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Evaluation of Copper /Zinc Ratio, Total Protein, Albumin and Reproductive Hormones among Infertile Women in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Infertility in females is a devastating problem whose solution has posed a lot of challenges due to the difficulty and complexity of identifying the exact cause and etiology among different individuals. This study evaluated copper/zinc ratio, total protein, albumin and reproductive hormones in infertile females in Port Harcourt. This was a case-control study, carried out among residents of Port Harcourt. A total of 130 female subjects within the age of 18 and 49 years were recruited for the study; 65 infertile subjects and 65 apparently healthy fertile control subjects. Approximately 10 mL of blood was collected using standard venipuncture technique from each subject on day 3 and 21 of the menstrual cycle. Samples for copper, zinc, total protein and albumin were analyzed using colorimetric methods, while the reproductive hormones were analyzed using Enzyme-Linked

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Immunosorbent Assay. Data generated were analyzed using Graph-Pad Prism version 8.0.2 and P≤0.05 was considered statistically significant. This study revealed a significant increase on day 3 and 21 in mean copper 27.68±10.78 and 20.29 ± 16.41 (P<0.001 and P=0.043) levels in infertile women when compared to the mean copper 16.82±4.52 and 15.20 ±5.29 of control group respectively. Copper/zinc ratio was also increased in the infertile group with mean levels of 11.40±1.82 and 3.27 ±0.59 on days 3 and 21 compared to the control group with mean levels of 4.53±0.76 and 1.46 ±0.12 (P=0.010 and P=0.004) respectively. There was significant decrease on day 3 of Menstrual cycle in Mean ± SD levels of Total Protein 72.62 ±7.84 in infertile group and 77.23 ±4.71 in control group (P< 0.001), and albumin 40.54 ±4.79 in infertile group and 44.82 ±3.64 in control group (P=< 0.001) respectively. There was no significant difference in mean zinc level (P=0.402) on day 3, but mean zinc level 10.74 ±4.58 was significantly decreased on day 21 in infertile females compared to the control 12.91 ±4.73 group (P=0.009). There was also significant increase in mean levels of Luteinizing hormone 8.64 ±4.32 infertile and 7.16 ±2.21 control (P=0.016), prolactin 27.02 ±18.55 infertile and 12.28 ±6.44 control (p<0.001), and testosterone 0.74 ±0.25 infertile and 0.38 ±0.18 control (P<0.001) respectively. There was a significant decrease in mean levels of estrogen 28.66 ±10.34 infertile and 41.79 ±4.55 control (P<0.001), and progesterone 7.71 ±6.38 infertile and 15.63 ±6.57 control (P<0.001) respectively. However, there was no significant difference in mean levels of follicle stimulating hormone (P=0.519). The findings in this study indicate that a high copper/zinc ratio is associated with alterations in levels of reproductive hormones. Hence, micronutrient supplementation to maintain optimum levels will be beneficial for infertile females.

Keywords: Women; infertility; reproductive hormones; copper; zinc; copper/zinc ratio.

1. INTRODUCTION

Female infertility is defined as childlessness among women of child-bearing age caused by female factors Murtaza *et al.,* [1]. Epidemiologically, it refers to “trying for “ or “time to “a pregnancy within female population subjected to a probability of conceiving [2]. Male infertility contributes to 20%-30% of infertility cases, 20%-35% are due to female infertility, while 25%-40% are as a result of both male and female problems [1].

Trace elements including copper and zinc are very important for normal life processes, and have been associated closely to infertility in females, spontaneous abortions and growth of the fetus [3]. Alterations in copper levels among infertile women have been associated with impaired structural and functional properties of the collagen found in graaffian follicle [4]. Low copper levels may result in inhibition of the movement of an ovum via the fallopian tubes. Zinc is required in reproduction as its deficiency is associated with infertility and abnormal gene function. It is important in the regulation of reproductive hormone functions [5]. Zinc has a significant function in spermatogenesis and sex differentiation, it is also important in the function of the organs responsible for reproduction [6,7].

Alterations in levels of protein have been associated with outcomes of fertility among humans and animals [8]. Studies have revealed that the quantity of dietary protein has an effect on the hypothalamo-pituitary axis and altered protein levels affects fertility [9]. Albumin is one of the major serum proteins that binds many lipophilic compounds including hormones. The sex steroids such as estrogen, progesterone and testosterone are transported by albumin and these hormones play a crucial role in homeostasis, development and reproduction [10].

The reproductive hormones are the hormones that controls puberty, reproduction and sexuality, and they are usually produced in the ovaries by females. The major female reproductive hormones include follicle stimulating hormones (FSH), luteinizing hormones (LH), estrogen (E_2) and progesterone (PRG).LH and FSH functions in the control of menstrual cycle. When there is an increased production of estrogen, the pituitary gland stops producing FSH, while the production of LH is encouraged. Prolactin and testosterone also affect female fertility as any abnormalities in their levels have been shown to contribute to infertility in females [11]. Prolactin stimulates production of breast milk. Prolactin levels have been shown to be a measure of sexual satisfaction in both sexes [11]. Progesterone functions in the induction of ovulation, facilitation of implantation and maintenance of pregnancy [12]. Excessive and low levels of androgens are involved in female infertility cases. Testosterone

plays a significant role in female libido [13]. The serum levels of testosterone have been known to decline with age, corresponding to the age-related decline in fertility rate [14]. Excess or absence of secretion of these reproductive hormones may result in hormonal imbalance, which can eventually cause irregular monthly menstrual cycles, anovulation and infertility [15].

In the context of female infertility in Port Harcourt, there exists a gap in understanding the interplay between copper/zinc ratio, total protein levels, albumin concentrations, and reproductive hormones. Despite the significance of these biomarkers in various physiological processes, their specific roles and associations with infertility among women in this region remain unclear. This study aim to bridge this gap and provide a foundation for future studies, enhancing the understanding of fertility challenges faced by women in this geographical area. Evaluating the levels of copper/zinc ratio, Total Protein, Albumin and Reproductive Hormones in infertile women may reveal potential diagnostic markers or patterns that can aid healthcare professionals in identifying underlying causes of infertility and tailoring treatment plans accordingly. The findings in this study will enrich the existing body of knowledge and provide a basis for further research in reproductive medicine.

2. MATERIALS AND METHODS

2.1 Study Design

A cross-sectional study design and a simple random sampling method was adopted for this study.

2.2 Study Population

This study was carried out among adult females who reside in Port Harcourt, for a minimum of two years. A total of one hundred and thirty (130) subjects aged 18-49 years were recruited for the study, comprising sixty five (65) apparently healthy fertile subjects who were used as the control subjects, and sixty five (65) infertile subjects.

The type of infertility, number of years of infertility was considered. The bio-data of the subjects were obtained using a well-structured questionnaire.

2.3 Sample Collection and Preparation

Approximately 10 mL of venous blood was drawn from the antecubital fossa of each subject on days 3 (follicular phase) and 21 (luteal phase) of the menstrual cycle respectively, using vacutainer into a sterile plain sample bottle. The blood sample collected was allowed to coagulate and then retracted and centrifuged to obtain serum. The serum was separated into another sterile plain sample bottle and stored frozen till the time of analysis.

2.4 Blood Sample Analysis

Day 3 samples of each subject was analyzed for Follicle stimulating hormone, luteinizing hormone, estradiol, testosterone, prolactin, albumin, total protein, zinc and copper, while day 21 sample was analyzed for progesterone, albumin, total protein, zinc and copper respectively. All the reproductive hormones were analyzed using enzyme-linked immunosorbent assay (ELISA) method, while copper, zinc, albumin and total protein were determined by colorimetric methods using semi auto-analyzer WP 21E.

2.5 Determination of Zinc Using Human Zinc Monoliquid Kit, Fortress Diagnostics, United Kingdom as Described by Eliasson [16]

Principle: Zinc present in the sample is chelated by 5-Br- P A P S 2-(5-bromo-2-pyridylazo)-5-(N—propyl-N-sulfopropylamino)- phenol in the reagent. The formation of this complex is measured at a wavelength of 560nm. The color intensity thus formed is directly proportional to the concentration of iron in the serum or plasma.

Procedure: All reagents were brought to room temperature before use. The assay was performed at room temperature. Four glass tubes were arranged and labelled as blank, standard, quality control (QC) and sample respectively. Fifty microlitre (50µL) of the sample was added only to the sample tube, 50µL of the QC was added to the QC tube, and then 50µL of the standard was added to the standard tube. One thousand microlitre (1000µL) of R1 zinc reagent was added to the blank, standard, QC and sample tubes. It was then mixed and incubated for 5 minutes at 37℃. The concentration of each sample was determined within 5 minutes using a semi-automatic analyzer set at 560nm.

2.6 Determination of Copper using Human Copper Kit, Fortress Diagnostics, United Kingdom as Described by Beckett *et al.,* [17]

Principle: At pH 4.7 (acidic buffer) copper, which is bound to ceruloplasmin is released by a

reducing agent. It then reacts with a specific color reagent, 4-(3.5-Dibromo-2-pyridylazo)-N-sulfopropylaniline, to form a stable colored chelate. The intensity of the color measured photometrically at 580nm is directly proportional to the amount of total copper in the sample.

Procedure: The assay was performed at room temperature. Three glass tubes were arranged and labelled as reagent blank, standard and sample respectively. Fifty microlitre (50µL) of distilled water was pipetted into the reagent blank tube, 50µL of standard was added to the standard tube and 50µL of sample was added to the sample tubes. One milliliter (1mL) of R1 was pipetted into all the tubes. It was then mixed and incubated for 5 minutes using a water bath at 20-25℃. The concentration of each sample was determined using a semi-automatic analyzer set at 580nm within 5 minutes after adding the working reagent.

2.7 Determination of Follicle Stimulating Hormone Using Abnova Human FSH Kit as Described by Ongaro *et al.,* [18]

Principle: The FSH in assay samples bind to wells of the microtiter plate coated with a polyclonal anti-FSH antibody. The enzyme Horseradish peroxidase (HRP) detects the bound FSH, which is visualized by adding the substrate TMB, giving a blue coloured product, measured at 4 50nm following the addition of a stop solution. The absorbance of the solution at 450nm is a measure of FSH concentration in the sample.

Procedure: The assay was performed at room temperature. One hundred microlitre (100 µL) of each of the calibrators and samples were added to the appropriate antibody pre-coated microtiter well, followed by the addition of 50 µL of the conjugate. It was mixed properly, covered and incubated at 37^0 c for 1hour. The microtiter plate was washed, then 50 µL of substrate A and B was added to each well respectively. It was covered and incubated for 10 minutes at 20-25^0c in a water bath. Subsequently, 50 µL of stop solution was added to each well and the absorbance of the resulting solution was read at 450nm using a microtiter plate reader after mixing properly.

2.8 Determination of Luteinizing Hormone using Abnova Human LH Kit as Described by Qin *et al.,* [19]

Principle: Luteinizing hormone determination is a solid phase enzyme-linked immunosorbent assay (ELISA) in which the microtiter wells are incubated with mouse monoclonal antibody directed towards an antigenic site on LH molecule. The assay sample which contains endogenous LH is incubated in the antibody coated well containing conjugate enzyme (anti-LH monoclonal antibody conjugated with HRP). The unbound conjugate is washed off after incubation.bthe quantity of bound peroxidase is proportional to LH concentration in the sample. After the addition of substrate solution, the colour intensity is proportional to concentration of LH in the assay sample.

Procedure: The assay was performed at room temperature and all reagents were prepared following the manufacturers instruction. Fifty microlitre (50 µL) of standard, controls and samples were dispensed into appropriate wells respectively, followed by the addition of 100 µL of enzyme conjugate reagent into each well. This was mixed properly and incubated at room temperature for 45 minutes. The microtiter plate was washed, 100 µL of TMB reagent was dispensed into each of the wells and mixed gently. It was incubated at room temperature for 20 minutes, followed by the addition of 100 µL of stop solution to each well. This was gently mixed for 30 seconds, yielding a yellow coloured product. The absorbance of the resulting coloured solution was read at 450nm with a microtiter plate reader.

2.9 Determination of Progesterone using Abnova Human PRG Kit as Described by Relave *et al.* [20]

Principle: The determination of progesterone through ELISA method is based on the principle of competitive binding between progesterone in the assay sample and progesterone-HRP conjugate for a constant amount of mouse anti-progesterone. In the incubation, IgG-coated wells are incubated with 25 µL progesterone standards, controls, patient samples, 100 µL progesterone-HRP Conjugate Reagent and 50 µL mouse anti-progesterone reagent at room temperature (18-25°C) for 90 minutes. During the incubation, a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard, control and assay

sample for binding sites of the specific progesterone antibody. Thus, the amount of progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of progesterone in the sample increases. A solution of TMB Reagent added results in the development of blue colour. The colour development is stopped with the addition of Stop Solution, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely proportional to the amount of progesterone in the sample.

Procedure: All reagents and samples were brought to room temperature and prepared following manufacturers instruction. Twenty five microlitres (25 µL) of standard, controls and samples were dispensed into the appropriate wells, 100 µL of conjugate reagent and 50 µL of anti-progesterone reagent were added respectively to each well. It was mixed thoroughly for 30 seconds, incubated at room temperature for 90 minutes and washed with distilled water. One hundred microlitres (100 µL) of TMB reagent was dispensed into each well, mixed and incubated for 20 minutes at room temperature. One hundred microlitres (100 µL) of stop solution was added, mixed properly and the absorbance of the resulting solution was read at 450nm with a microtiter plate reader.

2.10 Determination of Estradiol using Abnova Human E_2 Kit as Described by Zhao *et al.* [20]

Principle: In the determination of Estradiol, the assay principle is based on a solid-phase enzyme immunoassay that utilizes competitive binding. The 17β-Estradiol(antigen) in the sample competes with the antigenic17β-Estradiol conjugated with horseradishperoxidase for binding to the limited number of antibodies (anti 17β-Estradiol) coated on the microplate (solid phase). Following incubation, the bound /free separation is performed by a simple solid-phase washing. Then the enzyme HRP in the bound-fraction reacts with theSubstrate (H2O2) and TMB Substrate to develop a blue coloured product that changes into yellow when the Stop Solution (H2SO4) is added. The colour intensity is inversely proportional to the 17β-Estradiol concentration in the sample.

Procedure: The reagents and samples were brought to room temperature. Fifty microlitres (50 µL) of standards, controls and samples were dispensed into the appropriate wells respectively, 100 µL of E_2 enzyme conjugate solution was added to each well except for the blank. The plate was incubated at 37^0 c for 2hours using water bath. It was washed properly using the wash solution contained in the kit. One hundred microlitres (100 µL) of TMB substrate was added into each well including the blank, then the plate was incubated for 20 minutes at room temperature. Fifty microlitres (50 µL) of stopping solution was added and mixed gently, the absorbance of the resulting solution was read at 450nm against blank with a microtiter plate reader.

2.11 Determination of Prolactin using Biovendor Human PRL Kit as Described by Ugwa *et al.* [22]

Principle: A monoclonal antibody specific for prolactin is immobilized onto the microplate well and another monoclonal antibody specific for a different region of prolactin is conjugated to horseradish peroxidase. Prolactin from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The colour intensity of the product formed by the enzymatic reaction is directly proportional to the concentration of prolactin in the sample.

Procedure: This assay was performed at room temperature. Twenty five microlitres (25 µL) of calibrator, control and sample were dispensed into correspondingly labeled wells, 100 µL of conjugate solution was added into each well and mixed gently, the microtiter plate was incubated at room temperature for 1hour. It was washed 3 times with wash buffer. One hundred and fifty microlitre (150 µL) of TMB substrate was added to each well and incubated for 10 minutes at room temperature, 50 µL of the stopping solution was added to each well and the absorbance of the solution was read with microwell plate reader at 450nm.

2.12 Determination of Testosterone using Abnova Human TESTO Kit as Described by Emad and Al-Dujaili [23]

Principle: In the determination of testosterone, the ELISA method is based on competitive

binding. Testosterone (antigen) in the sample competes with the antigenic Testosterone conjugated with horseradish peroxidase (HRP) present in the Conjugate for binding to the anti-testosterone coated on the microplates (solid phase). After the incubation, the bound/free separation is performed by a simple solid-phase washing. The enzyme HRP in the bound-fraction reacts with the Substrate (H2O2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution (H2SO4) is added. The colour intensity is inversely proportional to the Testosterone concentration in the sample.

Procedure: The assay was performed at room temperature. Ten microlitres (10 µL) of standard, controls and sample was dispensed into the appropriate wells, 100 µL of testosterone- HRP conjugate reagent was added to each well and 50 µL of anti-testosterone reagent was added as well. It was mixed thoroughly and incubated for 90 minutes at 37^0 c. The wells were washed with deionized water. One hundred microlitres(100µL) of TMB reagent was dispensed into each well and gently mixed. It was incubated at room temperature for 20 minutes. One hundred microlitres of stop solution was added to each well, it was gently mixed and the absorbance of the resulting yellow coloured solution was read with a microtiter well reader at 450nm.

2.13 Determination of total protein using Randox Total Protein kit as Described by Basil *et al.* [24]

Principle: Compound that contains two -CONH2 in the molecule react with alkaline copper solution to form a purple complex, which is known as the biuret reaction. Peptide bonds (-CONH-) in protein molecules present in the assay sample react with alkaline copper solution to form a purple coloured complex.

Procedure: All reagents and samples were brought to room temperature. Fifty microlitres (50 µL) of protein standard and sample was dispensed into the corresponding test tubes respectively and 2500 µL of biuret reagent was added to all the test tubes including blank. It was mixed thoroughly and incubated at 37^0 c for 30 minutes, the absorbance of the resulting solution was read at 540nm.

2.14 Determination of Albumin using Randox Albumin kit as Described by Rodkey [25]

Principle: Bromocresol green (BCG) combines with albumin in pH 3.5-4.2 to form an albumin-BCG complex, which has a blue-green colour. The color intensity is proportional to the concentration of albumin in the sample. The absorbance of the colour produced is measured spectrophotometrically at 630nm wavelength.

Procedure: The assay was performed at room temperature. Ten microlitres (10 µL) of standard, quality control and assay sample was dispensed into the corresponding test tubes respectively, 1000 µL of the working reagent (BCG reagent) was added to each tube, mixed and incubated at room temperature for 10 minutes. Absorbance of the resulting solution was read at 630nm.

Statistical Analysis: The generated data were expressed as Mean ± Standard Deviation and analyzed using Graphpad prism version 8.0.2. Comparisons of means and standard deviation values were made for the various parameters using t-test. Results were considered statistically significant at 95% confidence interval ($P \leq 0.05$).

3. RESULTS

The levels of copper/zinc ratio, total protein, albumin and reproductive hormones in infertile women were compared with those in fertile women treated as control.

4. DISCUSSION

Infertility is one of the frequently encountered gynecological problemwith different pathophysiological mechanisms in individuals, thereby making the treatment and management process a challenging factor. This study was carried out among 130 females of mean age33±7 years and 32±6 years for infertile and control subjects respectively as shown in Table 1.

In this study, serum Cu^{2+}level was significantly high on day 3 and 21 of menstrual cycle in infertile group compared to the control group. The increase in serum Cu^{2+}levels seen in this study agrees with the findings of Hamad *et al.,* [26] and Matsubayashi *et al.,* [27]. This may be due to the hormonal changes associated with menstrual cycle.

There was no significant difference in Zn^{2+} level between the infertile and fertile subjects on day 3 of the menstrual cycle. This finding disagrees with the report of Garner *et al.,* [28]. However significantly lowZn^{2+} level was observed in infertile group on day 21. This agrees with the findings of Nasiadek*et al.,* [29] in women with PCOS. Foods of animal origin are the richest source of adequately absorbable Zn^{2+} [30]. The consumption of such foods can affect Zn^{2+} levels. More so, during day 3 of the menstrual cycle there is loss of blood [29]. This may be responsible for the differences in Zn^{2+}level following days of the menstrual cycle as seen in Table 2 and 3.

The serum level of Cu/Zn ratio was significantly higher in infertile women compared to the fertile group. Cu/ Zn ratio, instead of absolute Zn or Cu alone is essential for optimal enzymatic functions [31]. The levels of serum Cu and Zn are closely regulated by mechanisms that tend to control them within specific ranges. Some mechanisms act to decrease serum Zn level while increasing serum Cu level, this phenomenon is seen in inflammatory mechanisms and age-related chronic conditions [32]. Serum concentration of Zn may be affected by dietary changes except in severe deficiency or during supplementation. However, serum Cu concentration is less sensitive to dietary changes [32]. An increase in Cu/Zn ratio is usually associated with a decrease in Zn and / or increase in Cu. In this study, the mean Cu/Zn ratio was significantly higher in infertile group compared to the control group on day 3 and 21 of the menstrual cycle. This disagrees with the findings of Cao *et al.,* [33] but agrees with the report of Al-Saraf *et al.,* [7]. Furthermore, a high Cu/Zn ratio has been reported to be a risk factor for implantation failure as Cu/Zn ratio is a better predictor of clinical pregnancy rates than Cu or Zn alone [27].

This study report a significant decrease in level of total protein in infertile group compared to control on day 3 and 21 of the menstrual cycle respectively. Protein metabolism is affected by several factors including hydration status, capillary permeability and diet, these invariably affects serum protein concentrations [34,35].

Table 1. Demographic data of subjects

	Infertile	**Control**
Mean Age (Years)	**33± 7**	**32 ±6**
Type of Infertility		
1. Primary	27	
2. Secondary	38	
Level of Education		
1. Secondary	26	30
2. Tertiary	39	35
Occupation		
1. Business	33	28
2. Civil/ Public servant	28	32
3. Student	4	5

Table 2. Mean levels of Cu^{2+}, Zn^{2+}, Cu/Zn ratio,total protein and albumin on day 3of menstrual cycle

	Cu (µmol/L)	**Zn^2(µmol/L)**	**Cu/ Zn Ratio**	**Total Protein (g/L)**	**Albumin (g/L)**
Infertile (n= 65)	27.68±10.78	7.12 ±6.70	11.40±1.82	72.62 ±7.84	40.54 ±4.79
Control (n= 65)	16.82±4.52	8.01 ±5.11	4.53±0.76	77.23 ±4.71	44.82 ±3.64
P- Value	<0.001	0.402	0.010	< 0.001	< 0.001
t- Value	1.989	1.484	3.480	4.059	5.729

Keys: Cu =Copper, Zn = Zinc, Cu/Zn Ratio = Copper/Zinc Ratio, P-Value = Probability Value, t-Value = test Statistic

Table 3. Mean levels of Cu^{2+}, Zn^{2+}, Cu/Zn ratio, total protein and albumin on day 21 of menstrual cycle

	Cu (µmol/L)	Zn (µmol/L)	Cu/ Zn Ratio	Total Protein (g/L)	Albumin (g/L)
Infertile	20.29 ± 16.41	10.74 ±4.58	3.27 ±0.59	50.35 ±9.66	35.73 ±8.94
Control	15.20 ±5.29	12.91 ±4.73	1.46 ±0.12	67.46 ±19.14	32.44 ±6.42
P- Value	0.043	0.009	0.004	< 0.001	0.018
t- Value	2.060	2.652	3.151	6.433	2.407

Keys: Cu =Copper, Zn = Zinc, Cu/Zn Ratio = Copper/Zinc Ratio, P-Value = Probability Value, t-Value = test Statistic

Table 4. Mean levels of reproductive hormones

	Infertile	Control	P- Value	t- Value
FSH(mIU/mL)	7.58 ±4.81	7.14 ±2.64	0.519	0.648
LH(mIU/mL)	8.64 ±4.32	7.16 ±2.21	0.016	2.458
E_2(pg/mL)	28.66 ±10.34	41.79 ±4.55	<0.001	9.377
PRL(ng/mL)	27.02 ±18.55	12.28 ±6.44	<0.001	5.968
TESTO(ng/mL)	0.74 ±0.25	0.38 ±0.18	<0.001	1.986
PRG(ng/mL)	7.71 ±6.38	15.63 ±6.57	<0.001	1.961

Key: FSH- Follicle stimulating hormone, LH- Luteinizing Hormone, E_2- Estradiol,PRL- Prolactin, TESTO- Testosterone, PRG- Progesterone

Table 5. Parameters according to type of infertility on day 3 of menstrual cycle

	Cu (µmol/L)	Zn (µmol/L)	Cu/Zn Ratio	Total Protein (g/L)	Albumin (g/L)
Primary Infertility (n= 27)	28.40± 11.80	6.85± 1.16	10.50 ±2.90	70.72 ±5.30	39.96 ±4.68
Secondary Infertility (n= 38)	27.20± 12.70	7.31 ±1.22	12.04± 2.49	73.98 ±9.06	40.95 ±4.89
P- Value	0.706	0.785	0.703	0.074	0.412
t- Value	0.380	0.275	0.380	1.819	0.826

Keys: Cu =Copper, Zn = Zinc, Cu/Zn Ratio = Copper/Zinc Ratio, P-Value = Probability Value, t-Value = test Statistic

Table 6. Parameters according to type of infertility on day 21 of menstrual cycle

	Cu (µmol/L)	Zn (µmol/L)	Cu/Zn Ratio	Total protein (g/L)	Albumin (g/L)
Primary Infertility	21.10 ±13.32	9.84 ±4.26	3.66± 0.90	47.81 ±10.13	33.82 ±8.86
Secondary Infertility	17.60± 14.60	11.37 ±4.76	2.99 ±0.79	52.16 ±9.02	37.08 ±8.85
P- Value	0.319	0.181	0.575	0.080	0.150
t- Value	1.007	1.353	0.560	1.784	1.460

Keys: Cu =Copper, Zn = Zinc, Cu/Zn Ratio = Copper/Zinc Ratio, P-Value = Probability Value, t-Value = test Statistic

Albumin is the major protein involved in transportation of ionic Cu from tissues. Cu transport by albumin involves the formation of copper-albumin complex which is taken to the liver through the circulating portal system, after gastrointestinal absorption [36]. The level of Total protein and albumin have not been appreciably explored in women with primary and secondary infertility generally. There was a statistically significant decrease in albumin within the infertile

group compared to control group on day 3 of the menstrual cycle, while on the contrary, there was a significant increase in albumin level within the infertile group compared to the control group on day 21 of menstrual cycle as seen in this study. These differences observed may be associated with hormonal changes during menstrual cycle, diet and / or metabolism Graham, [35] Carol [34].

There was no significant difference in level of follicle stimulating hormone in the infertile women compared to control as seen in Table 4. This finding aligns the finding of Digban*et al.* [37] but disagrees with the report of Owiredu*et al.*[38] in Ghanaian women. However, follicle stimulating hormone is important in follicular development and steroidogenesis in the ovary.

Prolactin and testosterone were significantly higher in the infertile group. This finding aligns with the findings of Braide *et al.* [39] and Digban*et al.* [37]. Half of the testosterone in women is produced mainly through peripheral conversion of androstenedione. Increased testosterone levels in women have been associated with PCOS and hirsutism [40]. These conditions contribute to infertility. Hence, the increased levels of prolactin and testosterone may be the contributory factors responsible for the infertility in these women. The high prolactin level observed may be due to interference with ovulation process.

Serum progesterone levels on day 21 of the menstrual cycle is used to determine ovulation. This study observed significant decrease in the level of progesterone in the infertile group. This finding correlates with the report of Rabiya *et al.* [41] Digban*et al. al.* [37] Ben-Chioma & Tamuno-Emine [42].

This study observed a significant increase in serum Estradiol (E_2)and luteinizing hormone (LH) in the fertile group compared to the infertile group. Higer levels of E_2 are reported to be associated with better ovarian response [43]. This finding agrees with the observation of Digban*et al.* [37] in Bida women.

Serum Cu, Zn, Cu/Zn ratio, Total protein and Albumin showed no significant difference in primary and secondary infertility following day 3 and 21 of menstrual cycle as displayed in Table 5 and 6 respectively.

Serum FSH, LH, E_2,TESTO, PRL and PRG levels showed no significant differences at P≥0.05 in primary and secondary infertility as shown in Table 7. This finding is consistent with the report of Ben-Chioma and Tamuno-Emine [42].

Table 7. Mean levels of reproductive hormones according to type of infertility

	Primary Infertility	Secondary Infertility	P- Value	t- Value
FSH(mIU/mL)	6.64 ±2.81	8.26 ±5.79	0.141	1.493
LH(mIU/mL)	7.99 ±3.30	9.11 ±4.92	0.278	1.094
E_2 (pg/mL)	27.83 ±10.41	29.24 ±10.39	0.592	0.538
PRL(ng/mL)	30.82 ±22.32	24.33 ±15.71	0.201	1.300
TESTO(ng/mL)	0.60 ±011	0.84 ±0.23	0.084	2.001
PRG(ng/mL)	8.17 ±6.43	7.39 ±6.22	0.633	2.003

Key: FSH- Follicle stimulating hormone, LH- Luteinizing Hormone, E_2- Estradiol, PRL- Prolactin, TESTO- Testosterone, PRG- Progesterone.

Table 8. Parameters according to duration of infertility on day 3 of menstrual cycle

	Cu µmol/L)	Zn (µmol/L)	Cu/Zn Ratio	Total Protein (g/L)	Albumin (g/L)
1-5 Yrs (n= 52)	28.48±11.74	7.19 ±1.05	13.90±2.70	72.66 ±8.04	40.05 ±4.84
6-10 Yrs (n=13)	27.91 ±15.29	6.85 ±3.37	12.44 ±5.63	72.49 ±7.31	42.51 ±4.19
P- Value	0.411	0.809	0.474	0.944	0.562
t- Value	0.841	0.243	0.731	0.072	0.587

Keys: Cu =Copper, Zn = Zinc, Cu/Zn Ratio = Copper/Zinc Ratio, P-Value = Probability Value, t-Value = test Statistic

Table 9. Mean levels of parameters according to duration of infertility on day 21 of menstrual cycle

	Cu (µmol/L)	Zn (µmol/L)	Cu/Zn Ratio	Total Protein (g/L)	Albumin (g/L)
1-5 Yrs	20.76 ±17.51	10.70 ±4.69	3.90 ±0.78	51.34 ±9.92	35.90 ±9.42
6-19 Yrs	18.42 ±11.38	10.88 ±4.31	2.76 ±1.00	46.39±7.63	35.04 ±6.95
P- Value	0.562	0.892	0.375	0.062	0.712
t- Value	0.587	0.137	0.900	1.959	0.373

Keys: Cu =Copper, Zn = Zinc, Cu/Zn Ratio = Copper/Zinc Ratio, P-Value = Probability Value, t-Value = test Statistic

Table 10. Reproductive hormones according to duration of infertility

	1-5 Yrs	6-10 Yrs	P- Value	t- Value
FSH (mIU/mL)	7.74 ±5.20	6.94 ±2.88	0.459	0.749
LH (mIU/mL)	8.67 ±4.32	8.52 ±4.53	0.914	0.109
E_2 (pg/mL)	39.03 ±25.11	34.31 ±16.49	0.419	0.821
PRL (ng/mL)	28.19 ±2.40	27.34 ±9.86	0.044	1.489
TESTO(ng/mL)	0.75 ±0.65	0.70 ±0.40	0.740	0.335
PRG (ng/mL)	13.17 ±2.50	24.10 ±6.26	0.012	1.621

Key: FSH- Follicle stimulating hormone, LH- Luteinizing Hormone, E_2- Estradiol, PRL- Prolactin, TESTO- Testosterone, PRG- Progesterone.

There were no significant differences in levels of Cu, Zn, Cu/Zn ratio, Total protein and Albumin according to the duration of infertility following day 3 and 21 of menstrual cycle as displayed in Table 8 and 9 respectively.

There were significant increase in the levels of progesterone and testosterone in 1-5 years duration of infertility compared to 6-10 years duration. However, there wassignificant decrease in level of progesterone in 1-5 years duration as shown in Table 10.For a successful implantation to occur in the uterus, there must be adequate progesterone effect on the endometrium and the embryo must be present during the window of uterine receptivity. An improper timing of window of uterine receptivity with respect to ovulation may result in implantation failure or infertility [42].

5. CONCLUSION

Female fertility can be affected by different factors which can be environmental, dietary, hormonal and psychosocial. Hence female fertility is vulnerable and must be closely assessed, monitored and treated timely to avoid the problem of infertility. This study reported significant increase in Copper, Copper/Zinc ratio on day 3 and 21 and a significant decrease in total protein on day 3 and 21.Albumin was significantly lower on day 3 but significantly higher on day 21 in infertile group. The reproductive hormones LH, PRL and TESTO were significantly higher while E2 and PRG were significantly lower in infertile group. No significant difference was seen in FSH. An increased copper/zinc ratio is a risk factor contributing to female infertility. Thus, this study is suggesting that copper and zinc levels should be routinely monitored as they invariably affect copper/zinc ratio. An increase in Cu/Zn ratio is usually associated with a decrease in Zn and / or increase in Cu. Hence they should be incorporated in the assessment, diagnosis, management and monitoring of infertility. An increase in copper level results in decrease in zinc level, thus care must be taken during supplementation of these elements to ensure optimal balance in their concentrations as it is required for a healthy reproductive life. The transport protein albumin, showed variable fluctuations following menstrual cycle phase, this may be attributed to differences in secretion and synthesis of reproductive hormones across different phases of the menstrual cycle, hence more studies should be done to establish the interaction between transport proteins and reproductive hormones following menstrual cycle phases.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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