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Occupational and environmental exposures to heavy metals: Risk factors for male infertility in Lebanon?

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Abstract

A case–control study was conducted to examine whether occupational or environmental exposures, particularly to heavy metals, are associated with male infertility in Lebanon, a war-torn country with a history of environmental degradation. Seventy-four infertile cases and 76 fertile controls were selected from 2 major fertility clinics in Beirut. Data collection involved risk-factor interviews, semen analysis, and blood collection for heavy metal analysis. Multiple regression analysis showed that men with reported occupational exposures were twice as likely to be infertile as unexposed men. However, none of the subcategories of infertile men (based on semen analysis results) had significantly higher whole blood concentrations of heavy metals when compared to fertile controls. Blood concentrations were well within the range for referent populations of healthy individuals. Thus, despite Lebanon's poor record of occupational and environmental stewardship, exposure to metal pollutants does not appear to represent an important risk factor for male infertility.

Keywords: Male infertility; Heavy metals; Occupational health; Environmental health; War; Lebanon

1. Introduction

Infertility affects between 50 and 80 million people worldwide. Globally, male factors, such as oligospermia (low sperm count) and/or asthenospermia (poor sperm motility), account for nearly half of all infertility cases [1]. Epidemiological studies providing detailed, multi-factorial assessments of possible risk factors for male infertility are relatively scarce, particularly in the developing world. There, male infertility is a potentially emasculating condition, which remains hidden and unidentified in many societies [2]. A range of logistical and methodological problems, including accurately identifying cases through standardized semen analysis, inhibit developing-world epidemiological research on this condition. As a result, estimation of the prevalence of male infertility, alone and in combination with female factors, is an issue of major uncertainty in developing countries [2,3].

In the Middle Eastern nations, it is estimated that 10–15% of all couples are infertile [4]. In this region, male infertility rates are not well determined, but reach in some studies as high as 60–70% of the total infertile population [2]. Furthermore, male infertility cases presenting to Middle Eastern in vitro fertilization (IVF) clinics are often of a serious nature, including many cases of azoospermia (lack of sperm in the ejaculate) and severe oligo-, astheno-, and teratozoospermia (poor sperm morphology). Few if any epidemiological studies of male infertility have been conducted in the Middle East, with the exception of those documenting fertility impairments among Euro-American male veterans of the First Gulf War and men exposed to mustard gas in the Iran–Iraq war [5–8]. Thus, assessing risk factors for male infertility among the local population in Middle Eastern communities seems imperative.

Lebanon is a Middle Eastern nation that has undergone severe environmental degradation over the past few decades [9–11]. In his article on "The Ecological Crisis in Lebanon", Fouad Ham-

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dan argues that the improper disposal of household, industrial, and hospital waste, industrial pollution, air pollution, and the use of chemicals in agriculture have compromised the quality of Lebanon's air, water, and soil [12]. These types of environmental disruptions may have long-term impacts on human health, particularly in a country with non-existent or un-enforced occupational safety and health standards.

Furthermore, much of Lebanon's environmental degradation is the direct result of more than 25 years of ongoing war and political violence, including the illegal importation of toxic wastes from abroad and dumping of those wastes on Lebanon's soil [12]. During the last war in July 2006, thousands of hectares of agricultural land and greenhouses were destroyed, huge quantities of toxic wastes were produced from destroyed buildings and structures, and 15 tonnes of oil were spilled into the Mediterranean Sea [9,10].

Heavy metal contamination is a cause for concern. A 2001 report by the Lebanese Ministry of the Environment reported that more than 2400 tonnes of industrial waste containing heavy metals is generated each year [11]. This is of particular concern, given that heavy metals are known or suspected to cause damage to the male reproductive system.

The presence of abnormal levels of Pb, Cd, Mn, Zn, Se, and As may affect spermatogenesis with regard to production, maturation, motility, and fertilizing capacity of human spermatozoa [13]. Observations that decreases in ejaculate volume and sperm concentration are more pronounced among men living in urban areas compared to rural districts have led to the suggestion that the reduction in sperm quality may be due to chronic, low-level exposures to contaminants in the environment, which are potential reproductive toxicants [14,15].

Given Lebanon's tumultuous political and environmental history, Lebanese men may be exposed in their daily lives to many environmental and occupational agents that are hazardous to their reproductive capacity [16–18]. To that end, a case–control study was conducted to examine whether occupational or environmental exposures, particularly to heavy metals, are associated with male infertility in Lebanon.

