Sokoto Journal of Medical Laboratory Science 2021; 6(3): 45 - 55

SJMLS - 6 (3) - 007

Assessment of Seminal Plasma Anti-oxidant capacities, Fructose Content, and Some Heavy Metal Concentrations among Infertile Males in Akwa Ibom State, Nigeria

Peter Edidong Umohinyang[1,2]; Ibioku Elekima[1*]; Donatus Onwuli[1]

Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria [1], State Public Health Laboratory, Uyo, Akwa Ibom State, Nigeria [2].

Author for Correspondence: asaboasa@rocketmail.com/ +234-904-727-3925/ 0000-0003-1524-6495*

https://dx.doi.org/10.4314/sokjmls.v6i3.7

Abstract

Males are found to be solely responsible for 20-30% of infertility cases and contribute to 50% of cases overall. This study evaluates the levels of fructose, testosterone and heavy metals such as selenium, lead, mercury, and arsenic in the seminal plasma of infertile males in Uyo Metropolis of Akwa Ibom Sate, Nigeria. This case-control study included a total of 124 males made of 32 azoospermic, 38, oligospermic, and 54, normospermic subjects. Semen specimens were collected after 3-5 days abstinence according to WHO standard while seminal plasma was obtained by centrifuging the semen at 4500rpm for 10 minutes and stored at -70^{0}C prior to laboratory analysis. Determination of heavy metals was by atomic absorption spectrometer (AAS). Testosterone and Glutathione (GSH) were done using ELISA methods while Total Antioxidant Capacity (TAC) and fructose assays were carried out using spectrophotometric methods. Results showed significantly lower antioxidant activities, selenium (Se) and testosterone levels in infertile males with oligospermic and azoospermic counts, abnormal sperm morphologies, and higher percentage of dead sperm cells compared to fertile males with normospermic count and normal sperm morphology. In addition, it was also observed that heavy metals such lead (Pb) and arsenic (As) were significantly higher in the infertile males while fructose was observed to fall significantly among older infertile males within age interval of 40-46 years compared to 22-27 years. In conclusion, the significant reduction in Se, TAC and GSH are suggestive of oxidative stress probably induced due the significant higher levels of heavy metals like Pb and As in the seminal plasma.

Keywords: Male infertility, Antioxidants, Heavy Metals, Fructose, Seminal Plasma, Akwa Ibom, Niger Delta

Introduction

Overpopulation and infertility have been documented as two extreme human problems in some countries of the world (Ombelet, 2011). While some countries are struggling to cope with rising population, many residents of these same countries are battling infertility problems (Ombelet, 2011). Developed countries are silently facing a reducing birth rate and this is also becoming evident in developing countries. Socioeconomic, clinical, and environmental factors have been fingered as prominent causative factors behind this decline especially in developing countries (Bongaarts, 2020; Kupis *et al.,* 2015; Cocuzza *et al.,* 2013). However, in most African societies like Nigeria, women are majorly blamed for the infertility in their homes, creating tension in such homes and increased pressure for the man to take a second wife (Uadia and Emokpae, 2015). Most of our African societies fail to realize that male infertility contributes hugely to infertility problems among couples (Barati *et al.,* 2020; Uadia and Emokpae, 2015). In Nigeria, male factor infertility accounts for up to 50% of all infertility cases and yet society blames females for every problem of childlessness (Uadia & Emokpae, 2015; Abarikwu, 2013). It was reported by Kumar and Singh, (2015), that up to 14-30% of couples at procreative age are infertile and male factors

contribute to 50% of the cases making male infertility an issue of global importance.

One of the major hormones associated with male fertility is testosterone and it is produced by the testes (Elekima and Nwachuku, 2019). The testis secretes several sex hormones, which are collectively called androgens including testosterone, dihydrotestosterone, and androstenedione (Elekima and Nwachuku, 2019). Testosterone is so much abundant than the others, that one can consider it the significant testicular hormone (Elekima and Nwachuku, 2019). Testosterone is the primary sex hormone of the male reproductive system and is involved with spermatogenesis, sexual differentiation and sexual maturation. Low levels of testosterone are associated with male infertility (Elekima and Nwachuku, 2019). Some studies have shown that oxidative stress and heavy metals can act as endocrine disruptors causing a reduction in the levels of the hormones (Darbandi *et al.,* 2018).

In addition, heavy metals and other toxic substances could cause infertility by oxidative stress damage and are endocrine disruptors which affect hormones responsible for sperm production. There is growing evidence of a link between oxidative stress (OS) and male infertility (Alahmar, 2019). Oxidative stress occurs when Reactive Oxygen Species (ROS) production overwhelms the antioxidant defense system, resulting in serious cellular damage if OS is prolonged and/or massive (Ben-Chioma *et al.,* 2020; Pizzino *et al.,* 2017). ROS are by-products of normal sperm processes and leucocyte activity which are necessary for hyperactivation and capacitation of spermatozoa and fighting bacteria (Ben-Chioma *et al.,* 2020; Pizzino *et al.,* 2017).

The rapid industrialization in Uyo, Akwa Ibom State of Nigeria has led to massive deposition of non-biodegradable heavy metals and other chemical toxicants which residents are exposed to. These toxicants can cause serious health problems among residents including infertility (Sukhn *et al.,* 2018; Orisakwe *et al.,* 2015). In Nigeria, it has been reported that male infertility in the South-East accounts for 56%, the South-South accounts for 46%, South-West 42% and North-West 40% of all fertility cases in couples (Uadia and Emokpae, 2015). Uadia and Emokpae, (2015), further documented that, there have been rapid decrease in normospermia (14.1%) and increasing cases of severe oligospermia (44.1%) and azoospermia (74.7%) in Nigeria. The reduction in male sperm count, lack or loss of sperm motility and morphological aberrations of sperm cells could have been due to significant association with exposure to heavy metals such as lead, mercury, cadmium and arsenic or even consumption of crude contaminated food or food products. Researches have been done to investigate the effect of oxidative stress and heavy metals on infertility in other regions of the country but study are quite scare in the South-South of Nigeria particularly Akwa Ibom State. This study is therefore designed to evaluate the antioxidant capacity, fructose content, and some heavy metals levels in seminal plasma of infertile men resident in Uyo metropolis of Akwa Ibom State.

Materials and Methods

Materials

Materials used in this study include binocular microscope (Olympus, Japan), Haier thermocool refrigerator (China), MPW bucket centrifuge Model 351 (MPW Medical Instruments, Poland), Stat Fax 4200 Microplate Reader (Awareness, USA), Nitric acid (HNO_3) and sulphuric acid (H_2SO_4) (Sigma Aldrich, Germany), and Atomic Absorption Spectrophotometer (AAS) Buck 158 Model 211, USA. Other materials used include Glutathione peroxidase (GSH) and Testosterone ELISA kits purchased from Bioassays Technology Laboratory (Shanghai, China) while fructose and Total Antioxidant Capacity (TAC) reagents were purchased from Fortress Diagnostics (Belfast Road, Antrim, United Kingdom).

Study Area

The study was carried out in Uyo metropolis in Akwa Ibom State, Nigeria. Semen specimens were collected from males attending the Urology/Fertility Clinic of the University of Uyo Teaching Hospital (UUTH), St. Luke's Hospital, Anua, and Ibom Specialist Hospital, Uyo, Akwa Ibom State. However, specimen analyses were done at the Department of Medical

Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Study Design and Subject Characterization

A case-control study design was used for this study. A total of 349 males indicate interest to partake in the study of which only 124 males participated. The participants were randomly recruited and were aged 25-45 years. This study was performed within a span of 2.5 years. Of the 124 participants, 70 were infertile males (clinically established for at least a year) attending the Urology or Fertility Clinic of UUTH or St. Luke's Hospital, Anua, or Ibom Specialist Hospital while 54 fertile males randomly recruited (who had at least a child within the time-frame) of the study were used as controls. The subjects were classified after semen analysis as normospermic (n=54), oligospermic (n=38) and azoospermic (n=32) according to the WHO classification (1992). The oligospermic group were further classified into oligospermic group 1 and oligospermic group 2 based on their sperm count of 11-19 x 10^6cells/ml and 1-10 x 10^6cells/ml respectively. More so, the infertile participants were also classified into 4 groups according to their sperm cell viability– those with more than 50% dead sperm cells (DSC>50%); those with up to 50% but more than 30% (DSC$\leq$ 50%>30%), those with up to 30% but more than 20% (DSC$\leq$ 30%>20%), and those with up to 20% dead sperm cells (DSC$\leq$20%).

Subject Selection Criteria

Inclusion criteria

A well-structured questionnaire was given to all participants to obtain demographic information, medical history and lifestyle. Those included in the study as infertile subjects were those attending urology/fertility clinic due to established male factor infertility without any confounding factor or presence of other diseases like obesity, diabetes mellitus, thyroid disorder, cardiovascular diseases, and so on as seen in their medical records. In addition, these subjects were non-smokers, non-alcohol and without any history of substance abuse as well as established sperm cell count of less than 0.1x 10^6 cells/ml. On the other hand, the control subjects were males that have had at least established pregnancy within the study period with sperm count of $\geq$20 x 10^6 cells/ml. They are also non-smokers, non-alcoholic as well as without history of any diseases as seen above.

Exclusion criteria

Subjects excluded from the study include those that did not give their consent, males with pus cells of >6 hpf in microscopic examination of their semen as well as those that did not abstain for 3-5 days or did spill in the course of semen collection. Also, males that were obese (those with BMI of > 30.0kg/m^2) as described by Elekima and Ugwu (2018); Elekima and Inokon (2019), diabetes mellitus (fasting blood sugar >7mmol/L), benign prostate hyperplasia, prostate cancer, thyroid disorders, cardiovascular diseases were also excluded from the study. Obesity was determined using the Weight (kg)/ to height (m^2) ratio. DM was screened using Accu-chek glucometer, hypertension was checked using Omron digital sphygmomanometer (Omron Healthcare Co., Ltd, Japan). Other medical information was obtained from subject records. In addition, subjects on anti-hypertensive or lipid lowering drugs or anti-diabetic drugs were also excluded.

Ethical Consideration and Approval

Prior to the commencement of study, subject engagement and recruitment, ethical clearance was sort and obtained from the Ethical Review Boards of the University of Uyo Teaching Hospital, Uyo and Akwa Ibom State Ministry of Health with approval file no of UUTH/AD/S/96/VOLI/401 and MH/PRS/99/Vol.V/923 respectively. In addition, informed written informed consent was given by the participants before being enrolled into the study. A standard questionnaire was also used to obtain relevant information.

Specimen collection and Preparation

Participants were educated before semen specimens were collected by means of masturbation after abstaining from sex for 3–5 days into a sterile universal container with subject lab number appropriately labeled as described by WHO (1992). The time of production, receiving, and examination were also noted. All semen specimen collected were allowed to liquefy in the incubator at 37^0C

examined within 45–60 minutes. The examination covered the determination of sperm volume, viscosity, concentration, motility, morphology and viability according to the WHO manual for semen analysis (WHO, 1992). Seminal plasma specimens were obtained by centrifuging the semen samples at 4500rpm for 10 minutes to separate the sperm cells from the seminal plasma. The seminal plasma specimens obtained were carefully pipetted into another clean, dry plain container and stored at -70^{0}C prior to analysis.

Laboratory Assays of Semen and Seminal Plasma

Volume and Viscosity of semen

Semen samples were examined macroscopically to determine volume and viscosity of the sample (WHO, 1992). Viscosity of the semen sample was estimated by observation of the thread formed when a glass rod is introduced into and withdrawn from the semen sample. The length of normal threads should not exceed 2cm as described by Vasan (2011).

a. Microscopic evaluation of Semen

A Wet preparation was made by placing a drop of semen with a Pasteur pipette from a well-mixed sample on a clean grease-free slide and covering it with a cover slip. It was then viewed microscopically using x40 objective lens. Examination of fields were carried out to check for the presence of pus cells, red blood cells, epithelial cells and to determine the motility, morphology, and viability of the sperm cells as described by WHO (1992). The methylene blue eosin staining procedure was used to determine the morphology the cells after incubation of the sample at 250C with trypsin for 10 minutes. A minimum of a hundred cells were examined at a magnification of x1000, to determine the percentage sperm cell motility as documented by WHO (1992).

Determination of Biochemical Parameters in Seminal Plasma

Heavy metals (Lead (Pb), Arsenic (As), Mercury (Hg), and Selenium (Se)) were estimated using atomic absorption spectrometer (AAS) Buck 158 Model 211, USA) with samples digestion done according to the method described by Kayne *et al.* (1978). The concentrations of heavy metals in the samples were read using standard curves produced from the respective heavy metal standards. Meanwhile, the concentration of testosterone and glutathione peroxidase enzyme in the seminal plasma was determined using ELISA technique as described in previous reports (Adele *et al.,* 2019; Engvall and Perlmann, 1971). The determination of fructose was carried out using the resorcinol Spectrophotometric method as documented by Yaphe and Arsenault (1965), while that of total antioxidant capacity (TAC) was determined as described by Mahfouz *et al.* (2009).

Statistical Analysis

Graphpad Prism 8.0.2 (California, USA) was the statistical software used for the analysis of bio-data obtained. Descriptive statistics like mean and standard deviation were used while One-Way ANOVA was used for the inferential statistics. Also, post-hoc was done using the Turkey's multiple comparison tests. Statistical significance was set at p=0.05.

Results

The comparative analyses of values of normospermic, oligospermic and azoospermia subjects as well as that of normal sperm morphology and abnormal sperm morphologies (like big head, double head, pin head, short tail, bent neck, middle piece defects, etc) indicated significant increase in Pb concentration of oligospermic, azoospermic subjects as well as those with abnormal sperm morphologies when compared against normospermic control subjects (p=0.05). Also, significant falls were seen in Se, TAC, GSH and Testosterone concentration in the oligospermic and azoospermic subjects as well as those with abnormal sperm head morphologies when compared against the normospermic controls. Though, higher values of Hg and As were seen in Oligospermic and azoospermic subject, no significant difference were seen compared to normospermic groups. Likewise, fructose did not also indicate any significant difference (p=.05) (table 1, 2, and 3).

More so, when various age groups bracket of oligospermic and azoospermic subjects were considered, fructose indicated significant fall in age

group of 40 -46 years compared to age group of 22-27 years (p=.05). However, no significant differences were seen in other parameters when the various age groups were compared (p>.05) (table 4).

Finally, when the percentage of dead sperm cells (DSC) were considered, significant reductions in the values of Pb and As were seen in groups with DSC≤ 30%>20% and DSC≤ 20% compared against groups with DSC>50% and DSC≤ 50%>30%. However, values of Se, TAC, GSH and Testosterone were seen to be significantly increased in groups with DSC≤ 30%>20% and DSC≤ 20% compared against groups with DSC>50% and DSC≤50%>30% (p=0.05).

Table 1: ANOVA of Heavy Metals, Anti-oxidative Enzymes, Fructose, and Testosterone in Normospermic, Oligospermic and Azoospermic Subjects

Parameters	Normospermic (≥2x10^6 cells/ml)	Oligospermic (1.1-1.9 x 10^{6}cells/ml)	Oligospermic (0.1-1.0 x 10^{6}cells/ml)	Azoospermic (<0.1 x 10^{6}cells/ml)	p-value	F-value	Remark
Pb (mg/L)	0.017±0.00 2^a	0.133±0.0 0 9^{bc}	0.163±0.00 2^{bcd}	0.107±0.0 0 1^{bcd}	0.0412	4 .529	S
Hg (mg/L)	0.002±0.00 1	0.004±0.003	0.003±0.001	0.003±0.002	0.7310	0.4315	NS
As (mg/L)	0.003±0.00 1	0.020±0.010	0.006±0.004	0.010±0.0 0 1	0 .1212	1.986	NS
Se (mg/L)	0.57±0.06 ^{a}	0.26±0.41 ^{bc}	0.15±0.03 ^{bcd}	0.02±0.01 ^{bcd}	0.0009	6.038	S
Fructose (mg/ml)	5.07±1.69	5.09±1.57	5.22±1.69	5.08±1.97	0.7320	0.5797	NS
TAC (mmol/L)	5.85±3.41 ^{a}	5.53±3.59 ^{ac}	2.47±2.32 ^{bde}	1.89±1.24 ^{bde}	<0.0001	10.40	S
GSH (mg /L)	8.04±1.47 ^{a}	6.70±1.79 ^{bc}	6.00±1.80 ^{bcd}	5.98±1.92 ^{bcd}	0.0001	7.769	S
TESTO (ng/ml)	2.15±2.16 ^{a}	0.70±0.58 ^{bc}	0.40±0.32 ^{bcd}	0.29±0.23 ^{bcd}	<0.0001	11.60	S

Post Hoc Analysis (Turkey's Test):
Hg, As & Fructose: Values in the same row with the same superscripts (a) do not differ significantly when the various groups were compared against each other.

Pb, Se, GSH &Testo: Values in the same row with the different superscripts (a, b) differ significantly when Normospermic (≤ 20x10^9 cells/ml) values were compared with Oligospermic (11-19 x 10^{9}cells/ml), Oligospermic (1-10 x 10^{9}cells/ml) and Azoospermic (0 x 10^{9}cells/ml). However, values in the same row with the same superscripts (c) do not differ significantly when Oligospermic (11-19 x 10^{9}cells/ml) values were compared with Oligospermic (1-10 x 10^{9}cells/ml) and Azoospermic (0 x 10^{9}cells/ml). In addition, values in the same row with the same superscripts (d) do not differ significantly when Oligospermic (1-10 x 10^{9}cells/ml) values were compared with Azoospermic (<0.1 x 10^{9}cells/ml). **TAC:** Values in the same row with the different superscripts (a, b) differ significantly when Normospermic (≤ 20x10^9 cells/ml) values were compared with Oligospermic (11-19 x 10^{9}cells/ml), Oligospermic (1-10 x 10^{9}cells/ml) and Azoospermia (0 x 10^{9}cells/ml). Also, values in the same row with different superscripts (c, d) differ significantly when Oligospermic (11-19 x 10^{9}cells/ml) values were compared with Oligospermic (1-10 x 10^{9}cells/ml) and Azoospermic ((<0.1 x 10^{9}cells/ml). However, values in the same row with the same superscripts (e) do not differ significantly when Oligospermic (1-10 x 10^{9}cells/ml) values were compared with Azoospermic (0 x 10^{9}cells/ml). Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone, S=Significant, NS=Not Significant at p=.05. n=No. of Samples

Table 2: ANOVA of Heavy Metals, Anti-oxidative Enzymes, Fructose, and Testosterone in Sperm Cells with Head Abnormalities

Parameters	Normal Head	Big head	Double head	Pin Head	Round Head	pvalue	Fvalue	Remark
Pb (mg/L)	0.017 ± 0.002^{a}	0.177 ± 0.053^{bc}	0.206 ± 0.094^{bcd}	0.092 ± 0.046^{acde}	0.169 ± 0.017^{bcde}	0.3721	4.627	S
Hg (mg/L)	0.002±0.001	0.003±0.001	0.004±0.002	0.002±0.001	0.003±0.001	0.5208	0.8106	NS
As (mg/L)	0.003±0.001	0.013±0.0001	0.007±0.002	0.022±0.005	0.005±0.002	0.3113	1.208	NS
Se (mg/L)	0.57 ± 0.45^{a}	0.20 ± 0.12^{bc}	0.13 ± 0.05^{bcd}	0.32 ± 0.19^{acde}	0.14 ± 0.02^{bcde}	0.0023	4.430	S
Fructose (mg/mL)	5.07±1.69	5.43±1.75	4.89±1.76	5.52±1.86	4.84±1.43	0.5797	0.7203	NS
TAC (mmol/L)	5.85 ± 3.41^{a}	4.98 ± 3.43^{ac}	5.08 ± 3.50^{acd}	4.72 ± 3.60^{acde}	1.49 ± 2.04^{bcde}	0.0006	5.255	S
GSH (mg/L)	8.04 ± 1.47^{a}	6.39 ± 1.90^{bc}	6.43 ± 1.97^{bcd}	5.37 ± 1.70^{bcde}	5.79 ± 1.99^{bcde}	<0.0001	7.312	S
Testo (ng/ml)	2.15 ± 2.16^{a}	0.64 ± 0.62^{bc}	0.69 ± 0.76^{bcd}	0.63 ± 0.75^{bcde}	0.76 ± 0.69^{bcde}	<0.0001	8.345	S

Post Hoc Analysis (Turkey's Test):

Pb, Hg, As & Fructose: Values in the same row with the same superscripts (a) do not differ significantly when the various groups were compared against each other. Pb, **Se, GSH &Testo:** Values in the same row with the different superscripts (a, b) differ significantly when Normal heads values were compared with big heads, double heads, pin heads and round heads. However, values in the same row with the same superscripts (c) do not differ significantly when big heads values were compared with double heads, pin heads, and round heads. In addition, values in the same row with the same superscripts (d) do not differ significantly when double heads values were compared with pin heads and round heads. Also, values in the same row with the same superscripts (e) do not differ significantly when pin heads values were compared with round heads. **TAC:** Values in the same row with the different superscripts (a, b) differ significantly when Normal heads values were compared with big heads, double heads, pin heads and round heads. Also, values in the same row with same superscripts (c) do not differ significantly when big heads values were compared with double heads, pin heads, and round heads. Again, values in the same row with same superscripts (d) do not differ significantly when double heads values were compared with pin heads, and round heads. Finally, values in the same row with the same superscripts (e) do not differ significantly when pin heads values were compared with round heads. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone, S=Significant, NS=Not Significant at p=.05. n=No. of Samples

Table 3: ANOVA of Heavy Metals, Anti-oxidative Enzymes, Fructose, and Testosterone in Sperm cells with Middle piece, Neck and Tail Abnormalities

Parameters	Normal sperm	Middle piece	Bent Neck	Short tail	Coiled tail	p-value	F-value	Remark
Pb (mg/L)	0.017 ± 0.002^{a}	0.169 ± 0.034^{bc}	0.173 ± 0.034^{bcd}	0.184 ± 0.036^{bcde}	0.241 ± 0.141^{bcde}	0.03534	5.893	S
Hg (mg/L)	0.002 ± 0.001^{a}	0.002 ± 0.001^{ab}	0.003 ± 0.002^{abc}	0.003 ± 0.002^{abcd}	0.541 ± 0.336^{abcd}	0.4305	0.9605	NS
As (mg/L)	0.003 ± 0.002^{a}	0.013 ± 0.010^{ab}	0.012 ± 0.011^{abc}	0.007 ± 0.010^{abcd}	0.014 ± 0.011^{abcd}	0.5692	0.7350	NS
Se (mg/L)	0.57 ± 0.36^{a}	0.19 ± 0.13^{bc}	0.23 ± 0.13^{bcd}	0.23 ± 0.16^{bcde}	0.23 ± 0.18^{bcde}	0.0032	4.126	S
Fructose (mg/ml)	5.07 ± 1.69^{a}	5.43 ± 1.72^{ab}	5.23 ± 1.69^{abc}	5.38 ± 1.73^{abcd}	5.10 ± 1.49^{abcd}	0.8289	0.3280	NS
TAC (mmol/L)	5.85 ± 3.41^{a}	3.20 ± 2.931^{bc}	2.15 ± 2.02^{bcd}	2.35 ± 2.11^{bcde}	2.31 ± 2.08^{bcde}	<0.0001	11.40	S
GSH (mg/L)	8.04 ± 1.47^{a}	5.20 ± 1.99^{bc}	5.25 ± 1.84^{bcd}	4.52 ± 1.81^{bcde}	4.27 ± 1.58^{bcde}	<0.0001	21.39	S
TESTO (ng/ml)	2.15 ± 1.16^{a}	0.85 ± 0.45^{bc}	0.97 ± 0.76^{bcd}	0.66 ± 0.35^{bcde}	0.73 ± 0.62^{bcde}	0.0006	5.186	S

Post Hoc Analysis (Turkey's Test):

Hg, As & Fructose: Values in the same row with the same superscripts (a) do not differ significantly

when the various groups were compared against each other. **Pb, Se, TAC, GSH &Testo:** Values in the same row with the different superscripts (a, b) differ significantly when normal sperm values were compared with middle piece, bent neck, short tail and coiled tail abnormalities. However, values in the same row with the same superscripts (c) do not differ significantly when middle piece values were compared with bent neck, short tail and coiled tail abnormalities. In addition, values in the same row with the same superscripts (d) do not differ significantly when bent neck values were compared with short tail and coiled tail abnormalities. Finally, values in the same row with the same superscripts (e) do not differ significantly when short tail values were compared with coiled tail abnormalities. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone, S=Significant, NS=Not Significant at p=.05. n=No. of Samples

Table 4: ANOVA of Heavy Metals, Anti-oxidative Enzymes, Fructose, and Testosterone on Varying Age Interval (yrs) in abnormal sperm (oligo & Azoospermic) subjects

Parameters	22-27 yrs	28-33 yrs	34-39 yrs	40-46 yrs	pvalue	Fvalue	Remark
Pb (mg/L)	0.214±0.143	0.07±0.031	0.151±0.134	0.128±0.108	0.9512	0.1146	NS
Hg (mg/L)	0.001±0.0004	0.002±0.001	0.003±0.002	0.004±0.003	0.4464	0.8996	NS
As (mg/L)	0.006±0.005	0.02±0.01	0.018±0.014	0.008±0.001	0.6496	0.5508	NS
Se (mg/L)	0.151±0.128	0.28±0.14	0.260±0.155	0.2026±0.156	0.8905	0.2080	NS
Fructose (mg/mL)	7.00 ± 2.25^{a}	4.85 ± 1.49^{ac}	5.39 ± 1.64^{acd}	4.74 ± 1.49^{bcd}	0.0303	3.1620	S
TAC (mmol/L)	5.09±3.34	3.65±1.422	5.35±3.55	3.55±2.80	0.0512	3.108	NS
GSH (mg/L)	5.77±1.47	4.50±1.44	5.75±1.98	5.07±2.05	0.2696	1.338	NS
Testo (ng/ml)	1.08±0.99	0.57±0.51	0.62±0.29	1.13±0.67	0.8534	0.2608	NS

Post Hoc Analysis (Turkey's Test):
Pb, Hg, As, Se, GSH, Testo & TAC: Values in the same row with the same superscripts (a) do not differ significantly when the various groups were compared against each other. **Fructose:** Values in the same row with the different superscripts (a, b) differ significantly when age 22-27 yrs values were compared with 28-33 yrs, 34-39 yrs and 40-46 yrs. However, values in the same row with the same superscripts (c) do not differ significantly when 28-33 yrs values were compared with 34-39 yrs and 40-46 yrs. In addition, values in the same row with the same superscripts (d) do not differ significantly when34-39 yrs values were compared with40-46 yrs. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone, S=Significant, NS=Not Significant at p=.05. n=No. of Samples.

Table 5: ANOVA of Heavy Metals, Anti-oxidative Enzymes, Fructose, and Testosterone on varying % of Dead Sperm cells

Parameters	DSC>50%	DSC≤50%>30%	DSC≤30%>20%	DSC≤20%	p-value	F-value	Remark
Pb (mg/L)	0.140 ± 0.012^{a}	0.174 ± 0.037^{ac}	0.014 ± 0.009^{bde}	0.032 ± 0.002^{bde}	0.0283	6.290	S
Hg (mg/L)	0.003±0.002	0.003±0.002	0.003±0.002	0.004±0.002	0.9478	0.1206	NS
As (mg/L)	0.021 ± 0.01^{a}	0.030 ± 0.010^{ac}	0.002 ± 0.001^{bde}	0.004 ± 0.001^{bde}	0.0405	4.5336	S
Se (mg/L)	0.23 ± 0.19^{a}	0.29 ± 0.14^{ac}	0.89 ± 0.59^{bde}	0.78 ± 0.50^{bde}	<0.0001	10.60	S
Fructose (mg/mL)	5.31±1.55	4.77±1.74	4.96±1.83	5.28±1.60	0.7063	0.4667	NS
TAC (mmol/L)	3.63 ± 2.66^{a}	2.68 ± 2.18^{ac}	8.84 ± 1.52^{bde}	6.42 ± 2.57^{bde}	<0.0001	17.25	S
GSH (mg/L)	5.24 ± 1.39^{a}	5.24 ± 1.71^{ac}	8.01 ± 1.33^{bde}	8.36 ± 1.11^{bde}	<0.0001	28.87	S
Testo (ng/ml)	1.80 ± 0.94^{a}	1.23 ± 0.52^{ac}	2.30 ± 1.56^{bde}	2.66 ± 1.57^{bde}	0.0463	5.196	S

Post Hoc Analysis (Turkey's Test): Hg, fructose & Testo: Values in the same row with the same superscripts (a) do not differ significantly when the various groups were compared against each other. **Pb, As, Se, TAC & Testo:** Values in the same row with different superscripts (a, b) differ significantly

when DSC>50% values were compared with DSC≤ 50%>30%, DSC≤ 30%>20% and DSC≤ 20%. Also, values in the same row with different superscripts (c, d) differ significantly when DSC≤ 50%>30%, values were compared with DSC≤ 30%>20% and DSC≤ 20%. Finally, values in the same row with the same superscripts (e) do not differ significantly when DSC≤ 30%>20% values were compared with and DSC≤ 20%. DC=Dead Sperm Cells Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone, S=Significant, NS=Not Significant at *p*=.05. n=No. of Samples.

Discussion

The study indicated a significantly higher values in Pb and morphological deformities (higher percentage of sperm cell deaths) in infertile males who were oligospermic and azoospermic counts. These observations are in line with the reports of Nsonwu-Anyanwu *et al* (2019). They documented that Pb was observed to be increased significantly in infertile males compared to fertile males. It was further observed in our study that As were significantly higher in values among infertile males with higher percentage of dead sperm cells (DSC). Heavy metals such as Pb and As have been reported to be strongly associated with the induction of oxidative stress as well as poisoning. Previous reports (Kumar, 2018; Kim and Kim, 2015) stated that Pb and As are major endocrine disruptors following some certain key molecular mechanisms which involves reduction of testosterone and gonadotrophins (LH and FSH), disruptions of steroidogenic enzymes (3β-HSD and 17β-HSD), negative regulations of LH and FSH through increased corticosterone levels, hindered sperm motility and viability following the direct binding to sperm, and apoptosis of sertoli cells. These metals are also associated with decline in sperm functions and spermatogenesis as a result of increase lipid peroxidation of sperm cell membrane (Rice *et al.,* 2014; Ghaffari and Motlagh, 2011). The presence of these heavy metals seen in higher concentration in seminal plasma could be attributed to high level of hydrocarbon pollution and consumption of crude contaminated seafoods within the Niger Delta and particularly in Akwa Ibom State which is host to several local and multinational oil and gas companies. Also, illegal crude oil refining and spillage have also added significantly to this burden. Elekima *et al.* (2020), also established the presence and high levels of heavy metals above WHO permissible limits in seafoods commonly consumed in the Niger Delta.

Furthermore, the significantly lower values were seen in selenium, testosterone, TAC and GSH in infertile males with oligospermic and azoospermic counts. Abnormal sperm morphologies, and higher percentage of dead sperm cells could be related to oxidative stress due to the significant presence of heavy metals such as Pb and As in the seminal plasma. Our finding disagrees with the reports of Bassey *et al*. (2013). They reported no significant differences in selenium in seminal plasma between fertile and infertile men. On the other hand, our finding concurs with the report of Turks and Kullisaar (2014), who reported significantly decreased selenium, TAC, and GSH levels in azoospermic and oligospermic subjects compared to normospermic subjects as well as in infertile males with abnormal sperm morphology and higher percentage dead sperm cells. Lower levels of Se, TAC, and GSH have been associated with increased incidence of sperm cells deaths, loss of sperm cell motility, morphological abnormalities and breakage at the middle piece. Selenium and other antioxidants such as glutathione appear to have a positive influence on the Sertoli and Leydig cells, therefore influencing the production of testosterone. In 2020, Ben-Chioma and Colleagues also reported in their study that selenium acts as a potent antioxidant in biological systems, scavenging ROS and radicals. Previous reports (Qazi *et al*., 2019; Moslemi and Tavanbakhsh, 2011) indicates that selenium and other antioxidants are vital for normal mammalian spermatogenesis and protection against oxidative DNA damage and this important role is mainly mediated by selenoproteins. The glutathione peroxidase also confers structural integrity on the sperm cells due to its role as a component of the membrane structure of sperm cells.

More so, when the various age intervals were considered, fructose was seen to be significantly reduced in infertile males within the age interval of 40-46 years compared against those of 20- 27 years. However, other seminal plasma parameters were observed to be not significant. Contrary to our findings, some other studies have reported lower seminal fructose levels in oligospermic and azoospermic men (Nsonwu-Anyanwu *et al.,* 2019) while others have reported higher seminal fructose levels in infertile men (Amidu *et al.,*1998). Seminal fructose is the major source of energy for spermatozoa and is produced by the ampulla of the ductus deferens and seminal vesicles. Though, the reason for this is not very clear but the significant fall in the content of fructose in these infertile males within 40-46 years could probably suggest that fructose content in seminal plasma tends to reduce as infertility progress with age.

Finally, the significantly lower values of testosterone in the infertile males could be as a cumulative influence of the poor antioxidant properties, inadequate selenium concentration, and significant presence of heavy metals in the seminal plasma. Our findings are in accordance with the reports of Morbat *et al.* (2018) and Walczak-Jedrzejowska *et al*. (2013). It has been recorded that multifunctional selenoprotein are expressed by germ cells in the testes, and represents vital link between testosterone production, sperm quality, and male fertility. Therefore, our results further suggest that infertile men may benefit from selenium supplementation due to their synergic infleunce on the testis and antioxidative properties particularly in region prone to heavy metal toxicity viz-a-viz hydrocarbon pollution.

Conclusion

Our study indicated a significantly lower antioxidant activities, selenium and testosterone levels in infertile males with oligospermic and azoospermic counts, abnormal sperm morphologies, and higher percentage of dead sperm cells compared to fertile males with normospermic count and normal sperm morphology. In addition, it was also observed that heavy metals such Pb and As were significantly higher in these infertile males.

Recommendation

It is recommended that antioxidant parameters and selenium in seminal plasma should be included in investigating male infertility. However, with respect to hydrocarbon pollution and large-scale oil spillage peculiar to the Niger Delta, it very vital that the concentration of heavy metals when investigating male infertility should not be overlooked or underestimated.

Competing Interests

Authors have declared that no competing interests exist.

References

Abarikwu, S.O. (2013). Causes and risk factors for male-factor infertility in Nigeria: a review. *African Journal of Reproductive Health; **17*(4)**: 150–166.

Adele, A. U., Iroh, G., Briggs, O. N., Waribo, A.H. & Elekima, I. (2019). Evaluation of Anti-oxidant Enzymes, Lipid Peroxidation, Lipid Profile and Liver Function in Albino Rats Orally Administered Tartrazine. *International Journal of Biochemistry Research & Review;* **29(5)**: 19-29.

Alahmar, A.T. (2019). Role of Oxidative Stress in Male Infertility: An Updated Review. *Journal of Human Reproductive Sciences;* ***12*(1)**: 4–18.

Amidu, N., Owiredu, W. K. B. A., Bekoe, M. A. T. & Quaye, L. (1998). The impact of seminal zinc and fructose concentration on thiols in polypeptides. *Biochemistry,* 37, 8965–8972.

Barati, E., Nikzad, H. & Karimian, M. (2020). Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cellular and Molecular Life Science;***77:** 93–113.

Bassey, I. E., Essien, O. E., Isong, I. K. P., Udoh, A. E. & Agbara, G. S. (2013). Seminal Plasma Chromium, Cadmium and Lead Levels in Infertile Men. Journal *of Medical Sciences;* **13**: 497-500.

Ben-Chioma, E. A., Sheudeen, A. A., Dornu, L. N., Isaac, I., Nwachuku, E. O., Aworu, M.A. & Elekima, I. (2020). Evaluation of

Antioxidants Supplementation on Renal, Hepatic and Cardiac Function Markers in Alloxan Induced Diabetes Mellitus Wister Rats. *Journal of Complementary and Alternative Medical Research;* **9(3)**: 23-39.

Bongaarts, J. (2020). Trends in fertility and fertility preferences in sub-Saharan Africa: the roles of education and family planning programs. *Genus;* **76:** 32-33.

Cocuzza, M., Alvarenga, C. & Pagani, R. (2013). The epidemiology and etiology of azoospermia. *Clinics (Sao Paulo, Brazil);* ***68*** **(1)**: 15–26.

Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R. & Sadeghi, M. R. (2018). Reactive oxygen species and male reproductive hormones. *Reproductive Biology and Endocrinology;* ***6*****(1)**: 87 - 88.

Elekima, I. & Inokon, A. (2019). A Study of Correlation of Anthropometric Data with Atherogenic Indices of students of Rivers State University, Port Harcourt, Nigeria *Asian Journal of Research in Medical and Pharmaceutical Sciences;* **6(1)**: 1-12.

Elekima, I., & Nwachuku, E. O. (2019). Evaluation of Acute and Chronic Toxicity of Tartrazine (E102) on Steriod Reproductive Hormones of Albino Rats. *Asian Journal of Research and Reports in Endocrinology;* **2(1)**: 1-15.

Elekima, I., Edookue, B.R., Pepple, F.N., Aworu, M.A., & Ben-Chioma, E.A. (2020). Evaluation of Some Heavy Metals in Selected Sea Foods Directly from the Creeks in Rivers State, Nigeria. *Journal of Advances in Medical and Pharmaceutical Sciences;* **22(10)**: 29-39.

Elekima, I. & Ugwu, C. J. (2018). A Pilot Correlative Study of Anthropometric Indices with Lipid Parameters in Normal Weight, Overweight and Obese Participants in Port Harcourt Metropolis. *Asian Journal of Medicine and Health;* **13(4)**: 1-10.

Engvall, E. & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry;* **8(9)**: 871–874.

Ghaffari, M. A. & Motlagh, B. (2011). In vitro effect of lead, silver, tin, mercury, indium and bismuth on human sperm creatine kinase activity: a presumable mechanism for men infertility. *Iranian Biomedical Journal;* ***15*****(1-2)**: 38–43.

Kayne, F.J., Komer, G., Laboda, H., Vanderline, R.E. (1978). Atomic Absorption Spectrophotometry of Chromium in semen and urine with a modified Perkin Elmer 603 Atomic Absorption Spectrophotometer. *Clinical Chemistry;* **24 (12)**: 2151-2154.

Kim, Y. J., & Kim, J. M. (2015). Arsenic toxicity in male reproduction and development. *Development & Reproduction;* ***19*****(4)**: 67–180.

Kumar, N. & Singh, A. K. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of Human Reproductive Sciences;* ***8*****(4)**: 191–196.

Kumar, S. (2018). Occupational and Environmental Exposure to Lead and Reproductive Health Impairment: An Overview. *Indian Journal of Occupational and Environmental Medicine;* ***22*****(3)**: 128–137.

Kupis, Ł., Dobroński, P. A. and Radziszewski, P. (2015). Varicocele as a source of male infertility - current treatment techniques. *Central European Journal of Urology;* ***68*****(3)**: 365–370.

Mahfouz, R., Sharma, R., Sharma, D., Sabanegh, E. and Agarwal, A. (2009). Diagnostic value of total antioxidant capacity (TAC) in human seminal plasma. *Fertility and Sterility;* **91(3)**: 805-811

Morbat, M. M., Hadi, M. A. & Hadri, H. D. (2018). Effect of Selenium in treatment of Male infertility. *Experimental Techniques in Urology and Nephrology;* **1(1)**: 1-4.

Moslemi, M. K. and Tavanbakhsh, S. (2011). Selenium-vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. *International Journal of General Medicine;* ***4****:* 99–104.

Nsonwu-Anyanwu, A. C., Raymond, E. E., Jeremiah, O. S., Francis, A. O., Chukwuma, O. O., Idiongo, U. E., Aleruchim, O. J., Rowland, E. F. and Usoro, O. A. C. (2019).

Heavy metals, biomarkers of oxidative stress and changes in sperm function: A case-control study. *International Journal of Reproductive Biomedicine;* ***17*****(3)**: 163–174.

Ombelet, W. (2011). Global access to infertility care in developing countries: a case of human rights, equity and social justice. *Facts, views & vision in Obstetrics and Gynecology;* 3**(4)**: 257–266.

Orisakwe, O. E., Mbagwu, H. O., Ajaezi, G. C., Edet, U. W. & Uwana, P. U. (2015). Heavy Metals in Seafood and Farm Produce from Uyo, Nigeria: Levels and health implications. *Sultan Qaboos University Medical Journal;* ***15*****(2)**: e275–e282.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity:* doi: 10.1155/2017/8416763.

Qazi, I. H., Angel, C., Yang, H., Zoidis, E., Pan, B., Wu, Z., Ming, Z., Zeng, C. J., Meng, Q., Han, H. & Zhou, G. (2019). Role of Selenium and Selenoproteins in Male Reproductive Function: A Review of Past and Present Evidences. *Antioxidants;* ***8*****(8)**: 268.

Rice, K. M., Walker, E. M (Jr.)., Wu, M., Gillette, C. & Blough, E. R. (2014). Environmental mercury and its toxic effects. *Journal of Preventive Medicine and Public Health;* ***47*****(2)**: 74–83.

Sukhn, C., Awwad, J., Ghantous, A. & Zaatari, G. (2018). Associations of semen quality with non-essential heavy metals in blood and seminal fluid: data from the Environment and Male Infertility (EMI) study in Lebanon. *Journal of Assisted Reproduction and Genetics;* ***35*****(9)**: 1691–1701.

Turk, S. & Kullsaar, T. (2014). Male infertility: Decreased levels of Selenium, Zinc, and antioxidants. *Journal of Trace Elements in Medicine & Biology;* **28(2)**: 179-185.

Uadia, O. P. and Emokpae, M. A. (2015). Male infertility in Nigeria: A Neglected reproductive health issue requiring attention. *Journal of Basic and Clinical Sciences,* 4(2), 45 – 53

Vasan, S. S. (2011). Semen analysis and sperm function tests: How much to test? *Indian Journal of Urology;* ***27*****(1)**: 41–48.

Walczak-Jedrzejowska, R., Wolski, J. K., & Slowikowska-Hilczer, J. (2013). The role of oxidative stress and antioxidants in male fertility. *Central European Journal of Urology;* ***66*****(1)**: 60–67.

World Health Organization (1992). Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 5 ?*h* Ed. Cambridge University Press, Cambridge, UK, 1992: 23.

Yaphe, W. and Arsenault, G. P. (1965). Improved resorcinol reagent for the determination of fructose, and of 3,6-anhydrogalactose in polysaccharide. *Analytical Biochemistry;* **13(1)**: 143-148

Citation: Peter Edidong Umohinyang, Ibioku Elekima, Donatus Onwuli. Assessment of Seminal Plasma Anti-oxidant capacities, Fructose Content, and Some Heavy Metal Concentrations among Infertile Males in Akwa Ibom State, Nigeria. ***Sokoto Journal of Medical Laboratory Science; 6(2): 45 - 55***.