

Serum Calcium Status among Fertile and Sub fertile Women in Northwestern Nigeria: A Comparative Hospital-Based Study

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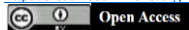
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ABSTRACT

Calcium plays an essential role in female reproductive physiology through its involvement in folliculogenesis, steroidogenesis, oocyte maturation, fertilization, and embryo development. However, evidence regarding the relationship between serum calcium status and female subfertility remains limited, particularly in sub-Saharan Africa. To compare serum calcium concentrations between fertile and subfertile women attending the Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria, and determine the association between serum calcium status and selected sociodemographic and clinical characteristics. A comparative hospital-based cross-sectional study was conducted among 200 women comprising 100 fertile and 100 subfertile participants. Sociodemographic and clinical information was obtained using a structured interviewer-administered questionnaire. Venous blood samples were collected for serum calcium estimation using the O-cresolphthalein Complexone colorimetric method. Data were analysed using IBM SPSS Statistics, with statistical significance set at $p < 0.05$. The mean serum calcium concentration was significantly higher among fertile women than subfertile women (2.12 ± 0.25 mmol/L vs. 1.93 ± 0.25 mmol/L; $p < 0.001$). Low serum calcium was significantly associated with subfertility, age, body mass index, religion, physical activity, and alcohol intake. Subfertile women were more frequently overweight or obese and were generally older than fertile women. Subfertile women had significantly lower serum calcium concentrations than fertile women, suggesting that altered calcium homeostasis may be associated with female subfertility. Assessment of nutritional status may complement conventional infertility evaluation. Further prospective studies are needed to establish causality and evaluate whether correction of calcium deficiency improves fertility outcomes.

Introduction

Infertility is a significant global public health problem affecting millions of couples and is associated with considerable medical, psychological, social, and economic consequences. 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. Recent estimates indicate that approximately one in six adults experiences infertility during their reproductive lifetime, highlighting its substantial global burden and the need for improved access to fertility care, particularly in low- and middle-income countries where diagnostic and treatment services remain limited (World Health Organization [WHO], 2023a, 2023b).

Infertility is a multifactorial disorder resulting from abnormalities affecting either or both partners. Female infertility commonly results from ovulatory disorders, tubal disease, uterine abnormalities, endometriosis, endocrine dysfunction, or diminished ovarian reserve, whereas male infertility is mainly related to impaired spermatogenesis, endocrine disorders, or obstruction of the reproductive tract. Despite advances in diagnostic techniques, a proportion of infertility cases remain unexplained, suggesting that additional biological and environmental factors contribute to impaired reproductive



function (Practice Committee of the American Society for Reproductive Medicine [ASRM], 2021; WHO, 2023a).

The burden of infertility is particularly pronounced in sub-Saharan Africa, where parenthood is highly valued socially and culturally. Women experiencing infertility frequently face stigma, marital instability, emotional distress, and reduced quality of life, irrespective of the underlying cause. Consequently, identifying potentially modifiable risk factors that may improve fertility outcomes remains an important public health priority (WHO, 2023a; Dyer & Patel, 2012).

Nutritional status has emerged as an important determinant of reproductive health. In addition to adequate energy intake, optimal reproductive function requires sufficient concentrations of essential micronutrients that regulate endocrine activity, follicular development, oocyte maturation, fertilization, implantation, and early embryonic development. Among these micronutrients, calcium is increasingly recognized as a key regulator of female reproductive physiology (Gaskins & Chavarro, 2018; Palomba et al., 2024).

Calcium is the most abundant mineral in the human body and functions as an essential intracellular second messenger involved in numerous physiological processes. Within the reproductive system, calcium signalling regulates folliculogenesis, granulosa cell proliferation, steroid hormone synthesis, meiotic progression, ovulation, oocyte activation, fertilization, embryo development, and implantation. Alterations in calcium homeostasis may impair ovarian steroidogenesis, disrupt oocyte maturation, compromise endometrial receptivity, and reduce reproductive potential. Experimental evidence further suggests that abnormal calcium signalling contributes to impaired mitochondrial function, oxidative stress, and defective embryo development, providing biological plausibility for an association between calcium deficiency and female subfertility (Bhardwaj et al., 2024; Clapham, 2007).