2. Materials and methods

2.1. Study design and population

Two hundred and twenty men seeking IVF treatment at two major IVF centers in Beirut, the American University of Beirut Medical Center (AUBMC) and FIRST IVF, were included in the study over a period of 8 months (January–August 2003). The patients were divided into 2 groups based on their semen analyses. The cases included 120 men who had had repeated abnormal semen analyses [19]. The control group included 100 men with repeated normal semen analyses, but who were seeking fertility treatment because of female factor infertility. The study was IRB approved and patients were not entitled to any financial reimbursement.

2.2. Data collection

2.2.1. Interviews

After obtaining the informed consent of subjects, detailed reproductive histories and risk factor assessments were carried out. Data were collected through a semi-structured interview schedule, which was administered verbally in either Arabic or English, depending upon the preference of each research subject. The interview included baseline information on demographics (age, religion, place of residence, education, income), and reproductive and sexual history (age at first sexual intercourse, number of sexual partners, age at marriage, number of marriages, endogamy, pregnancies and births). Men were then asked about exposures to possible reproductive risk factors in five areas of interest: (a) reproductive illnesses and traumas, (b) consanguinity, (c) substance use (i.e., tobacco, caffeine, and alcohol consumption), (d) war (e.g., participation in fighting, injury, close residential proximity to bombing), and (e) occupational and environmental exposures.

With regard to the last category, men were asked about their contact with chemical substances or physical agents at their workplaces and in their home environments. Common occupational exposures reported by the men included work with gasoline (mostly leaded), pesticides, paints, solvents, and chemicals used in manufacturing and construction. A number of men in the study worked as professional drivers and reported extensive exposure to car and truck exhaust, including diesel fuel. In addition, men were asked to indicate whether they lived in residential areas noted for ambient air pollution or environmental toxicity (e.g., near cement factories or electricity companies). This was important, as a significant number of men in the study (46% of cases, 35% of controls) resided in Beirut, a city known for its poor air quality.

2.2.2. Blood collection

Approximately 4 mL of blood were drawn from all consenting subjects on the day of the interview and then frozen for later heavy metal analysis in the environmental health sciences laboratory at the University of Michigan School of Public Health. The blood samples were analyzed using a method described elsewhere [20]. Briefly, a known volume of each sample was digested with nitric acid/hydrogen peroxide in a Teflon tube using a graphite heating block. The resulting clear solution was made up to a known volume and then analyzed using an Agilent 7500c series inductively coupled plasma/mass spectrometer (ICP-MS) equipped with a collision cell. Instrumental operating conditions recommended by the manufacturer were used. Each batch of 12 blood samples included a reagent blank (dilutent with no blood), a duplicate sample, and a standard reference blood sample (NIST 1640 or Seronorm^{TM}) mixed with the digesting solution. The precision (RSD) of the method at different levels of each metal was better than ±4% and the recovery was greater than 90% for each metal. The detection limits, calculated as three times the standard deviation of the blank, was <0.05 μg/L for Pb, Cd and As; <0.1 μg/L for Se and Mn; and <0.5 μg/L for Se, Cu and Zn.

2.2.3. Semen analysis

Subjects underwent semen analysis at the time of the study, generally on the day of study recruitment at the IVF center to confirm the results of previous analyses. Semen analysis was reliable and standardized to reflect current WHO guidelines [19]. In most cases, numerical results for both sperm count and sperm motility were obtained, and cases of poor sperm morphology and azoospermia were also noted. Outcomes of interest in this study were oligospermia (i.e., sperm count <20 million/mL^3) and asthenospermia (i.e., sperm motility <40%). Men with one or both of these outcomes (oligoasthenospermia) were considered infertile for the purposes of this study, as were azoospermic men with no viable sperm in the ejaculate.

Of the 220 men interviewed, 70 were ultimately excluded from this analysis because of incomplete sperm analysis data ($n = 18$), refusal to provide a blood sample ($n = 13$), or because of reported dietary zinc, selenium, or multi-vitamin supplementation ($n = 38$). Those subjects eliminated from the study due to vitamin supplementation were more likely to be cases (94%) than controls (6%), as infertile men are often prescribed dietary supplements including zinc and selenium to enhance sperm parameters. In the final analysis, 74 infertile men (cases) and 76 fertile men (controls) were included for a total sample size of 150. There were no systematic differences between the subjects included in the study and those excluded from the final analysis.

