




# Seminal Plasma Levels of Heavy Metals (Lead, Cadmium and Arsenic) and Oxidative Status in Asthnozoospermic Men

Mehran Dorostghoal <sup>1,2,\*</sup>, Fahimeh Izadi<sup>1</sup>, Mojdeh Gholami<sup>1</sup>, Saleh Zahraei<sup>1</sup>

<sup>1</sup> Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

<sup>2</sup> Biotechnology and Bioscience Research Center, Shahid Chamran University of Ahvaz, Ahvaz, Iran

\*Corresponding Author: Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran. Email: dorostghoal@gmail.com

Received: 7 October, 2024; Revised: 14 December, 2024; Accepted: 18 December, 2024

## Abstract

**Background:** Recent evidence suggests a decline in sperm quality due to exposure to environmental toxicants. Several studies have reported the adverse effects of heavy metals on human reproductive efficiency.

**Objectives:** This study analyzed the seminal levels of heavy metals (lead, cadmium, and arsenic) and oxidative status, evaluating their relationships with sperm parameters in men from Ahvaz, southwest Iran.

**Methods:** Semen quality, seminal lipid peroxidation, and antioxidant enzyme activity were assessed in 100 men attending the Narges Medical Genetics and Prenatal Diagnosis Laboratory in Ahvaz, southwest Iran. Sperm concentration, motility, and normal morphology were analyzed following the guidelines of the World Health Organization (WHO, 2010). Seminal levels of lead, cadmium, and arsenic were determined using a flame atomic absorption spectrophotometer.

**Results:** Higher seminal levels of lead and cadmium were observed in asthnozoospermic men compared with normozoospermic individuals. Seminal levels of lead, cadmium, and arsenic exhibited significant negative correlations with sperm concentration, total motility, and normal morphology. Additionally, higher concentrations of seminal lead and cadmium were associated with increased seminal levels of malondialdehyde (MDA) and decreased activity of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx).

**Conclusions:** The findings suggest an association between sperm quality and seminal oxidative status with seminal levels of lead and cadmium in men from Ahvaz, southwest Iran.

**Keywords:** Heavy Metals, Semen Quality, Oxidative Stress, Asthnozoospermic Men, Male Infertility

## 1. Background

In recent decades, numerous studies have confirmed a decline in semen quality among men, contributing to decreased male fertility worldwide (1). This decline in sperm quality can result from improper diets, increased obesity, and exposure to environmental and occupational pollutants (2). Environmental factors, including pesticides, external estrogens, and heavy metals, have been shown to adversely affect male fertility (3).

Heavy metals are among the most significant and serious environmental pollutants contributing to reproductive dysfunction (4, 5). Humans are exposed to metal particles such as lead, cadmium, and arsenic through occupational and environmental sources (3).

These metals naturally exist in the earth's crust and are introduced into the environment through natural forces. However, industrial activities have significantly increased their release, resulting in greater exposure to these metals via food, water, and air pollution (6).

High concentrations of heavy metals have been linked to impaired sperm quality (7). Exposure to lead is associated with abnormalities in sperm motility (asthenozoospermia), morphology (teratozoospermia), and DNA integrity (8-11). Cadmium, another highly toxic heavy metal, is found in various industrial products and contaminated drinking water (12). Exposure to cadmium is negatively associated with sperm concentration, motility, and morphology, adversely affecting male fertility (13, 14). Similarly, arsenic exposure has been implicated in reducing sperm count,

motility, and normal morphology (15-17). Although the exact mechanism of toxicity remains poorly understood, heavy metals are known to cause cellular damage through the production of reactive oxygen species (ROS) (18).

Ahvaz, the capital of Khuzestan Province, is a metropolitan city in southwest Iran, bordering Iraq, Kuwait, and Saudi Arabia. With coordinates of 31°20'N and 48°40'E, a surface area of 528 km<sup>2</sup>, and a population of 1.112 million, Ahvaz is one of Iran's major industrial cities (19). The city's air pollution is exacerbated by the presence of numerous oil, gas, petrochemical, metal, and non-metal industries. Furthermore, dust pollution is another significant issue in Ahvaz, primarily attributed to the city's proximity to western deserts and fine dust sources in neighboring countries such as Iraq and Saudi Arabia (1).

## 2. Objectives

The present study aimed to investigate the seminal levels of lead, cadmium, and arsenic, as well as oxidative status, in men residing in Ahvaz, southwest Iran.

## 3. Methods

### 3.1. Subjects

This study was approved by the Ethics Committee of Shahid Chamran University of Ahvaz (EE/1400.2.24.25679/scu.ac.ir). The nature and purpose of the study were explained to all participants, and written informed consent was obtained from each of them. A total of 100 men attending the Narges Medical Genetics and Prenatal Diagnosis Laboratory in Ahvaz, southwest Iran, from April 2021 to May 2022, were recruited for semen analysis. The sample size was determined based on related studies (20, 21).