Although hormonal disorders and structural reproductive abnormalities have been extensively investigated in infertility, comparatively little attention has been given to calcium status as a potentially modifiable determinant of reproductive health. Published studies evaluating serum calcium concentrations among infertile women have reported inconsistent findings. While some investigators have demonstrated significantly lower calcium concentrations among infertile women than fertile controls, others have reported weak or non-significant associations. These inconsistencies may reflect differences in study populations, dietary patterns, laboratory methods, and study designs (Bhardwaj et al., 2024; Harzif et al., 2023).

Evidence from sub-Saharan Africa remains particularly limited. Most available studies have been conducted in Asia, Europe, and the Middle East, making it difficult to generalize their findings to African populations, where nutritional practices, socioeconomic conditions, environmental exposures, and reproductive health characteristics differ substantially. In Nigeria, infertility is one of the commonest indications for gynaecological consultation; however, research has predominantly focused on hormonal abnormalities, tubal disease, uterine pathology, and infectious causes, with relatively few studies examining serum calcium despite its established physiological importance (Okonofua, 2003; WHO, 2023a).

Given that calcium deficiency is potentially preventable and amenable to nutritional intervention, understanding its relationship with female fertility could provide a simple and cost-effective adjunct to infertility evaluation and management, particularly in resource-limited settings. Furthermore, there is a paucity of comparative studies evaluating serum calcium status among fertile and subfertile women in northwestern Nigeria (Palomba et al., 2024; Bhardwaj et al., 2024).

Therefore, this study compared serum calcium concentrations between fertile and subfertile women attending the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. The findings are expected to contribute to the growing evidence on the role of calcium in female reproductive health and provide locally relevant data that may inform clinical practice and future research on nutritional factors associated with subfertility.

1.1 Aim

To compare serum calcium status between fertile and subfertile women attending the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

1.2 Specific Objectives

1. To determine the mean serum calcium concentration among fertile women attending UDUTH, Sokoto.
2. To determine the mean serum calcium concentration among subfertile women attending UDUTH, Sokoto.
3. To compare serum calcium concentrations between fertile and subfertile women.
4. To determine the association between serum calcium status and selected sociodemographic and clinical characteristics among the study participants.

Methodology

Study Setting

The study was conducted at the Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria. UDUTH is a major tertiary referral hospital serving Sokoto State and neighbouring states in northwestern Nigeria. The hospital provides comprehensive specialist services in Obstetrics and Gynaecology, Internal Medicine, Surgery, Paediatrics, and other medical specialties.

Participants were recruited from the Gynaecology Clinic and the Postnatal Clinic (PNC). Women undergoing evaluation and treatment for infertility were enrolled from the Gynaecology Clinic, while fertile controls were recruited from women attending the Postnatal Clinic six weeks after spontaneous delivery. Laboratory analyses were conducted in the Department of Chemical Pathology, UDUTH, where serum calcium estimation was performed using standardized laboratory protocols under the supervision of qualified laboratory scientists.

Study Design

This was a hospital-based comparative cross-sectional study designed to compare serum calcium concentrations between fertile and infertile women attending UDUTH, Sokoto.

Study Population

The study population consisted of women of reproductive age attending the Gynaecology and Postnatal Clinics of UDUTH during the study period.

The participants comprised two groups:

Group I (Infertile women):

Women diagnosed with infertility who attended the Gynaecology Clinic for evaluation and management.

Group II (Fertile women):

Women who conceived spontaneously, delivered at UDUTH, and attended the Postnatal Clinic six weeks after delivery.

Before enrolment, the study objectives, procedures, potential benefits, and possible risks were explained to all eligible participants in either English or Hausa. Written informed consent was subsequently obtained before recruitment.

Eligibility Criteria

Group I (Infertile Women)

Women were classified as infertile according to the World Health Organization (WHO) definition of infertility as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.