2.3. Data management and analysis

Following data collection, data were analyzed using SPSS (Version 12) and SAS statistical software. Univariate analysis consisted of frequency and percentage distributions for the different categorical variables in the study. Means,

standard deviations, and ranges were computed for the different continuous variables, with checking for normality and outliers.

Bivariate analysis utilized Chi-Square-Fisher's exact test for categorical variables, and the *t*-test and analysis of variance for continuous variables, in order to examine the association between the main outcome variable (male infertility) and the various exposure and confounding variables. The purpose of this analysis was to examine crude associations and to check for potential confounders and effect modification.

Multiple logistic regression analysis was used to produce odds ratios (ORs) and 95% confidence intervals for the association between fertility status, semen parameters, and exposure variables, adjusting for confounding factors. Confounding factors were identified using univariate logistic regression analysis for each metal separately and for all of the metals together. Factors were considered confounding if their inclusion in the model modified the estimate of the beta by more than 10%.

For the logistic regression analysis involving the metal concentrations as the exposure of interest, an interquartile was used as the unit of measurement. The resulting interpretation of the beta (OR) would be as follows: for a change equivalent to the value of one interquartile, the log odds (odds) of infertility increase/decrease by *X*. The purpose of using this unit of measurement was to enable easier interpretation of the change in odds associated with an increase or decrease in each of the metals.

Odds ratios, *p*-values, and confidence intervals were computed at a type I error-alpha of 5%. The final model incorporated the exposure and confounding variables that displayed the most significant odds ratios. All *p*-values were two-sided, and noted to be significant at $p < 0.05$ (and of borderline significance at $p < 0.10$, given the relatively small sample size).

3. Results

There were no significant differences between cases and controls in terms of socio-demographic background (Table 1). The average age in both groups was 38, and most subjects had completed high school. The average monthly income in both groups was around US$ 1700. Cases had a slightly lower average income than controls, although cases averaged slightly more years of education. Controls were more likely to be white-collar professionals; however, the professional background of both groups was relatively similar. Reflecting Lebanon's comparatively high educational levels, around half of cases and controls held professional sector jobs, including physicians, engineers, professors, and businessmen. The rest of the men in this study worked in occupations in which exposure to hazardous materials was common (e.g., manufacturing, agriculture, painting and construction, long-distance driving).

Infertile men (57%) were more likely to report occupational exposure to hazardous materials than were fertile men

Table 1
Socio-demographic characteristics of the study sample

	Fertile ($N = 76$)	Infertile ($N = 74$)
Age (mean) in years	38.7	38.0
Salary/month (mean ± S.D.)	1725 ± 2205	1690 ± 1801
Years of education (mean ± S.D.)	13.77 ± 5.8	14.07 ± 4.4
Occupational exposure		
Yes	34	42
No	42	32
Occupational exposure duration (mean) in years	4.8	5.5

Significant difference between fertile and infertile groups at $p < 0.05$.

Table 2
Mean semen parameters and mean metal concentrations by fertility status and occupational exposure (mean ± S.D.)[a]

	Sperm count (10^6 mL^{-1})	Motility (%)	Arsenic (μg/L)	Manganese (μg/L)	Copper (μg/L)	Cadmium (μg/L)	Lead (μg/L)	Zinc (μg/L)	Selenium (μg/L)
Fertile: environment only $N = 42$ "F-E"	68.2 ± 35.6	66.4 ± 13.4	1.34 ± 1.5	7.64 ± 3.0	683.9 ± 95.6	0.50 ± 0.52	46.6 ± 20.1	5802.1 ± 1047	106.9 ± 25.6
Fertile: environmental and occupational $N = 34$ "F-EO"	69.7 ± 28.6	64.8 ± 14.4	0.87 ± 1.2	8.58 ± 3.8	754.5 ± 287	0.44 ± 0.40	52.4 ± 20.1	6905.9 ± 2397**	112.4 ± 42.3
Infertile: environment only $N = 32$ "INF-E"	18.5 ± 39.5**	41.2 ± 26.6**	1.05 ± 1.2	7.69 ± 2.3	696.7 ± 101	0.66 ± 0.96	42.9 ± 14.6	6507.4 ± 1237**	107.9 ± 21.2
Infertile: environmental and occupational $N = 42$ "INF-EO"	13.8 ± 24.2*	31.6 ± 20.9**	0.84 ± 0.71*	7.42 ± 2.8	705.1 ± 191	0.41 ± 0.31	54.0 ± 21.5	6336.8 ± 1624*	102.0 ± 25.8

* Significantly different from F-E at $p < 0.10$.
** Significantly different from F-E at $p < 0.05$.
[a] Minus 12 azoospermic men.

