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REPRODUCTIVE PHYSIOLOGY AND DISEASE

Associations of semen quality with non-essential heavy metals in blood and seminal fluid: data from the Environment and Male Infertility (EMI) study in Lebanon

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Abstract

Background Human exposure to environmental pollutants is widespread. It was suggested that exposure to non-essential heavy metals may adversely affect semen development in men.

Purpose To evaluate associations between non-essential heavy metals in blood and seminal fluid and semen quality parameters in men.

Methods Male partners of heterosexual couples were included. The following elements were measured in blood and seminal fluid: lead (Pb), cadmium (Cd), arsenic (As), barium (Ba), mercury (Hg), and uranium (U) using ion-coupled plasma-mass spectrometry.

Setting The fertility clinic at the American University of Beirut Medical Center.

Main outcome measures Semen quality parameters (volume, concentration, total count, progressive motility, viability, and normal morphology).

Results We found that participants with low-quality semen had significantly higher Cd and Ba concentrations in the seminal fluid than participants with normal-quality semen. We also observed significant associations between low sperm viability and higher blood Cd and Ba, as well as higher seminal Pb, Cd, Ba, and U. Furthermore, U concentrations in the seminal fluid were associated with increased odds ratios for below-reference progressive sperm motility and normal morphology.

Conclusions Environmental exposures to Pb, Cd, Ba, and U appear to adversely influence sperm development in men. In non-occupationally exposed men, measurements of heavy metals in the seminal fluid may be more predictive of below-reference sperm quality parameters than in blood.

Keywords Non-essential heavy metals · Semen quality · Male infertility · Environmental pollution

Introduction

A direct consequence of the global industrial revolution has been the exponential inevitable increase in environmental pollutants, more significantly in countries with poorer regulations [1, 2]. Some of these pollutants are believed to carry substantial toxic implications on various aspects of life on earth [3–6]. Metals, such as lead (Pb), cadmium (Cd), arsenic (As), barium (Ba), mercury (Hg), and uranium (U), are non-essential xenobiotics that have been increasingly detected in humans [2, 7, 8]. Because of their widespread cumulative burden, there is growing concern for their adverse effects on reproductive health even at low-level exposures.

Joint first authorship with equal contribution (Carol Sukhn and Johnny Awwad).

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In recent years, studies reported that human sperm quality has been on the decline in a number of countries around the world [9–12]. A corresponding decline in fertility rates has also been observed and has been linked to environmental toxic exposures, including heavy metals [13–21]. Toxicants, in general, are believed to offset the fine tuning of biochemical reactions [22] that regulate the normal functioning of the male reproductive system. The adverse effects of high-level exposure of non-essential heavy metals on male reproduction have been clearly demonstrated in experimental animal models and in occupational human exposure studies [23–26]. Information on the effects of non-occupational exposure over male reproductive health remains, nonetheless, controversial and at best inconclusive [27]. Available studies present several shortcomings and are often limited by their retrospective design, low number of observations, and/or lack of adjustment to potential confounders [27]. Given that heavy metals have variable distributions in body compartments [28], the common practice of measuring these elements in blood may not necessarily be truly reflective of the actual exposure of the male reproductive system. Since some metals have preferential predisposition to male reproductive organs [29, 30], it is then plausible to entertain the premise that the seminal fluid could represent a more suitable body compartment to measure these elements.

Lebanon has been the scene of wars over the past 30 years, which led to several demolition and reconstruction cycles. As a consequence, dust residues containing heavy metals have likely been released in the environment, contaminating various aspects of human life. The characterization of heavy metal exposure of the Lebanese population remains widely unexplored. Only one study performed in the same center sought to determine levels of heavy metals in the blood of Lebanese men and to examine associations with male infertility [31].

In the present study, we aimed to bridge the existing information gap by (a) investigating associations between non-occupational non-essential heavy metal exposures and semen quality parameters, (b) exploring whether the seminal fluid represents a more suitable body compartment in men to measure these elements than blood, and (c) determining a baseline platform of heavy metal concentrations in the blood and seminal fluid of Lebanese men.

Materials and methods

Study population

The Environment and Male Infertility (EMI) study is a prospective cohort study of environmental determinants of male infertility, conducted between January 2003 and December 2009. Male partners of infertile heterosexual couples who attended the fertility clinic at the American University of Beirut Medical Center were recruited. All men with diabetes, endocrine disease, fertility-related genetic disorders, obstructive azoospermia, cryptorchidism, varicocele, hydrocele, orchitis, epididymitis, and history of testicular injury or surgery were excluded from consideration. All participants were 18 to 55 years of age, had a BMI of 18 to 30 kg/m^2, and have not been on any hormone therapy for the past 6 months. This study was approved by the American University of Beirut Institutional Review Board, and all eligible participants signed an informed consent form prior to enrollment. About 40% of interviewed subjects either declined participation despite eligibility (~ 12%) or were excluded because they did not meet the eligibility criteria (~ 28%).

Study design

Participants were categorized into two groups, low- and normal-quality semen groups, according to the World Health Organization (WHO) 4th edition reference value of semen quality [32]. Participants with a semen volume < 1.5 mL, sperm concentration < 15 million/mL, total count < 39 million, progressive motility < 32%, viability < 58%, and/or normal WHO morphology < 30% were assigned to the low-quality semen group A. Participants whose semen analyses expressed better results in all the above parameters were assigned to the normal-quality semen group B. A questionnaire was completed by each participant. Questions included information on socio-demographic characteristics, personal lifestyle (tobacco, alcohol, and diet), occupational exposure, medication intake, and medical history.