Inclusion Criteria

Participants were eligible if they:

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

Exclusion Criteria

Women were excluded if they:

- had chronic renal disease;
- had chronic liver disease;
- had metabolic bone disease;
- had parathyroid disorders;



- were receiving medications known to interfere with calcium metabolism, including corticosteroids, anticonvulsants or hydroxychloroquine;
- were taking calcium supplementation;
- had isolated male-factor infertility.

Group II (Fertile Women)

Women were eligible as controls if they:

- conceived spontaneously;
- delivered a live infant;
- attended the Postnatal Clinic six weeks postpartum;
- provided written informed consent.

Exclusion Criteria

Women were excluded if they:

- had chronic kidney disease;
- had chronic liver disease;
- had metabolic bone disease;
- had parathyroid disorders;
- were receiving medications affecting calcium metabolism;
- were using calcium supplements.

Sample Size Determination

The minimum sample size was calculated using the formula for comparing the means of two independent populations.

The calculation was based on previously reported mean serum calcium concentrations among fertile and infertile women, assuming a 95% confidence level, 80% statistical power, and equal allocation between study groups.

An additional allowance was made for possible non-response and incomplete data.

A final sample size of **200 participants** comprising **100 infertile women and 100 fertile women** was recruited for the study. This sample size provided adequate statistical power to detect clinically meaningful differences in serum calcium concentrations between the two groups.

Sampling Technique

A systematic random sampling technique was employed for participant recruitment.

Infertile Women

Eligible women attending the Gynaecology Clinic during the study period constituted the sampling frame. Approximately ten eligible women were seen weekly.

Based on the recruitment target, four participants were selected each week using systematic random sampling. The sampling interval was determined by dividing the estimated weekly clinic attendance by the required weekly recruitment.

The first participant each week was selected by simple random sampling through balloting. Thereafter, every third eligible participant was recruited until the weekly sample size was achieved.

Participants previously enrolled in the study were excluded from subsequent recruitment.

Fertile Women

The same systematic random sampling procedure was used among women attending the Postnatal Clinic.

Eligible women presenting for their routine six-week postnatal visit constituted the sampling frame.

Following random selection of the first participant each week by balloting, every third eligible woman was recruited until the required sample size was obtained.

Using identical sampling procedures for both study groups minimized selection bias and ensured comparability between groups.

Study Variables

Primary Outcome

The primary outcome measure was the mean serum calcium concentration among fertile and infertile women.

Secondary Outcomes

Secondary outcomes included:

- comparison of serum calcium concentrations between fertile and infertile women;
- assessment of the relationship between serum calcium concentration and selected sociodemographic characteristics;
- evaluation of the association between serum calcium concentration and infertility-related clinical characteristics.

Study Procedure

Eligible participants were identified during routine clinic attendance.

Following confirmation of eligibility, the study objectives and procedures were explained in detail. Participants who agreed to participate signed written informed consent forms.

Each participant was assigned a unique study identification number to ensure anonymity and confidentiality.

Information was collected using a structured interviewer-administered questionnaire covering:

- sociodemographic characteristics;
- reproductive history;
- infertility history (for Group I);
- obstetric history (for Group II);
- relevant medical history;
- lifestyle characteristics.

Following completion of the questionnaire, venous blood samples were collected under aseptic conditions for laboratory estimation of serum calcium concentration.

Blood Sample Collection and Laboratory Analysis

Approximately 5 mL of venous blood was collected from each participant into plain specimen tubes under aseptic conditions.

Samples were allowed to clot before centrifugation at 3,000 rpm for approximately 10 minutes.

Separated serum was transferred into labelled cryovials and analysed immediately or stored appropriately until laboratory analysis.

Serum calcium concentration was determined 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 intensity of the colour produced is directly proportional to the calcium concentration in the sample.

Absorbance was measured spectrophotometrically at **578 nm**, and serum calcium concentration was calculated by comparing the absorbance of the specimen with that of a standard solution according to the manufacturer's instructions.

Quality Assurance

Strict quality assurance procedures were maintained throughout specimen collection, storage and laboratory analysis.

