

Evaluating the Effects of Heavy Metals on Seminal Fluid Anti Oxidants Status and Semen Parameters among Males with Infertility at Tertiary Health Centre in Nigeria

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Abstract

Introduction: There are evidences of a reduction in male fertility potential globally. This, coupled with the limitation of the gold standard investigative tool of male infertility, has become necessary that focus should be on elucidating the aetiopathogenic causes of male fertility. This includes the impact of environmental toxic products such as levels of heavy metals that have been proposed to impact both the sperm quality and seminal antioxidant status. The study assessed how the seminal concentrations of specific heavy metals such as cadmium, lead and Iron affect the seminal fluid analysis (SFA) parameters and seminal antioxidant status of males exhibiting abnormal parameters of sperm, analysing infertile male patients consecutively in a cross-sectional manner, with at least one abnormal SFA parameter, at a tertiary health centre. **Materials and Methods:** One hundred and thirty consenting males who have one or several anomalies with sperm characteristics were selected as subjects. Semen samples were collected, processed and centrifuged. The supernatants were analysed for the heavy metals using Buck Scientific 210/211VGP Flame Atomic Absorption Spectrophotometers 220GF Graphite Furnace and 220AS Autosampler and seminal Vitamins C and E, using spectrophotometric method and glutathione peroxidase, whereas catalase was analysed using ELISA method. **Results:** Subjects' seminal levels of Cd, Fe and Pb were notably greater than those of controls, respectively. In addition, individuals' levels of seminal antioxidants were noticeably lower than controls. Seminal antioxidant status and Seminal Cd, Fe and Pb were positive and negative significantly correlated with sperm count and active motility, respectively. **Conclusion:** Laboratory quantification of the seminal concentrations of these heavy metals in the evaluation of males with infertility, especially those still regarded as idiopathic, because of limitations of investigative tools and biomarkers, will be of clinical utility, in both the management and prevention of sperm quality deterioration.

Keywords: Antioxidants, heavy metals, male infertility, spermatozoa

INTRODUCTION

Over the last 50 years, evidence suggests a global reduction in male fertility potential. This is associated with declining spermatozoa quality and quantity associated with modernisation, especially industrialisation.^[1] Nigeria is a developing country, experiencing her fair share of industrialisation in telecommunications, agriculture, automobiles, rail transportation system and petrochemical exploration and refining, etc. Despite the economic advancement benefits of industrialisation, there are hazardous health complications directly and indirectly linked to industrialisation.^[2]

Many issues arise when a huge population gathers in a city, especially for the impoverished. For instance, many rural

migrants move to urban slum areas with their families, bringing with them domesticated animals of all kinds, including livestock and pets. All migrants become susceptible to circulating infectious diseases, thereby creating an urban transmission cycle following the inflow of people and animals.

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In addition, the majority of urban poor people reside in uncontrolled slums that are crowded, dirty, close to open sewers and limited to dangerous physical locations such as hillsides, riverbanks and water basins that are vulnerable to flooding, landslides or industrial hazards. The spread of both infectious and non-infectious diseases, pollution, inadequate diet, traffic and other issues are all caused by these variables.^[3-5] Other city people are affected by the issues that the impoverished encounter. This spillover effect grows and becomes more global as the trend of urbanisation continues, affecting an increasing portion of the world's population.^[6]

Amongst the main health issues brought on by urbanisation are inadequate nutrition, illnesses linked to pollution, communicable diseases, unsanitary housing conditions and other connected health issues, from factors such as pesticides, heavy metals and industrial toxic wastes. These directly affect people's quality of life while putting a strain on public health systems and resources.^[6] There are about 7% of males with infertility globally. Increasing number of couples are presenting with infertility, about 40% of the cases are attributable to male factors and about 20% of causes of male infertility are still referred to as idiopathic.^[6] Multiple non-communicable disorders, including infertility, have been linked to environmental contaminants and heavy metals in their aetiopathogenesis.^[8]

