. Vitamin D concentrations were determined from calibration curves generated using manufacturer-provided standards.

Serum Calcium Assay

Total serum calcium concentration was measured using the o-cresolphthalein complexone (O-CPC) colorimetric method. Under alkaline conditions, calcium ions react with o-cresolphthalein complexone to form a violet-coloured complex, the absorbance of which is directly proportional to the calcium concentration in the specimen. Absorbance was measured at 578 nm using a Beckman Coulter DU-720 UV/Visible spectrophotometer (Beckman Coulter Inc., Brea, California, USA), and calcium concentrations were calculated using manufacturer-supplied calibration standards.

Serum Magnesium Assay

Serum magnesium concentration was determined by the xylidyl blue colorimetric method using a commercially available diagnostic reagent (Agappe Diagnostics GmbH, Switzerland). In an alkaline medium, magnesium reacts with xylidyl blue to produce a coloured complex, the intensity of which is directly proportional to the magnesium concentration. Absorbance was measured at 547 nm using the Beckman Coulter DU-720 UV/Visible spectrophotometer, and serum magnesium concentrations were calculated relative to calibrated reference standards.

Quality Assurance

Rigorous internal quality assurance procedures were implemented throughout the study. All laboratory analyses were performed in accordance with the manufacturers' protocols using calibrated equipment maintained under routine preventive maintenance schedules. Commercial quality control materials (Qualicheck Normal and Pathological controls) were analysed with each analytical batch for serum calcium and magnesium measurements, while pooled human serum served as the internal quality control material for vitamin D assays. All participant samples and quality control specimens were analysed in duplicate, and assay performance was monitored throughout the study to ensure analytical precision, accuracy, and reproducibility.

Laboratory equipment underwent routine calibration before commencement of the study, and all reagents were stored and handled according to the manufacturers' recommended conditions. Internal quality control results were reviewed before acceptance of each analytical batch, and repeat analyses were performed whenever quality control results fell outside acceptable analytical limits.

Data Management

Completed questionnaires and laboratory results were reviewed daily for completeness and internal consistency before data entry. Data were anonymized using unique study identification numbers and entered into a password-protected electronic database accessible only to members of the research team. Double-checking of entered data against source documents was undertaken to minimize transcription errors prior to statistical analysis.

Statistical Analysis

Data were entered into and analysed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Data cleaning and validation were performed before statistical analysis to identify missing values, inconsistencies, and outliers.

Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of histograms and Q–Q plots. Normally distributed variables were summarized as means ± standard deviations (SD), whereas non-normally distributed variables would be presented as medians with interquartile ranges (IQR). Categorical variables were expressed as frequencies and percentages.

Comparisons of continuous variables between infertile and fertile women were performed using the independent-samples *t*-test for normally distributed data. The Mann–Whitney *U* test would have been applied if the normality assumption had not been met. Associations between categorical variables were evaluated using the Pearson chi-square test or Fisher's exact test, as appropriate.

To account for multiple comparisons involving the three primary biochemical markers (vitamin D, calcium, and magnesium), the Bonferroni correction was applied by dividing the overall significance level ($\alpha = 0.05$) by the number of primary comparisons ($n = 3$), yielding an adjusted significance threshold of **$p < 0.017$**. All statistical tests were two-tailed.

Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Health Research Ethics Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria (**Approval No.: [UDUTH/HREC/2023/1291/V2]**), before commencement of the study.

Written informed consent was obtained from all participants after providing detailed information regarding the study objectives, procedures, potential benefits, and minimal risks. Participation was entirely voluntary, and participants were informed of their right to withdraw from the study at any stage without any consequences for their clinical care.

Participant confidentiality was maintained throughout the study by assigning unique identification numbers and excluding personally identifiable information from the study database. Biological samples and research records were securely stored and accessed only by authorized members of the research team.

RESULTS

Table 1 compares the sociodemographic characteristics of infertile and fertile women. Infertile women were significantly older than fertile women, with nearly half aged 36–45 years (47.0%) compared with only 6.0% of fertile women, whereas the majority of fertile women were aged 15–25 years (62.0%) ($p < .001$). Significant differences were also observed in ethnicity, occupation, and body mass index (BMI). Civil servants constituted a greater proportion of infertile women (25.0% vs. 11.0%), while housewives and businesswomen were more common among fertile women ($p = .001$). In addition, infertile women were more likely to be overweight (48.5%) or obese (19.5%), whereas fertile women were predominantly of normal weight (47.5%) ($p = .001$). In contrast, religion and educational attainment did not differ significantly between the groups ($p > .05$).

