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Hormonal Profile and Trace Metal Contents of Blood and Semen in Men with Infertility Attending University College Hospital, Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. The author OIO conceptualized and designed the work, interpreted and prepared the manuscript. Author OJ carried out the analysis while author ASA recruited the participants and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to determine the relationship between blood hormonal profile and trace metal contents of blood and semen in men with infertility.
Study Design: This was a case-control study conducted using 33 male participants. Cases comprised of male patients presenting with infertility and the controls comprised apparently healthy males without any history of fertility problems.
Place and Duration of Study: University College Hospital, Ibadan, Nigeria.
Methodology: Ten millilitres of venous blood samples were collected for the determination of trace metals and male reproductive hormones using Enzyme Linked Immunosorbent Assay (ELISA). Atomic absorption spectrophotometry measurement of the trace elements concentration in plasma samples was done. All analyses were performed using standard laboratory procedures while data were analysed using Statistical Package for Social Science (SPSS) version 20.0.

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Results: No significant difference was observed between the dietary history and lifestyle factors between these two groups. There was no significant difference in median cadmium and zinc between cases and controls. Median seminal plasma Zn concentration was significantly lower than controls. The median FSH of infertile men was significantly higher than that of fertile controls.
Conclusion: There was no significant difference in the toxic trace metal content of both serum and seminal plasma between cases and controls possibly because of the similarity in their sociodemographic and environmental characteristics. There was a significant difference in the essential trace metals of both serum and seminal plasma between cases and controls. Oxidative stress due to other factors other than Pb and Cd may be a possible contributory factor to infertility as indicated by similar levels of these metals in both cases and controls and a significantly reduced level of the essential trace metals which are important components of the antioxidant enzyme system in the body.

Keywords: Male infertility; toxic and essential metals in blood; toxic and essential metals in semen; hormonal profile; oxidative stress.

1. INTRODUCTION

Infertility is defined as 'the inability of couples to achieve conception despite regular unprotected sexual intercourse for 1 year' [1]. It is a problem of public health importance in Nigeria and many other developing nations because of its high prevalence and its serious social implications on affected couples and families. It is a common clinical problem affecting 10–15% of the general population [2]. The prevalence varies between developed and underdeveloped countries, being higher in the latter in which limited resources for diagnosis and treatment exist [3]. Problem of infertility may be traceable to either the male or female and sometimes both factors.

Male infertility refers to the inability of a man to impregnate a woman after 12 months of regular and unprotected sexual intercourse [4]. Male infertility is considered when identifiable female causes of infertility are excluded and semen quantity and quality fail to fulfill the World Health Organization (WHO) criteria [5]. Infertility can be classified as primary when the man has never impregnated a woman and secondary when he has impregnated a woman before, regardless of the outcome. The male factor is associated with a greater percentage of cases of primary rather than secondary infertility [6]. The WHO sponsored multicentre collaborative investigation of infertility study indicated that Africa might have a pattern of infertility quite different from that of other regions. While primary infertility is commoner in other regions, secondary infertility is predominant in Africa [7].

Epidemiologically, male factor alone is approximately 20% and contributory in another 30–40% of couples; as such, a male factor is implicated in more than 50% of couples attempting to conceive [8]. The incidence and the causes of male infertility and male reproductive ill-health are important issues that remain poorly characterised. Factors like congenital or acquired conditions such as urogenital abnormalities, varicocele, infections of the genital tract, genetic abnormalities, endocrine disturbances, testicular failure, immunologic problems, cancer, systemic diseases, altered lifestyle, and exposure to gonadotoxic substances have been implicated in male infertility [9]. The cause of fertility impairment cannot be determined in several cases, despite the advances in diagnosis by the introduction of novel sophisticated tests. It has long been suggested that, at least, one half of the cases of human male infertility of unknown aetiology may be attributed to various environmental and occupational exposures [10] alongside hormonal imbalances.

Exposure to toxic metals at trace concentrations either voluntarily through supplementation or involuntarily through occupation or intake of contaminated/polluted food and water or contact with contaminated/polluted soil, dust, or air.