Thirteen of the 35 naturally occurring metals have an atomic weight larger than 40.04 and a high specific density above 5 g/cm³, making them what are known as heavy metals.^[9,10] When present in excess of necessary amounts, certain heavy metals, including cobalt, chromium, copper, magnesium, iron, molybdenum, manganese, selenium, nickel and zinc, can lead to deficiency diseases or syndromes; when present in large enough concentrations, however, they can cause acute or chronic toxicities.^[11] Excessive or insufficient amounts of iron can impair certain functions in the body. An abundance of iron deposits in essential organs raises the risk of heart failure, diabetes mellitus, depression, osteoarthritis, osteoporosis, hypothyroidism, hypogonadism and liver illness (cirrhosis and cancer).^[12,13] Male infertility has also been linked to heavy metal exposure due to testicular apoptosis and oxidative damage in sperm. Partners of males employed in lead battery factories have reported increased infertility and increased rates of miscarriage, suggesting that heavy metals have detrimental impacts on the success of reproduction.^[14] Environmental toxins with heavy chemicals also sometimes referred to as endocrine-disrupting chemicals are a large group of chemicals implicated in testicular dysgenesis syndromes, including pesticides and herbicides. There is a lack of consistency regarding the effects of heavy metal exposure on biological systems, which led to the belief that semen was a less useful indicator of occupational exposure. In addition, it is acknowledged that the detection of heavy metals in spermatozoa cells does not provide more insightful information about occupational (or non-occupational) exposure than biological and conventional monitoring using urine and blood.^[15,16] However, a recent study found that since males

who live in high-impact environments have been shown to have greater amounts of Zn, Cr and Cu in their semen, semen may be an early biomarker of environmental exposure to these elements.^[17]

The major antioxidative defence in the seminal plasma can be of the enzymatic type, which includes superoxide dismutase, catalase (CAT), glutathione peroxidase (GPx), Vitamin C (ascorbic acid), Vitamin E (tocopherol) and Zinc (Zn). This represents the total antioxidant capacity of the seminal plasma. The spermatozoa are well endowed with an array of antioxidants that act as free radical scavengers to protect them against oxidative stress.^[18,19]

The objective of this research was to evaluate the effects on sperm characteristics and seminal antioxidant status of a few specific heavy metals – Cd, Fe and Pb – that humans commonly come into contact with in the environment.

MATERIALS AND METHODS

The study was carried out at the Chemical Pathology and Immunology Department, Federal Medical Centre, Abeokuta, Ogun State, Nigeria. Male partners of infertile couples who are asked to perform seminal fluid analysis (SFA) were included in the study population at the Microbiology Laboratory, Federal Medical Centre, Abeokuta, Ogun State. Participants with abnormal SFA parameters served as subjects, whereas participants with normal sperm count and motility served as controls.

An analytical cross-sectional study 130 male infertility patients were conducted, with at least one abnormal SFA parameter. Sample size was determined using modified Fisher's formula.^[7] Participant selection was by simple random sampling method. Consenting male patients were referred to the Medical Microbiology laboratory for SFA from the gynaecology, urology and general outpatient department clinics. One hundred and thirty men with normal semen analysis parameters were recruited as controls, whereas those with at least one defective semen analysis parameter were recruited as subjects.

This study excluded patients with aspermia, azoospermia (lack of spermatozoa in the semen), cryptorchidism (undescended testes) or atrophic testes as determined by clinical examination, and patients receiving oral antioxidant therapy or supplements.

Before patient samples were collected, the study's protocol and purpose were explained, and then written consent was sought for study participation. The Federal Medical Centre Abeokuta ethics committee provided IRB/ethical clearance with Protocol Number: FMCA/470/HRE/10; NREC Assigned Number: NHREC/08/10-2015.

After a minimum of 3 days and a maximum of 6 days of abstinence from sexual intercourse, the patients used coitus interruptus or masturbation to collect semen samples into a sterile, wide-mouthed container. After being collected, the samples were brought to the research facility in less than an

hour. The samples were centrifuged at $1000 \times g$ for 20 min at 2°C – 8°C . Sediments were collected and assayed right away, or they might be used within 7 days if kept between 2 and 8°C .