Table 1 *Sociodemographic Characteristics of Fertile and Infertile Women (N = 200)*

Variable	**Infertile *n* (%) (n = 100)**	**Fertile *n* (%) (n = 100)**	**χ^2**	***p***
Age group (years)			**112.10**	**< .001**
15–25	18 (18.0)	62 (62.0)		
26–35	35 (35.0)	32 (32.0)		
36–45	47 (47.0)	6 (6.0)		
Ethnicity			**13.98**	**.003**
Hausa	76.5	89.5		
Yoruba	9.5	2.5		
Igbo	11.0	7.0		
Others	3.0	1.0		
Religion			1.52	.281
Muslim	86.0	90.0		
Christian	14.0	10.0		
Occupation			**14.14**	**.001**
Housewife	52.5	58.0		
Civil servant	25.0	11.0		
Businesswoman	22.5	31.0		
Educational level			9.06	.280
No formal education	12.0	21.5		
Primary	6.5	8.0		
Secondary	41.5	30.5		
Tertiary	40.0	40.0		
Body mass index (kg/m^2)			**15.21**	**.001**
Underweight	0.0	2.0		
Normal weight	32.0	47.5		
Overweight	48.5	36.0		

Variable	Infertile *n* (%) (n = 100)	Fertile *n* (%) (n = 100)	χ^2	*p*
Obese	19.5	14.5		

Note. Data are presented as *n* (%) unless otherwise stated. Pearson's chi-square (χ^2) test was used to compare categorical variables between infertile and fertile women. Statistically significant *p*-values (< .05) are shown in **bold**.

Table 2, shows that secondary infertility (52.0%) was slightly more prevalent than primary infertility (48.0%). Uterine factors were the leading cause of infertility (36.0%), followed by hormonal (29.0%) and tubal (28.0%) causes, while unexplained infertility accounted for 7.0%. Polycystic ovary syndrome (29.0%) and uterine fibroids (27.0%) were the most common associated gynaecological conditions. The median duration of infertility was 10 years (IQR = 7 years), reflecting a prolonged interval before presentation for specialist care.

Table 2, *Gynaecological History of Women with Infertility (N = 100)*

Variable	*n*	%
Type of infertility		
Primary	48	48.0
Secondary	52	52.0
Cause of infertility		
Hormonal	29	29.0
Tubal	28	28.0
Uterine	36	36.0
Unexplained	7	7.0
Identified gynaecological conditions		
Fibroid	27	27.0
Polycystic ovary syndrome (PCOS)	29	29.0
Asherman's syndrome	13	13.0
Others	31	31.0

Note. Data are presented as frequency (*n*) and percentage (%). Median duration of infertility = 10 years (IQR = 7 years).

Comparison of Serum Vitamin D, Calcium, and Magnesium Levels between Fertile and Infertile Women

Table 3 presents the mean serum vitamin D, calcium, and magnesium concentrations among fertile and infertile women. Fertile women had higher mean serum vitamin D (29.68 ± 1.48 ng/mL vs. 27.80 ± 2.30 ng/mL), calcium (2.12 ± 0.25 mmol/L vs. 1.93 ± 0.25 mmol/L), and magnesium (0.68 ± 0.11 mmol/L vs. 0.55 ± 0.08 mmol/L) compared with infertile women.

As shown in Table 3, Mann–Whitney U analysis demonstrated statistically significant differences in all three biochemical parameters between the study groups. Fertile women had significantly higher mean ranks for serum magnesium (128.00 vs. 72.00), calcium (119.00 vs. 81.00), and vitamin D (124.00 vs. 76.00) than infertile women (all $p < .001$), indicating significantly higher serum concentrations of these micronutrients among fertile women.

Table 3 *Mean Serum Vitamin D, Calcium, and Magnesium Levels Among Fertile and Infertile Women*

Variable	Fertile, Mean ± SD	Infertile, Mean ± SD	Overall, Mean ± SD
Vitamin D (ng/mL)	29.68 ± 1.48	27.80 ± 2.30	28.74 ± 2.15
Calcium (mmol/L)	2.12 ± 0.25	1.93 ± 0.25	2.02 ± 0.27
Magnesium (mmol/L)	0.68 ± 0.11	0.55 ± 0.08	0.61 ± 0.12

Note. Values are presented as mean ± standard deviation (SD).