Commercially prepared normal and pathological quality control sera were analysed with every batch of samples.

Equipment calibration was performed according to the manufacturer's recommendations.

All laboratory analyses were carried out by trained laboratory personnel following standardized operating procedures.

Data Collection Instrument

Data were collected using a structured interviewer-administered questionnaire developed specifically for the study.

The questionnaire contained sections on:

- sociodemographic characteristics;
- reproductive history;
- infertility history;
- obstetric history;
- medical history;
- laboratory findings.

Completed questionnaires were reviewed daily for completeness and consistency before data entry.

Data Management and Statistical Analysis

Completed questionnaires were checked for completeness, coded, and entered into IBM SPSS Statistics (IBM Corp., Armonk, NY, USA) for analysis.

Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed variables were summarized using means and standard deviations, while skewed data were summarized using medians and interquartile ranges.

Categorical variables were presented as frequencies and percentages.

Comparisons of categorical variables between fertile and infertile women were performed using the Chi-square test or Fisher's exact test where appropriate.

Differences in mean serum calcium concentrations between the two groups were assessed using the independent-samples *t*-test.

Statistical significance was set at $p < 0.05$, and all tests were two-tailed.

Results

Sociodemographic Characteristics of the Study Participants

A total of 200 women participated in the study, comprising 100 fertile and 100 subfertile women. Participants were aged between 15 and 45 years, with a mean age of 29.1 ± 6.8 years. The largest proportion of participants were aged 15–25 years (80, 40.0%), followed by those aged 26–35 years (67, 33.5%) and 36–45 years (53, 26.5%). All participants were married. More than half were housewives (110, 55.0%), while 54 (27.0%) were businesswomen and 36 (18.0%) were civil servants.

Most participants were Muslims (176, 88.0%), whereas 24 (12.0%) were Christians. Regarding educational attainment, 80 (40.0%) had tertiary education, 72 (36.0%) had secondary education, 33 (16.5%) had no formal education, and 15 (7.5%) had primary education. The majority of participants were Hausa (166, 83.0%), followed by Igbo (18, 9.0%), Yoruba (12, 6.0%), and other ethnic groups (4, 2.0%) (Table 1).

Table 1

Sociodemographic Characteristics of the Study Participants (N = 200)

Sociodemographic Characteristics of the Study Participants (N = 200)	Frequency (n)	Percentage (%)
Age group (years)		
Mean $\pm$ SD	29.1 $\pm$ 6.8	
15–25	80	40.0
26–35	67	33.5
36–45	53	26.5
Ethnicity		
Hausa	166	83.0
Yoruba	12	6.0
Igbo	18	9.0
Others	4	2.0
Religion		
Muslim	176	88.0
Christian	24	12.0
Occupation		
Housewife	110	55.0
Civil servant	36	18.0
Businesswoman	54	27.0
Educational level		
No formal education	33	16.5*
Primary	15	7.5*
Secondary	72	36.0
Tertiary	80	40.0

Clinical Characteristics of Subfertile Women

Among the 100 subfertile women, secondary subfertility was slightly more common than primary subfertility, accounting for 52 (52.0%) and 48 (48.0%) cases, respectively. Uterine factors were the leading identified cause of subfertility (36.0%), followed by hormonal (29.0%) and tubal factors (28.0%), while unexplained subfertility accounted for 7.0% of cases. Regarding associated gynaecological conditions, polycystic ovary syndrome (PCOS) was the most frequently identified condition (29.0%), followed by uterine fibroids (27.0%) and Asherman's syndrome (13.0%). Other gynaecological conditions constituted 31.0% of cases. The median duration of subfertility was **10 years** (interquartile range, 7 years) (Table 2).

