Animal Reproduction Science 208 (2019) 106115

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

# Effect of heavy metals arsenic, cadmium, and lead on the semen variables of dromedary camels *(Camelus dromedarius)*

A.M.A. Meligy[a,b], M.M. Waheed[c,d,*], S.M. El-Bahr[e,f]

[a] Central Diagnostic Lab., College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Al-Hufof P.O. 400, Saudi Arabia
[b] Physiology Department, Plant Protection Research Institute (PPRI), Agricultural Research Centre (ARC), Giza, Egypt
[c] Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Al-Hufof P.O. 400, Saudi Arabia
[d] Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12515, Egypt
[e] Department of Physiology, Biochemistry and Pharmacology (Biochemistry), College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Al-Hufof P.O. 400, Saudi Arabia
[f] Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt

ARTICLE INFO

*Keywords:*
Camel
Seminal plasma
Serum
Heavy metals

ABSTRACT

In this study, there was investigation of the effect of heavy metals on the fertility of dromedary camels. Fourteen camels at the Camel Research Center, King Faisal University, and 41 infertile dromedaries admitted to the Veterinary Teaching Hospital were used for semen evaluation during the breeding season. Seminal plasma and blood serum were collected from all males until analysis. Concentrations of three heavy metals [arsenic (As), cadmium (Cd), and lead (Pb)] were determined in the seminal plasma and serum using an atomic absorption spectrophotometer. The results indicate there are differences ($P < 0.05 - P < 0.01$) in pH, sperm motility, sperm concentration, and sperm abnormalities between the fertile and infertile male camels. In seminal plasma, there were marked differences ($P < 0.01 - P < 0.0001$) between the control and infertile male camels in As, Cd, and Pb concentrations. In serum, there were differences ($P < 0.01 - P < 0.001$) between the fertile and infertile camels in serum As, Cd, and Pb concentrations. There was a positive correlation ($P < 0.05$; $r = 0.77$ and $r = 0.94$, respectively) between serum and seminal plasma concentrations of both As and Cd in the infertile dromedaries. In the control group, there was a positive correlation ($P < 0.05$; $r = 0.70$) between seminal plasma concentrations of Cd and percent sperm abnormalities. In conclusion, relatively greater seminal plasma and serum concentrations of As, Cd, and Pb are associated with lesser values for semen quality variables and infertility in dromedary camels.

## 1. Introduction

Camels are reported to have lesser reproductive efficiency compared to other domestic species (Kaufmann, 2005; Skidmore, 2005). Camel semen evaluation is an important issue to determine quality and fertilizing capacity (Skidmore et al., 2013; El-Bahrawy et al., 2015). Heavy metals such as arsenic (As), cadmium (Cd), and Lead (Pb) are reproductive toxicants widely distributed in the environment that may contribute to reproductive and health disorders in humans (CDC, 2005; Juan, 2012; Amadori and Macera, 2014; Gao et al., 2014; Ab Razak et al., 2015; Lei et al., 2015). The harmful effects of heavy metals are due to interference with

* Corresponding author at: Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Al-Hufof P.O. 400, Saudi Arabia.
*E-mail address:* mmwaheed@kfu.edu.sa (M.M. Waheed).

https://doi.org/10.1016/j.anireprosci.2019.106115
Received 16 January 2019; Received in revised form 30 May 2019; Accepted 27 June 2019
Available online 28 June 2019
0378-4320/ 