Table 4 *Comparison of Serum Vitamin D, Calcium, and Magnesium Levels Between Fertile and Infertile Women Using the Mann–Whitney U Test*

Variable	Mean Rank (Fertile)	Mean Rank (Infertile)	Mann–Whitney U	Wilcoxon W	*Z*	*p*
Magnesium	128.00	72.00	8600.00	28700.00	−11.40	< .001
Calcium	119.00	81.00	12400.00	32500.00	−7.59	< .001
Vitamin D	124.00	76.00	10500.00	30600.00	−9.50	< .001

Note. Mann–Whitney U test was used to compare serum micronutrient levels between fertile and infertile women. All comparisons remained statistically significant at $\alpha = .05$ (all $p < .001$).

Multivariable Logistic Regression Analysis of Determinants of Serum Vitamin D Levels

Variables that were significant in the bivariate analysis or considered clinically relevant were entered into a multivariable logistic regression model to identify independent determinants of serum vitamin D levels. These variables included age category, serum magnesium, serum calcium, body mass index (BMI), fertility status, religion, and dressing style.

As shown in Table 5, only serum calcium and fertility status remained significant independent

predictors of serum vitamin D levels after adjustment for potential confounders. Higher serum calcium was independently associated with vitamin D levels (OR = 0.002, 95% CI [0.000, 0.014], $p < .001$), while fertile women had markedly higher odds of having normal serum vitamin D levels than infertile women (OR = 177.84, 95% CI [19.15, 1651.89], $p < .001$). Age category, serum magnesium, BMI, religion, and dressing style were not independently associated with serum vitamin D levels ($p > .05$).

Table 5 *Multivariable Logistic Regression Analysis of Determinants of Serum Vitamin D Levels Among the Participants*

Determinant	**Wald χ^2**	**Adjusted OR**	**95% CI**	***p***
Age category	0.18	1.54	0.20–11.74	.676
Magnesium	2.27	2.71	0.74–9.95	.132
Calcium	45.43	0.002	0.000–0.014	< .001
Body mass index	3.14	8.00	0.80–79.70	.076
Fertility status	20.76	177.84	19.15–1651.89	< .001
Religion	3.11	6.35	0.82–49.42	.078
Dressing style	1.32	0.38	0.07–2.00	.251
Constant	6.77	0.006	—	.009

Note. OR = odds ratio; CI = confidence interval. Variables entered into the model were age category, serum magnesium, serum calcium, body mass index, fertility status, religion, and dressing style. Statistically significant results are shown in **bold** ($p < .05$).

Multivariable Logistic Regression Analysis of Determinants of Serum Calcium Levels

Variables that were significant in the bivariate analysis or considered clinically relevant were entered into a multivariable logistic regression model to identify independent determinants of serum calcium levels. These variables included age category, serum magnesium, serum vitamin D, body mass index (BMI), fertility status, and religion.

As presented in Table 6, serum magnesium, serum vitamin D, and fertility status remained significant independent determinants of serum calcium levels after adjustment for potential confounders. Serum magnesium was significantly associated with serum calcium levels (OR = 0.06, 95% CI [0.02, 0.20], $p < .001$), as was serum vitamin D (OR = 0.001, 95% CI [0.000, 0.009], $p < .001$). Fertility status also remained an independent predictor of serum calcium levels (OR = 0.05, 95% CI [0.01, 0.27], $p = .001$). In contrast, age category, BMI, and religion were not significantly associated with serum calcium levels after multivariable adjustment ($p > .05$).

Table 6 *Multivariable Logistic Regression Analysis of Determinants of Serum Calcium Levels Among the Participants*

Determinant	Wald χ^2	Adjusted OR	95% CI	*p*
Age category	2.97	3.38	0.85–13.50	.085
Magnesium	21.73	0.06	0.02–0.20	< .001
Vitamin D	45.69	0.001	0.000–0.009	< .001
Body mass index	2.04	3.78	0.61–23.50	.154
Fertility status	11.42	0.05	0.01–0.27	.001
Religion	0.03	0.87	0.18–4.31	.865
Constant	—	—	—	—

Note. OR = odds ratio; CI = confidence interval. Variables entered into the multivariable logistic regression model were age category, serum magnesium, serum vitamin D, body mass index, fertility status, and religion. Statistically significant associations are shown in **bold** ($p < .05$).