Increase interest has arisen in the evaluation of essential trace elements present in different concentrations in the human body fluids and their correlation to health. These trace elements, including chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn), are essential for good health but may be harmful above certain levels [11,12,13]. Aside from this, a number of metals are reproductive toxicants and suspected endocrine disruptors [14]. Although, exposure to several of these toxic metals is prevalent, human studies of exposure to metals and altered hormone levels to date are quite limited. Alterations in reproductive hormone levels, even at degrees that are considered subclinical, may

be associated with or lead to declined fertility and reproductive health, increased risk of endocrine-related cancers, or other adverse effects. The focus of this work was thus on effects of these environmental contaminants on the male reproductive system.

Since the focus of this work was on interplay between trace metals and male reproductive hormones, the physiological roles of some of the trace metals in male reproduction have been shown to be varied. For example, between Pb and/or Cd and Zn for the same binding sites in enzymes, proteins, and transporters, can change enzyme activity. On the other hand, essential metals such as Zn and Se may decrease the absorption and retention of toxic metals thus preventing their toxic effect [15]. Moreover, metals have a significant role in the antioxidant system, adaptive response, and genetic repair system. Therefore, the interaction between various toxic and/or essential metals could be particularly important for the final health outcomes of metal exposure [16].

Results of many studies on the effects of Pb and Cd on hormone concentration, male infertility and sperm parameters have been equivocal [17]. Not much has been documented on the possible contribution of trace metals (toxic and essential) to male infertility. This becomes relevant in the face of uncontrolled exposure to these metals especially in our quest for industrialisation. Also, expanding knowledge about exposure to environmental substances that could adversely affect male fertility has great importance in maintaining a man's health, his family life, and the health of his progeny.

The ever-increasing presence of these confounding environmental factors coupled with the increasing incidence of infertility and its attendant sociocultural consequences Informed this study. The aim of this study is, therefore, to determine the relationship between blood hormonal profile and trace metal contents of blood and semen in men with infertility attending University College Hospital, Ibadan, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design and Selection of Participants

This is a case-control study conducted using 33 age-matched male participants (aged 30-39 years). Informed written consent was obtained from each participant before sample collection after ethical approval for the study was obtained from the ethics committee of the Joint University of Ibadan/University College Hospital, Institutional Review Committee (UI/UCH IRC), Ibadan.

2.2 Selection Criteria

Cases comprised of male patients presenting with infertility at the Urology Clinic of the Surgery Outpatient Department, University College Hospital, Ibadan. Cases were subjects without medical/surgical complications (as listed in the introductory passage) screened by the Consultant Urologist in the research team.

The controls comprised apparently healthy males without any history of fertility problems, the partners of whom had a spontaneous pregnancy within one year of regular unprotected intercourse and were pregnant at the time of the male's inclusion into the study.

2.3 Data Collection

Each participant completed an extensive questionnaire regarding his occupation, residence, social status, diet, water source, and smoking habits. Detailed medical history was taken from all participants with special emphasis on reproductive history. They were also subjected to thorough general medical and genital examination by the Consultant Urologist in the team.

2.4 Sample Collection and Analysis

Ten millilitres of venous blood samples were collected from each participant into plain bottles from which serum was obtained for the determination of trace metals and male reproductive hormones. The blood samples were centrifuged at 3000rpm for 10 min and the plasma was stored at -20°C until analysis

Reproductive hormones (LH, FSH and testosterone) were determined by Enzyme Linked Immunosorbent Assay (ELISA) based on antigen-antibody interaction using Monobind Inc. FSH, LH and Testosterone ELISA kit (USA).

Atomic Absorption Spectrophotometry (AAS) measurement of the trace elements [lead (Pb), cadmium (Cd), zinc (Zn) and selenium (Se)] concentration in plasma samples was performed on Buck Scientific 210 VGP (Germany). Working

standards solutions of Pb, Cd, Zn and Se were prepared by diluting the stock standards of each with deionised water and the required part per million (ppm) used for the standardisation of the corresponding trace elements. The frozen plasma samples were thawed and diluted with 0.1N hydrochloric acid, HCL (1:20) to release bound trace metals to enhance accurate measurement. The digested samples were then determined for the various metals on Buck Scientific Atomic Absorption Spectrophotometer (Model 210VGP) at their different wavelength after calibration of the equipment.

Quality Control (QC) check: QC check was ensured with the use of QC materials supplied along with the ELISA kit (purchased from Monobind Incorporated, USA) for the hormonal assays while QC materials for the trace metals routinely used on the AAS were those obtained from the Environmental Research Laboratory, Texas South Western University, Houston, USA. Values obtained in all the analysis (ELISA and AAS) were within the acceptable range as stated by the respective manufacturers.

2.5 Statistical Analysis

All completed questionnaires were collected and screened for completeness. Because most of the laboratory data were not normally distributed as determined by Shapiro-Wilk test, descriptive statistics such as median (interquartile range) was used to summarise and present the results. Differences in data obtained from questionnaire and laboratory parameters in case and control groups were analysed by Chi square and Mann-Whitney U test. Spearman's correlation analysis was used to determine relationship between parameters. A two-sided P-value of less than 0.05 was considered to be statistically significant. All analyses were performed using Statistical Package for Social Science (SPSS) version 20.0.

3. RESULTS

Table 1 shows the comparison of occupational and residential characteristics of infertile and fertile participants. There were no significant differences in their occupational and residential characteristics. 36.4% and 24.2% of the infertile and fertile participants has worked between 2-5yrs while 21.2% and 18.2% has worked between 6-9 yrs respectively.

Table 2 shows the dietary history and lifestyle factors of the infertile and fertile participants. No significant differences were observed between the dietary history and lifestyle factors between these two groups. 9.1% each of the infertile and fertile participants consumed alcohol. Also, 3% of the infertile participants smoked while none of the fertile participants smoked. The difference was not significant.

Table 3 shows the comparison of age and BMI of cases and controls. The median age (IQR) obtained for the two groups were 33.50 years (33.00-39.00years) and 32.00 (30.00-35.00 years) respectively. No significant difference was observed ($p>0.05$). The BMI values were 21.82 kgm^{-2} (19.33-24.32 kgm^{-2}) and 22.61 kgm^{-2} (19.44-24.49 kgm^{-2}) for cases and controls respectively. No significant difference was observed between the two group ($p>0.05$).

Table 4 shows the comparison of median toxic and essential trace metals levels in serum of cases and controls. There were no significant differences in median cadmium and zinc levels between cases and controls but the serum Se level estimated showed a median value of 1.14 (0.49-1.81) µmol/L and 1.92 (1.52-2.22) µmol/L in cases and controls groups respectively. The difference was statistically significant ($p = 0.005$). The median Cd levels were 0.0005 nmol/L (IQR=0.0004-0.0113) and 0.0005 nmol/L (IQR=0.0028-0.0063) in cases and controls respectively while levels of Pb in cases and controls were below limit of detection. There was no difference in Cd levels in cases and controls.

Table 5 shows the comparison of toxic and essential trace elements in seminal plasma of cases and controls. Median seminal plasma Zn concentration was significantly lower in cases compared to that of controls ($p = 0.014$). A significantly lower seminal plasma Se ($p = 0.000$) was also observed in cases compared to controls. The median Cd levels were 0.0004 nmol/L (IQR=0.0003-0.0004) and 0.0004 nmol/L(IQR=0.0003-0.014) in cases and controls respectively while levels of Pb in cases and controls were below limit of detection. There was no difference in Cd levels in cases and controls.

Table 6 shows comparison of male reproductive hormones between cases and controls. There were no significant differences between the median LH and testosterone level between cases and control. However, the median FSH of infertile men was significantly higher than that of fertile controls ($p= 0.044$).

Table 7 shows the Spearman's correlation of levels of trace metals in both serum and seminal plasma with those of male reproductive hormones in control participants. None of the parameters correlated significantly with one another.

Table 1. Comparison of occupational and residential characteristics between cases and controls

Characteristic	Cases (%)	Controls (%)	x^2	p-value
Job and residential characteristics				
House near factory	0	0		
Yes	57.6	42.4		
No				
House near filling station	0	0		
Yes	57.6	42.4		
No				
Source of drinking water	18.2	6.1	1.446	0.485
Well	18.2	12.1		
Piped	21.2	24.2		
Others (sachets water)				
Description of work environment	39.4	24.2	1.613	.656
Indoor	9.1	12.1		
Outdoor	6.1	6.1		
Workshop	3.0	0.0		
Others				
Length of period of occupation	0	0	0.122	.727
<2yrs	36.4	24.2		
2-5yrs	21.2	18.2		
6-9yrs	0	0		
>10yrs				

**Statistically significant at p < 0.05. Indoor (Civil servants), Outdoor (Labourers), Workshop (Artisans), Others (Drivers, filling station attendants)*

Table 2. Comparison of dietary and lifestyle factors between cases and controls (Chi square test)

Variable	Cases	Controls	x^2	p-value
Dietary history				
Vegetables and fruits				
Daily	9.1	9.1	1.551	0.461
Weekly	24.2	24.2		
Occasionally	24.2	9.1		
Nutritional supplements				
Daily	0	0		
Weekly	0	0		
Occasionally	0	0		
Sea foods				
Daily	0	0		
Weekly	0	0		
Occasionally	57.6	42.4		
Lifestyle factors				
Smoke				
Yes	0	0		
No	57.6	42.4		
Alcohol				
Yes	9.1	9.1	0.172	0.678
No	48.5	33.3		

**Statistically significant at p < 0.05*

Table 3. Comparison of age (yrs) and BMI (kg/m^2) between cases and controls. (Median) (Mann-Whitney U Test)

Parameter	Cases (n=18) Median (IQR)	Controls (n=15) Median(IQR)	Z	P-value
Age (yrs)	33.50 (33.00-39.00)	32.00 (30.00-35.50)	-1.705	0.088
BMI (kg/m^2)	21.82 (19.33-24.32)	22.61(19.44-24.49)	-0.495	0.621

**statistically significant at p < 0.05. z = Mann-Whitney U value, IQR = Interquartile range, BMI = Body mass index*

Table 4. Comparison of median toxic and essential trace metals in serum between cases and controls (Median) (Mann-Whitney U Test)

Parameter	Cases (n=18) Median(IQR)	Controls (n=15) Median(IQR)	Z	P-value
Pb (µmol/L)	<LOD	<LOD		
Cd (nmol/L)	0.0005 (0.0004-0.0113)	0.0005 (0.0028-0.0063)	-1.263	0.207
Zn (µmol/L)	81.81 (62.50-116.17)	95.55 (84.45-126.30)	-1.114	0.254
Se (µmol/L)	1.14 (0.49-1.81)	1.92 (1.52-2.22)	-2.830	0.005*

**statistically significant at p < 0.05. z = Mann-Whitney U value, IQR = Interquartile range, LOD = Limit of detection.*

Table 5. Comparison of median Toxic and Essential Elements in Seminal Plasma between cases and controls (Median) (Mann-Whitney U Test)

Parameter	Cases (n=18) Median(IQR)	Controls (n=15) Median(IQR)	Z	P-value
Pb (µmol/L)	<LOD	<LOD		
Cd (nmol/L)	0.0004 (0.0003-0.0004)	0.0004 (0.0003-0.0140)	-0.918	0.359
Zn (µmol/L)	40.00 (23.55-63.17)	97.90 (50.40-135.50)	-2.467	0.014*
Se (µmol/L)	0.77 (0.25-1.37)	1.72 (1.17-2.33)	-3.567	0.000*

**statistically significant at p < 0.05. z = Mann-Whitney U value, IQR = Interquartile range. LOD = Limit of detection*

Table 6. Comparison of median serum male reproductive hormones between cases and controls (Median) (Mann-Whitney U Test)

Parameter	Cases (n=18) Median (IQR)	Controls (n=15) Median (IQR)	Z	P-value
LH (mIU/ml)	8.68 (6.97-11.90)	10.54 (8.35-11.55)	-1.012	0.271
FSH (mIU/ml)	5.84 (4.84-7.78)	4.44 (3.57-5.15)	-2.013	0.044*
Testosterone (ng/ml)	3.23 (2.71-4.92)	3.56 (2.56-5.32)	-0.057	0.955

**statistically significant at p < 0.05. z = Mann-Whitney U value, IQR = Interquartile range*

Table 8 shows correlation of serum and seminal plasma trace metals with reproductive hormones in the infertile males. None of the parameters correlated significantly with one another.

Table 9 shows a correlation matrix of serum and seminal plasma trace metals of clinically diagnosed men with infertility. A positive correlation was observed between serum Zn and serum Cd (r= 0.486, p= 0.041). Serum Se and seminal plasma Se were also positively correlated (r= 0.843, p=0.000).

Table 10 shows a correlation matrix of serum and seminal plasma trace metals of fertile controls. There was a significant positive correlation between serum Zn and serum Cd (r = 0.533, p = 0.050) and between seminal plasma Zn and seminal plasma Cd (r = 0.611, p = 0.015).

Table 7. Relationships between essential and toxic metals in serum and seminal plasma with the Male reproductive hormones in infertile males

	LH (mIU/ml) r, p-value	FSH (mIU/ml) r, p-value	Testosterone (ng/ml) r, p-value
Serum Pb (µmol/L)	<LOD	<LOD	
Serum Cd (nmol/L)	.261, .295	.393, .107	-.101, .690
Serum Zn (µmol/L)	-.129, .610	-.086, .737	-.207, .409
Serum Se (µmol/L)	-.007, .977	-.212, .399	.616, .006*
Seminal Pb (µmol/L)	<LOD	<LOD	
Seminal Cd (nmol/L)	-.439, .068	-.185, .462	-.259, .300
Seminal Zn (µmol/L)	-.329, 183.	-.200, .426	-.199, 0.428
Seminal Se (µmol/L)	.048, .849	-.206, .412	.290, .339

**Statistically significant at p < 0.05., only correlations that have p-values less than 0.05 are asterisked (95% confidence interval), r = Spearman's correlation coefficient, FSH = follicle stimulating hormone, LH = luteinizing hormone, LOD = Limit of detection*

Table 8. Relationships between essential and toxic metals in serum and seminal plasma with the Male reproductive hormones in controls

	LH (mIU/ml) r, p-value	FSH (mIU/ml) r, p-value	Testosterone (ng/ml) r, p-value
Serum Pb (µmol/L)	<LOD	<LOD	
Serum Cd (nmol/L)	-.187, .52	-.040, .892	-.003, .991
Serum Zn (µmol/L)	-.459, .099	-.273, .345	-.048, .870
Serum Se (µmol/L)	-.070, .813	-.241, .406	-.192, .512
Seminal Pb (µmol/L)	<LOD	<LOD	
Seminal Cd (nmol/L)	.037, .901	.277, .339	-.235, .418
Seminal Zn (µmol/L)	.065, .825	.223, .444	-.150, .609
Seminal Se (µmol/L)	-.286, .322	.026, .930	-.483, .081

**Statistically significant at p < 0.05., only correlations that have p-values less than 0.05 are asterisked (95% confidence interval), r = Spearman's correlation coefficient, FSH = follicle stimulating hormone, LH = luteinizing hormone, LOD = Limit of detection*

Table 9. Correlation matrix of serum and seminal plasma trace metals of infertile males

	Serum Cd r	Serum Zn r	Serum Se r	Seminal Cd r	Seminal Zn r
Serum Zn (µmol/L)	.486* p=.041				
Serum Se (µmol/L)	-.375	-.404			
Seminal Cd (nmol/L)	-.153	-.021	-.135		
Seminal Zn (µmol/L)	-.223	.221	-.088	.048	
Seminal Se (µmol/L)	-.281	-.172	.843* p=.000	-0.142	.335

**Statistically significant at p < 0.05., only correlations that have p-values less than 0.05 are asterisked (95% confidence interval), r = Spearman's correlation coefficient*

Table 10. Correlation matrix of serum and seminal plasma trace metals in fertile controls

	Serum Cd r	Serum Zn r	Serum Se r	Seminal Cd r	Seminal Zn r
Serum Zn (µmol/L)	.533* p=.050				
Serum Se (µmol/L)	-.093	-.069			
Seminal Cd (nmol/L)	-.351	-.422	-.226		
Seminal Zn (µmol/L)	-.368	.177	.206	.611* p=.015	
Seminal Se (µmol/L)	-.184	-.190	-.046	-.226	.206

**Statistically significant at p < 0.05., only correlations that have p-values less than 0.05 asterisked (95% confidence interval), r = Spearman's correlation coefficient*

4. DISCUSSION

The incidence and causes of male infertility and male reproductive ill-health are important issues that remain poorly characterized. The cause of fertility impairment cannot be determined in several cases, despite the advances in diagnosis by the introduction of novel sophisticated tests. Certain environmental factors may influence the endocrine system in male, thus, playing a role in increasing the male infertility problem. In addition, metals can cause hormonal imbalance by affecting the neuroendocrine system, disrupting the secretion of androgens from Leydig cells or Inhibin B from Sertoli cells [18]. Similarly, heavy metals have been shown to induce modifications of neurotransmitters in the central nervous system and impair the pulsatile, hypothalamic release of gonadotropin-releasing hormone (GnRH) [19]. Furthermore, the major changes in the levels of toxic elements in seminal fluid have been related to abnormal spermatozoa function and fertilising capacity [20]. The seminal plasma has the important function as a vehicle for the transportation of the spermatozoa through the epididymis, the vas deference, and urethra and into the vagina [21, 22]. One factor influencing function of the seminal plasma on the sperm metabolism is the trace elements content of the fluid [23].

In this study, there was no significant difference in the level of reproductive hormones between clinically diagnosed infertile men and fertile controls. Although still within normal range, the FSH of the cases was significantly higher than that of the controls. FSH is physiologically involved in the maintenance of spermatogenic epithelium by stimulating Sertoli cells in the male and is responsible for the early growth of ovarian follicles in the female. This finding is consistent with the finding of Emopkae et al. who observed an increased level of FSH in azoospermic men [24]. Hence, the increase in serum FSH level in the infertile men may reflect a decreased testicular activity resulting in changes in normal feedback mechanism between the testes and the hypothalamic-pituitary axis.

Serum and seminal plasma Pb in this study was below detection limit for both cases and controls respectively. Similarly, there was no significant difference in both serum and seminal plasma Cd between cases and controls. Similarities in environmental and dermographic factors may be responsible for the observed non-significant differences. Although this might indicate that low-dose exposure to lead does not cause gross abnormalities in male reproductive function as observed by others [25], however, low to moderate lead exposure has been associated with reduced sperm concentration, poor semen motility and viability, and abnormal sperm morphology [26,27,28,29]. The observed non-significant difference in Pb and Cd concentrations in fertile and infertile subjects in this study could also be informed by the inclusion criteria which deliberately excluded smokers and others routinely exposed to these toxic metals. Some studies also reported seminal plasma Pb value to be similar between infertile and fertile male [30,31] while Keck et al. did not find any significant difference in seminal plasma Cd concentration in infertile and fertile males [32]. In contrast, in other studies, seminal plasma Cd concentration was found to be increased in infertile compared to fertile men [33,34]. Because the serum and seminal plasma concentration of Pb was below detection limit, this study could not determine the relationship between serum hormone levels and Pb in both body fluids of cases and controls. However, other studies have not found any association between low lead exposure and semen quality or endocrine function in men [35,36,37,38].

The physiological function of Zn in male reproductive health necessitated its inclusion in the analysis. The human prostate gland contains higher concentrations of zinc than any other organ in the body, which suggests that this element has an important role in male reproduction. There is evidence that zinc is required for normal testicular development [39,40,41]. Zinc is secreted predominantly by the prostate gland and is known to influence a number of functional properties of spermatozoa [42]. Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation. Thus, it seems that seminal plasma, because of its high content of zinc; exert protective, antioxidant like activity sufficient to cope with the excessive amount of superoxide anions [43]. In this study, the median seminal plasma zinc concentration of infertile men was significantly higher than that of infertile men. Although, the median serum zinc concentration of infertile men was lower in infertile men compared with that of fertile men, the difference was not statistically significant. Hence, despite the physiological importance of this trace metal in male reproductive health, the difference in observed levels in fertile and

infertile men was not significant despite the reduced levels. This underscores systemic relativity as different from statistical relativity. No significant difference was found in mean Zn levels in fertile and infertile patients, and between normospermic and dyspermic infertile men [44], nor between idiopathic infertile and normal men [45].