Table 2

Clinical Characteristics of Subfertile Women (N = 100)

Variable	Frequency (n)	Percentage (%)
Type of subfertility		
Primary	48	48.0
Secondary	52	52.0
Cause of subfertility		
Hormonal	29	29.0
Tubal	28	28.0
Uterine	36	36.0
Unexplained	7	7.0
Associated gynaecological conditions		
Polycystic ovary syndrome (PCOS)	29	29.0
Uterine fibroids	27	27.0
Asherman's syndrome	13	13.0
Others	31	31.0
Duration of subfertility (years)	Median (IQR)	10 (7)

Comparison of Sociodemographic Characteristics between Fertile and Subfertile Women

Table 3 compares the sociodemographic characteristics of fertile and subfertile women. Age distribution differed significantly between the two groups ($\chi^2 = 112.103$, $p < 0.001$), with subfertile women predominantly aged 36–45 years (47.0%), whereas fertile women were mainly within the 15–25 years age group (62.0%). Ethnicity also differed significantly between the groups ($\chi^2 = 13.981$, $p = 0.003$), with Hausa ethnicity predominating in both groups.

Occupation was significantly associated with fertility status ($\chi^2 = 14.137$, $p = 0.001$). A higher proportion of subfertile women were civil servants (25.0%) compared with fertile women (11.0%), whereas housewives and businesswomen were more common among fertile women. Body mass index (BMI) also differed significantly between the groups ($\chi^2 = 15.213$, $p = 0.001$). Subfertile women were more frequently overweight (48.5%) or obese (19.5%), while fertile women were predominantly of normal weight (47.5%). There were no statistically significant differences in religion ($p = 0.281$) or educational attainment ($p = 0.280$) between the two groups (Table 3).

Table 3

Comparison of Sociodemographic Characteristics between Fertile and Subfertile Women

Variable	Subfertile n = 100 n (%)	Fertile n = 100 n (%)	χ^2	p-value
Age group (years)			112.103	<0.001
15–25	18.0	62.0		
26–35	35.0	32.0		
36–45	47.0	6.0		
Ethnicity			13.981	0.003
Hausa	76.5	89.5		
Yoruba	9.5	2.5		
Igbo	11.0	7.0		
Others	3.0	1.0		
Religion			1.515	0.281



Muslim	86.0	90.0		
Christian	14.0	10.0		
Occupation			14.137	0.001
Housewife	52.5	58.0		
Civil servant	25.0	11.0		
Businesswoman	22.5	31.0		
Educational level			9.060	0.280
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/m²)			15.213	0.001
Underweight	0.0	2.0		
Normal weight	32.0	47.5		
Overweight	48.5	36.0		
Obese	19.5	14.5		

Mean Serum Calcium Levels among Fertile and Subfertile Women

The mean serum calcium level was higher among fertile women (2.12 ± 0.25 mmol/L) than among subfertile women (1.93 ± 0.25 mmol/L). The overall mean serum calcium level for all participants was 2.02 ± 0.27 mmol/L (Table 4).

Table 4

Mean Serum Calcium Levels among Fertile and Subfertile Women

Variable	Fertile (n = 100)	Subfertile (n = 100)	Total (N = 200)
Serum calcium (mmol/L), Mean $\pm$ SD	2.12 ± 0.25	1.93 ± 0.25	2.02 ± 0.27

Comparison of Serum Calcium Levels between Fertile and Subfertile Women

There was a statistically significant difference in serum calcium levels between fertile and subfertile women. Fertile women had a significantly higher mean rank (119.00) than subfertile women (81.00), indicating higher serum calcium levels among fertile women (Mann–Whitney U = 12,400.000, $Z = -7.592$, $p < 0.001$) (Table 5).

Table 5

Comparison of Serum Calcium Levels between Fertile and Subfertile Women

Variable	Mean Rank (Fertile)	Mean Rank (Subfertile)	Mann–Whitney U	Wilcoxon W	Z	p-value
Serum calcium	119.00	81.00	12,400.000	32,500.000	-7.592	<0.001

Sociodemographic and Clinical Factors Associated with Low Serum Calcium among the Study Participants

Table 6 presents the association between selected sociodemographic and clinical characteristics and serum calcium status among the study participants. Participants aged 26–35 years had the highest prevalence of low serum calcium (40.7%), followed by those aged 36–45 years (34.3%) and 15–25 years (25.0%). This association was statistically significant ($\chi^2 = 39.428$, $p < 0.001$).