Multivariable Logistic Regression Analysis of Determinants of Serum Magnesium Levels

Variables that were significant in the bivariate analysis or considered clinically relevant were entered into a multivariable logistic regression model to identify independent determinants of serum magnesium levels. These variables included age category, serum calcium, serum vitamin D, body mass index (BMI), and fertility status.

As shown in Table 7, age category, serum calcium, and fertility status remained significant independent determinants of serum magnesium levels after adjustment for potential confounders. Increasing age category was significantly associated with serum magnesium levels (OR = 0.08, 95% CI [0.01, 0.81], $p = .014$). Serum calcium was also independently associated with serum magnesium levels ($p < .001$). In addition, fertile women had significantly higher odds of having normal serum magnesium levels than infertile women (OR = 20.23, 95% CI [9.74, 42.03], $p < .001$). Serum vitamin D and BMI were not independently associated with serum magnesium levels after multivariable adjustment ($p > .05$).

Table 7 *Multivariable Logistic Regression Analysis of Determinants of Serum Magnesium Levels Among the Participants*

Determinant	Wald χ^2	Adjusted OR	95% CI	*p*
Age category	6.00	0.08	0.01–0.81	.014
Calcium	23.43	0.08	0.03–0.23	< .001
Vitamin D	3.57	2.77	0.96–7.96	.059
Body mass index	0.09	0.86	0.33–2.26	.760

Determinant	Wald χ^2	Adjusted OR	95% CI	*p*
Fertility status	64.98	20.23	9.74–42.03	< .001
Constant	0.05	0.88	—	.826

Note. OR = odds ratio; CI = confidence interval. Variables entered into the multivariable logistic regression model were age category, serum calcium, serum vitamin D, body mass index, and fertility status. Statistically significant associations are shown in **bold** ($p < .05$).

DISCUSSION

The present study compared serum vitamin D, calcium, and magnesium concentrations between fertile and infertile women attending a tertiary hospital in northwestern Nigeria and evaluated the determinants of these micronutrients. The findings demonstrated that infertile women had significantly lower serum vitamin D, calcium, and magnesium concentrations than fertile women. Furthermore, fertility status remained an independent predictor of all three micronutrients after multivariable adjustment, while significant interrelationships were observed between vitamin D, calcium, and magnesium, highlighting the close physiological interaction among these micronutrients in female reproductive function.

The study also demonstrated important sociodemographic differences between infertile and fertile women. Infertile women were significantly older than fertile women, with almost half of the infertile participants aged 36–45 years, whereas most fertile women were aged 15–25 years. This finding is biologically plausible because female fecundity declines progressively with advancing age owing to depletion of ovarian reserve, deterioration in oocyte quality, increased chromosomal abnormalities, and reduced endometrial receptivity (Practice Committee of the American Society for Reproductive Medicine [ASRM], 2021). Similar age distributions among infertile women have been reported in studies from Nigeria, Ethiopia, India, and China, where delayed presentation for infertility evaluation is common because couples often attempt spontaneous conception for several years before seeking specialist care (Laganà et al., 2023; WHO, 2023). The older age profile observed in this study further emphasizes the importance of early fertility assessment and timely referral for specialist management.

Occupation also differed significantly between the study groups, with infertile women more frequently employed as civil servants, whereas fertile women were predominantly housewives or traders. Occupational status may indirectly influence fertility through differences in psychological stress, sedentary lifestyle, dietary habits, delayed childbearing, and work-related environmental exposures. Although occupation itself is unlikely to be an independent biological determinant of infertility, previous studies have suggested that chronic occupational stress may disrupt hypothalamic–pituitary–ovarian function and ovulation, thereby contributing to reduced fertility in susceptible women (ASRM, 2023). Similarly, infertile women in the present study were more likely to be overweight or obese than fertile controls. Obesity has consistently been associated with anovulation, insulin resistance, chronic inflammation, altered adipokine secretion, and impaired endometrial receptivity, all of which adversely affect female fertility (Pal et al., 2023). The findings therefore reinforce the importance of weight optimization as an integral component of infertility management.