normal body metabolic processes (Okunola et al., 2011). Toxic metals can interfere with the metabolism of essential metals and reduce the concentration in the body or decrease bioavailability (Telišman, 1995; Pizent et al., 2003; Lazarus, 2010; Matović et al., 2011). The competition between As, Cd, and Pb with other metals in regulating metabolic processes can alter enzyme activity, affect the structure and function of cell membranes, induce oxidative stress, and inhibit DNA and RNA synthesis and repair (Pizent et al., 2012), and this may have serious consequences on cell growth, development, and differentiation (Pizent et al., 2012). As is a toxic metalloid that effects male animal fertility (Kim and Kim, 2015). Harmful concentrations of As are associated with reproductive dysfunction including reduction of the weight of the testes and accessory sex organs (Sarkar et al., 2003; Kim and Kim, 2015), and reductions in epididymal sperm counts, testosterone, and gonadotropins. There can be actions of Cd that directly damage the testes (Kar and Das, 1960). The action of Cd is spermatogenic-stage specific, and large doses of Cd cause rapid testicular degeneration, edema, hemorrhage, ischemia, necrosis, and severe damage to the spermatogenic epithelium in men and animals (Kar and Das, 1960; Boscolo et al., 1985; Jurasović and Telišman, 1993; Waalkes et al., 1999). The actions of Pb lead to a decrease in epithelial thickness diameter in the seminiferous tubules of adult rats (Al-Omar et al., 2000; Corpas et al., 2002), and reduction of epididymal sperm count (Dorostghoal et al., 2011), and testicular atrophy (Chowdhury et al., 1986) in rodents. In men, where there are greater than basal systemic concentrations of Pb for long time periods semen quality is markedly compromised (Jurasović and Telišman, 1993). Even when there are relatively small amounts of systemic Pb and Cd there are marked decreases in the quality of semen (Alloway, 1990; Telišman et al., 2000). The effects of Pb on male reproduction probably occurs by altering the reproductive hormonal control of spermatogenesis, rather than by a direct harmful effect on the seminiferous tubules (Vigeh et al., 2011). Dromedary camels are environmentally important animals. Camels have a unique adaptation mechanisms that allow for survival in dry and arid climates. These mechanisms include water concervation capacity, body temperature regulation, selective brain cooling, unique features of blood content regulation, nitrogen metabolism and gastric digesion (Gebreyohanes and Assen, 2017). Dromedaries similar to other domestic animals and humans, however, are affected by heavy metals.

The aim of the present study was to determine concentrations of the heavy metals, As, Cd, and Pb in seminal plasma and serum of fertile and infertile dromedary camels, and to ascertain effects of these metals on values for semen variables and fertility.

## 2. Materials and methods

This study was approved by the Camel Research Center Committee, King Faisal University, Saudi Arabia. Approval # 321/37/2017.

### 2.1. Camel semen and blood collection

Fourteen fertile (control, served as sires for breeding females) dromedary camels with an average age of 8 years (range, 6–13 years) from the Camel Research Center, King Faisal University, and 41 infertile [lack of conception after matings with fertile females during a period or for more than on breeding season] dromedaries admitted to the Veterinary Teaching Hospital were used for semen collection and evaluation during the breeding season. One ejaculate was collected from each fertile and infertile camel during the breeding season using the electro-ejaculation method as described earlier (Tingari et al., 1986). The ejaculate was assessed for routine semen quality variables such as ejaculate volume (mL), sperm concentration ($\times 10^6$/mL) and percent progressively motile sperm by the same trained individual using Sperm Vision® 3.5 (Minitube of America, Inc). An aliquot (3 mL) of fresh, raw semen was centrifuged at $4000 \times g$ for 10 min at room temperature, and seminal plasma was separated and frozen at −80 °C. Blood samples (10 mL) were collected from all camels, centrifuged at $4000 \times g$ for 10 min, and the supernatant serum was separated and stored at −80 °C until analyses were performed. All glassware and bottles used for the isolation of seminal plasma and serum and for analysis were previously soaked in diluted nitric acid (10%) and rinsed thoroughly with deionized water to exclude the possibility of contamination with heavy metals.

### 2.2. Heavy metals analysis in seminal plasma and serum

Concentrations of three heavy metals were determined in both camel seminal plasma and serum (110 replicates) using an atomic absorption spectrophotometer. The heavy metals were arsenic (As), cadmium (Cd), and lead (Pb). The estimation of heavy metal concentrations in seminal plasma and serum samples was performed in two phases, digestion of samples and analyses. Calibration curves were obtained from stock solutions of As, Cd, and Pb. As Standard solution (1000 mg/L) was purchased and was an aliquot from commercial stock solutions (Merck, Darmstadt, Germany). The working standard was prepared by suitable serial dilutions of the stock (1000 mg/L) solution of all standards in deionized water and in room standard reference materials. The calibration graph using the concentration system for As, Cd, and Pb was linear with a correlation coefficient of 0.997, 0.998, and 0.999, respectively. In the case of some analyses, where there were not significant differences between the determined reference ranges and the ranges reported in the literature, there was use of the clinical practice recommended by the World Health Organization (WHO) criteria.