The prevalence of low serum calcium was significantly higher among Muslim participants (83.3%) than Christians (16.7%) ($\chi^2 = 8.586$, $p = 0.003$). Participants who were overweight (57.4%) or obese (31.9%) were more likely to have low serum calcium than those with normal body weight, and this association was statistically significant ($\chi^2 = 168.613$, $p < 0.001$).

Subfertile women had a significantly higher prevalence of low serum calcium (68.6%) than fertile women (31.4%) ($\chi^2 = 57.783$, $p < 0.001$). Physical activity ($p = 0.025$) and alcohol intake ($p = 0.030$) were also significantly associated with serum calcium status. No statistically significant associations were observed for ethnicity ($p = 0.094$), occupation ($p = 0.832$), educational level ($p = 0.071$), or mode of delivery ($p = 0.440$) (Table 6).

Table 6

Sociodemographic and Clinical Factors Associated with Low Serum Calcium among the Study Participants

Variable	Low Calcium (%)	Normal Calcium (%)	χ^2	df	p-value
Age group (years)			39.428	2	<0.001
15–25	25.0	55.6			
26–35	40.7	26.0			
36–45	34.3	18.4			
Ethnicity			6.310	3	0.094
Hausa	78.9	87.2			
Yoruba	6.4	5.6			
Igbo	11.8	6.1			
Others	2.9	1.0			
Religion			8.586	1	0.003
Muslim	83.3	92.9			
Christian	16.7	7.1			
Occupation			0.368	2	0.832
Housewife	55.9	54.6			
Civil servant	18.6	17.3			
Businesswoman	25.5	28.1			
Educational level			7.024	3	0.071
No formal education	20.1	13.3			
Primary	5.9	8.7			
Secondary	38.7	33.2			
Tertiary	35.3	44.9			
Mode of delivery			1.241	1	0.440
Spontaneous vaginal delivery	93.8	97.1			
Caesarean section	6.2	2.9			
Alcohol intake			5.852	1	0.030
Yes	2.9	0.0			
No	97.1	100.0			
Body mass index			168.613	3	<0.001
Underweight	0.0	2.0			
Normal weight	10.8	69.9			
Overweight	57.4	26.5			
Obese	31.9	1.5			
Physical activity			5.141	1	0.025
Yes	33.8	44.9			
No	66.2	55.1			
Fertility status			57.783	1	<0.001
Fertile	31.4	69.4			
Subfertile	68.6	30.6			

χ^2 = Chi-square test.

Discussion

This comparative hospital-based study evaluated serum calcium status among fertile and subfertile women attending the Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, and examined selected sociodemographic and clinical factors associated with serum calcium status. The principal finding was that subfertile women had significantly lower mean serum calcium concentrations than fertile women, with low serum calcium significantly associated with fertility status, age, and body mass index (BMI). These findings suggest that calcium homeostasis may play an important role in female reproductive health and provide valuable evidence from northwestern Nigeria, where studies evaluating nutritional determinants of female subfertility remain limited.

The mean age of participants was 29.1 ± 6.8 years, although subfertile women were significantly older than fertile controls. This observation is consistent with the well-established decline in female reproductive potential with advancing age, which is primarily attributable to progressive depletion of ovarian follicular reserve, declining oocyte quality, increasing chromosomal abnormalities, and reduced endometrial receptivity (Practice Committee of the American Society for Reproductive Medicine [ASRM], 2021). The age distribution observed in this study therefore reflects the natural epidemiology of female fertility and reinforces the importance of early fertility assessment and timely intervention among women experiencing delayed conception.

The majority of participants were married, Hausa, Muslim, and housewives, reflecting the sociodemographic characteristics of women seeking reproductive healthcare in northwestern Nigeria. Although religion and educational attainment did not differ significantly between fertile and subfertile women, significant differences were observed in ethnicity and occupation. These findings are likely to reflect variations in socioeconomic circumstances, healthcare-seeking behaviour, and cultural practices rather than direct biological determinants of fertility. Similarly, secondary subfertility was slightly more common than primary subfertility, with uterine pathology, hormonal disorders, and tubal disease representing the predominant identified causes. The prolonged median duration of subfertility before presentation further highlights the persistent challenges of delayed diagnosis and limited access to specialized fertility services in low-resource settings.