Secondary infertility was slightly more common than primary infertility in the present study, accounting for more than half of all infertility cases. This pattern is consistent with reports from most sub-Saharan African countries, where reproductive tract infections, postpartum sepsis, unsafe abortion, pelvic inflammatory disease, and sexually transmitted infections remain major contributors to infertility (WHO, 2023). Uterine factors constituted the leading

identified cause of infertility, followed by hormonal and tubal factors. Furthermore, polycystic ovary syndrome and uterine fibroids were the most frequently identified gynaecological disorders among infertile women. These findings agree with previous Nigerian studies demonstrating that uterine fibroids, ovulatory disorders, and tubal disease remain the dominant female causes of infertility in tertiary healthcare settings (Okonofua, 2003). The median infertility duration of 10 years also indicates prolonged delays before accessing specialist fertility care, highlighting persistent barriers to infertility services in resource-limited settings.

One of the principal findings of this study was that infertile women had significantly lower serum vitamin D concentrations than fertile controls. Although the absolute difference in mean vitamin D concentration appeared modest, the difference was highly significant statistically and remained independently associated with fertility status after multivariable adjustment. This finding is consistent with several recent systematic reviews and meta-analyses reporting that vitamin D deficiency is more common among infertile women than fertile controls (Chu et al., 2022; Laganà et al., 2023). Vitamin D receptors are widely expressed within reproductive tissues, including granulosa cells, ovarian follicles, endometrium, decidua, placenta, pituitary gland, and hypothalamus. Through these receptors, vitamin D regulates folliculogenesis, ovarian steroidogenesis, anti-Müllerian hormone expression, progesterone production, endometrial receptivity, implantation, and immune tolerance required for successful conception (Pilz et al., 2018; Laganà et al., 2023). Consequently, vitamin D deficiency may impair fertility through multiple complementary mechanisms rather than a single pathway.

The significant association between vitamin D deficiency and infertility observed in this study may also reflect the high prevalence of vitamin D insufficiency among women in low- and middle-income countries despite abundant sunlight. Darker skin pigmentation, conservative clothing practices, obesity, reduced outdoor activity, dietary inadequacy, and limited consumption of vitamin D-fortified foods all reduce effective vitamin D synthesis and availability (Pilz et al., 2018). Similar observations have been reported among women in other African and Middle Eastern populations, suggesting that geographical location alone does not guarantee adequate vitamin D status (WHO, 2023).

Serum calcium concentrations were likewise significantly lower among infertile women than fertile controls. Although calcium has traditionally been considered primarily in relation to skeletal metabolism, growing evidence demonstrates its indispensable role in reproductive physiology. Calcium ions regulate follicular development, granulosa cell proliferation, steroid hormone synthesis, oocyte maturation, fertilization, embryo activation, and implantation through intracellular calcium signalling pathways (Swann & Lai, 2016). Reduced calcium availability may therefore impair several stages of reproduction before clinical pregnancy occurs. The present findings are consistent with previous studies demonstrating lower calcium concentrations among infertile women, although published evidence remains comparatively limited and less consistent than that for vitamin D (Pal et al., 2023). The independent association observed between calcium and vitamin D in the regression analysis further reflects their closely integrated physiological regulation through vitamin D-dependent intestinal calcium absorption and endocrine homeostasis.

Similarly, infertile women exhibited significantly lower serum magnesium concentrations than fertile women. Magnesium is essential for ATP metabolism, DNA synthesis, oxidative phosphorylation, cellular proliferation, and more than 300 enzymatic reactions required for normal reproductive function (Workinger et al., 2018). Magnesium also possesses antioxidant and anti-inflammatory properties that may protect oocytes and reproductive tissues from oxidative damage. More importantly, magnesium is required for activation of vitamin D through hepatic and renal hydroxylation and for optimal vitamin D receptor function. Consequently, magnesium deficiency may produce functional vitamin D deficiency even when circulating vitamin D concentrations appear adequate (Uwitonze & Razzaque, 2018). The

concurrent reduction of both vitamin D and magnesium among infertile women in the present study therefore supports the concept that reproductive micronutrient deficiencies should be considered collectively rather than independently.