Specimen collection

Whole venous blood samples were collected using stainless steel needles into tubes containing ethylene diamine tetraacetic acid. Blood and semen specimens were stored at − 70 °C for subsequent metal analysis.

Following an abstinence period of 3–7 days, each participant provided a semen sample into a sterile wide-mouth metal-free polypropylene container at the Fertility Center by masturbation. Participants were asked to wear metal-free gloves while providing semen samples. All metal components in the semen collection room were carefully covered with plastic material. Semen samples were handled and analyzed by two qualified laboratory technologists according to the WHO Laboratory Manual for the Examination of Human Semen and Sperm-Mucous Interaction [33]. The semen volume (mL) was measured using a metal-free polypropylene graduated pipette. Sperm concentration (million/mL) and progressive motility (%) were determined manually using a Makler® counting chamber (Irvine Scientific, CA). Total sperm count (million) was calculated as sperm concentration × semen volume. Sperm morphology was determined by high-power magnification (× 1000) on air-dried smears stained with a Wright-

Giemsa stain based on the WHO guidelines (1999) guidelines [33]. Laboratory technicians analyzed two slides for each participant with no less than 100 sperms per slide. External quality controls were used, and discrepancies in results were reported to be insignificant. The overall laboratory CV for inter-observer determinations of sperm morphology was ~ 17%.

Metal analyses

Whole blood samples (2 mL) were digested with 70% nitric acid (HNO_3) and oxygen peroxide (H_2O_2), filtered and diluted to 10 mL. Seminal fluid aliquots (0.5 mL) were digested with 70% nitric acid (HNO_3) (Fisher Scientific, USA), filtered, and diluted to 5 mL. Digestion was performed in a closed vessel microwave system (temperature program reaching 120 °C and sustained for 10 min at 1000 W). Double distilled purified deionized water from Milli-Q system was used for dilution before performing the analyses. All glassware was washed and immersed in concentrated HNO_3 overnight and then rinsed with deionized water before use.

Metal levels (Pb, Cd, As, Hg, Ba, and U) in blood and seminal fluid were analyzed using an ion-coupled plasma-mass spectrometry (ICP-MS; Agilent 7500ce, Agilent Technologies, Germany) equipped with a cell dynamic range (CDR). For quality control, certified reference materials were used as per manufacturer, and measurements of analytes found to be within standard range. Quantification of analytes was done by the standard addition of 0, 1, 5, and 10 ppb of heavy metals. Responses were measured for the series of the standards added, and results were plotted then extrapolated to $y = 0$ to get the value of the metal. To assess instrument performance, the standard reference material was analyzed after each 20 samples. One blank tube containing deionized water was added to each batch of samples. Metal levels in all blank samples were lower than the limits of detection (LOD). The LOD for Hg was 0.5 and 1 μg/L for all other metals. For statistical purposes, no censoring of concentrations below the detection limit was implemented [34]. Machine read values were analyzed as described and were reported in micrograms per liter.

Statistical analyses

Descriptive statistics [mean, median, standard deviation (SD) and standard error of the mean (SE), proportion (%)] were used to describe the population demographic characteristics, distributions of blood/seminal fluid metals and semen quality outcome measures (Table 1). Since continuous metal variables did not follow a normal distribution, the Mann-Whitney U and Wilcoxon signed rank tests were conducted to compare the difference in central tendency between independent and paired groups (Tables 2 and 3).

Metal concentrations in blood and seminal fluid were categorized into quartiles in order to investigate dose-dependent relationships, with the lowest quartile considered as the reference group. Associations between quartiles and continuous semen quality outcome measures were assessed using multivariable linear regression for each metal separately. Data for volume, concentration, total count, progressive motility, viability, and normal morphology were modeled untransformed as they displayed normal distribution. Multivariable logistic regression models were used to assess the correlations between single metal categories and dichotomous outcomes. Participants were dichotomized on the basis of semen quality parameters as per WHO reference level values described above [31]. Participants who had values for all semen quality parameters equal or greater than the reference levels were considered as the comparison group. Tests of trends in ordinal metal categories were performed using regression models with integer values.

Potential confounders were assessed on the basis of statistical and biological considerations. Univariate regression correlations were used to explore the relevance of confounders on semen quality measures. Men age and cigarette consumption were found, respectively, to be negatively correlated with low normal morphology and low sperm viability. Alcohol consumption and period of sexual abstinence were not statistically significant confounders but were retained in the multivariable models as co-variates because of biological significance [35, 36]. Education and monthly income were not retained for the lack of statistical significance.

All statistical analyses were conducted using SPSS version 24 (IBM SPSS, USA). For all analyses, a p value < 0.05 was regarded as statistically significant for two-tailed significance tests.

Results

Characteristics of participants

According to the WHO reference values, 61 participants (53%) had at least one semen quality measure (volume, concentration, total count, progressive motility, viability, and/or normal morphology) inferior to the reference values (low-quality semen group A) (Table 1). Another 55 participants (47%) had all six measures equal to or superior to the reference values (normal-quality semen group B). No statistically significant differences in the age of participants, their

Table 1 Demographic and semen characteristics of participants in the EMI study stratified by semen quality reference value

Variables	Study group A (low-quality semen)	Study group B (normal-quality semen)
Demographic characteristics		
No. of participants [*n* (%)]	61 (53)	55 (47)
Age (years) (mean ± SE)	37.4 ± 0.8	37.6 ± 0.8
Education		
Elementary and intermediate [*n* (%)]	17 (28)	18 (33)
High school [*n* (%)]	9 (15)	10 (18)
College and university [*n* (%)]	35 (57)	27 (49)
Income		
Low (less than 1000 USD/month) [*n* (%)]	13 (22)	8 (15)
Medium (1000 to 3000 USD/month) [*n* (%)]	44 (71)	41 (74)
High (above 3000 USD/months) [*n* (%)]	4 (7)	7 (12)
Smoking history		
No [*n* (%)]	29 (47.5%)	24 (43.6%)
Yes [*n* (%)]	28 (45.9%)	26 (47.3%)
Missing [*n* (%)]	4 (6.6%)	5 (9.1%)
Cigarettes/week (mean ± SE)	38.2 ± 9.6	30.5 ± 8.2
Alcohol consumption		
No [*n* (%)]	32 (52.5%)	18 (32.7%)
Yes [*n* (%)]	25 (41.0%)	32 (58.2%)
Missing [*n* (%)]	4 (6.6%)	5 (9.1%)
Drinks/week (mean ± SE)	2.5 ± 1.0	3.1 ± 1.2
Sex frequency/week (mean ± SE)	2.7 ± 0.2	2.4 ± 0.3
Abstinence (days) (mean ± SE)	4.5 ± 0.3	4.5 ± 0.3
Semen characteristics		
Liquefaction time (s) [mean ± SE (median)]	*68.1 ± 7.4 (30)**	*50.9 ± 6.4 (30)**
Volume (mL) [mean ± SE (median)]	3.1 ± 0.2 (2.8)	3.4 ± 0.2 (3.5)
Concentration (million/mL) [mean ± SE (median)]	*16.2 ± 2.3 (7.0)**	*64.5 ± 5.0 (52.0)**
Total count (million) [mean ± SE (median)]	*51.5 ± 10.0 (13.5)**	*216.5 ± 22.1 (170.0)**
Progressive motility (%) [mean ± SE (median)]	*34.7 ± 2.8 (40.0)**	*56.6 ± 1.5 (60.0)**
Viability (%) [mean ± SE (median)]	*28.9 ± 2.6 (30.0)**	*52.2 ± 2.4 (50.0)**
Normal morphology (%) [mean ± SE (median)]	*31.3 ± 3.0 (30.0)**	*51.5 ± 1.5 (50.0)**

Student *t* test (italicized characters)

*P value < 0.05

education status, and their monthly income were observed between both groups. Furthermore, the reported smoking history and alcohol consumption status did not differ significantly.

Blood and seminal fluid metals

Analyte concentrations stratified by semen quality reference values were compared in the blood and seminal fluid of participants (Table 2). Analytes measured in blood did not significantly differ between groups on the basis of semen quality. Conversely, we found that participants with low-quality semen had significantly higher Cd and Ba concentrations in the seminal fluid than participants with normal-quality semen. For the remaining metals (Pb, As, Hg, and U), no significant differences were found in the seminal fluid of either groups. Spearman's rank correlation analyses showed positive correlations between blood and seminal fluid concentrations of analytes measured, with the strongest correlation coefficients observed for Cd ($r = 0.67$) and Ba ($r = 0.58$).

When comparing analyte concentrations in the blood and seminal fluid within same participants (Table 3), we found Pb to be significantly more concentrated in blood than in the seminal fluid. Conversely, both As and U were significantly more concentrated in the seminal fluid than in the blood of participants. We detected no significant differences between both compartments for the remaining elements (Cd, Ba, and Hg).

Blood/seminal fluid metals and semen quality

When the semen quality parameters were modeled as dichotomous variables in single-element multivariate logistic models (Table 4), Cd and Ba quartiles in blood were significantly associated with increased odds ratios (ORs) for below-reference sperm viability after accounting for multiple confounders (p trend < 0.05). Ba levels in blood were also significantly associated with below-reference normal morphology albeit a borderline insignificant p trend of 0.08. In accordance with the findings of the logistic models, Ba in blood remained inversely correlated with sperm viability in the single-element linear models adjusted for confounders ($P < 0.05$).

Table 2 Comparison of blood and seminal fluid concentrations of heavy metals in participants in the EMI study stratified by semen quality reference value

Variable	Study group A (low-quality semen) ($n = 61$)	Study group B (normal-quality semen) ($n = 55$)
Blood		
Pb	32.57 (51.98 ± 7.08)	30.98 (35.75 ± 2.51)
Cd	6.80 (16.93 ± 5.08)	6.04 (25.51 ± 7.16)
As	12.10 (19.70 ± 2.32)	12.29 (21.55 ± 3.97)
Ba	9.30 (134.78 ± 52.74)	7.40 (63.40 ± 22.82)
Hg	12.50 (20.54 ± 3.82)	10.40 (90.54 ± 51.14)
U	0.60 (1.39 ± 0.40)	0.90 (1.10 ± 0.15)
Seminal fluid		
Pb	5.88 (16.26 ± 4.46)	4.70 (12.85 ± 3.50)
Cd	*6.22 (55.69 ± 29.84)**	*3.67 (11.35 ± 2.76)**
As	17.80 (43.26 ± 7.54)	17.87 (31.15 ± 4.78)
Ba	*11.00 (64.16 ± 22.90)**	*8.60 (39.17 ± 23.29)**
Hg	10.61 (50.54 ± 13.66)	10.90 (54.89 ± 25.62)
U	1.70 (2.79 ± 0.46)	0.95 (5.44 ± 2.58)

Values in micrograms per liter are median (mean ± SE)

*Significance levels (p value < 0.05) when comparing low-quality semen study group A and normal-quality semen study group B, using Mann-Whitney U non-parametric test (italicized characters)

In the multivariate logistic models (Table 5), we found Pb, Cd, Ba, and U quartiles in the seminal fluid to be significantly associated with increased risk of below-reference sperm viability after adjusting for multiple confounders (p trend < 0.05). U categories in the seminal fluid were also significantly correlated with increased ORs for below-reference sperm progressive motility and normal morphology (p trend < 0.05). The associations of Cd, Ba, and U with decreasing sperm viability in a dose-dependent manner were confirmed in the single-element linear models adjusted for confounders.

Discussion

In this study of non-occupationally exposed male partners of heterosexual couples attending an infertility clinic in Lebanon, we observed significant inverse associations between heavy metals and semen quality. The findings involving Pb, Cd, Ba, and U were consistent across the different statistical modeling strategies described and showed significant trends for increased ORs for below-reference semen quality parameters in multivariate logistic models. Cd and Ba in blood, as well as Pb, Cd, Ba, and U in the seminal fluid were associated with increased risks for low sperm viability. U levels in the seminal fluid were also associated with increased risks for low progressive motility and low normal morphology. Many of these relationships were maintained in the multivariable linear regression models.

Lead

In the present study, we found significant associations of seminal Pb concentrations with below-reference sperm viability. Such relationship could not be demonstrated for blood Pb, however, despite the detection of significantly higher concentrations of lead in blood compared to seminal fluid. Current evidence indicates that associations between non-occupational lead exposure and semen quality remain inconclusive in men. Consistent with our findings, Mendiola et al. observed significant positive associations between seminal Pb and low sperm motility in a Spanish clinic-based population of infertile men [28]. Doubling in seminal Pb was also found to be associated with a 47% lower total motile sperm count [37]. These findings were not reproducible for Pb levels in blood. In a Mexican low-level exposure community-based study, Hernández-Ochoa et al. also supported an impairment of sperm progressive motility and morphology with elevated

Table 3 Comparison between blood and seminal fluid heavy metal concentrations within same participants in the EMI study

	All participants (low-quality and normal-quality semen) ($n = 116$)		
Variable	Blood	Seminal fluid	p value
Pb	*31.21 (44.43 ± 42.96)*	*5.40 (14.64 ± 30.87)*	*0.00*[a]
Cd	6.55 (20.96 ± 45.81)	4.98 (34.48 ± 168.39)	0.50
As	*12.10 (20.57 ± 23.89)*	*17.87 (37.53 ± 48.97)*	*0.00*[b]
Ba	9.00 (101.58 ± 319.37)	9.75 (52.31 ± 175.66)	0.20
Hg	11.05 (54.27 ± 260.39)	10.85 (52.60 ± 151.33)	0.20
U	*0.61 (1.26 ± 2.44)*	*1.50 (4.03 ± 13.27)*	*0.00*[b]

Values in micrograms per liter are median (mean ± SD). Significance levels (p value < 0.05) when comparing blood and seminal fluid heavy metals, using Wilcoxon signed rank paired non-parametric test) (italicized characters)

[a] Based on positive ranks (blood > seminal fluid levels)

[b] Based on negative ranks (blood < seminal fluid levels)

Table 4 Associations between semen quality parameters and quartiles of heavy metal concentrations in the blood of participants in the EMI study

Variable Quartile range (μg/L)	Volume (< 1.5 mL) (*n* = 15) Adjusted[a] OR (95% CI)	Concentration (< 15 M/mL) (*n* = 39)	Total count (< 39 M) (*n* = 38)	Progressive motility (< 32%) (*n* = 23)	Viability (< 58%) (*n* = 38)	WHO morphology (< 30%) (*n* = 30)
Pb						
1 (LOD–21.99)	1.00	1.00	1.00	1.00	1.00	1.00
2 (22.00–32.56)	0.53 (0.11, 2.44)	0.51 (0.16, 1.63)	0.36 (0.11, 1.18)	0.70 (0.19, 2.62)	0.44 (0.14, 1.39)	0.50 (0.15, 1.66)
3 (32.57–53.57)	0.24 (0.02, 2.24)	1.17 (0.37, 3.73)	0.83 (0.26, 2.65)	0.78 (0.19, 3.19)	0.68 (0.21, 2.21)	0.93 (0.28, 3.10)
4 (≥ 53.58)	1.32 (0.33, 5.26)	1.58 (0.53, 4.68)	1.35 (0.46, 3.96)	1.47 (0.43, 5.02)	1.35 (0.46, 3.96)	0.84 (0.26, 2.66)
p trend	0.26	0.26	0.15	0.66	0.23	0.68
Cd						
1 (LOD–1.19)	1.00	1.00	1.00	1.00	1.00	1.00
2 (1.20–6.55)	0.63 (0.14, 2.75)	0.76 (0.24, 2.32)	1.25 (0.40, 3.89)	2.09 (0.46, 9.41)	1.68 (0.47, 6.00)	1.99 (0.56, 6.99)
3 (6.56–18.26)	0.53 (0.11, 2.62)	0.71 (0.22, 2.24)	1.19 (0.37, 3.80)	4.06 (0.95, 17.29)	*4.20 (1.21, 14.54)*	2.22 (0.62, 7.89)
4 (≥ 18.27)	0.65 (0.13, 3.24)	1.37 (0.45, 4.13)	1.65 (0.53, 5.16)	1.82 (0.39, 8.59)	*3.60 (1.04, 12.48)*	1.54 (0.42, 5.70)
p trend	0.84	0.64	0.85	0.24	*0.05*	0.61
As						
1 (LOD–7.80)	1.00	1.00	1.00	1.00	1.00	1.00
2 (7.81–12.10)	0.92 (0.17, 5.02)	0.41 (0.13, 1.24)	0.47 (0.15, 1.45)	0.70 (0.17, 2.95)	0.90 (0.28, 2.88)	1.11 (0.32, 3.86)
3 (12.11–24.40)	0.66 (0.10, 4.35)	0.57 (0.19, 1.72)	0.55 (0.18, 1.71)	1.95 (0.54, 7.03)	1.74 (0.56, 5.41)	1.85 (0.55, 6.24)
4 (≥ 24.41)	3.29 (0.74, 14.59)	0.64 (0.21, 1.98)	0.89 (0.29, 2.71)	1.46 (0.38, 5.60)	2.01 (0.63, 6.36)	1.75 (0.50, 6.06)
p trend	0.11	0.45	0.50	0.45	0.42	0.67
Ba						
1 (LOD–4.30)	1.00	1.00	1.00	1.00	1.00	1.00
2 (4.31–9.00)	0.47 (0.09, 2.53)	0.69 (0.22, 2.10)	1.11 (0.362, 3.41)	2.18 (0.56, 8.54)	2.82 (0.81, 9.76)	*5.10 (1.22, 21.25)*
3 (9.01–67.37)	1.57 (0.34, 7.12)	1.26 (0.41, 3.83)	1.70 (0.54, 5.34)	1.31 (0.29, 5.95)	2.56 (0.70, 9.30)	3.79 (0.85, 16.80)
4 (≥ 67.38)	0.69 (0.11, 4.08)	0.96 (0.32, 2.84)	1.30 (0.43, 3.96)	2.63 (0.69, 10.05)	*6.00 (1.76, 20.46)*	*5.10 (1.22, 21.25)*
p trend	0.68	0.78	0.81	0.45	*0.03*	0.08
Hg						
1 (LOD–4.35)	1.00	1.00	1.00	1.00	1.00	1.00
2 (4.36–11.05)	2.57 (0.42, 15.61)	0.95 (0.30, 3.02)	0.67 (0.21, 2.14)	1.53 (0.32, 7.15)	1.20 (0.35, 4.11)	0.82 (0.23, 2.98)
3 (11.06–21.47)	3.95 (0.68, 22.89)	1.67 (0.52, 5.29)	1.18 (0.38, 3.72)	2.31 (0.51, 10.54)	2.92 (0.87, 9.77)	1.23 (0.34, 4.37)
4 (≥ 21.48)	0.07 (0.01, 4.44)	1.46 (0.47, 4.56)	1.22 (0.40, 3.75)	3.66 (0.86, 15.59)	2.53 (0.77, 8.33)	2.17 (0.66, 7.20)
p trend	0.06	0.70	0.72	0.26	0.18	0.37
U						
1 (LOD–0.20)	1.00	1.00	1.00	1.00	1.00	1.00
2 (0.21–0.61)	1.44 (0.35, 5.95)	1.41 (0.47, 4.21)	1.20 (0.40, 3.57)	1.97 (0.53, 7.29)	1.14 (0.37, 3.51)	1.11 (0.35, 3.53)
3 (0.62–1.50)	0.19 (0.02, 1.80)	0.51 (0.17, 1.54)	0.51 (0.17, 1.54)	1.20 (0.32, 4.47)	0.81 (0.27, 2.43)	0.80 (0.26, 2.50)
4 (≥ 1.51)	1.50 (0.33, 6.81)	0.62 (0.19, 1.97)	0.62 (0.19, 1.97)	1.33 (0.33, 5.31)	1.46 (0.47, 4.50)	0.61 (0.17, 2.18)
p trend	0.24	0.28	0.42	0.76	0.78	0.81

Entries in italics indicate statististical significance

[a] ORs adjusted for age, cigarette smoking, alcohol intake, and period of sexual abstinence

seminal Pb concentrations but failed to associate Pb in blood with semen quality measures [16].

It should be noted nonetheless that the use of seminal Pb measurements as a biological marker of toxicant exposure in men is not universal. Data on this subject are controversial particularly when considering the nature of the population exposure. In their study of lead smelter workers in British Columbia, Alexander et al. concluded that lead concentrations in the blood of occupationally exposed men were more consistently associated with indicators of spermatogenesis than in seminal plasma [38]. Similarly, Robins et al. reported no associations between semen lead and sperm quality in South African Lead Acid Battery Plant workers [39]. Telisman et al. also concluded that blood indicators of lead exposure are superior to seminal fluid indicators in industrial workers with regard to predicting decreased semen quality [19]. Taken together, these findings suggest that non-occupational low-level lead exposure in the reproductive system may not be best reflected by blood measurements and that Pb would be more appropriately assessed in the seminal fluid in this population of men. Additional research is nonetheless required to confirm this contention.

Animal experimental studies demonstrated dose-dependent reductions in testicular alkaline phosphatase and sodium-potassium ATPase activities in testicular tissues of rats exposed to lead [40]. Production of reactive oxygen radicals has also been detected leading to increased lipid peroxidation, loss of plasma membrane fluidity, and decreased sperm motility [41].