(45%), although this difference was not statistically significant (Table 1). Infertile men also reported longer average occupational exposures than controls, 5.5 years as opposed to 4.8 years.

Men in the study were then divided into four groups based on their fertility and exposure status (Table 2): (a) Fertile and Environmentally Exposed (F-E); (b) Fertile and Environmentally and Occupationally Exposed (F-EO); (c) Infertile and Environmentally Exposed (INF-E); and (d) Infertile and Environmentally and Occupationally Exposed (INF-EO). Occupationally exposed men were those reporting contact with chemicals and other potentially hazardous substances at work. Environmentally exposed men were those living in areas of known environmental pollution (i.e., Beirut, where the majority of the subjects resided) or who had extensive contact with car/truck exhaust and particulate matter in the air on a daily basis. The vast majority of the men in the study experienced some type of environmental or occupational exposure, making comparison with a non-exposed group difficult.

When testing for associations between occupational/environmental exposures and heavy metals in the blood, the F-EO exposed subjects had lower blood concentrations of arsenic and cadmium than the F-E group (Table 2). However, the concentrations of manganese, copper, lead, zinc and selenium were all higher in the occupationally and environmentally exposed fertile subjects (F-EO) compared to the environmentally only exposed fertile subjects (F-E). For infertile subjects, those with environmental and occupational exposures (INF-EO) had lower concentrations of arsenic, manganese, selenium and cadmium than infertile subjects exposed via the environment alone (INF-E). However, the INF-EO group had higher concentrations on average of copper, lead and zinc.

All of the occupationally exposed subjects were divided into three groups based on their reported duration of occupational exposure (Table 3). The first group ($N = 20$) consisted of those with 1–7 years of exposure, the second group ($N = 22$) with 8–14 years of exposure, and the third group ($N = 16$) with more than 14 years of exposure. The differences between the groups in mean

Table 3
Mean semen parameters and whole blood concentrations of metals by occupational exposure duration[a]

	Sperm count (10^6 mL^{-1})	Motility (%)	Arsenic (μg/L)	Manganese (μg/L)	Copper (μg/L)	Cadmium (μg/L)	Lead (μg/L)	Zinc (μg/L)	Selenium (μg/L)
Duration of occupational exposure									
1–7 years ($N = 20$)	32.2 ± 34.6	45.7 ± 21.7	0.96 ± 0.95	7.65 ± 2.46	728.0 ± 228	0.43 ± 0.38	55.5 ± 23.1	6406 ± 2002	106.6 ± 31.7
8–14 years ($N = 22$)	44.1 ± 44.8	51.4 ± 24.0	1.08 ± 1.20	7.80 ± 3.78	749.8 ± 352	0.38 ± 0.35	46.8 ± 21.4	6502 ± 2028	108.7 ± 45.5
14 + years ($N = 16$)	41.5 ± 37.4	50.9 ± 26.9	0.38 ± 0.37*	7.22 ± 2.56	678.6 ± 91.5	0.49 ± 0.36	55.8 ± 19.3	6717 ± 2594	104.8 ± 24.0

* Significantly different from other two groups at $p < .10$.
[a] Not including 12 azoospermic men.

Table 4
Results of logistic regression of occupational exposure on three outcome variables[a]

Outcome	Odds ratio (95% confidence interval)	Significance
Infertility (fertile, infertile)		
Crude OR	1.828 (0.93–3.61)	$p = 0.082$
Adjusted OR[b]	2.00 (0.88–4.57)	$p = 0.100$
Sperm count (>20 × 10^6, ≤20 × 10^6)		
Crude OR	1.744 (0.86–3.55)	$p = 0.125$
Adjusted OR[b]	1.569 (0.59–4.17)	$p = 0.367$
Motility (>40%, ≤40%)		
Crude OR	1.700 (0.78–3.69)	$p = 0.079$
Adjusted OR[b]	1.992 (0.74–5.36)	$p = 0.173$

[a] Not including 12 azoospermic men.
[b] Model for the effect of occupational exposure on three outcome variables controlling for age, salary, education, years of marriage, tobacco and alcohol consumption, consanguinity, family history of infertility, history of reproductive illness, war exposures.

Table 5
Mean semen parameters and mean heavy metal concentrations in whole blood by fertility status (mean ± S.D.)