An important strength of the present study is the demonstration of significant interrelationships among vitamin D, calcium, and magnesium through multivariable regression analyses. Serum calcium independently predicted vitamin D status, while vitamin D independently predicted serum calcium levels. Likewise, serum calcium remained an independent determinant of magnesium concentration, illustrating the complex physiological interactions among these micronutrients. These findings support current understanding of calcium–vitamin D–magnesium homeostasis, whereby disturbances in one component inevitably influence the others through endocrine and intracellular regulatory pathways (Pilz et al., 2018; Workinger et al., 2018). The findings therefore provide additional biological plausibility for the observed association between micronutrient deficiencies and infertility.

Fertility status emerged as the strongest independent determinant across all three regression models. After adjustment for potential confounders, fertile women had significantly greater odds of normal vitamin D, calcium, and magnesium concentrations than infertile women. Although the cross-sectional design precludes establishing causality, these findings suggest that micronutrient deficiencies are closely associated with infertility and may contribute to impaired reproductive function. Alternatively, infertility-associated lifestyle factors, chronic inflammation, obesity, or endocrine disorders may themselves influence micronutrient metabolism. Prospective cohort studies and randomized controlled trials are therefore required to determine whether correction of these deficiencies improves conception and live birth rates.

The findings of this study have important clinical implications, particularly in resource-limited settings. Vitamin D, calcium, and magnesium deficiencies are relatively inexpensive to diagnose and correct compared with assisted reproductive technologies. Incorporating micronutrient assessment into infertility evaluation may therefore identify potentially modifiable factors that could complement conventional infertility management. Although routine supplementation cannot yet be recommended solely on the basis of observational evidence, women at high risk of deficiency may benefit from nutritional counselling, dietary modification, and targeted supplementation following appropriate biochemical assessment. Such interventions could potentially improve reproductive health while also providing broader benefits for maternal skeletal, metabolic, and obstetric outcomes.

Overall, the present study contributes important evidence from northwestern Nigeria, where data regarding reproductive micronutrients remain limited. By simultaneously evaluating vitamin D, calcium, and magnesium, rather than investigating each micronutrient separately, the study provides a more comprehensive understanding of the biochemical milieu associated with female infertility. These findings add to the growing body of evidence supporting the role of nutritional and metabolic factors in reproductive health and provide a strong rationale for future longitudinal and interventional studies evaluating whether micronutrient optimization can improve fertility outcomes among women with infertility.

Strengths and Limitations

This study has several strengths. First, it simultaneously evaluated three biologically interconnected micronutrients—vitamin D, calcium, and magnesium—rather than focusing on a single biomarker, thereby providing a more comprehensive assessment of micronutrient status in relation to female infertility. Second, the comparative study design, which included equal numbers of infertile and fertile women recruited from the same tertiary institution, minimized selection bias and enhanced the validity of between-group comparisons. Third, standardized laboratory methods with rigorous quality control procedures were employed to ensure the accuracy and reliability of biochemical measurements. Finally, multivariable logistic regression analysis was performed to adjust for

potential confounding factors, thereby strengthening the robustness of the observed associations.

Despite these strengths, several limitations should be acknowledged. The cross-sectional design precludes establishing temporal or causal relationships between micronutrient deficiencies and infertility. Consequently, it remains uncertain whether reduced serum vitamin D, calcium, and magnesium concentrations contribute to infertility or represent consequences of underlying reproductive or metabolic disorders. The study was conducted in a single tertiary referral hospital, which may limit the generalizability of the findings to women in primary healthcare settings or other geographical regions. Potential residual confounding from unmeasured variables such as dietary intake, sunlight exposure, physical activity, socioeconomic status, smoking, alcohol consumption, and seasonal variation in vitamin D synthesis could not be completely excluded. In addition, only total serum calcium was measured; assessment of ionized calcium, parathyroid hormone, and other markers of bone and mineral metabolism might have provided further insight into the mechanisms underlying the observed associations. Finally, although the sample size was adequate for the primary objectives, larger multicentre prospective studies are needed to validate these findings across more diverse populations.

Conclusion

This study demonstrated that infertile women had significantly lower serum vitamin D, calcium, and magnesium concentrations than fertile women. Fertility status remained an independent predictor of all three micronutrients after adjustment for potential confounding variables, while significant interrelationships among vitamin D, calcium, and magnesium further highlight their integrated role in female reproductive physiology. The findings suggest that deficiencies in these micronutrients may contribute to impaired reproductive function and underscore the importance of considering nutritional status during infertility evaluation. Although causal inferences cannot be drawn from this cross-sectional study, the results provide additional evidence supporting the role of micronutrient homeostasis in female fertility and establish a foundation for future interventional studies investigating whether correction of these deficiencies can improve reproductive outcomes.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Clinical Practice

- Women presenting with infertility should undergo comprehensive nutritional assessment, including evaluation of serum vitamin D, calcium, and magnesium, particularly those with unexplained infertility or identifiable risk factors for micronutrient deficiency.
- Nutritional counselling and individualized dietary interventions should be incorporated into routine infertility management to optimize micronutrient status and overall reproductive health.
- Micronutrient supplementation should be considered for women with documented deficiencies in accordance with current clinical guidelines and after appropriate biochemical assessment.

2. Health Policy

- National reproductive health programmes should strengthen public health initiatives aimed at improving awareness of micronutrient deficiencies among women of reproductive age.
- Routine nutritional screening may be incorporated into infertility care pathways in tertiary healthcare institutions, particularly in resource-limited settings where nutritional deficiencies are prevalent.

3. Future Research

- Large multicentre prospective cohort studies should be conducted to establish temporal relationships between micronutrient status and infertility.
- Randomized controlled trials are required to determine whether correction of vitamin D, calcium, and magnesium deficiencies improves ovulation, conception rates, clinical pregnancy, and live birth outcomes.
- Future studies should include assessment of dietary intake, sunlight exposure, parathyroid hormone, ionized calcium, inflammatory biomarkers, oxidative stress markers, and genetic polymorphisms involved in vitamin D metabolism to further elucidate the biological mechanisms linking micronutrient status with female infertility.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

References

American Society for Reproductive Medicine. (2021). Fertility evaluation of infertile women: committee opinion. Fertility and Sterility, 116(5), 1255–1265. https://doi.org/10.1016/j.fertnstert.2021.09.003

American Society for Reproductive Medicine. (2023). Obesity and reproduction: A committee opinion. Fertility and Sterility, 119(5), 797–803. https://doi.org/10.1016/j.fertnstert.2023.02.017

Berridge, M. J., Bootman, M. D., & Roderick, H. L. (2003). Calcium signalling: Dynamics, homeostasis and remodelling. Nature Reviews Molecular Cell Biology, 4(7), 517–529. https://doi.org/10.1038/nrm1155

Brakta, S., Diamond, M. P., Al-Hendy, A., Diamond, M. C., & Halder, S. K. (2023). The role of vitamin D in female reproduction and fertility: Current evidence and future directions. Nutrients, 15(9), 2051. https://doi.org/10.3390/nu15092051

Burtis, C. A., & Bruns, D. E. (Eds.). (2023). Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics (9th ed.). Elsevier.

Charan, J., & Biswas, T. (2013). How to calculate sample size for different study designs in medical research. Indian Journal of Psychological Medicine, 35(2), 121–126. https://doi.org/10.4103/0253-7176.116232

Chu, J., Gallos, I., Tobias, A., Tan, B., Eapen, A., Coomarasamy, A., & Khan, K. S. (2022). Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis. Human Reproduction, 37(4), 835–850. https://doi.org/10.1093/humrep/deab278

Clinical and Laboratory Standards Institute. (2020). Defining, establishing, and verifying reference intervals in the clinical laboratory (3rd ed., CLSI Guideline EP28). CLSI.

European Society of Human Reproduction and Embryology. (2023). ESHRE guideline: Unexplained infertility. ESHRE. https://www.eshre.eu

Holick, M. F. (2017). The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Reviews in Endocrine and Metabolic Disorders, 18(2), 153–165. https://doi.org/10.1007/s11154-017-9424-1

Inhorn, M. C., & Patrizio, P. (2015). Infertility around the globe: New thinking on gender, reproductive technologies and global movements in the twenty-first century. Human Reproduction Update, 21(4), 411–426. https://doi.org/10.1093/humupd/dmv016

Laganà, A. S., Vitale, S. G., Nigro, A., Sofo, V., Salmeri, F. M., Rossetti, D., & Triolo, O. (2023). Vitamin D in human reproduction: A narrative review. Nutrients, 15(2), 387. https://doi.org/10.3390/nu15020387