An important finding of this study was the significant association between BMI and fertility status. Subfertile women were more frequently overweight or obese than fertile controls. Excess adiposity has been consistently associated with endocrine dysfunction, insulin resistance, chronic low-grade inflammation, altered adipokine secretion, and disruption of the hypothalamic–pituitary–ovarian axis, all of which may impair follicular development, ovulation, fertilization, and implantation (Gaskins & Chavarro, 2018). Although the present study was not designed to establish causal relationships, the observed association supports current evidence emphasizing the importance of maintaining healthy body weight as part of comprehensive fertility management.

The principal biochemical finding was the significantly lower mean serum calcium concentration among subfertile women compared with fertile women. This observation supports the hypothesis that calcium may contribute to normal female reproductive function. Calcium serves as a universal intracellular second messenger regulating numerous reproductive processes, including folliculogenesis, granulosa cell proliferation, steroidogenesis, meiotic progression, oocyte activation, fertilization, embryo cleavage, implantation, and early embryonic development (Berridge, 2016; Bhardwaj et al., 2024; Clapham, 2007). During fertilization, repetitive intracellular calcium oscillations initiate oocyte activation and embryogenesis, while disturbances in calcium signalling have been shown experimentally to impair spindle organization, mitochondrial function, chromosomal segregation, and embryo viability. These biological mechanisms provide strong plausibility for the association observed between reduced serum calcium concentrations and female subfertility in the present study.

The present findings are broadly consistent with previous reports demonstrating lower serum calcium concentrations among women with infertility compared with fertile controls. Although published evidence remains inconsistent, several investigators have suggested that inadequate calcium availability may impair ovarian steroidogenesis, increase oxidative stress, reduce endometrial receptivity, and compromise oocyte competence. Conversely, other studies have reported no independent association after adjustment for confounding variables such as age, nutritional status, endocrine disorders, and dietary intake. These discrepancies likely reflect differences in study populations, laboratory methods, nutritional patterns, sample size, and infertility aetiology (Harzif et al., 2024; Pandey et al., 2025). The present study therefore contributes additional evidence from a sub-Saharan African population, where comparable data remain scarce.

Another notable observation was the significant association between low serum calcium status and increased BMI. Women who were overweight or obese demonstrated a higher prevalence of low serum calcium than women with normal BMI. This finding suggests a possible interaction between calcium metabolism and metabolic health. Obesity has been associated with altered vitamin D metabolism, insulin resistance, chronic inflammation, and endocrine dysregulation, all of which may influence calcium homeostasis and reproductive function. Although these pathways were not directly evaluated in

the present study, they provide biologically plausible explanations for the observed relationship and warrant further investigation.

Age was also significantly associated with serum calcium status, with women aged 26–35 years demonstrating the highest prevalence of low serum calcium. While reproductive ageing is primarily characterized by declining ovarian reserve and oocyte quality, nutritional and metabolic factors may further influence reproductive competence during this period. The observed association highlights the importance of integrating nutritional assessment into fertility evaluation, particularly among women presenting with delayed conception or additional metabolic risk factors.

The findings of this study have important clinical implications. Conventional infertility assessment focuses predominantly on ovulatory function, tubal patency, uterine abnormalities, and male-factor infertility. However, the observed association between reduced serum calcium concentrations and subfertility suggests that nutritional and biochemical factors may also contribute to impaired reproductive potential. Although the present findings do not support routine measurement of serum calcium in all women undergoing infertility evaluation, they underscore the importance of individualized nutritional assessment and counselling as part of comprehensive reproductive healthcare, particularly in settings where micronutrient deficiencies remain prevalent.