In an attempt to reconcile discrepancies with reported studies, we compared the concentrations of lead in our study with published data from populations with environment low-level exposures. In the current study, the observed mean blood Pb concentrations in Lebanese men attending an infertility clinic were 44.43 ± 42.96 μg/L. These values were lower than those reported by Mendiola et al. (101 μg/L) on a similar clinic-

Table 5 Associations between semen quality parameters and quartiles of heavy metal concentrations in the seminal fluid of participants in the EMI study

Variable Quartile range (μg/L)	Volume (< 1.5 mL) (*n* = 15) Adjusted[a] OR (95% CI)	Concentration (< 15 M/mL) (*n* = 39)	Total count (< 39 M) (*n* = 38)	Progressive motility (< 32%) (*n* = 23)	Viability (< 58%) (*n* = 38)	WHO morphology (< 30%) (*n* = 30)
Pb						
1 (LOD–2.48)	1.00	1.00	1.00	1.00	*1.00*	1.00
2 (2.49–5.40)	0.86 (0.16, 4.67)	1.57 (0.50, 4.92)	1.66 (0.51, 5.46)	4.36 (0.83, 22.81)	*8.00 (1.59, 40.30)*	3.83 (0.924, 15.90)
3 (5.41–11.85)	1.34 (0.25, 7.17)	1.99 (0.62, 6.38)	3.33 (1.01, 10.99)	6.35 (1.21, 33.19)	*12.00 (2.34, 61.52)*	*6.57 (1.57, 27.43)*
4 (≥ 11.86)	2.07 (0.37, 11.51)	1.94 (0.59, 6.35)	2.00 (0.58, 6.85)	2.40 (0.39, 14.49)	*10.15 (1.95, 52.92)*	2.02 (0.426, 9.55)
p trend	0.95	0.64	0.24	0.09	*0.006*	0.06
Cd						
1 (LOD–1.57)	1.00	1.00	1.00	1.00	*1.00*	1.00
2 (1.58–5.01)	1.00 (0.19, 5.25)	2.00 (0.62, 6.45)	2.06 (0.61, 6.96)	4.10 (0.74, 22.61)	*10.21 (1.98, 52.69)*	*5.55 (1.31, 23.45)*
3 (5.02–16.29)	0.22 (0.02, 2.16)	2.57 (0.81, 8.13)	3.66 (1.12, 12.00)	6.88 (1.32, 35.82)	*9.53 (1.86, 48.91)*	*4.41 (1.04, 18.70)*
4 (≥ 16.30)	2.21 (0.54, 9.09)	1.76 (0.55, 5.62)	2.15 (0.65, 7.11)	3.71 (0.68, 20.34)	*14.00 (2.76, 71.05)*	3.51 (0.82, 15.03)
p trend	0.14	0.43	0.18	0.10	*0.003*	0.09
As						
1 (LOD–13.60)	1.00	1.00	1.00	1.00	1.00	1.00
2 (13.61–17.95)	0.43 (0.07, 2.70)	1.25 (0.40, 3.89)	2.50 (0.77, 8.13)	2.09 (0.46, 9.41)	1.06 (0.33, 3.36)	2.60 (0.69, 9.83)
3 (17.96–38.50)	0.41 (0.06, 2.55)	1.12 (0.35, 3.57)	1.40 (0.41, 4.80)	2.68 (0.61, 11.78)	1.12 (0.35, 3.57)	2.31 (0.60, 8.91)
4 (≥ 38.51)	2.19 (0.52, 9.22)	2.07 (0.66, 6.52)	3.07 (0.92, 10.25)	2.98 (0.67, 13.18)	2.07 (0.66, 6.52)	3.09 (0.81, 11.84)
p trend	0.10	0.59	0.21	0.48	0.54	0.37
Ba						
1 (LOD–5.00)	1.00	1.00	1.00	1.00	1.00	1.00
2 (5.01–10.10)	1.27 (0.22, 7.14)	1.30 (0.44, 3.86)	1.80 (0.59, 5.52)	1.09 (0.28, 4.28)	2.40 (0.68, 8.39)	1.91 (0.57, 6.38)
3 (10.11–26.90)	2.75 (0.59, 12.82)	1.00 (0.33, 3.06)	1.39 (0.44, 4.37)	2.13 (0.59, 7.62)	*3.52 (1.02, 12.14)*	2.03 (0.60, 6.79)
4 (≥ 26.91)	1.47 (0.26, 8.37)	1.14 (0.37, 3.51)	1.87 (0.59, 5.92)	1.26 (0.32, 5.01)	*5.67 (1.62, 19.88)*	1.28 (0.35, 4.63)
p trend	0.51	0.96	0.68	0.62	*0.04*	0.61
Hg						
1 (LOD–5.25)	1.00	1.00	1.00	1.00	1.00	1.00
2 (5.26–11.14)	1.04 (0.13, 8.26)	1.30 (0.42, 3.96)	1.54 (0.50, 4.80)	0.58 (0.14, 2.35)	0.56 (0.17, 1.80)	0.77 (0.22, 2.72)
3 (11.15–37.46)	2.26 (0.39, 13.25)	0.89 (0.29, 2.77)	1.25 (0.40, 3.89)	0.72 (0.19, 2.74)	0.53 (0.16, 1.71)	1.08 (0.33, 3.58)
4 (≥ 11.47)	4.13 (0.71, 24.02)	1.26 (0.40, 3.92)	1.26 (0.39, 4.06)	1.57 (0.45, 5.42)	2.03 (0.66, 6.22)	1.52 (0.46, 5.02)
p trend	0.24	0.89	0.90	0.46	0.07	0.74
U						
1 (LOD–0.50)	1.00	1.00	1.00	1.00	*1.00*	1.00
2 (0.51–1.50)	0.27 (0.05, 1.51)	1.32 (0.40, 4.33)	1.91 (0.57, 6.38)	8.00 (0.89, 71.57)	*6.75 (1.30, 34.94)*	*4.33 (1.02, 18.25)*
3 (1.51–3.20)	0. 22 (0.03, 1.51)	3.14 (1.00, 9.89)	3.28 (1.00, 10.73)	*24.00 (2.83, 203.68)*	*15.75 (3.08, 80.40)*	*6.35 (1.52, 26.45)*
4 (≥ 3.21)	0.68 (0.16, 2.81)	2.65 (0.82, 8.56)	3.24 (0.97, 10.82)	5.60 (0.58, 53.94)	*15.95 (3.08, 82.70)*	3.57 (0.81, 15.74)
p trend	0.22	0.14	0.15	*0.002*	*0.000*	*0.05*