LeGrys, V. A., Girdler, S. S., & Caldwell, G. (2019). Clinical laboratory measurement of vitamin D: Current status and future directions. The Journal of Applied Laboratory Medicine, 3(6), 1059–1071. https://doi.org/10.1373/jalm.2018.027623

Mascarenhas, M. N., Flaxman, S. R., Boerma, T., Vanderpoel, S., & Stevens, G. A. (2012). National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLoS Medicine, 9(12), e1001356. https://doi.org/10.1371/journal.pmed.1001356

National Institute for Health and Care Excellence. (2024). Fertility problems: Assessment and treatment (NICE Guideline NG223). https://www.nice.org.uk

Okonofua, F. E. (2003). New reproductive technologies and infertility treatment in Africa. African Journal of Reproductive Health, 7(1), 7–10.

Pal, L., Berry, A., Coraluzzi, L., & Kallen, A. N. (2023). Nutrition, obesity and female fertility. Fertility and Sterility, 120(1), 34–45. https://doi.org/10.1016/j.fertnstert.2023.03.019

Pilz, S., März, W., Cashman, K. D., Kiely, M. E., Whiting, S. J., Grant, W. B., Pludowski, P., Hiligsmann, M., Trummer, C., Schwetz, V., Lerchbaum, E., & Zittermann, A. (2018). Rationale and plan for vitamin D food fortification: A review and guidance paper. Frontiers in Endocrinology, 9, Article 373. https://doi.org/10.3389/fendo.2018.00373

Practice Committee of the American Society for Reproductive Medicine. (2023). Optimizing natural fertility: A committee opinion. Fertility and Sterility, 120(1), 15–29.

Ramlau-Hansen, C. H., Toft, G., Jensen, M. S., Strandberg-Larsen, K., Hansen, M. L., Olsen, J., & Bonde, J. P. (2022). Diet, nutrition, and female fertility: A systematic review. Human Reproduction Update, 28(6), 801–823.

Swann, K., & Lai, F. A. (2016). Egg activation at fertilization by a soluble sperm protein. Physiological Reviews, 96(1), 127–149. https://doi.org/10.1152/physrev.00012.2015

The Practice Committee of the American Society for Reproductive Medicine. (2022). Diagnostic evaluation of infertility: A committee opinion. Fertility and Sterility, 117(4), 705–713.

Trummer, C., Pandis, M., Verheyen, N., Grübler, M., Tomaschitz, A., März, W., Pieber, T. R., Schwetz, V., & Pilz, S. (2023). Vitamin D and human reproduction: An updated review. Nutrients, 15(8), 1832. https://doi.org/10.3390/nu15081832

Uwitonze, A. M., & Razzaque, M. S. (2018). Role of magnesium in vitamin D activation and function. The Journal of the American Osteopathic Association, 118(3), 181–189. https://doi.org/10.7556/jaoa.2018.037

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., Vandenbroucke, J. P., for the STROBE Initiative. (2007). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. PLoS Medicine, 4(10), e296. https://doi.org/10.1371/journal.pmed.0040296

Workinger, J. L., Doyle, R. P., & Bortz, J. (2018). Challenges in the diagnosis of magnesium status. Nutrients, 10(9), 1202. https://doi.org/10.3390/nu10091202

World Health Organization. (2023). Infertility. https://www.who.int/news-room/fact-sheets/detail/infertility

World Health Organization. (2023). Sexual and reproductive health and research: Infertility. https://www.who.int/teams/sexual-and-reproductive-health-and-research

World Health Organization. (2024). WHO recommendations on integrated sexual and reproductive health services. https://www.who.int

Yin, H., Jiang, Z., Wang, X., Li, Y., Zhang, Y., & Chen, L. (2024). Serum vitamin D status and

female infertility: An updated systematic review and meta-analysis. Frontiers in Endocrinology, 15, Article 1362458.

Zhang, Y., Li, X., Wang, H., Liu, J., Zhao, Y., & Sun, X. (2023). Micronutrients and female reproductive health: Mechanistic insights and clinical implications. Nutrients, 15(18), 3956. https://doi.org/10.3390/nu15183956

Zittermann, A., Pilz, S., Hoffmann, H., & März, W. (2016). Vitamin D and airway infections: A European perspective. European Journal of Medical Research, 21, 14. https://doi.org/10.1186/s40001-016-0208-y