Laboratory procedures

Assay of seminal levels of lead, cadmium and iron

Principle of method

All the three trace metals (Pb, Cd and Al) were analysed using Buck Scientific 210/211 VGP Flame Atomic Absorption Spectrophotometers 220GF Graphite Furnace and 220AS Autosampler that utilise principle of atomic absorption spectrophotometry method. When using atomic absorption spectrophotometry, the sample solution is aspirated into a flame, which transforms the analyte of interest into gaseous phase atoms. Because of the low temperature during atomisation, the majority of the atoms remain in the ground state, where they can absorb characteristic radiation from the radiation source made from the analyte element. As a result, light from the hollow cathode lamp enters the flame and is partially absorbed by the ground-state atoms in the flame, resulting in a net decrease in the intensity of the lamp's beam.^[20]

Thus, the amount of light absorbed as the incident light passed through the sample was related mathematically to the concentration of the analyte in question. Pb, Cd and Fe were read at their characteristic radiation wavelengths.

Quality control

The following quality control and quality assurance techniques were applied during the analysis:

- Blanks were used to check for contamination during sample preparation
- Replicate measurements for each sample were taken to ensure precision
- Ultra-pure water was used to flush the instrument for about 10 min to prevent contamination.

Assay of seminal glutathione peroxidase

Elabscience human glutathione peroxidase (GPX1) ELISA assay kit was used.^[21]

Test principle

Sandwich-ELISA is the method used with this ELISA kit. An antibody to Human GPX1 has already been pre-coated on the micro ELISA plate included in this package. After being mixed with the appropriate antibody, standards or samples are put into the micro ELISA plate wells. After that, each microplate well receives a sequential addition of a biotinylated detection antibody specific for human GPX1 and an incubation period for the Avidin-Horseradish Peroxidase (HRP) combination. Components that are free are removed. Each well is filled with the substrate solution. Avidin-HRP conjugate, biotinylated detection antibody and Human GPX1 are the only components that will cause any well to become blue. The colour turns yellow as a stop solution is added, ending the enzyme-substrate process. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional

to the concentration of Human GPX1. You can calculate the concentration of Human GPX1 in the samples by comparing the OD of the samples to the standard curve.

Assay of seminal catalase

Elabscience colorimetric assay kit was used.^[22]

Assay principle

Ammonium molybdate can swiftly halt the reaction that CAT uses to break down hydrogen peroxide. The remaining hydrogen peroxide then combines with ammonium molybdate to form a yellowish complex, which can be used to determine CAT activity. This complex is produced at 405 nm.

Assay of seminal Vitamin C

Elabscience colorimetric assay kit was used.^[23]

Detection principle

The most evident chemical action of VC is the reduction of Fe^{3+} to Fe^{2+} , which then facilitates intestinal iron absorption and increases iron storage and use. Fe^{3+} and reducing ascorbic acid react instantly to produce Fe^{2+} . Phenanthroline and Fe^{2+} then react, resulting in the reaction that develops colour. One can ascertain the sample's Vitamin C concentration And determine the indirect VC content by measuring the OD value.

Assay of seminal Vitamin E

Detection principle

The presence of ferroin allows VE to deoxidise Fe^{3+} to Fe^{2+} . Under some circumstances, phenanthroline and Fe^{2+} can react to generate a pink molecule. Following a colorimetric experiment, the VE content can be computed using a formula or by referencing the standard curve.^[24]

Statistical analysis

SPSS, a statistical analysis software package, version 20.0 (SPSS Inc., Chicago, Illinois, USA), was used for the statistical analysis. Data that were not regularly distributed were expressed as a median and interquartile range, whereas properly distributed data were expressed as mean $\pm$ standard deviation. The transformation of logarithms was utilised for data that was not regularly distributed. Analysis of variance was used to compare data that were regularly distributed. By utilising Pearson's correlation analysis, the link between the variables was evaluated. Statistics were deemed significant when parameters showed $P < 0.05$.

RESULTS

Table 1 depicts sociodemographic features of participants in the study, who were majorly within the age range of 41–50 years and mainly urban-dwelling civil servants. About 43.8% and 42.3% of subjects and controls, respectively, took alcohol whereas 56.2% and 57.7% of subjects and controls smoked cigarettes, respectively. Table 2 shows that study controls had more statistically significant sperm counts and active motility than subjects, whereas the study subjects had a higher mean percentage of immotile sperm cells than fertile controls. No statistically significant differences in mean normal morphology between subjects and controls as shown in Table 3.

Mean seminal concentrations of lead, cadmium and iron were significantly higher amongst subjects than controls as shown in Table 4, whereas in Table 5, the antioxidant status or mean seminal levels of Vitamin C, Vitamin E, glutathione peroxidase and CAT, were considerably greater in the controls than in the standards. There were significant moderate negative correlations between sperm count as well as that of active sperm motility with seminal concentrations of heavy metals; however, no such relationship exists with sperm morphology, as shown in Tables 6-8. In addition, as indicated by Tables 9-11, the levels of seminal antioxidants (Vitamins C and E, glutathione peroxidase and CAT) were all positively linked with sperm count and motility whereas not with sperm morphology.

DISCUSSION

The sociodemographic distribution of our research participants showed that the majority of them were inhabitants of Abeokuta, an urban mainly civil servants state, with a large population of artisans and surrounded by villages resided by farmers, who all access the study site for secondary and tertiary healthcare.

Although there were no statistically significant differences in the two groups' normal sperm morphology, the mean sperm count and the percentage of actively motile sperm were substantially higher in the controls than in the subjects. This was in line with the study selection criteria and consistent with the WHO recommendation of a less significant role of normal sperm morphology, in fertility compared to sperm motility and count, as 5% normal morphology is considered adequate.

In this investigation, the seminal cadmium concentration was statistically substantially higher in study individuals compared to controls (0.012 ± 0.010 vs. 0.004 ± 0.002 mg/L, $P \leq 0.001$). The results of several research that used the blood and semen of infertile men are comparable to this one.^[25-28] There was an inverse correlation found between the concentration of seminal cadmium and both sperm count and active motility ($r = -0.334$, $P < 0.001$ and $r = -0.357$, $P < 0.001$, respectively). Excessive cadmium intake has been associated with a reduction in testicular size, reduced testosterone synthesis, deranged spermatogenesis and histological alterations of the testicular tissues in mammals.^[29]

The overproduction of reactive oxygen species (ROS) is the suggested mechanism through which cadmium damages tissue. Glutathione and sulfhydryl (-SH) groups bonded to proteins are depleted by cadmium, which sets off a chain reaction that produces ROS and damages testicles through lipid and DNA peroxidation.^[30] Cadmium has also been implicated in the damage of testicular vascular endothelium and blood-testis barrier, resulting in testicular tissue inflammation and apoptosis.^[31,32]

Comparing participants to controls, the mean Seminal lead concentration was significantly higher (0.432 ± 0.105 vs. 0.222 ± 0.087 mg/L, $P < 0.001$). In addition, there was a strong inverse correlation ($r = -0.573$, $P < 0.001$ and

Table 1: Sociodemographic distribution of participants

Variable	Frequency (%)	
	Control	Subject
Age (years)		
21–30	29 (22.3)	34 (26.2)
31–40	40 (30.8)	42 (32.3)
41–50	52 (40.0)	49 (37.7)
>50	9 (6.9)	5 (3.8)
Occupation		
Artisan	34 (26.2)	33 (25.4)
Civil servant	54 (41.5)	38 (29.2)
Farmer	29 (22.3)	38 (29.2)
Student	9 (6.9)	20 (15.4)
Trading	4 (3.1)	1 (0.8)
Settlement		
Rural	53 (40.8)	51 (39.2)
Urban	77 (59.2)	79 (60.8)

Table 2: Social history of research participants

Variable	Frequency (%)	
	Control	Subject
Alcohol consumption		
Yes	55 (42.3)	57 (43.8)
No	75 (57.7)	73 (56.2)
Cigarette smoking		
Yes	59 (45.4)	41 (31.5)
No	71 (54.6)	89 (68.5)

Table 3: Comparison of means of seminal fluid analysis parameters

Variable	Control, mean $\pm$ SD	Subject, mean $\pm$ SD	<i>t</i>	<i>P</i>
Sperm count ($\times 10^6$ /mL)	65.38 $\pm$ 26.28	10.65 $\pm$ 7.87	22.748	<0.001*
Active motility (%)	49.91 $\pm$ 12.98	21.97 $\pm$ 12.66	17.575	<0.001*
Immotile (%)	21.77 $\pm$ 13.18	63.66 $\pm$ 15.24	-23.707	<0.001*
Normal morphology (%)	72.69 $\pm$ 18.83	80.53 $\pm$ 82.95	-1.051	0.294