	Sperm count (10^6 mL^{-1})	Motility (%)	Arsenic (μg/L)	Manganese (μg/L)	Copper (μg/L)	Cadmium (μg/L)	Lead (μg/L)	Zinc (μg/L)	Selenium (μg/L)
Fertile $N = 76$	68.9 ± 32.5	65.6 ± 13.8	1.13 ± 1.4	8.06 ± 3.4	715.5 ± 206	0.47 ± 0.47	49.2 ± 20.2	6295.9 ± 1853	109.3 ± 33.9
Infertile $N = 74$									
Oligospermia $N = 26$	2.47 ± 5.0*	65.1 ± 18.1	0.87 ± 0.68	7.21 ± 2.9	682.2 ± 130	0.39 ± 0.25	49.2 ± 15.7	6361.2 ± 1182	104.1 ± 20.2
Asthenospermia $N = 14$	66.1 ± 43.4	34.1 ± 6.0*	0.63 ± 0.66	8.76 ± 2.2	753.0 ± 254	0.99 ± 1.23	52.6 ± 27.6	6848.2 ± 2195	116.8 ± 33.7
Oligoasthenospermia $N = 22$	5.25 ± 5.6*	18.3 ± 14.2*	1.19 ± 1.29	7.13 ± 2.3	692.2 ± 112	0.37 ± 0.27	48.0 ± 18.8	6176.4 ± 1207	96.9 ± 18.2
Azoospermia $N = 12$	0*		1.04 ± 0.70	7.54 ± 2.6	698.9 ± 158	0.46 ± 0.67	60.3 ± 37.8	6205.8 ± 1954	95.6 ± 20.2

* Significantly different from Fertile group at $p < 0.05$.

semen parameters and metal concentrations were tested using ANOVA. The analysis of variance revealed that one exposure duration group was different from the others for arsenic. Bonferroni results revealed that the longest exposure duration group (>14 years) had lower mean arsenic concentrations than both the 1–7 year and 8–14 year groups at $p < 0.10$, although this finding is the opposite of what is expected and might be spurious. In general, there was no difference between groups for any of the other metals or semen parameters.

The multivariate logistic regression analysis demonstrated that occupational exposure is a moderately significant risk factor for infertility (Table 4). In the crude regression of occupational exposure on fertility, the odds of being infertile increased 83% due to occupational exposure (OR = 1.83). This result was significant at $p < 0.10$. Controlling for other variables in the model, the adjusted odds of being infertile increased two-fold due to occupational exposure (OR = 2.0). This result was significant at $p = 0.10$.

The infertile men in the study ($n = 74$) were then divided into 4 groups based on the type of infertility (i.e., oligospermia, asthenospermia, oligoasthenospermia, azoospermia), in order to examine both occupational exposures and heavy metal concentrations among subgroups of infertile men (Table 5).

Among oligospermic men, the odds of having a sperm count <20 million/mL^3 increased by 74% in the crude analysis and by 57% in the adjusted model for those men occupationally exposed to physical and chemical agents. However, these results were not statistically significant. Subjects with oligospermia demonstrated lower whole blood concentrations of arsenic, manganese, copper, cadmium, and selenium than those subjects with normal sperm counts (>20 million/mL^3). Lead levels were equal, and only zinc concentrations were higher among oligospermic men as compared to fertile men.

For subjects with asthenospermia, the odds of having sperm motility <40% increased by 70% with occupational exposure to physical and chemical agents in the unadjusted analysis, a result that was significant at $p < 0.10$. In the adjusted model, the odds increased by 99%, but the result was not statistically significant. Among asthenospermic men, whole blood metal concentrations were higher than those of fertile men for manganese, copper, cadmium, lead, zinc and selenium. Arsenic, however, was found in lower concentrations in men with asthenospermia than in fertile controls.

For men with oligoasthenospermia (both low sperm count and poor sperm motility), average whole blood concentrations of manganese, copper, cadmium, lead, zinc and selenium were lower than those of fertile controls. Only the average concentration of arsenic was greater in men with oligoasthenospermia than in fertile controls, but the result was not statistically significant.

Men with no sperm output (azoospermia) had lower average concentrations of arsenic, manganese, copper, cadmium, zinc and selenium than fertile controls. Only the average lead concentration was higher in azoospermic men than in fertile men, but not at a statistically significant level.