The results of the present study showed that concentration of Se in infertile patients was significantly lower when compared with that of fertile controls in both serum and seminal plasma. Results from this study were similar to those of Behne et al. [46], Xu et al. [47] and Shinohara et al. [48] who observed significant decrease in seminal plasma Se concentration in infertile patients. In contrast Saaranen et al. [49], and Akinloye et al. [33] observed significant increase of Se concentration in azoospermic patients when compared with oligospermic subjects and controls. Comparatively, in animals, selenium has been shown to be an essential element for normal male reproductive function.

The biological functions of selenium in mammals appear to be expressed through different biologically active compounds, including glutathione peroxidase [50] and other selenoproteins in serum and tissues [51]. However, one role of glutathione peroxidase, which is present in both animal and human semen, is to remove hydrogen peroxide and lipid peroxides and thus protect tissues including spermatozoa, from peroxidative damage [52]. Hence, Se, an antioxidant and as a component of selenoproteins and selenoenzymes is involved in spermatogenesis by protecting spermatozoa from ROS. It may therefore be inferred that the observed reduced Se level in both serum and seminal plasma of cases may precipitate an increased peroxidative damage in spermatozoa of cases eventually resulting in infertility. This might have been accentuated by the reduced Zn level observed in seminal plasma of cases. Our reports corroborated findings of Oluboyo *et al.,* [53] who concluded that there is a relationship between the serum levels of zinc, selenium and testosterone in infertile males. It may therefore be inferred that Se play an important role in leydig cell function in reproduction. The reduced Se level may also precipitate hypoactivity of the leydig cells which produces testosterone; thus, underscoring the important role of Se in leydig cell function previously stated.

Metals can interact additively, synergistically, or antagonistically thus affecting each other's absorption, distribution, and excretion. Toxic metals can interfere with the metabolism of essential metals and reduce their concentration in the organism or decrease their bioavailability [54,55]. This observation underscores the synergistic or possibly antagonistic function of trace metals as stated above. The antagonistic function may be a result of an early defense response against the reproductive toxicity of cadmium in this group to ensure fertility in the presence of the toxic metal, Cd. It could also be synergistic by potentiating the antioxidant capability of the seminal plasma to ensure the effectiveness of its function. Zinc has a protective role against lead and cadmium induced toxicity in rats [56,57]. Therefore, the positive correlation in the levels of Zn and Cd in the test and control group underscores the dual role of Zn either as an antagonist to Cd toxicity in controls or synergistically in the test group where its reduced level may facilitate the damaging toxic effect of Cd and other toxic metals resulting in impaired fertility.

5. CONCLUSION

In summary, it may be concluded from the outcome of this study that; Oxidative stress due to other factors other than Pb and Cd may be a possible contributory factor to infertility in the studied group. Endocrine disruption and hormonal imbalance may not be the only cause of infertility in the studied group; thus, the need to look beyond hormonal imbalance and examine presence of oxidative stress, trace/toxic elements imbalance especially in subjects that are occupationally vulnerable becomes imperative.

6. LIMITATION OF STUDY

This study is limited by the inability to control for some confounding factors which may be responsible for some of the observations. Also, the type of analytical sample involved in this study happened to be responsible for a reduced participation rate and lower statistical power.

7. RECOMMENDATION

The use of multiple end points, including biomarkers to assess exposure to other toxicants and outcome may be necessary. Further studies involving a larger number of participants are required to give a clearer picture and improve the statistical sensitivity to small associations.

CONSENT

Informed written consent was obtained from each participant before sample collection.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the ethics committee of the Joint University of Ibadan/University College Hospital, Institutional Review Committee (UI/UCH IRC), Ibadan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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