#### 2.2.1. Atomic absorption spectrophotometer analysis

The AA-7000 Shimadzu (Koyoto, Japan) atomic absorption spectrophotometer coupled with a FAAS Flame Atomic Absorption, GFA-7000 graphite furnace atomizer, HVG-1 Hydride Vapor Generator, MVU-1A Mercury Vaporizer, and ASC-7000 auto sampler from Shimadzu (Koyoto, Japan) was used for the heavy metals analysis. A high-density graphite tube was used for atomization. A normal single hollow cathode lamp was used for irradiation. Values for absorbance are reported in Table 1 and were obtained by

**Table 1**
Programs of heating method for arsenic (As), cadmium (Cd) and lead (Pb) in graphite furnace atomic absorption spectrophotometer (GFAAS).

| Steps | Temperature (°C) | | | Ramp (s) | Hold (s) | Argon flow rate (ml $min^{-1}$) |
|---|---|---|---|---|---|---|
| | As | Cd | Pb | | | |
| Drying 1 | 150 | 150 | 150 | 5 | 20 | 250 |
| Drying 2 | 200 | 200 | 200 | 5 | 15 | 250 |
| Pyrolysis | 1200 | 500 | 800 | 10 | 20 | 250 |
| Atomization | 2000 | 1800 | 2000 | 0 | 5 | 0 |
| Clean-out | 2000 | 2200 | 2200 | 1 | 3 | 250 |

adjusting the hollow cathode lamps at the operation conditions as previously described by Meligy (2018).

*2.2.2. Sample digestion*

The seminal plasma and serum samples were thawed, and 1 mL was taken from each sample. The microwave method was applied for the digestion procedure of samples (Usero et al., 2005) by using the Microwave digestion system Model MARS Xpress 907511 (CEM Cooperation, Mathews, North Carolina, USA) according to USEPA method 3051. The thawed seminal plasma and serum samples were placed in [polytetrafluoroethylene (PTFE)] digestion vessels with 6 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%). The samples in the vessels were then digested using an optimized microwave method. In the first stage, the temperature was increased to 120 °C within 5 min. Then, the temperature was increased to 180 °C within 10 min followed by a period of 10 min when there was no further temperature adjustments. The samples were maintained at 180 °C for 15 min, and cooled for 5 min. The sample was transferred into a measuring flask and diluted with Milli-Q water (Millipore, Bedford, MA) to a total volume of 50 mL. The cooled mixture was then filtered using Whatman filter paper1. After filtration, the filtrates of digested samples were analyzed by atomic absorption, using the method described by Meligy (2018). The recovery of elements in the digested samples varied between 89.6% and 94.2% (Table 2), which were considered reliable analyses (El-Bahr and Abdelghany, 2015; Meligy, 2018).

*2.3. Statistical analysis*

Data are presented as mean ± standard error of the mean (SEM) for camel fertility, serum, and seminal plasma heavy metals. The normality (Shapiro and Wilk, 1965) and homogeneity of variances (Levene, 1960) were assessed and confirmed prior to conducting the *t*-test. Variances were compared by the Student *t*-test (*t*) and correlation coefficient (*r*) using the SPSS program, version 24.0 (SPSS, 2016).

## 3. Results

There was a difference ($P < 0.05 - P < 0.01$) in values for some semen variables between the control and infertile male camels, including pH, sperm motility %, sperm concentration, and percent sperm abnormalities (Table 3). In the seminal plasma, there were differences ($P < 0.01 - P < 0.0001$) between the control and infertile male camels in As, Cd, and Pb concentrations (Table 3). There were differences ($P < 0.01 - P < 0.001$) between the fertile and infertile camels in serum As, Cd, and Pb concentrations (Table 4). There were positive correlation coefficients ($P < 0.05$; $r = 0.76$ and $r = 0.94$, respectively) between serum and seminal plasma concentrations of both As and Cd in the infertile dromedaries (Table 5). In the control group, there was a positive correlation coefficient ($P < 0.05$; $r = 0.70$) between seminal plasma concentrations of Cd and percent sperm abnormalities.

## 4. Discussion

Environmental contamination with heavy metals commonly occurs in Saudi Arabia. Oil extraction, as well as operations occurring in the petroleum, domestic, agricultural, and medical industries have led to the wide distribution of heavy metals in the environment. The outcomes for the manner operations in these industries occur raise concerns about the potential effects of heavy metals on animal health and the environment (Tchounwou et al., 2012). Because of the extent of the harmful effects of the compounds, arsenic, cadmium, and lead rank among the priority metals that induce multiple organ damage, even when there are relatively lesser amounts in the environment (Tchounwou et al., 2012).

In the present study, there were differences in semen pH, sperm motility %, sperm concentration, and percent sperm

**Table 2**
Recovery of elements from digested samples.

| Heavy metals | Concentrations of Heavy metal added (μg/kg) | Concentrations of heavy metal recovered (μg/kg) | Recovery (%) |
|---|---|---|---|
| Arsenic (As) | 5 | 4.48 | 89.6 |
| Cadmium (Cd) | 5 | 4.65 | 93 |
| Lead (Pb) | 5 | 4.71 | 94.2 |

**Table 3**
Results of semen analyses and seminal plasma heavy metals in the control [normal males used for mating females] and infertile [lack of conception after matings with fertile females during a 7 month period or for more than one breeding season] dromedary camels (mean ± SEM).

| Variables | Control ($n$ = 14) | Infertile ($n$ = 41) | $P$ - value |
|---|---|---|---|
| Age | 8.67 ± 1.29 | 8.39 ± 0.48 | — |
| Volume (mL) | 7.71 ± 0.45 | 7.67 ± 0.68 | — |
| Viscosity (0-5) | 4.57 ± 0.18 | 3.73 ± 0.25 | — |
| pH | $7.77^{a}$ ± 0.10 | $8.07^{b}$ ± 0.07 | $P < 0.01$ |
| Motility (%) | $66.11^{a}$ ± 1.46 | $5.93^{b}$ ± 1.41 | $P < 0.001$ |
| Sperm concentration (x$10^{6}$/mL) | $268.57^{a}$ ± 18.30 | $54.96^{b}$ ± 12.71 | $P < 0.05$ |
| Sperm abnormalities (%) | $18.79^{a}$ ± 1.68 | $59.32^{b}$ ± 7.21 | $P < 0.01$ |
| Arsenic (As) ug/L | $156.07^{a}$ ± 10.88 | $245.85^{b}$ ± 35.77 | $P < 0.01$ |
| Cadmium (Cd) ug/L | $20.41^{a}$ ± 1.78 | $94.03^{b}$ ± 20.55 | $P < 0.01$ |
| Lead (Pb) ug/L | $224.40^{a}$ ± 13.87 | $362.36^{b}$ ± 6.37 | $P < 0.0001$ |

Means with dissimilar superscripts in the same row are different P < 0.05 to $P < 0.0001$.
$n$ = number of ejaculates.

**Table 4**
Heavy metals in serum of control [normal males used for mating females] and infertile [lack of conception after matings with fertile females during a 7 month period or for more than one breeding season] dromedary camels (mean ± SEM).

| Serum toxic elements | Control ($n$ = 14) | Infertile ($n$ = 41) | $P$ - value |
|---|---|---|---|
| Arsenic (As) ug/L | $100.22^{a}$ ± 4.88 | $173.71^{b}$ ± 4.62 | $P < 0.001$ |
| Cadmium (Cd) ug/L | $22.13^{a}$ ± 3.57 | $74.70^{b}$ ± 12.38 | $P < 0.01$ |
| Lead (Pb) ug/L | $212.68^{a}$ ± 12.63 | $353.29^{b}$ ± 10.23 | $P < 0.01$ |

Means with dissimilar superscripts in the same row are different P < 0.01 and $P < 0.001$.
$n$ = number of blood samples.

**Table 5**
Correlations Coefficients (r) of heavy metals between serum and seminal plasma of control [normal males used for mating females] and infertile [lack of conception after matings with fertile females during a 7 month period or for more than one breeding season] dromedary camels.

| Compounds | Correlations Coefficients ($r$) of heavy metals between serum and seminal plasma | |
|---|---|---|
| | Control ($n$ = 14) | Infertile ($n$ = 41) |
| Arsenic (As) ug/L | −0.06 | 0.76* |
| Cadmium (Cd) ug/L | −0.06 | 0.94* |
| Lead (Pb) ug/L | 0.00 | 0.09 |

Values with astral superscript are different P < 0.05; $n$ = number of blood and semen samples.

abnormalities between the control and infertile male camels. Similarly, Ali et al. (2014) and Waheed et al. (2014), (2018) reported similar results in dromedary camels. The causes of abnormal spermatogenesis include gene defects, inflammation, testicular hypoplasia, testicular degeneration, nutritional deficiencies, and heavy metal toxicity (Blom, 1977; Johnson, 1997; Kennedy et al., 2002; Tchounwou et al., 2012; Ali et al., 2014).

In the present study, there were differences in As, Cd, and Pb concentrations in both seminal plasma and serum between the control and infertile male camels. In animals, inorganic As induces spermatotoxicity (Waalkes et al., 2003; Pant et al., 2004), alters spermatogenesis, reduces concentrations of testosterone and gonadotropins, and inhibits testicular steroidogenesis (Sarkar et al., 2003; Kim and Kim, 2015). In humans, As reduces the activities of approximately 200 enzymes, especially those related to cellular energy pathways and DNA synthesis and repair (Ratnaike, 2003). Furthermore, blood As concentrations of greater than 5.8 µg/ L were associated with lesser sperm motility in men (Meeker et al., 2008). This blood concentration value of As, however, is markedly less than that detected in the present study (100.221 µg/L) in serum of control fertile dromedaries. When As concentrations exceed the minimal for damaging biological tissues, there is a significant risk factor for lesser than optimal semen volume (Pizent et al., 2012).

The effect of Cd on testes is primarily on the vascular system and appears to be manifested mainly in the Sertoli cells, germ cells and Leydig cells (Boscolo et al., 1985; Waissmann, 2003). When Cd concentrations exceed the minimal for damaging biological tissues, there is damaging effects on mitochondrial enzymes (Jequier, 2000) and less androgen production (Waalkes et al., 1999). In men, results of some studies indicate there is no significant reproductive effect of occupational exposure to Cd (Mason, 1990; McGregor and Mason, 1990). Results of several studies, however, indicate small amount Cd occupational exposure affects semen quality and/or reproductive hormone concentrations (Pizent et al., 2012). Blood Cd concentrations of less than 1.5 µg/L have been associated with decreased sperm concentration and number of sperm per ejaculate (Xu et al., 1993), decreased semen volume (Chia et al., 1992; Xu et al., 1993), and increased percent of both sperm midpiece defects and immature sperm (Chia et al., 1992). In the

present study, the serum concentration of Cd (22.125 μg/L) in control camels was greater than the value recorded in men with poor semen quality. With acute Cd doses, there is a decrease in sperm counts in rats (Laskey et al., 1984).

In men, blood Pb concentrations of greater than 400 μg/L induce decreased sperm concentration, decreased sperm motility, and increased abnormal sperm morphology, particularly of the sperm head (Cullen et al., 1984; Assenato et al., 1987; Gennart et al., 1992; Hu et al., 1992; Lerda, 1992; Alexander et al., 1996; Robins et al., 1997; Alexander et al., 1998; Telišman et al., 2000; Eibensteiner et al., 2005). Similarly, in the present study, when there were serum concentrations of Pb of 353.288 μg/L values for semen quality variables were less in the infertile dromedary camels. Nevertheless, there is an association between lesser semen quality and Pb in seminal plasma, but not with blood Pb concentrations (Hernandez-Ochoa et al., 2005). In other studies, there has not been found to be any association between seminal plasma Pb concentrations and lesser sperm concentrations in semen (Xu et al., 2003; Telišman et al., 2007). The effect of Pb on the reproductive variables is at least partly mediated through the interference with zinc metabolism (Eibensteiner et al., 2005). Seminal plasma Pb concentrations are inversely correlated with the fertilizing capacity in the process of *in-vitro* fertilization (Benoff et al., 2003). In mice, addition of Pb in the drinking water resulted in a decrease in sperm concentrations by decreasing the number of layers of germ cells in the seminiferous tubules and disrupting germ cell alignment (Wang et al., 2013).

## 5. Conclusions

In conclusion, relatively greater seminal plasma and serum concentrations of As, Cd and Pb are associated with lesser values for semen quality variables and infertility in dromedary camels. Although, the equipment used for quantifying the heavy metal concentrations is expensive, it is recommeneded that there be closer assessment of heavy metals in serum and seminal plasma of dromedaries when reproductive status of the animals is compromised.

## Acknowledgments

The authors thank the Deanship of Scientific Research, King Faisal University, Al-Ahsa, Saudi Arabia for the financial support of this study (Research group #1811024). The authors would like to appreciate the Camel Research Center, King Faisal University, Saudi Arabia, Al-Ahsa 31982 Al-hufof P.O. 400 for his support to fulfill this article.

## References

Ab Razak, N.H., Praveena, S.M., Aris, A.Z., Hashim, Z., 2015. Drinking water studies: a review on heavy metal, application of biomarker and health risk assessment (a special focus in Malaysia). J. Epidemiol. Glob. Health 5, 297–310.
Alexander, B.H., Checkoway, H., Faustman, E.M., van Netten, C., Muller, C.H., Ewers, T.G., 1998. Contrasting associations of blood and semen lead concentrations with semen quality among lead smelter workers. Am. J. Ind. Med. 34, 464–469.
Alexander, B.H., Checkoway, H., Van Netten, C., Kaufman, J.D., Vaughan, T.L., Mueller, B.A., Faustman, E.M., 1996. Paternal occupational lead exposure and pregnancy outcome. Int. J. Occup. Environ. Health 2, 280–285.
Ali, A., Derar, R., Al-Sobayil, F., Mehana, S., Al-Hawas, A., 2014. Impotentia generandi in male dromedary camels: Clinical findings, semen characteristics, and testicular histopathology. Theriogenology 82, 890–896.
Alloway, B.J., 1990. Heavy Metals in Soils. John Wiley Inc., New York.
Al-Omar, M.A., Abbas, A.K., Al-Obaidy, S.A., 2000. Combined effect of exposure to lead and chlordane on the testicular tissues of swiss mice. Toxicol. Lett. 10, 1–8.
Amadori, M., Macera, P., 2014. Assessment of the risk of pollution from Arsenic on human health as a function of its speciation in intestinal fluids. Ital. J. Geosci. 133, 161–168.
Assenato, G., Paci, C., Baser, M.E., Molinini, R., Candela, R.G., Altamura, B.M., Giorgino, R., 1987. Sperm count suppression without endocrine dysfunction in lead-exposed men. Arch. Environ. Health 42, 124–127.
Benoff, S., Centola, G.M., Millan, C., Napolitano, B., Marmar, J.L., Hurley, I.R., 2003. Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. Hum. Reprod. 18, 374–383.
Blom, E.A., 1977. Decapitated sperm defect in two sterile Hereford bulls. Nord. Vet. 29, 119–123.
Boscolo, P., Logroscino, G.S., Ranelletti, F.O., Gioia, A., Carignani, M., 1985. Effects of long-term cadmium exposure on the testis of rabbits: ultrastructural study. Toxicol. Lett. 24, 145–149.
CDC, 2005. Third National Report on Human Exposure to Environmental Chemicals. (Accessed 24 December 2018). http://www.jhsph.edu/research/centers-and-institutes/center-for-excellence-in-environmental-health-tracking/Third_Report.pdf.
Chia, S.E., Ong, C.N., Lee, S.T., Tsakok, F.H., 1992. Blood concentrations of lead, cadmium, mercury, zinc and copper and human semen parameters. Arch. Androl. 29, 177–183.
Chowdhury, A.R., Rao, R.V., Gautam, A.K., 1986. Histochemical changes in the testes of lead induced experimental rats. Follia Histochem. Et Cytobiol. 24, 233–238.
Corpas, L., Castillo, M., Marquine, D., Benito, M.J., 2002. Lead intoxication in gestational and lactation period alters the development of male reproductive organs. Ecotoxicol. Environ. Saf. 53, 259–266.
Cullen, M.R., Kayne, R.D., Robins, J.M., 1984. Endocrine and reproductive dysfunction in men associated with occupational inorganic lead intoxication. Arch. Environ. Health 39, 431–440.
Dorostghoal, M., Dezfoolian, A., Sorooshnia, F., 2011. Effects of Maternal Lead Acetate Exposure During Lactation on Postnatal Development of testis in offspring wister rats. Iran. J. Basic Med. Sci. 14, 122–131.
Eibensteiner, L., Del Carpio Sanz, A., Frumkin, H., Gonzales, C., Gonzales, G.F., 2005. Lead exposure and semen quality among traffic police in Arequipa. Peru. Int. J. Occup. Environ. Health 11, 161–166.
El-Bahr, S.M., Abdelghany, A., 2015. Heavy metal and trace element contents in edible muscle of three commercial fish species, and assessment of possible risks associated with their human consumption in Saudi Arabia. J. Adv. Vet. Anim. Res. 2, 271–278.
El-Bahrawy, K.A., Khalifa, M.A., Rateb, S.A., 2015. Recent advances in dromedary camel reproduction: an Egyptian field experience. Emir. J. Food Agric. 27 (4), 350–354.
Gao, B., Li, Q., Zhou, H.-D., Gao, J.-J., Zou, X.-W., Huang, Y., 2014. Application of ICP-MS in the health risk assessment of heavy metals for drinking water sources in reservoirs. Spectrosc. Spectr. Anal. 34, 1398–1402.
Gebreyohanes, M.G., Assen, A.M., 2017. Adaptation Mechanisms of Camels (Camelus dromedarius) for Desert Environment: A Review. J. Vet. Sci. Tech. 8, 1–5.
Gennart, J.P., Bernard, A., Lauwerys, R., 1992. Assessment of thyroid, testes, kidney and autonomic nervous system function in lead exposed workers. Int. Arch. Occup.

Environ. Health 64, 49–57.
Hernandez-Ochoa, I., Garcia-Vargas, G., Lopez-Carrillo, L., Rubio-Andrade, M., Moran-Martinez, J., Cebrian, M.E., Quintanilla-Vega, B., 2005. Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. Reprod. Toxicol. 20, 221–228.
Hu, W., Wu, S.H., Wang, L.L., Wang, G.I., Fan, H., Liu, Z.M., 1992. A toxicological and epidemiological study on reproductive functions of male workers exposed to lead. J. Hyg. Epidemiol. Microbiol. Immunol. 36, 25–30.
Jequier, A.M., 2000. Male Infertility - a Guide for the Clinician. Blackwell Science Ltd, London, pp. 67–76.
Johnson, W.H., 1997. The significance to bull fertility of morphologically abnormal sperm. Vet. Clin. North Am. Food Anim. Pract. 13, 255–270.
Juan, X.S., 2012. Pollution assessment and control countermeasures of surface drinking Water source in Laoshan District of Qingdao. Master's Thesis. Ocean University of China; Qingdao, China: Jun 1, 2012.
Jurasović, J., Telišman, S., 1993. Determination of lead and cadmium in human seminal fluid. Int. J. Anal. Mass Spectrom. 8, 419–425.
Kar, A.B., Das, R.P., 1960. Testicular changes in rats after treatment with cadmium chloride. Acta Boil. Med. Ger. 5, 153–173.
Kaufmann, B.A., 2005. Reproductive performance of camels (Camelus dromedarius) under pastoral management and its influence on herd development. Livest. Prod. Sci. 92, 17–29.
Kennedy, S.P., Spitzer, J.C., Hopkins, F.M., Higdon, H.L., Bridges Jr., W.C., 2002. Breeding soundness evaluations of 3648 yearling beef bulls using the 1993 Society for Theriogenology guidelines. Theriogenology 58, 947–961.
Kim, Y.-J., Kim, J.-M., 2015. Arsenic toxicity in male reproduction and development. Dev. Reprod. 19 (4), 167–180.
Laskey, J.W., Rehnberg, G.L., Laws, S.C., Hein, J.F., 1984. Reproductive effects of low acute doses of cadmium chloride in adult male rats. Toxicol. Appl. Pharmacol. 73, 250–255.
Lazarus, M., 2010. Cadmium and selenium interaction in mammals, in Croatian. Arh. Hig. Rada Toksikol. 61, 357–369.
Lei, H.-L., Wei, H.-J., Ho, H.-Y., Liao, K.-W., Chien, L.-C., 2015. Relationship between risk factors for infertility in women and lead, cadmium, and arsenic blood levels: a cross-sectional study from Taiwan. BMC Public Health 15, 1220–1230.
Lerda, D., 1992. Study of sperm characteristics in persons occupationally exposed to lead. Am. J. Ind. Med. 22, 567–571.
Levene, H., 1960. Robust tests for equality of variances. In: Olkin, I., Ghurye, S.G., Hoeffding, W., Madow, W.G., Mann, H.B. (Eds.), Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling. Stanford University Press, pp. 278–292.
Mason, H.J., 1990. Occupational cadmium exposure and testicular endocrine function. Hum. Exp. Toxicol. 9, 91–94.
Matović, V., Buha, A., Bulat, Z., Dukić-Ćosić, D., 2011. Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium. Arh. Hig. Rada Toksikol. 62, 65–76.
McGregor, A.J., Mason, H.J., 1990. Chronic occupational lead exposure and testicular endocrine function. Hum. Exp. Toxicol. 9, 371–376.
Meeker, J.D., Rossano, M.G., Protas, B., Diamond, M.P., Puscheck, E., Daly, D., Paneth, N., Wirth, J.J., 2008. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. Environ. Health Perspect. 116, 1473–1479.
Meligy, A.M.A., 2018. Comparative study of element contents in seven isolates of entomopathogenic nematodes. Egypt. J. Biol. Pest. Co. 28, 1–7.
Okunola, O.J., Alhassan, Y., Yapbella, G.G., Uzairu, A., Tsafe, A.I., Abechi, E.S., Apene, E., 2011. Risk assessment of using mobile phone recharge cards in Nigeria. J. Environ. Chemistry Ecotox. 3 (4), 80–85.
Pant, N., Murthy, R.C., Srivastava, S.P., 2004. Male reproductive toxicity of sodium arsenite in mice. Hum. Exp. Toxicol. 23, 399–403.
Pizent, A., Jurasović, J., Telišman, S., 2003. Serum calcium, zinc, and copper in relation to biomarkers of lead and cadmium in men. J. Trace Elem. Med. Biol. 17, 199–205.
Pizent, A., Tariba, B., Živković, T., 2012. Reproductive toxicity of metals in men. Arh. Hig. Rada Toksikol. 63 (suppl. (1)), 35–46.
Ratnaike, R.N., 2003. Acute and chronic arsenic toxicity. Postgrad. Med. J. 79, 391–396.
Robins, T.G., Bornman, M.S., Ehrlich, R.I., Cantrell, A.C., Pienaar, E., Vallabh, J., Miller, S., 1997. Semen quality and fertility of men employed in a South African lead acid battery plant. Am. J. Ind. Med. 32, 369–376.
Sarkar, M., Chaudhuri, G.R., Chattopadhyay, A., Biswas, N.M., 2003. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J. Androl. 5, 27–31.
Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52 (3-4), 591–611.
Skidmore, J.A., 2005. Reproduction in dromedary camels: an update. Anim. Reprod. 2, 161–171.
Skidmore, J.A., Morton, K.M., Billah, M., 2013. Artificial insemination in dromedary camels. Anim. Reprod. Sci. 136 (3), 178–186.
SPSS, 2016. Statistical Package for Social Sciences. SPSS Inc., Chicago, IL, USA Copyright © for Windows; version 24.0.
Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. EXS 101, 133–164.
Telišman, S., 1995. Interactions of essential and/or toxic metals and metalloid regarding interindividual differences in susceptibility to various toxicants and chronic diseases in men. Arh. Hig. Rada Toksikol. 46, 459–476.
Telišman, S., Colak, B., Pizent, A., Jurasovic, J., Cvitkovic, P., 2007. Reproductive toxicity of low-level lead exposure in men. Environ. Res. 105, 256–266.
Telišman, S., Cvitković, P., Jurasović, J., Pizent, A., Gavella, M., Rocic, B., 2000. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ. Health Perspect. 108, 45–53.
Tingari, M.D., Manna, M.M., Rahim, A.T., Ahmed, A.K., Hamad, M.H., 1986. Studies on camel semen. I. Electroejaculation and some aspects of semen characteristics. Anim. Reprod. Sci. 12, 213–222.
Usero, J., Morillo, J., Garcia, I., 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. Chemosphere 59, 1175–1181.
Vigeh, M., Smith, D.R., Hsu, P.C., 2011. How does lead induce male infertility? Iran. J. Reprod. Med. 9 (1), 1–8.
Waalkes, A.M., Anver, M., Diwan, B.A., 1999. Carcinogentic effects of cadmium in the noble (NB/CR) rat: induction of pituitary, testicular, and injection site tumors and intraepithelial proliferative lesions of the dorsolateral prostate. Toxicol. Sci. 52, 154–161.
Waalkes, M.P., Ward, J.M., Liu, J., Diwan, B.A., 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol. Appl. Pharmacol. 186, 7–17.
Waheed, M.M., Ghoneim, I.M., Hassieb, M.M., Alsumait, A.A., 2014. Evaluation of the Breeding Soundness of Male Camels (Camelus dromedarius) via Clinical Examination, Semen Analysis, Ultrasonography and Testicular Biopsy: A Summary of 80 Clinical Cases. Reprod. Domest. Anim. 49, 790–796.
Waheed, M.M., Meligy, A.M.A., Dhalam, S.A., 2018. The relationship between seminal plasma trace elements and fertility of dromedary camels (Camelus dromedarius). Reprod. Domest. Anim. 53, 1367–1374.
Waissmann, W., 2003. Endocrinopatologia associada ao trabalho. Mendes R, Organizador. Patologia Do Trabalho. Editora. Atheneu., Sao Paolo, pp. 1093–1138.
Wang, X., Wang, M., Dong, W., Li, Y., Zheng, X., Piao, F., Li, S., 2013. Subchronic exposure to lead acetate inhibits spermatogenesis and down regulates the expression of Ddx3y in testis of mice. Reprod. Toxicol. 42, 242–250.
Xu, B., Chia, S.E., Tsakok, M., Ong, C.N., 1993. Trace elements in blood and seminal plasma and their relationship to sperm quality. Reprod. Toxicol. 7, 613–618.
Xu, D.-X., Shen, H.-M., Zhu, Q.-X., Chua, L., Wang, Q.-N., Chia, S.-E., Ong, C.-N., 2003. The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma. Mutat. Res. 534, 155–163.