In the final multivariate logistic regression analysis (Table 6), research subjects were divided into three groups: fertile, oligospermic, and asthenospermic (with oligoasthenospermic

Table 6
Mean metal concentration in fertile and infertile subjects with multiple logistic regression of metals on three outcomes[a]

	Arsenic (μg/L)	Manganese (μg/L)	Copper (μg/L)	Cadmium (μg/L)	Lead (μg/L)	Zinc (μg/L)	Selenium (μg/L)
Fertility							
Fertile ($n=76$) (mean ± S.D.)	1.13 ± 1.4	8.06 ± 3.4	715.5 ± 206	0.47 ± 0.47	49.2 ± 20.2	6295.9 ± 1853	109.3 ± 33.9
Infertile ($n=62$) (mean ± S.D.)	0.93 ± 0.9	7.53 ± 2.6	701.7 ± 160	0.51 ± 0.66	49.5 ± 19.7	6405.6 ± 1472	104.4 ± 24.1
Crude OR	0.84 (0.58–1.2)	0.79 (0.50–1.2)	0.94 (0.71–1.2)	1.05 (0.84–1.3)	1.02 (0.63–1.6)	1.07 (0.75–1.5)	0.83 (0.57–1.2)
Adjusted OR[b]	0.64 (0.38–1.09)*	0.86 (0.51–1.5)	0.86 (0.63–1.2)	1.22 (0.93–1.6)	1.07 (0.58–2.0)	1.02 (0.66–1.6)	0.81 (0.52–1.3)
Adjusted OR[c]	0.61 (0.35–1.06)*	1.02 (0.53–1.9)	0.80 (0.48–1.3)	1.23 (0.92–1.6)	1.31 (0.67–2.6)	1.53 (0.82–2.9)	0.74 (0.34–1.6)
Sperm count							
$>20 \times 10^6$ ($n=90$) (mean ± S.D.)	1.05 ± 1.3	8.17 ± 3.2	721.3 ± 213	0.55 ± 0.66	49.7 ± 21.3	6381.8 ± 1907	110.5 ± 33.8
$\leq 20 \times 10^6$ ($n=48$) (mean ± S.D.)	1.02 ± 1.0	7.17 ± 2.6	686.8 ± 120	0.38 ± 0.25	48.6 ± 17.0	6276.5 ± 1185	100.8 ± 19.4
Crude OR	0.94 (0.67–1.4)	0.62 (0.38–1.04)*	0.83 (0.59–1.2)	0.72 (0.49–1.06)*	0.92 (0.56–1.5)	0.93 (0.64–1.4)	0.66 (0.42–1.04)*
Adjusted OR[b]	0.66 (0.36–1.2)	0.63 (0.32–1.2)	0.84 (0.58–1.2)	0.46 (0.21–1.007)*	1.02 (0.49–2.1)	0.86 (0.50–1.5)	0.60 (0.33–1.1)
Adjusted OR[c]	0.63 (0.32–1.2)	0.70 (0.38–1.9)	0.94 (0.51–1.7)	0.48 (0.21–1.08)*	1.18 (0.52–2.7)	1.54 (0.71–3.3)	0.54 (0.21–1.4)
Motility							
>40% ($n=102$) (mean ± S.D.)	1.06 ± 1.3	7.84 ± 3.3	707.0 ± 190	0.45 ± 0.42	49.2 ± 19.0	6312.6 ± 1701	108.0 ± 31.0
≤40% ($n=36$) (mean ± S.D.)	0.97 ± 1.1	7.76 ± 2.4	715.9 ± 180	0.61 ± 0.84	49.8 ± 22.4	6437.6 ± 1666	104.6 ± 26.8
Crude OR	0.92 (0.61–1.4)	0.97 (0.58–1.6)	1.04 (0.78–1.4)	1.19 (0.93–1.5)	1.04 (0.61–1.8)	1.08 (0.73–1.6)	0.88 (0.57–1.4)
Adjusted OR[b]	0.74 (0.38–1.4)	1.10 (0.59–2.1)	1.02 (0.69–1.5)	1.25 (0.93–1.7)	0.95 (0.43–2.1)	1.12 (0.68–1.9)	0.95 (0.56–1.6)
Adjusted OR[c]	0.73 (0.36–1.4)	1.18 (0.57–2.5)	0.99 (0.54–1.8)	1.25 (0.92–1.7)	0.99 (0.42–2.3)	1.38 (0.64–3.0)	0.75 (0.32–1.8)

* Significant at $p<.10$.

[a] Not including 12 azoospermic men.

[b] