

Cobalt and Copper Levels in the Seminal Plasma of Infertile Men Living in Port Harcourt Metropolis

Donatus Onukwufor Onwuli^{1,*}, Gospel Ajuru²

¹Department of Medical Laboratory Science, Chemical Pathology Unit, Faculty of Science, Rivers State University of Science and Technology, NkpoluOroworukwo, Port Harcourt, Rivers State, Nigeria

²Anatomical Pathology Department, Faculty of Basic medical science, University of Port Harcourt Choba, Port Harcourt, Nigeria
*Corresponding author: onwuli.donatus@Yahoo.Com

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Abstract The seminal plasma levels of cobalt and copper were assessed in men living in Port Harcourt metropolis using Atomic Absorption Spectrophotometer. These subjects were divided into two groups based on accepted criteria for fertility in males. The subjects in group 1 (fertile men) had $67.30 \pm 10.52\%$ as the percentage motility; $67.70 \pm 18.20 \times 10^6$ Spermcells/L as cell count, $10.04 \pm 2.27\mu\text{g/L}$ and $4.14 \pm 2.60\mu\text{g/L}$ as seminal plasma cobalt and copper levels respectively. The subjects in group 2 (infertile men) had $30.60 \pm 16.60\%$ as percentage motility; $16.80 \pm 4.5 \times 10^6$ Spermcells/L as cell count, $11.82 \pm 2.83\mu\text{g/L}$ and $7.05 \pm 3.27\mu\text{g/L}$ as seminal plasma cobalt and copper levels respectively. The percentage motility and sperm cell count were significantly higher in the fertile group than the infertile group ($P < 0.05$). The seminal plasma level of copper was significantly higher in the infertile men than in the fertile men ($P < 0.05$), while cobalt levels in both groups did not show any significant difference, although there was an increase observed in the non-fertile group. The result of this study shows that men presenting with infertility have higher copper levels in their seminal plasma, a finding that could be useful in the management of male infertility.

Keywords: copper, cobalt, fertility

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1. Introduction

Male infertility is a situation where a man is unable to get a fertile woman pregnant after at least one year of consistent unprotected sex. In humans it accounts for about 40 – 50% of infertility in a couple [5,19].

Male infertility is commonly due to deficiencies in the semen and semen quality is therefore used as a surrogate measure of male infertility [8]. The volume, approximate number of total sperm count, sperm motility, forward progression and percentage of sperm cells with normal morphology are often measured in most common type of fertility testing. Male infertility may be caused by a lot of factors ranging from complete azospermia, oligospermia, asthenospermia to malformed sperm cells and other factors. Infertility affects about 8-12% of the world's population [22], and in the above half of the cases, men are either the single cause or contribute to the couples infertility [3].

Some factors that may reduce the quality of sperm cells in a man's sperm include the consumption of heavy alcohol, use of certain drugs, cigarette smoking, hormonal imbalance, treatment with certain chemotherapy for cancer, age and environmental toxicants, such as pesticides and heavy metals, which may act as endocrine

disruptors. Exposure to toxic levels of trace elements may be deleterious to health. The general population is exposed to metals at low concentrations either voluntarily through supplementation or involuntary through intake of contaminated food, water or contact with contaminated soil or air. Exposure to environmental contaminants has been suggested to play a role in the pathology of adverse reproductive health effects, including decreased semen quality, sub fertility, change in birth sex ratio and an increase in the prevalence of developmental abnormalities of the male reproductive tract [7,20,24]. Because widespread human exposure and body burden has been demonstrated for some metals, there is growing concern for adverse health effects associated with low level exposures encountered in the environment [20]. Human and animal evidence suggest that these metals may have adverse impact on male reproductive health at relatively low levels e.g. Cadmium has been linked with poor human sperm quality and DNA damage [32]. Lead may adversely affect sperm shape, motility and DNA integrity [13]. The unabated pollution of the environment is considered to be a major reason for the decline in human sperm quality over the years [27]. Occupational, industrial, environmental decay and therapeutic exposures to a wide range of chemicals and heavy metals have been reported to have harmful effect on human fertility [6,26].

Damage to human fertility, specifically a decline in the male reproductive capacity has been reported by many researchers and the influence of environmental factors including chemical substances and other pollutants in the air, water and soil have been documented [9,16]. Copper and Cobalt have been reported to have beneficial effect on human health. Suboptimal levels or toxic levels of essential trace elements have been reported in some abnormal metabolic situations. Supplementations of the trace metal have been known to correct the deficiency disorder while reduction of the toxic levels has also been shown to improve health in toxicity situations. Trace metals play vital role in the metabolic processes of the body, either by acting as co-factors in metabolic pathways or they may be an integral part of enzyme system that catalyze specific biochemical reactions. Presently there have been reports of unexplained causes of infertility even where the routine semen parameters may be normal, it is upon this backdrop that this research work was designed with the objective of accessing the level of cobalt and Copper in the seminal plasma of infertile men living in Port Harcourt metropolis and ascertaining if their levels may be a contributory factor to the infertility experienced by these individuals. The research also hopes to improve the dearth of information on the seminal plasma levels of trace elements of individuals in this part of the world. Information gathered here can serve as baseline for further research of trace elements level in seminal plasma.

2. Materials and Methods

2.1. Study Group.

The study population consisted of 18 subject's attending the fertility unit of Braithwaite Memorial Specialist Hospital, 18 Out patients of the urology unit of university of Port Harcourt teaching hospital in Port Harcourt, and 20 apparently healthy control subjects (evidenced by having at least 2 children) drawn from the city of Port Harcourt.

A total of 56 semen samples were collected from these subjects into plastic tubes after an informed oral consent was obtained from the subjects. Specimen collection was by masturbation and semen analysis was performed immediately after liquefaction had taken place on each specimen, using WHO recommended approach (10, 31) (described below). After the analysis, the semen samples were emptied into plastic centrifuge tubes and spun at 3000rpm for 3 minutes. The supernatant (seminal plasma) was transferred into a sterile plastic tube and stored frozen until chemical analysis was done.

2.2. Semen Analysis

2.2.1. Volume

The volume of the semen samples were measured using a small graduated measuring cylinder.

2.2.2. Percentage Motility

One drop of well mixed, liquefied semen was placed on a clean dry slide and covered with a cover slip. Using x40 objective, with the condenser iris closed sufficiently to give a good contrast, several fields were examined for

motile spermatozoa. Then the approximate percentage of actively motile spermatozoa was reported. The addition of a drop of eosin to the preparation assisted in the differentiation of the viable spermatozoa from the non-viable ones. Non-viable spermatozoa stain red while the viable ones remain unstained.

2.2.3. Sperm Cell Count

Using a graduated tube, the semen was diluted 1 in 20 as follows: The tube was filled to the 1ml mark with the well-mixed liquefied semen, then sodium bicarbonate-formalin diluting fluid was added to the 20 ml mark and then mixed very well.

Using a Pasteur pipette, an improved Neubauer counting chamber was charged with the diluted semen and allowed to stand for 3-5 minutes for the spermatozoa to settle.

Using x10 objective lens, with the condenser iris closed sufficiently to give a good contrast, the number of the spermatozoa in a 2 square millimeter (ie 2 large squares) were counted. The number of spermatozoa was then calculated by multiplying the number counted by 100,000.

2.2.4. pH

The pH of the semen samples were determined using Multistix urine stripes. The strips were dipped in the semen sample and the excess drained off the strip and colour change compared against a range of colour shades.

2.2.5. Morphology

The morphological examination of the semen was done as follows: A thin smear of the liquefied well mixed semen was made on a slide while the samples were still wet; the smear was fixed with 95% v/v ethanol for 5-10 minutes and then allowed to air dry. The smear was washed with sodium bicarbonate-formalin solution to remove any mucus which may be present and then rinsed with several changes of water. The smear was covered with dilute carbolfuchsin (1 in 20) and allowed to stain for 3 minutes, and the stain was then washed off. This was counterstained with dilute Loeffler's methylene blue for 2 minutes and then washed off with water, drained and allowed to air dry. Using the oil and x 40 objective and the condenser iris closed gently to give a good contrast, the slide was examined for normal and abnormal spermatozoa.

2.3 Sample Preparation for AAS

2.3.1. Glassware

All the plastic tubes were washed in three changes of deionized, distilled water, soaked overnight in 50% v/v nitric acid and then rinsed thrice in deionized, distilled water to remove all traces of trace element present that might present as a contaminant.

2.3.2. Sample Digestion

The samples were prepared by digestion according to the method of [21]. 0.5mls of the sample were placed in a fused silica coated tube and 0.5mls of concentrated nitric acid and 30% hydrogen peroxide respectively were added. The tube were capped tightly and then incubated at 80°C overnight. The tubes were then allowed to come to room temperature and the final volume made up to 2.5mls with deionized distilled water.

2.3.3 Analytical Determinations

Atomic Absorption Spectrophotometry: The method is based on the principle that atoms of an element in the ground state or unexcited state absorb light of the same wavelength as that emitted by the element in the excited state. Each element has its own characteristic absorption or resonance lines pattern.

2.4. Statistical Analysis

The results were expressed in mean, and standard deviation. The comparison of the means of the two groups of subjects was done using the student's t-test to determine if the differences are statistically significant. The Results were considered statistically significant when p is less than 0.05 ($P < 0.05$).

3. Results, Discussion and Conclusion

3.1. Results

The results of the percentage motility, cell count, Copper and Cobalt levels for the study groups are shown in the table below.

It was found in this study (Table 1) that percentage motility for the fertile men of $67.30 \pm 10.52\%$ is in agreement with the WHO reference of 50% and above. The cell count of $67.70 \pm 58.20 \times 10^6$ sperm cells/L was also found to be in agreement with the WHO's count of 20×10^6 sperm cells/L cells and above. The seminal plasma level of copper was recorded as $4.41 \pm 2.60 \mu\text{g/L}$ and cobalt $10.04 \pm 2.74 \mu\text{g/L}$ respectively.

This work reveals the results for the infertile men as $30.60 \pm 26.60\%$ for percent motility and $16.80 \pm 45.10 \times 10^6$ sperm cells/L as cell count. Both the percentage motility and sperm cell count recorded here for infertile men were in agreement with the WHO levels for men presenting with infertility.

The value of $7.05 \pm 3.27 \mu\text{g/L}$ for seminal plasma Copper and $11.82 \pm 2.83 \mu\text{g/L}$ for seminal plasma Cobalt levels respectively were recorded.

Table 1. Total sperm count, percentage motility, copper and cobalt Levels in the study groups (Mean \pm SD)

Parameters	Fertile men (n=20)	Infertile men (n=36)
Total count	$67.70 \pm 58.20 \times 10^6$	$16.80 \pm 45.10 \times 10^6$ ($P < 0.05$).
% motility	67.30 ± 10.52	30.60 ± 26.60 ($P < 0.05$).
Copper	$4.14 \pm 2.60 \mu\text{g/l}$	$7.05 \pm 3.27 \mu\text{g/l}$ ($P < 0.05$).
Cobalt	$10.54 \pm 2.74 \mu\text{g/l}$	$11.82 \pm 2.83 \mu\text{g/l}$ ($P > 0.05$).

Table 1 and Figure 1 compares the values in the two groups. It can be observed that the percentage motility and the cell count of fertile men (Group 1) was significantly higher than those of infertile men (Group 2) ($P < 0.05$). On the contrary, the copper levels in the seminal plasma of fertile men were found to be significantly lower than those of the infertile men ($P < 0.05$). The seminal plasma levels of cobalt did not show any significant difference ($P > 0.05$) in the two groups studied.

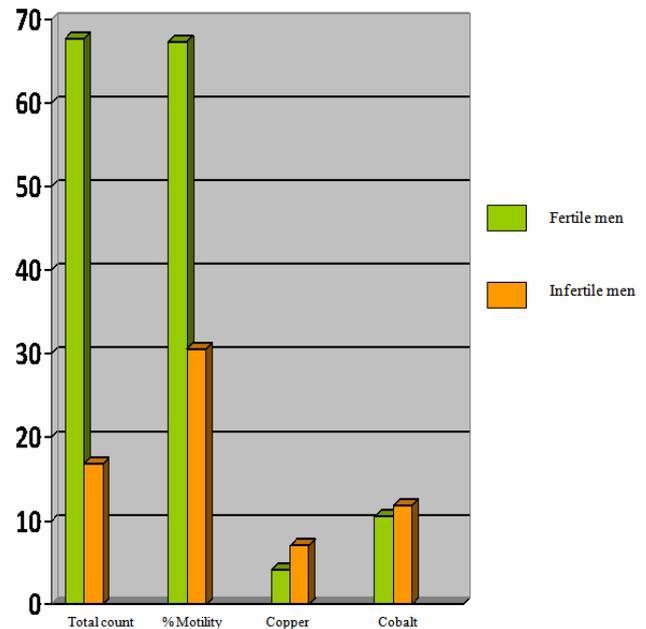


Figure 1. Comparison of parameters in fertile (group I) and infertile men (Group II)

3.2. Discussion

The finding that seminal plasma levels of Copper are significantly higher in infertile men when compared with the fertile men is at variance with the report of [30] who reported that the concentrations of magnesium, calcium, zinc and copper in blood and seminal plasma were not different between fertile and sub-fertile men. It is also not in agreement with the report of [11] who also found that there was no significant difference between copper levels in fertile and infertile men. Some other works, such as [15,23,28] also held similar view.

However, this finding agrees with that of [14] who reported a significantly higher seminal plasma copper concentration between normospermic, and azospermic, oligospermic and asthenospermic males. It is also in conformity with the report of [29] who found higher concentrations of copper in the seminal plasma of infertile men.

That copper level is high in the seminal plasma of infertile men suggests that copper may be toxic to spermatozoa, and this may explain the reason behind the use of copper wire as a component of intra uterine contraceptive device (IUCD) in the family planning units of our Hospitals [17,25]. The reason for the high levels of copper recorded for the infertile group is not certain. Although both groups of individuals belong to the same geographical location, the consumption of copper containing foods (such as oysters, sesame seeds, cocoa powder, nuts, and lobsters etc.) by the infertile group may be different from those of the fertile group.

Copper is a very common substance that occurs naturally in the environment and spreads through the environment by natural phenomena. Humans widely use copper. For instance it is applied in the industries and in agriculture. Due to this, copper quantities in the environment have increased and this basically means that more and more copper ends up in the environment especially in industrialized cities such as Port Harcourt with a number of companies that make use of copper such

as power generating companies, fertilizer companies and petroleum and petrochemical industries where copper is used for the sweetening of the petroleum products while refining.

Depending on choice of food preference, the infertile men may have been more affected, if they consume more of sea foods from contaminated river. This is because rivers can deposit sludge (contaminated with copper) on their banks, due to the disposal of copper-containing waste into water, hence Copper enters the water body.

When acidic foods are cooked in unlined copper cookware or in lined cookware, where the lining has worn through, toxic amounts of copper can leach into the foods being cooked, so also is the consumption of foods preserved in copper containing packages. This can also be another source of copper for the infertile men.

This effect is exacerbated if the copper has corroded, creating reactive salts [4]. Sometimes, actual cooking may not be required for copper to leach into acidic liquids if they are stored in copper pots for a period of time [18]. Many countries and states prohibit or restrict the sale of unlined copper cookware. Copper oxide glazed on cups, used for hot liquid might also be a concern, as well as copper pipes for conveying water to the home.

Because copper is released both naturally and through human activities, it is very widespread in the environment. People that live in houses that still have copper plumbing are exposed to higher levels of copper than most people, because copper is released into their drinking water through corrosion of pipes.

Biounavailability can also occur due to a deficiency of the copper-binding proteins, such as ceruloplasmin or metallothionein. This may also be a possibility for the infertile men, because without sufficient binding proteins, unbound copper may circulate freely in the body, where it may accumulate primarily in the liver, brain and organs. When copper is biounavailable, one may have symptoms of copper toxicity. When this occurs, free copper ions will be available in blood and other body fluids and this is the portion of the element that is involved in toxicity reactions.

This research work also reports that seminal plasma levels of Cobalt in both groups did not show any significant difference. Although higher levels of Cobalt were observed in the infertile men, the levels were not statistically significant. This may be suggestive of the fact that at such seminal plasma levels of Cobalt as recorded here, Cobalt in seminal plasma has little or no contribution to determining male infertility.

In literature, adequate information is lacking on the levels of Cobalt in seminal plasma and its implications in semen quality are not fully known. But the result of this work is corroborated by the report of [1] who reported that environmental exposure to heavy metals such as lead do not significantly contribute to male infertility. However, as with most metals, excessive levels of cobalt may be toxic to reproductive cells as reported by [2] and [12]. When an individual is exposed to toxic levels of any metal, the body burden of the metal will increase and it will most likely affect the metabolic processes especially reproduction.

3.3. Conclusion

There is evidence from the result of this work that high levels of Copper in seminal fluid have an adverse effect on male fertility. The higher levels of Copper recorded for the infertile men may be caused by exposure of these individuals to sources of copper from the environment either from drinking water contaminated with toxic levels of copper, occasioned by use of copper pipes for domestic water delivery or by consumption of contaminated food from foods cooked in copper utensils or preserved in copper containing materials which can easily leach toxic quantities of copper into the food. This work has also shown that unlike copper, cobalt levels in seminal plasma were similar for both the fertile and infertile men.

3.4. Recommendations

From the findings in this study, the authors recommend that potable water should be analysed regularly to ascertain the levels of heavy metals present. Individuals should also avoid activities that can expose them to toxic levels of heavy metals.

The laboratory diagnosis for infertility should be expanded to include some trace element analysis (in order to get a definitive diagnosis) especially for those that may likely be exposed to toxic levels of these metals.

Further research in the involvement of trace metals in reproduction is hereby recommended as this will expose salient trace elements that may adversely affect reproduction.

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