The findings should nevertheless be interpreted in light of the study limitations. The comparative cross-sectional design precludes determination of temporal or causal relationships between serum calcium status and subfertility. Total serum calcium was measured without assessment of ionized calcium, vitamin D, parathyroid hormone, serum albumin, dietary calcium intake, renal function, or other determinants of calcium homeostasis. Furthermore, the study was conducted at a single tertiary hospital, which may limit the generalizability of the findings to other populations. Despite these limitations, the study employed standardized laboratory methods, comparable study groups, and systematic participant recruitment, thereby strengthening the reliability of the findings and contributing valuable locally generated evidence to the limited literature on calcium status and female subfertility in Nigeria.

Overall, this study demonstrates that subfertile women had significantly lower serum calcium concentrations than fertile women and that low serum calcium was associated with fertility status, age, and BMI. These findings reinforce the growing recognition that nutritional status represents an important component of female reproductive health and support further prospective, multicentre, and interventional studies to clarify the causal role of calcium in female fertility and evaluate whether correction of calcium deficiency may improve reproductive outcomes.

Conclusion

This comparative hospital-based study demonstrated that subfertile women attending the Usmanu Danfodiyo University Teaching Hospital, Sokoto, had significantly lower mean serum calcium concentrations than fertile women. Low serum calcium status was significantly associated with subfertility, increasing age, and higher body mass index, suggesting that calcium homeostasis may be associated with female reproductive health. These findings support the growing evidence that nutritional and metabolic factors may contribute to female subfertility and should be considered alongside conventional clinical evaluation.

Although the study design does not permit causal inference, the findings provide important baseline evidence from northwestern Nigeria, where information on the relationship between serum calcium and female fertility is limited. Further well-designed prospective and interventional studies are required to clarify the causal relationship between calcium status and reproductive function and to determine whether correction of calcium deficiency can improve fertility outcomes.

Strengths of the study

This study has several notable strengths. It employed a comparative hospital-based design with equal numbers of fertile and subfertile women, enabling direct comparison of serum calcium concentrations between the two groups. Standardized participant selection criteria and laboratory procedures enhanced the validity and reliability of the findings. The study also evaluated relevant sociodemographic, anthropometric, and clinical variables alongside biochemical measurements, allowing a comprehensive assessment of factors associated with serum calcium status. Furthermore, it

provides valuable locally generated evidence from northwestern Nigeria, addressing an important gap in the literature on nutritional determinants of female subfertility.

Limitations of the study

The findings should be interpreted in light of several limitations. The comparative cross-sectional design precludes establishment of temporal or causal relationships between serum calcium status and subfertility. Total serum calcium was measured without assessment of ionized calcium, serum albumin, vitamin D, parathyroid hormone, dietary calcium intake, renal function, or other determinants of calcium homeostasis, which may have influenced the observed associations. In addition, participants were recruited from a single tertiary healthcare institution, which may limit the generalizability of the findings to other settings. Future multicentre prospective studies incorporating comprehensive nutritional and endocrine assessment are therefore recommended.

Clinical implications

The findings suggest that nutritional assessment may complement conventional infertility evaluation, particularly among women with suspected nutritional deficiencies or metabolic risk factors. Although routine measurement of serum calcium cannot be recommended solely on the basis of the present study, clinicians should recognize the potential contribution of nutritional status to female reproductive health and consider individualized nutritional assessment and counselling as part of comprehensive fertility care.

Recommendations

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

1. Women presenting with subfertility should receive individualized nutritional assessment where clinically indicated, particularly those with additional metabolic or nutritional risk factors.
2. Prospective cohort studies are required to determine the temporal relationship between serum calcium status and female subfertility.
3. Randomized controlled trials should evaluate whether correction of calcium deficiency improves fertility outcomes among women with subfertility.
4. Future studies should include ionized calcium, vitamin D, parathyroid hormone, serum albumin, dietary calcium intake, and other micronutrients to provide a more comprehensive assessment of calcium homeostasis.
5. Multicentre studies involving larger and more diverse populations should be undertaken to improve the external validity of the findings and inform evidence-based clinical guidelines.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of the 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 prior to enrolment. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki

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