$P \leq 0.05$ is statistically significant. SD: Standard deviation

Table 4: Comparison of means of seminal heavy metals

Variable	Control, mean $\pm$ SD	Subject, mean $\pm$ SD	<i>t</i>	<i>P</i>
Cadmium (mg/L)	0.004 $\pm$ 0.002	0.012 $\pm$ 0.010	-7.525	<0.001*
Lead (mg/L)	0.222 $\pm$ 0.087	0.432 $\pm$ 0.105	-17.419	<0.001*
Iron (mg/L)	2.11 $\pm$ 0.75	3.85 $\pm$ 1.15	-14.488	<0.001*

$P \leq 0.05$ is statistically significant. SD: Standard deviation

$r = -0.519$, $P < 0.001$) between the seminal Pb content and both sperm count and active motility. This is in line with findings from related studies on mammals in general and humans in particular.^[33-35] Similar to cadmium, lead can be exposed through the air, water one drinks, food, batteries,

Table 5: Comparison of means of seminal antioxidants (log transformation)

Variable	Control, mean±SD	Subject, mean±SD	<i>t</i>	<i>P</i>
Vitamin C (mg/100 mL)	54.55±2.78	21.74±2.30	7.165	<0.001*
Vitamin E (mg/100 mL)	14.73±1.62	5.62±2.17	12.020	<0.001*
GPx (IU/L)	136.14±2.79	74.28±2.63	4.895	<0.001*
CAT (IU/L)	185.57±1.24	102.28±1.44	16.020	<0.001*

GPx: Glutathione peroxidase, CAT: Catalase, SD: Standard deviation

Table 6: Correlation of heavy metals with sperm count

Variable	<i>r</i>	<i>P</i>
Cadmium (mg/L)	-0.334	<0.001*
Lead (mg/L)	-0.573	<0.001*
Iron (mg/L)	-0.565	<0.001*

r: Pearson correlation coefficient**Table 7: Correlation of heavy metals with active motility**

Variable	<i>r</i>	<i>P</i>
Cadmium (mg/L)	-0.357	<0.001*
Lead (mg/L)	-0.519	<0.001*
Iron (mg/L)	-0.519	<0.001*

r: Pearson correlation coefficient**Table 8: Correlation of heavy metals with sperm morphology**

Variable	<i>r</i>	<i>P</i>
Cadmium (mg/L)	-0.089	0.153
Lead (mg/L)	-0.045	0.474
Iron (mg/L)	-0.083	0.181

r: Pearson correlation coefficient**Table 9: Correlation of sperm count with antioxidant status**

Variable	<i>r</i>	<i>P</i>
Vitamin C (mg/100 mL)	0.408	<0.001*
Vitamin E (mg/100 mL)	0.496	<0.001*
GPx (IU/L)	0.262	<0.001*
CAT (IU/L)	0.586	<0.001*

r: Pearson correlation coefficient. GPx: Glutathione peroxidase, CAT: Catalase**Table 10: Correlation of seminal antioxidants with sperm active motility**

Variable	<i>r</i>	<i>P</i>
Vitamin C (mg/100 mL)	0.315	<0.001*
Vitamin E (mg/100 mL)	0.419	<0.001*
GPx (IU/L)	0.205	0.001*
CAT (IU/L)	0.531	<0.001*

r: Pearson correlation coefficient. GPx: Glutathione peroxidase, CAT: Catalase

soil and paint chips. The overproduction of ROS and the reduction of antioxidant defence capacity are also linked to both heavy metals,^[26,36] similar to cadmium, it combines at high concentration with -SH groups in proteins and rapid glutathione depletion and increases susceptibility to ROS damage.