Entries in italics indicate statististical significance

[a] ORs adjusted for age, cigarette smoking, alcohol intake, and period of sexual abstinence

based population of Spanish men [28] and by Hernandez-Ochoa et al. (93 μg/L) on a community-based population of men from Mexico [16]. Variations between studies could be accounted for not only by variable exposure levels but also by differences in methodologies and laboratory measurements techniques. Taken together, the relatively lower lead blood levels found in Lebanese men indicate that lead exposure in the country ranks low on the priority scale of public health perspectives.

Cadmium

In the present study, we found Cd in blood and seminal fluid to be significantly associated with low sperm viability. While the relationship between non-occupational Cd exposure and semen function remains controversial, numerous observational studies found significant negative associations of seminal Cd and sperm motility/viability [15, 28, 42, 43]. Fewer studies reported similar correlations between blood Cd and low sperm concentration [44]. In disagreement with the preceding, other investigators however failed to validate any significant relationships between blood and/or seminal Cd and any sperm quality parameters [37, 45–47].

Experimental animal models, on the other hand, demonstrated that Cd exposure is toxic to spermatogenesis leading to decreased sperm motility and concentration [48–50]. Cadmium, by competing with calcium for calmodulin binding, is believed to interfere with protein tyrosine phosphorylation [41]. Also, by increasing peroxidation of membrane lipids, Cd was shown to reduce the phosphorylation of axonemal proteins. Experimental human studies laid further evidence in favor of an adverse effect of Cd on sperm function. When a cohort of 210 fertile and infertile men was exposed to cadmium, significant drops in sperm motility and viability

were observed in a time- and dose-dependent fashion [51]. A detrimental effect of Cd on sperm metabolism is found and is believed to be mediated by inhibition of glycogen phosphorylase, glucose-6-phosphatase, Mg-dependent ATPase, and succinic acid dehydrogenase [52].

Blood Cd concentrations were determined to be 0.4–4 μg/L in occupationally non-exposed men [53] and to exceed 10 μg/L in environmentally contaminated areas [53]. The mean blood Cd in the present study was 20.96 ± 45.81 μg/L, which is clearly indicative of a significant environmental exposure. Sources of contamination in Lebanon may be traced back not only to catastrophic cycles of demolitions as a result of repeated wars over the past decades but also to the ongoing construction boom in the country with an exponential growth of unregulated quarries amidst populated areas with the aim of increasing demands for construction aggregates. Taking into account the prolonged estimated biological half-life of Cd of 10–40 years in humans and its slow 0.01% daily body burden excretion [6], these data underline an important public health issue and constitute an important database platform on which future governmental regulations could be based.

Barium

In the current study, Ba levels in blood and seminal fluid of participants correlated negatively with low sperm viability. There is, to date, no reliable information about reproductive health hazards in men or experimental animals exposed to barium. Disruptions to spermatogenesis have been observed in rats exposed to inhaled Ba carbonate [54]. Disturbances appeared reversible in nature and involved decreased sperm count, motility, and osmotic resistance. In contrast, rats and mice subjected to intermediate and chronic oral barium chloride exposure had no associated histopathological changes in their testes and no alterations in epididymal sperm counts, motility, or morphology [55].

Barium is an alkaline metal ubiquitously present in nature, including volcanic earth crust and drinking water. Soluble barium compounds ($BaCl_2$ and $BaCO_3$) are known for their acute general health toxicity. Epidemiological data examining the effects of chronic low-level Ba exposure on spermatogenesis are sparse despite the increased use of this compound in industry and medicine. The current study represents one of very few human studies examining the effects of chronic low dose Ba exposure on reproduction in men. More research nonetheless is needed to develop a more explicit understanding of barium accumulation in male reproductive organs and its potential reproductive implications.

Uranium

The findings of this study indicate notable uranium exposure of the participants, with significantly more measurable U concentrations in the seminal fluid than in blood. Also, significant associations of seminal U levels were observed with below-reference progressive sperm motility, viability, and normal morphology. In contrast, blood U levels did not show similar relationships with any sperm quality parameters. While toxicity as a result of natural uranium is extremely rare, exposure to depleted uranium, a man-made by-product of the uranium enrichment process, has become a significant public health concern lately, mainly in war-inflicted zones [56]. While the U by-product is partially depleted of its radioactivity, it retains its chemical toxicity as a non-essential heavy metal. The findings of the present study are exceptional, in that very few other studies investigating the effects of environmental uranium exposure on the reproductive system of men are available. In their research of veterans wounded at the First Gulf War, Squibb and McDiarmid failed to demonstrate any effects of depleted uranium exposure on sperm concentration and motility in men [56]. Whereas experimental studies evaluating the effects of natural U on the male reproductive system in rodents have demonstrated a clear association with testicular atrophy and reduced spermatogenesis [57], animal data derived from depleted U exposure are far more controversial. In one study, depleted U in rodents was found to pass through the blood-testis barrier by modulating the gene expression of molecules involved in the regulation of tight junctions and to accumulate in reproductive organs, seminal fluid and spermatozoa [58]. In the same study and in others [58–60], the presence of depleted U in the testes of adult rats did not induce oxidative stress [58] and did not correlate with any defects in spermatogenesis namely sperm concentration and velocity [59, 60]. An endocrine-mediated effect was nonetheless observed in rats as testicular testosterone production decreased along with depleted U exposure [58]. In contrast, other investigators demonstrated a dose-dependent increase in sperm abnormalities in rodents exposed to depleted U, with evidence of histologic atrophy of convoluted tubules, reduction in spermatogenic cells, and increase in sperm DNA damage [61].

In Lebanon, possible sources of human exposure are derived from the use of depleted uranium munitions during repeated rounds of war violence. Once fired, these munitions produce small dust particles and large shrapnel fragments, contaminating air and soil. While air inhalation is considered the main route of human exposure, more recent reports have favored soil as a vehicle of contamination of the food chain and underground waters [62]. This study suggests that the use of blood U measurement as indicator of chronic body burden may underestimate the duration and nature of exposure. Since depleted U has been shown to accumulate in body organs, namely in kidneys and testes, seminal fluid levels seem to be more appropriate for the estimation of cumulative chronic exposures [62]. From a public health point of view, it is unclear how low-dose chronic exposure should be managed at the population level bearing in mind (a) the absence of a clear

definition of what would constitute such exposure in humans and (b) the non-existence of a comprehensive approach for large-scale detoxification. As more humans are being exposed to depleted U, there is a fundamental need for large epidemiologic studies with the aim to examine the nature of the threat to general and reproductive health.

Special considerations

Some of the conflicting results of human studies may conceivably be due to the assumption that not all men have similar predispositions to the reproductive toxic effects of pollutants, including non-essential heavy metals. It is possible that damage to the male reproductive system is determined by alterations in the efficiency of the protective mechanisms operational at any specified target in any given individual. Such differential susceptibility may be not only cell- and organ-specific but also individual-dependent. Individuals may therefore be expected to respond differently to the same toxic stimulus. Despite evidence on mechanisms mediating metal-induced reproductive toxicity, less information is available on pathways which determine the susceptibility of male reproductive organs to toxic injury. These pathways may involve the expression of metal transporter mechanisms and specific regulatory proteins in the testis [63–65]. Polymorphisms have been identified in genes coding for thiol-containing metal-binding proteins, known as metallothioneins (MTs), which protect cells from metal toxicity [66–68]. Several isoforms of MTs have been acknowledged and found to be cell-type specific. The differential tissue expression of these metal carriers could therefore be responsible for organ and individual vulnerability to the toxic effect of these elements [66–68].

The major strength of this study was the concurrent measurements of non-essential heavy metals in two bodily compartments, blood and seminal fluid, designed to explore the best estimate of the exposure status of the male reproductive system. Several limitations nonetheless should be mentioned. First, the cross-sectional design of the study previewed a single specimen collection per participant. Single measurements of elements may fail to account for variable time exposures and elimination half-lives of metals, often leading to exposure misclassification and attenuation of risk estimates. Second, we recruited participants from a fertility clinic, which limits the generalization of the findings to the general population and potentially leads to the inflation of risk estimates. Third, the absence of speciated measurements of arsenic does not take into account the differential toxicity of organic and inorganic species, which may also lead to exposure misclassification. Finally, the cross-sectional design of the study does not allow to establish temporal relationships between exposure and outcome, which restricts the feasibility of establishing causal relationships.

Further consideration has been made to the limitations of the cross-sectional design of the present study, namely its failure to appraise the latency of effects and to monitor changes in the endpoints of interest over time. A long-term follow-up of the same participant cohort is currently under consideration in order to establish the traceability of heavy metal accumulation in body organs, namely blood and semen over the timeline, and investigate any additive effects of age, metal dose, and time exposure on spermatogenesis and other related health outcomes.

Conclusions

This study corroborates the reproductive toxicity of non-essential heavy metal exposures in men at non-occupational levels. Our findings suggest that environmental exposures to Pb, Cd, Ba, and U are associated with below-reference sperm quality parameters in Lebanese men. In non-occupationally exposed men, measurements of non-essential heavy metals in the seminal fluid may be more predictable of semen quality than conventional blood measurements. Additional well-designed human epidemiologic studies are required to eliminate inconsistencies and confirm the effects of heavy metals at human-relevant low dose levels on human male reproductive function. A concerted effort involving epidemiologists, clinicians, and basic scientists is best sought to meet these challenges.

Compliance with ethical standards

This study was approved by the American University of Beirut Institutional Review Board, and all eligible participants signed an informed consent form prior to enrollment.

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