Smokers, drug addicts, alcohol consumers, and men with a history of serious systemic diseases, chronic illnesses, or male reproductive disorders such as sexually transmitted diseases, prostatitis, varicocele, urinary tract infections, cryptorchidism, or previous genital surgery were excluded from the study (22).

### 3.2. Semen Collection

After collecting semen samples in wide-mouth sterile containers, semen analysis was conducted 30 minutes after liquefaction at room temperature, following the guidelines of the World Health Organization (WHO) (23). The semen samples were centrifuged at 15,000 g for 15 minutes. The supernatant was separated and used as

seminal plasma, which was then frozen at -20°C until further analysis.

### 3.3. Semen Analysis

A trained technician analyzed sperm concentration, motility, and morphology in duplicate samples. Subjects were considered normal if they met the following criteria:  $\geq 39 \times 10^6$  total sperm count,  $\geq 15 \times 10^6$  sperm count per mL,  $\geq 40\%$  total motile sperm (progressive and non-progressive),  $\geq 32\%$  progressive motility,  $\geq 4\%$  normal morphology, and  $< 1 \times 10^6$  WBC/mL.

An improved Neubauer hemocytometer (Hamburg, Germany) was used to determine sperm concentration. The total sperm count was calculated by multiplying the semen volume by the sperm concentration. The motility of at least 200 sperm was assessed and classified into progressive motile, non-progressive motile, and immotile categories, after which total sperm motility was calculated.

For sperm morphology assessment, semen smears were stained using the Papanicolaou method, and at least 200 sperm were evaluated at 1000× magnification according to WHO guidelines (23).

### 3.4. Seminal Heavy Metal (Lead, Cadmium and Arsenic) Analysis

Semen samples were analyzed to determine the levels of lead, cadmium, and arsenic in seminal plasma. A flame atomic absorption spectrophotometer (Thermo Fischer, USA) was used to measure the concentrations of these heavy metals in the seminal plasma.

### 3.5. Seminal Lipid Peroxidation and Antioxidant Enzymes Activity Analysis

Lipid peroxidation levels and antioxidant enzyme activities were analyzed in seminal plasma. Malondialdehyde (MDA) levels, as an indicator of lipid peroxidation, were measured using the thiobarbituric acid test at 532 nm and expressed as nmol/mL of seminal plasma. Seminal glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were evaluated using colorimetric assay kits (Randox Ltd., United Kingdom).

### 3.6. Statistical Analysis

SPSS version 16 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Differences between normozoospermic and asthenozoospermic men were assessed using the non-parametric Mann-Whitney test. Correlations between various parameters were

**Table 1.** Characteristic and Semen Parameters of Normozoospermic and Asthnozoospermic Men in Ahvaz, Southwest Iran <sup>a, b, c</sup>

Parameters	Normozoospermic (n = 58)	Asthnozoospermic (n = 42)	P-Value
Age (y)	33.0 (29.0 - 38.2)	34.0 (30.0 - 38.5)	0.997
BMI (kg/m <sup>2</sup> )	23.3 (22.3 - 23.8)	23.1 (22.1 - 23.6)	0.234
Semen volume (mL)	3.0 (2.5 - 4.0)	2.5 (2.5 - 3.5)	0.049
Sperm count (million/mL)	30.0 (29.5 - 39.2)	25.0 (18.0 - 35.0)	< 0.001
Total motility (%)	49.5 (45.0 - 55.0)	32.5 (28.0 - 38.0)	< 0.001
Normal morphology (%)	8.0 (6.5 - 12.0)	5.0 (4.0 - 8.0)	0.001

Abbreviation: BMI: Body Mass Index.

<sup>a</sup> Results expressed as median values and interquartile ranges.

<sup>b</sup> Mann-Whitney U-test was done as the test of significant.

<sup>c</sup> The level of significance was set at  $P < 0.05$ .

evaluated using the Spearman test. A P-value of  $< 0.05$  was considered statistically significant.

## 4. Results

### 4.1. Semen Analysis

Subjects were divided into normozoospermic and asthenozoospermic groups based on their semen quality. Semen characteristics for normozoospermic and asthenozoospermic individuals are presented in [Table 1](#). Significant differences were observed in semen volume, sperm concentration, total motility (progressive and non-progressive), and normal morphology between patients and healthy individuals ([Table 1](#)).

### 4.2. Seminal Heavy Metal (Lead, Cadmium and Arsenic) Analysis

Seminal concentrations of lead were significantly higher ( $P = 0.015$ ) in asthenozoospermic patients compared to normozoospermic individuals ([Figure 1A](#)). Significant negative relationships were observed between seminal lead concentrations and sperm count ( $r = -0.360$ ,  $P < 0.001$ ), motility ( $r = -0.257$ ,  $P = 0.010$ ), and normal morphology ( $r = -0.300$ ,  $P = 0.002$ ) ([Table 2](#)).

Additionally, significantly higher levels of cadmium ( $P < 0.001$ ) were detected in the semen of asthenozoospermic men compared to normozoospermic individuals ([Figure 1B](#)). Seminal cadmium levels were negatively associated with sperm count ( $r = -0.461$ ,  $P < 0.001$ ), motility ( $r = -0.432$ ,  $P < 0.001$ ), and normal morphology ( $r = -0.476$ ,  $P < 0.001$ ) ([Table 2](#)).

However, there was no significant difference ( $P = 0.457$ ) in seminal arsenic levels between asthenozoospermic men and normozoospermic

individuals ([Figure 1C](#)). Despite this, a significant negative relationship was identified between arsenic concentration and sperm count ( $r = -0.211$ ,  $P = 0.035$ ), motility ( $r = -0.285$ ,  $P = 0.004$ ), and normal morphology ( $r = -0.219$ ,  $P = 0.028$ ) ([Table 2](#)).

### 4.3. Seminal Lipid Peroxidation and Antioxidant Enzymes Activity Analysis

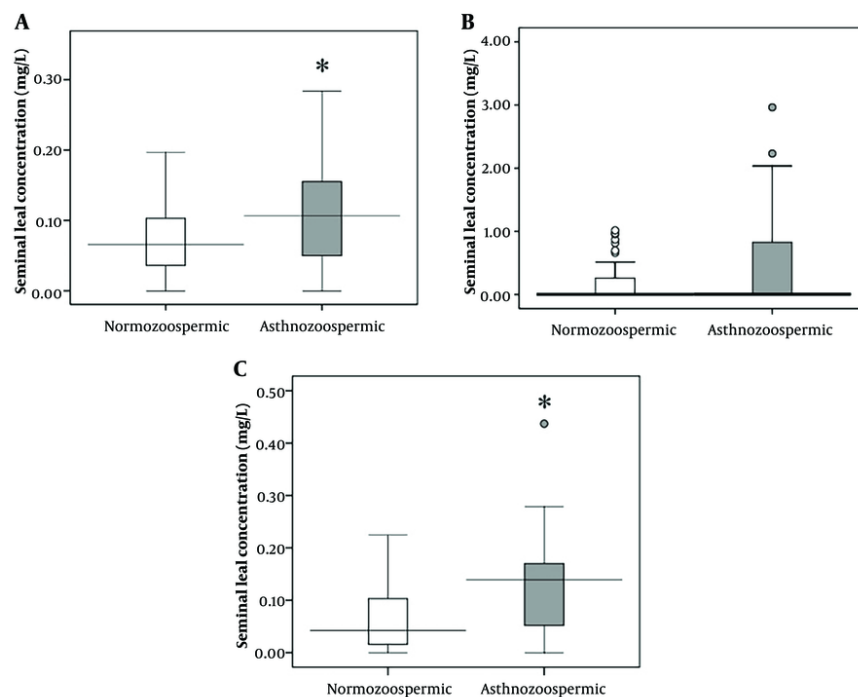
Significantly higher seminal levels of MDA ( $P < 0.001$ ) and lower activity of SOD and GPx enzymes were observed in asthenozoospermic men compared to normozoospermic men ([Figure 2A - C](#)). Significant associations were identified between sperm count, total motility, normal morphology, and seminal MDA levels, as well as SOD and GPx enzyme activity ([Table 3](#)).

A significant correlation was observed between seminal lead concentration and seminal MDA levels ( $r = 0.339$ ,  $P = 0.001$ ), as well as SOD ( $r = -0.383$ ,  $P < 0.001$ ) and GPx ( $r = -0.372$ ,  $P < 0.001$ ) enzyme activity ([Table 4](#)). Seminal cadmium levels were significantly associated with seminal MDA levels ( $r = 0.545$ ,  $P < 0.001$ ) and SOD ( $r = -0.623$ ,  $P < 0.001$ ) and GPx ( $r = -0.586$ ,  $P < 0.001$ ) enzyme activity ([Table 4](#)).

No significant associations were found between seminal arsenic concentration and seminal MDA levels or the activity of SOD and GPx enzymes ([Table 4](#)).

## 5. Discussion

With the expansion of industrial activities worldwide, pollution caused by heavy metals has significantly impacted human health ([24](#)). Ahvaz has been reported as one of the most polluted cities in Iran and globally, with air pollution adversely affecting the health of its residents ([25](#)). In recent years, the industrial development of Ahvaz, combined with population growth and increased vehicle traffic, has contributed to



**Figure 1.** A - C, seminal levels of heavy metals in normozoospermic (n = 58) and asthnozoospermic (n = 42) men in Ahvaz, southwest Iran. Mann-Whitney U-test was done as the test of significant and the results expressed as median. \*The level of significance was set at  $P < 0.05$ .

**Table 2.** Spearman's Correlation Between Semen Quality and Seminal Concentration of Heavy Metals in Ahvaz, Southwest Iran (n = 100)<sup>a</sup>

Parameters	Lead (mg/L)	Cadmium (mg/L)	Arsenic (mg/L)
Semen volume (mL)	$r = 0.089, P = 0.379$	$r = 0.065, P = 0.410$	$r = -0.113, P = 0.261$
Sperm count (million/mL)	$r = -0.360, P < 0.001$	$r = -0.461, P < 0.001$	$r = -0.211, P = 0.035$
Total motility (%)	$r = -0.257, P = 0.010$	$r = -0.432, P < 0.001$	$r = -0.285, P = 0.004$
Normal morphology (%)	$r = -0.300, P = 0.002$	$r = -0.476, P < 0.001$	$r = -0.219, P = 0.028$

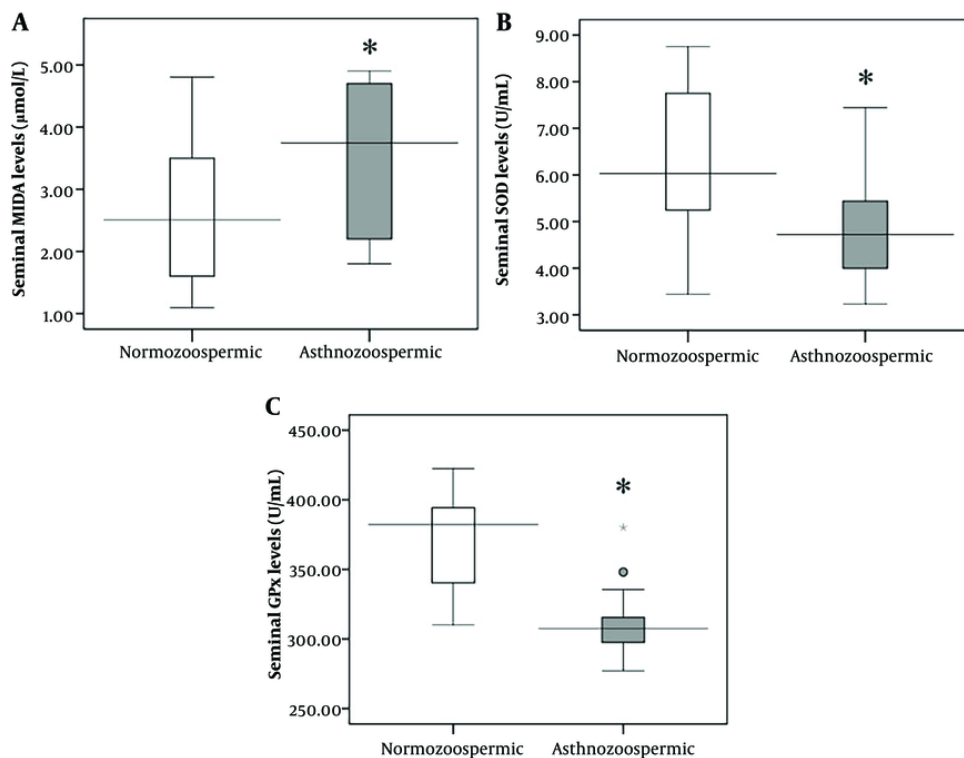
<sup>a</sup> The level of significance was set at  $P < 0.05$ .

the deposition of heavy metals in dust particles (26). Additionally, dust is a significant air pollutant in Ahvaz, due to its proximity to deserts and dust sources in neighboring countries (1). The intensive activity of oil, gas, and metal industries, along with light and heavy traffic, has resulted in higher-than-background levels of heavy metals such as lead, zinc, copper, chromium, cadmium, and arsenic in the surface soils of Ahvaz (27).

The present study evaluated seminal levels of heavy metals, including lead, cadmium, and arsenic, as well as oxidative status, and investigated their relationships with sperm quality in men from Ahvaz, southwest Iran.

Our findings revealed higher seminal levels of lead in asthnozoospermic men compared to normozoospermic individuals. A significant negative relationship was observed between lead concentration and sperm count, motility, and normal morphology. Higher seminal lead concentrations were also associated with decreased activity of seminal antioxidant enzymes and increased levels of lipid peroxidation.

Nandi et al. reported that lead levels in the seminal plasma of normozoospermic individuals were lower compared to infertile men (28). Similarly, Famurewa and



**Figure 2.** A - C, seminal levels of lipid peroxidation and antioxidant enzymes activity in normozoospermic (n = 58) and asthnozoospermic (n = 42) men in Ahvaz, southwest Iran. MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase. Mann-Whitney U-test was done as the test of significant and the results expressed as median. \*The level of significance was set at  $P < 0.05$ .

**Table 3.** Spearman's Correlation Between Semen Quality and Seminal Lipid Peroxidation Levels and Antioxidant Enzymes Activity in Ahvaz, Southwest Iran (n = 100)<sup>a</sup>

Parameters	MDA (nmol/mL)	SOD (U/mL)	GPx (mU/mL)
Semen volume (mL)	$r = -0.082, P = 0.417$	$r = 0.090, P = 0.375$	$r = 0.150, P = 0.137$
Sperm count (million/mL)	$r = -0.472, P < 0.001$	$r = 0.481, P < 0.001$	$r = 0.479, P < 0.001$
Total motility (%)	$r = -0.422, P < 0.001$	$r = 0.490, P < 0.001$	$r = 0.740, P < 0.001$
Normal morphology (%)	$r = -0.430, P < 0.001$	$r = 0.398, P < 0.001$	$r = 0.401, P < 0.001$

Abbreviations: MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase.

<sup>a</sup> The level of significance was set at  $P < 0.05$ .

Ugwuja found that lead levels in the seminal plasma of azoospermic and oligozoospermic men were higher than in normozoospermic individuals, correlating with unfavorable decreases in parameters such as sperm concentration, motility, and normal morphology (29).

According to the findings of Tanricut et al., exposure to low levels of environmental lead alters semen quality and affects sperm chromatin density (30). Similarly, Pant et al. observed a significant negative relationship

between lead concentration and sperm motility and concentration in infertile individuals compared to normozoospermic men (15). In this context, an analysis of semen quality in battery factory workers revealed high levels of lead in their semen, which was associated with reduced sperm concentration (31). However, Plechaty et al. found no relationship between sperm concentration, motility, and lead concentration in South African battery workers (32).

**Table 4.** Spearman's Correlation Between Seminal Concentrations of Heavy Metals and Seminal Lipid Peroxidation Levels and Antioxidant Enzymes Activity in Ahvaz, Southwest Iran (n = 100)<sup>a</sup>

Parameters	Lead (mg/L)	Cadmium (mg/L)	Arsenic (mg/L)
MDA (nmol/mL)	r = 0.339, P = 0.001	r = 0.545, P < 0.001	r = 0.183, P = 0.068
SOD (U/mL)	r = -0.383, P < 0.001	r = -0.623, P < 0.001	r = -0.142, P = 0.159
GPx (mU/mL)	r = -0.372, P < 0.001	r = -0.586, P < 0.001	r = -0.125, P = 0.215

Abbreviations: MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase.

<sup>a</sup> The level of significance was set at P < 0.05.

Additionally, higher levels of cadmium were observed in the semen of asthenozoospermic men compared to normozoospermic individuals. Seminal cadmium levels were negatively associated with sperm count, motility, and normal morphology. Higher concentrations of seminal cadmium were also linked to decreased activity of seminal antioxidant enzymes and increased levels of lipid peroxidation. Nandi et al. reported that cadmium significantly reduces seminal fluid volume (28). Famurewa and Ugwuja found that cadmium adversely affects sperm concentration, motility, and normal morphology (29). Zhao et al. demonstrated that cadmium decreases the total percentage of motile sperm in humans and mice in a dose- and time-dependent manner, reducing sperm motility and impairing sperm penetration into the egg, as well as embryo development (12). It has also been shown that cadmium induces inflammation in the testes of adult mice, leading to a decline in sperm parameters (33).

However, Keck et al. and Meeker et al. found no correlation between seminal cadmium levels and sperm parameters (34, 35). Xu et al. reported that cadmium adversely affects the prostate gland, which secretes seminal plasma, resulting in an inverse relationship between cadmium concentration and seminal fluid volume (7).

Our data indicated higher seminal levels of arsenic in asthenozoospermic men compared to normozoospermic individuals. A significant negative relationship was observed between arsenic concentration and sperm count and motility. Higher concentrations of seminal arsenic were also associated with decreased activity of seminal antioxidant enzymes and increased levels of lipid peroxidation. Nandi et al. reported that individuals with high arsenic concentrations had significantly higher percentages of sperm with abnormal motility (28). Similarly, a study conducted on men referred to infertility clinics in Michigan, United States, found that smoking men had a significantly increased risk of low sperm motility due to

prolonged arsenic exposure (35). Li et al. demonstrated that arsenic disrupted spermatogenesis in mice, leading to a decline in sperm quality (36).

Heavy metals are known to damage cells by inducing oxidative stress through the production of ROS (37). In this study, higher levels of lipid peroxidation and lower antioxidant enzyme activity were observed in asthenozoospermic men compared to normozoospermic individuals. Significant negative relationships were identified between seminal concentrations of lead, arsenic, and cadmium and the activity of antioxidant enzymes. Reduced antioxidant enzyme activity and increased lipid peroxidation, resulting from excessive ROS production, have been linked to impaired spermatogenesis (38).

Lead exposure induces testicular oxidative stress by increasing MDA levels and reducing the activity of antioxidant enzymes such as SOD, GPx, and catalase, thereby decreasing sperm count and motility (39). Similarly, cadmium exposure has been associated with excessive ROS production and oxidative stress. Leite et al. reported that even environmentally realistic doses of cadmium reduced testicular antioxidant status in Wistar rats (40). Sperm motility is impaired by excessive ROS production, which decreases axonemal protein phosphorylation and/or adenosine triphosphate levels and increases membrane lipid peroxidation (41).

Additionally, arsenic exposure has been linked to impaired spermatogenesis, reduced fertilization ability, and decreased sperm count and progressive motility. Like other heavy metals, arsenic exposure increases ROS and MDA levels while reducing the activity of glutathione (GSH) and SOD in the testes (42).

### 5.1. Conclusions

In conclusion, the present study demonstrates that higher levels of seminal heavy metals, along with decreased antioxidant enzyme activity and increased lipid peroxidation, were observed in asthenozoospermic men in Ahvaz, southwest Iran. These findings suggest that heavy metals adversely affect semen quality by

inducing oxidative stress. Further studies with larger sample sizes are required to validate these findings and provide more comprehensive insights.

## Acknowledgements

The authors wish to thank the Vice Chancellor for Research of Shahid Chamran University of Ahvaz for the research grant #SCU.SB1401.311.

## Footnotes

**Authors' Contribution:** M. D. contributed to the study conception and design. F. I., M. Gh., and S. Z. performed material preparation, data collection, and analysis. All authors contributed to the writing-original draft, review and editing.

**Conflict of Interests Statement:** No potential conflict of interest relevant to this article was reported.

**Data Availability:** Data supporting the findings of this study are available upon reasonable request from the corresponding author.

**Ethical Approval:** This study was approved by the Ethics Committee of Shahid Chamran University of Ahvaz (EE/1400.2.24.25679/scu.ac.ir).

**Funding/Support:** The vice chancellor for research of Shahid Chamran University of Ahvaz provided the research grant #SCU.SB1401.311 for present study.

**Informed Consent:** The nature and purpose of the study were explained to all participants, and written informed consent was obtained from each of them.

## References

- Lopez-Botella A, Velasco I, Acien M, Saez-Espinosa P, Todoli-Torro JL, Sanchez-Romero R, et al. Impact of Heavy Metals on Human Male Fertility-An Overview. *Antioxidants (Basel)*. 2021;**10**(9). [PubMed ID: 34573104]. [PubMed Central ID: PMC8468047]. <https://doi.org/10.3390/antiox10091473>.
- Mann U, Shiff B, Patel P. Reasons for worldwide decline in male fertility. *Curr Opin Urol*. 2020;**30**(3):296-301. [PubMed ID: 32168194]. <https://doi.org/10.1097/MOU.0000000000000745>.
- Sheweita SA, Tilmisany AM, Al-Sawaf H. Mechanisms of male infertility: role of antioxidants. *Curr Drug Metab*. 2005;**6**(5):495-501. [PubMed ID: 16248841]. <https://doi.org/10.2174/138920005744330594>.
- Kumar R, Pant N, Srivastava SP. Chlorinated pesticides and heavy metals in human semen. *Int J Androl*. 2000;**23**(3):145-9. [PubMed ID: 10844539]. <https://doi.org/10.1046/j.1365-2605.2000.00218.x>.
- Gopalkrishnan K. Characteristics of semen parameters in a selected population of Indian men over a period of 10 years. *Current Sci*. 1998;**75**(5):939-42.
- Wirth JJ, Mijal RS. Adverse effects of low level heavy metal exposure on male reproductive function. *Syst Biol Reprod Med*. 2010;**56**(2):147-67. [PubMed ID: 20377313]. <https://doi.org/10.3109/19396360903582216>.
- Xu B, Chia SE, Tsakok M, Ong CN. Trace elements in blood and seminal plasma and their relationship to sperm quality. *Reprod Toxicol*. 1993;**7**(6):613-8. [PubMed ID: 8118112]. [https://doi.org/10.1016/0890-6238\(93\)90038-9](https://doi.org/10.1016/0890-6238(93)90038-9).
- Eibensteiner L, Del Carpio Sanz A, Frumkin H, Gonzales C, Gonzales GF. Lead exposure and semen quality among traffic police in Arequipa, Peru. *Int J Occup Environ Health*. 2005;**11**(2):161-6. [PubMed ID: 15875892]. <https://doi.org/10.1179/oeh.2005.11.2.161>.
- Hernandez-Ochoa I, Garcia-Vargas G, Lopez-Carrillo L, Rubio-Andrade M, Moran-Martinez J, Cebrian ME, et al. Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. *Reprod Toxicol*. 2005;**20**(2):221-8. [PubMed ID: 15907657]. <https://doi.org/10.1016/j.reprotox.2005.01.007>.
- Jurasovic J, Cvitkovic P, Pizent A, Colak B, Telisman S. Semen quality and reproductive endocrine function with regard to blood cadmium in Croatian male subjects. *Biometals*. 2004;**17**(6):735-43. [PubMed ID: 15689116]. <https://doi.org/10.1007/s10534-004-1689-7>.
- Telisman S, Colak B, Pizent A, Jurasovic J, Cvitkovic P. Reproductive toxicity of low-level lead exposure in men. *Environ Res*. 2007;**105**(2):256-66. [PubMed ID: 17632096]. <https://doi.org/10.1016/j.envres.2007.05.011>.
- Zhao LL, Ru YF, Liu M, Tang JN, Zheng JF, Wu B, et al. Reproductive effects of cadmium on sperm function and early embryonic development in vitro. *PLoS One*. 2017;**12**(11). e0186727. [PubMed ID: 29095856]. [PubMed Central ID: PMC5667747]. <https://doi.org/10.1371/journal.pone.0186727>.
- Pant N, Kumar G, Upadhyay AD, Gupta YK, Chaturvedi PK. Correlation between lead and cadmium concentration and semen quality. *Andrologia*. 2015;**47**(8):887-91. [PubMed ID: 25228328]. <https://doi.org/10.1111/and.12342>.
- Lamas CA, Cuquetto-Leite L, do Nascimento da Silva E, Thomazini BF, Cordeiro GDS, Predes FS, et al. Grape juice concentrate alleviates epididymis and sperm damage in cadmium-intoxicated rats. *Int J Exp Pathol*. 2017;**98**(2):86-99. [PubMed ID: 28581201]. [PubMed Central ID: PMC5485367]. <https://doi.org/10.1111/iepp.12227>.
- Pant N, Murthy RC, Srivastava SP. Male reproductive toxicity of sodium arsenite in mice. *Hum Exp Toxicol*. 2004;**23**(8):399-403. [PubMed ID: 15346721]. <https://doi.org/10.1191/0960327104ht4670a>.
- Sarkar M, Chaudhuri GR, Chattopadhyay A, Biswas NM. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. *Asian J Androl*. 2003;**5**(1):27-31.
- Centeno JA, Mullick FG, Martinez L, Page NP, Gibb H, Longfellow D, et al. Pathology related to chronic arsenic exposure. *Environ Health Perspect*. 2002;**110** Suppl 5(Suppl 5):883-6. [PubMed ID: 12426152]. [PubMed Central ID: PMC1241266]. <https://doi.org/10.1289/ehp.02110S5883>.
- Sanchez T. Effects of mercury, lead, arsenic and zinc to human renal oxidative stress and functions: a review. *Archivos De Medicina*. 2018;**4**(2).
- Neisi A, Vosoughi M, Shirmardi M, Idani E, Goudarzi G, Hazrati S, et al. Concentration of air pollutants as toxic matter in urban and rural areas of Ahvaz. *Toxin Rev*. 2017;**37**(3):243-50. <https://doi.org/10.1080/15569543.2017.1337796>.
- Pant N, Banerjee AK, Pandey S, Mathur N, Saxena DK, Srivastava SP. Correlation of lead and cadmium in human seminal plasma with seminal vesicle and prostatic markers. *Hum Exp Toxicol*. 2003;**22**(3):125-8. [PubMed ID: 12723892]. <https://doi.org/10.1191/0960327103ht3360a>.
- Emokpae MA, Christian A, Ibadin K. Seminal plasma levels of lead and mercury in infertile males in Benin city, Nigeria. *Int J Med Res*

- Health Sci. 2016;5(1). <https://doi.org/10.5958/2319-5886.2016.00001.1>.
22. Dorostghoal M, Galehdari H, Hemadi M, Davoodi E. Sperm miR-34c-5p Transcript Content and Its Association with Sperm Parameters in Unexplained Infertile Men. *Reprod Sci.* 2022;29(1):84-90. [PubMed ID: 34494232]. <https://doi.org/10.1007/s43032-021-00733-w>.
  23. World Health Organisation. *WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed.* Geneva, Switzerland: World Health Organization; 2010. Available from: <https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://iris.who.int/handle/10665/44261&ved=2ahUKEwiC8M6ugNmKAXWnRkEAHUUGHQMqFnoECBsQAQ&usq=AovVaw3vydVULXjhtcLNgmjFWHLU>.
  24. Kaydan Z, Nazarpour A, Ghanavati N. Evaluation of Soil Pollution with Heavy Metals (Pb, Zn, Cu, Cr, Ni and V) in Ahvaz Parks (2016). *J Health.* 2019;10(2):228-39. <https://doi.org/10.29252/j.health.10.2.228>.
  25. Goudarzi G, Alavi N, Geravandi S, Idani E, Behrooz HRA, Babaei AA, et al. Health risk assessment on human exposed to heavy metals in the ambient air PM(10) in Ahvaz, southwest Iran. *Int J Biometeorol.* 2018;62(6):1075-83. [PubMed ID: 29464337]. <https://doi.org/10.1007/s00484-018-1510-x>.
  26. Geravandi S, Goudarzi G, Mohammadi MJ, Taghavirad SS, Salmanzadeh S. Sulfur and Nitrogen Dioxide Exposure and the Incidence of Health Endpoints in Ahvaz, Iran. *Health Scope.* 2015;4(2). <https://doi.org/10.17795/jhealthscope-24318>.
  27. Kianpor M, Payandeh K, Ghanavati N. Environmental Assessment of Some Heavy Metals Pollution in Street Dust in the Industrial Areas of Ahvaz. *Jundishapur J Health Sci.* 2019;11(3). <https://doi.org/10.5812/jjhs.87212>.
  28. Nandi P, Varghese AC, Das MC, Banerjee P, Murmu N, Banerjee SK. Lead, cadmium and arsenic content in seminal plasma and its effects on seminogram. *Int J Sci Eng Technol.* 2015;3(3):204-7.
  29. Famurewa AC, Ugwuja EI. Association of blood and seminal plasma cadmium and lead levels with semen quality in non-occupationally exposed infertile men in Abakaliki, South East Nigeria. *J Fam Reproductive Health.* 2017;11(2):97.
  30. Tanrikut E, Karaer A, Celik O, Celik E, Otlu B, Yilmaz E, et al. Role of endometrial concentrations of heavy metals (cadmium, lead, mercury and arsenic) in the aetiology of unexplained infertility. *Eur J Obstet Gynecol Reprod Biol.* 2014;179:187-90. [PubMed ID: 24966000]. <https://doi.org/10.1016/j.ejogrb.2014.05.039>.
  31. Assennato G, Paci C, Baser ME, Molinini R, Candela RG, Altamura BM, et al. Sperm Count Suppression without Endocrine Dysfunction in Lead-Exposed Men. *Arch Environm Health: An Int J.* 1987;42(2):124-7. <https://doi.org/10.1080/00039896.1987.9935808>.
  32. Plechaty MM, Noll B, Sunderman FW. Lead concentrations in semen of healthy men without occupational exposure to lead. *Ann Clin Lab Sci.* 1977;7(6):515-8.
  33. de Freitas ML, Dalmolin L, Oliveira LP, da Rosa Moreira L, Roman SS, Soares FA, et al. Effects of butane-2,3-dione thiosemicarbazone oxime on testicular damage induced by cadmium in mice. *J Toxicol Sci.* 2012;37(5):899-910. [PubMed ID: 23038000]. <https://doi.org/10.2131/jts.37.899>.
  34. Keck C, Bramkamp G, Behre HM, Müller C, Jockenhövel F, Nieschlag E. Lack of correlation between cadmium in seminal plasma and fertility status of nonexposed individuals and two cadmium-exposed patients. *Reproductive Toxicol.* 1995;9(1):35-40. [https://doi.org/10.1016/0890-6238\(94\)00053-y](https://doi.org/10.1016/0890-6238(94)00053-y).
  35. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, et al. Cadmium, Lead, and Other Metals in Relation to Semen Quality: Human Evidence for Molybdenum as a Male Reproductive Toxicant. *Environm Health Perspectives.* 2008;116(11):1473-9. <https://doi.org/10.1289/ehp.11490>.
  36. Li X, Wang W, Hou Y, Li G, Yi H, Cui S, et al. Arsenic interferes with spermatogenesis involving Rictor/mTORC2-mediated blood-testis barrier disruption in mice. *Ecotoxicol Environm Safety.* 2023;257. <https://doi.org/10.1016/j.ecoenv.2023.114914>.
  37. Koyama H, Kamogashira T, Yamasoba T. Heavy Metal Exposure: Molecular Pathways, Clinical Implications, and Protective Strategies. *Antioxidants (Basel).* 2024;13(1). [PubMed ID: 38247500]. [PubMed Central ID: PMC10812460]. <https://doi.org/10.3390/antiox13010076>.
  38. El-Magd MA, Kahilo KA, Nasr NE, Kamal T, Shukry M, Saleh AA. A potential mechanism associated with lead-induced testicular toxicity in rats. *Andrologia.* 2017;49(9). [PubMed ID: 28000947]. <https://doi.org/10.1111/and.12750>.
  39. 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. [PubMed ID: 31812049]. <https://doi.org/10.1016/j.chemosphere.2019.125498>.
  40. Leite R, Peloso EF, Gadelha FR, Dolder MA. Environmentally Realistic Doses of Cadmium as a Possible Etiologic Agent for Idiopathic Pathologies. *Biol Trace Elem Res.* 2015;168(1):133-40. [PubMed ID: 25850543]. <https://doi.org/10.1007/s12011-015-0322-7>.
  41. Wang YX, Sun Y, Feng W, Wang P, Yang P, Li J, et al. Association of urinary metal levels with human semen quality: A cross-sectional study in China. *Environ Int.* 2016;91:51-9. [PubMed ID: 26908166]. <https://doi.org/10.1016/j.envint.2016.02.019>.
  42. Huang Q, Luo L, Alamdar A, Zhang J, Liu L, Tian M, et al. Integrated proteomics and metabolomics analysis of rat testis: Mechanism of arsenic-induced male reproductive toxicity. *Sci Rep.* 2016;6:32518. [PubMed ID: 27585557]. [PubMed Central ID: PMC5009432]. <https://doi.org/10.1038/srep32518>.