In addition, the subjects' serum iron concentration was substantially greater than the controls' (3.85 ± 1.15 vs. 2.11 ± 0.75 mg/L, $P < 0.001$). Seminal iron also showed a substantial inverse connection ($r = -0.565$, $P < 0.001$ and $r = -0.519$, $P < 0.001$) with sperm count and active motility, in line with the findings in Pb and Cd. This is consistent with other research' conclusions.^[37,38] In one study, infertile patients had much greater seminal iron levels than controls, although there was no significant difference between the two groups' serum iron levels.^[39] As with previously discussed metals, iron also induces sperm damage by generation of ROS, leading to lipid peroxidation of the sperm cells.^[35,40] Fe-binding proteins and lactoferrin on the sperm membrane prevent iron-induced ROS generation at lower iron concentrations in the semen.

For the three heavy metals evaluated in this study, there was no significant correlation established with sperm morphology. This was contrary to findings in some other studies and still in line with narratives of opposing conclusions from similar studies.^[41,42]

A systematic review of these similar works will harmonise facts and put data into proper perspectives. Genetic modifications of responses to different environmental toxins may also be a possible explanation.

Enzymatic (CAT and glutathione peroxidase) and non-enzymatic (which include Vitamins C and E) constituents of the seminal fluid antioxidant system are intended to guard against the harmful effects of heavy metals and pesticides on the male reproductive systems. Consistent with findings in numerous other investigations, such as case-control studies and randomised control trials, mean seminal levels of Vitamins C and E were significantly greater in controls than in subjects. In addition, these levels demonstrated strong positive relationships with sperm count and active motility.^[43-47] Defending properties, When Vitamins C and E are studied separately and in combination with other antioxidants, they have been shown to be effective in preventing the possibility of significant cellular damage caused by excessive ROS production. They do this by activating pathways to scavenge excess ROS and allow a balance to be achieved between beneficial oxidant generation and damaging oxidative stress.^[48,49] Both vitamins have been associated with the improvement of oligospermia, asthenozoospermia, teratozoospermia and improvement of sperm DNA fragmentation and improved life births in Artificial reproductive technology. Studies have also established that a dose-dependent oral supplementation of these vitamins also improves sperm quality.^[50]

Table 11: Correlation of seminal antioxidants status with sperm morphology

Variable	<i>r</i>	<i>P</i>
Vitamin C (mg/100 mL)	0.066	0.289
Vitamin E (mg/100 mL)	0.013	0.829
GPx (IU/L)	0.028	0.657
CAT (IU/L)	-0.061	0.323

r: Pearson correlation coefficient. GPx: Glutathione peroxidase, CAT: Catalase

Similarly, seminal fluid glutathione peroxidase and CAT levels were significantly higher amongst controls than subjects and correlate positively with sperm quality, through their scavenging activities on hydrogen peroxide radicals, consistent with findings in some other studies.

Another study, however, found no correlation between glutathione peroxidase and sperm count but rather established a negative correlation between its seminal concentration, sperm motility and morphology.

Lower levels of seminal antioxidants and sperm quality were linked to seminal amounts of cadmium, lead and iron.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Swan SH, Elkin EP, Fenster L. The question of declining sperm density revisited: An analysis of 101 studies published 1934-1996. *Environ Health Perspect* 2000;108:961-6.
- Swan SH. Does our environment affect our fertility? Some examples to help reframe the question. *Semin Reprod Med* 2006;24:142-6.
- Alirol E, Getaz L, Stoll B, Chappuis F, Loutan L. Urbanisation and infectious diseases in a globalised world. *Lancet Infect Dis* 2011;11:131-41.
- Harpham T, Stephens C. Urbanization and health in developing countries. *World Health Stat Q* 1991;44:62-9.
- Moore M, Gould P, Keary BS. Global urbanization and impact on health. *Int J Hyg Environ Health* 2003;206:269-78.
- Krausz C. Male infertility: Pathogenesis and clinical diagnosis. *Best Pract Res Clin Endocrinol Metab* 2011;25:271-85.
- Brugh VM 3rd, Lipshultz LI. Male factor infertility: Evaluation and management. *Med Clin North Am* 2004;88:367-85.
- Zhang W, Jiang F, Ou J. Global pesticide consumption and pollution: With China as a focus. *Proc Int Acad Ecol Environ Sci* 2011;1:125-44.
- Roy P, Phukan PK, Changmai D, Boruah S. Pesticides, insecticides and male infertility. *Int J Reprod Contracept Obstet Gynecol* 2017;6:3387-91.
- Duffus JH. Heavy metals – A meaningless term? *Pure Appl Chem* 2002;74:793-807.
- Li F, Qiu Z, Zhang J, Liu W, Liu C, Zeng G. Investigation, pollution mapping and simulative leakage health risk assessment for heavy metals and metalloids in groundwater from a typical brownfield, Middle China. *Int J Environ Res Public Health* 2017;14:768.
- Beckman LE, Van Landeghem GF, Sikström C, Wahlin A, Markevörn B, Hallmans G, *et al.* Interaction between haemochromatosis and transferrin receptor genes in different neoplastic disorders. *Carcinogenesis* 1999;20:1231-3.
- Parkkila S, Niemelä O, Savolainen ER, Koistinen P. HFE mutations do not account for transfusional iron overload in patients with acute myeloid leukemia. *Transfusion* 2001; 41:828-31.
- Martin S, Griswold W. Human health effects of heavy metals. *Environ Sci Technol Briefs Citizens* 2009;15:1-6.
- López-Botella A, Velasco I, Acién M, Sáez-Espinoza P, Todolí-Torró JL, Sánchez-Romero R, *et al.* Impact of heavy metals on human male fertility-an overview. *Antioxidants (Basel)* 2021;10:1473.
- Figà-Talamanca I, Traina ME, Urbani E. Occupational exposures to metals, solvents and pesticides: Recent evidence on male reproductive effects and biological markers. *Occup Med (Lond)* 2001;51:174-88.
- Aitken RJ. The amoroso lecture. The human spermatozoon – A cell in crisis? *J Reprod Fertil* 1999;115:1-7.
- Bergamo P, Volpe MG, Lorenzetti S, Mantovani A, Notari T, Cocca E, *et al.* Human semen as an early, sensitive biomarker of highly polluted living environment in healthy men: A pilot biomonitoring study on trace elements in blood and semen and their relationship with sperm quality and RedOx status. *Reprod Toxicol* 2016;66:1-9.
- Agarwal A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. *J Androl* 2005;26:654-60.
- Available from: <https://www.thermofisher.com/ng/en/home/industrial/spectroscopy-elemental-isotope-analysis/spectroscopy-elemental-isotope-analysis-learning-center/trace-elemental-analysis-tea-information/atomic-absorption-aa-information.html>. [Last assessed on 2023 Aug 25].
- Available from: <https://u2.elabscience.com/PDF/Cate61/E-EL-H5410-Elabscience.pdf>. [Last assessed on 2023 Aug 25].
- Available from: https://file.elabscience.com/Manual/biochemical_kits/E-BC-K031-M-Elabscience.pdf. [Last assessed on 2023 Aug 25].
- Available from: https://file.elabscience.com/Manual/biochemical_kits/E-BC-K034-S-Elabscience.pdf. [Last assessed on 2023 Aug 25].
- Available from: https://file.elabscience.com/Manual/biochemical_kits/E-BC-K033-S-Elabscience.pdf. [Last assessed on 2023 Aug 25].
- Pant N, Upadhyay G, Pandey S, Mathur N, Saxena DK, Srivastava SP. Lead and cadmium concentration in the seminal plasma of men in the general population: Correlation with sperm quality. *Reprod Toxicol* 2003;17:447-50.
- Kiziler AR, Aydemir B, Onaran I, Alici B, Ozkara H, Gulyasar T, *et al.* High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biol Trace Elem Res* 2007;120:82-91.
- Chia SE, Xu B, Ong CN, Tsakok FM, Lee ST. Effect of cadmium and cigarette smoking on human semen quality. *Int J Fertil Menopausal Stud* 1994;39:292-8.
- Akinloye O, Arowojolu AO, Shittu OB, Anetor JI. Cadmium toxicity: A possible cause of male infertility in Nigeria. *Reprod Biol* 2006;6:17-30.
- Nna VU, Ujah GA, Mohamed M, Etim KB, Igba BO, Augustine ER, *et al.* Cadmium chloride-induced testicular toxicity in male Wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal. *Biomed Pharmacother* 2017;94:109-23.
- Liu J, Shen HM, Ong CN. Role of intracellular thiol depletion, mitochondrial dysfunction and reactive oxygen species in *Salvia miltiorrhiza*-induced apoptosis in human hepatoma HepG2 cells. *Life Sci* 2001;69:1833-50.
- Siu ER, Wong EW, Mruk DD, Sze KL, Porto CS, Cheng CY. An occludin-focal adhesion kinase protein complex at the blood-testis barrier: A study using the cadmium model. *Endocrinology* 2009;150:3336-44.
- Kishimoto T, Oguri T, Yamabe S, Tada M. Effect of cadmium injury on growth and migration of cultured human vascular endothelial cells. *Hum Cell* 1996;9:43-8.
- Gennart JP, Buchet JP, Roels H, Ghyselen P, Ceulemans E, Lauwerys R. Fertility of male workers exposed to cadmium, lead, or manganese. *Am J Epidemiol* 1992;135:1208-19.
- Naha N, Chowdhury AR. Inorganic lead exposure in battery and paint factory: Effect on human sperm structure and functional activity. *J UOEH* 2006;28:157-71.
- Bonde JP, Joffe M, Apostoli P, Dale A, Kiss P, Spano M, *et al.* Sperm count and chromatin structure in men exposed to inorganic lead: Lowest adverse effect levels. *Occup Environ Med* 2002;59:234-42.
- He Y, Zou L, Luo W, Yi Z, Yang P, Yu S, *et al.* Heavy metal exposure, oxidative stress and semen quality: Exploring associations and mediation

- effects in reproductive-aged men. *Chemosphere* 2020;244:125498.
37. Aydemir B, Kiziler AR, Onaran I, Alici B, Ozkara H, Akyolcu MC. Impact of Cu and Fe concentrations on oxidative damage in male infertility. *Biol Trace Elem Res* 2006;112:193-203.
38. Huang YL, Tseng WC, Cheng SY, Lin TH. Trace elements and lipid peroxidation in human seminal plasma. *Biol Trace Elem Res* 2000;76:207-15.
39. Ammar O, Houas Z, Mehdi M. The association between iron, calcium, and oxidative stress in seminal plasma and sperm quality. *Environ Sci Pollut Res Int* 2019;26:14097-105.
40. Thérond P, Auger J, Legrand A, Jouannet P. alpha-tocopherol in human spermatozoa and seminal plasma: Relationships with motility, antioxidant enzymes and leukocytes. *Mol Hum Reprod* 1996;2:739-44.
41. Omu AE, Fatinikun T, Mannazhath N, Abraham S. Significance of simultaneous determination of serum and seminal plasma alpha-tocopherol and retinol in infertile men by high-performance liquid chromatography. *Andrologia* 1999;31:347-54.
42. Lewis SE, John Aitken R, Conner SJ, Iuliis GD, Evenson DP, Henkel R, *et al.* The impact of sperm DNA damage in assisted conception and beyond: Recent advances in diagnosis and treatment. *Reprod Biomed Online* 2013;27:325-37.
43. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: Protective role of Vitamin E. *J Androl* 1996;17:530-7.
44. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, *et al.* Sperm oxidative stress and the effect of an oral Vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003;49:83-94.
45. Majzoub A, Agarwal A. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. *Arab J Urol* 2018;16:113-24.
46. Paradiso Galatioto G, Gravina GL, Angelozzi G, Sacchetti A, Innominato PF, Pace G, *et al.* May antioxidant therapy improve sperm parameters of men with persistent oligospermia after retrograde embolization for varicocele? *World J Urol* 2008;26:97-102.
47. Abad C, Amengual MJ, Gosálvez J, Coward K, Hannaoui N, Benet J, *et al.* Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. *Andrologia* 2013;45:211-6.
48. Gual-Frau J, Abad C, Amengual MJ, Hannaoui N, Checa MA, Ribas-Maynou J, *et al.* Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. *Hum Fertil (Camb)* 2015;18:225-9.
49. Agarwal A, Prabhakaran SA, Sikka SC. Clinical relevance of oxidative stress in patients with male factor infertility: Evidence-based analysis. *AUA Update Ser* 2007;26:1-12.
50. Tremellen K. Oxidative stress and male infertility – A clinical perspective. *Hum Reprod Update* 2008;14:243-58.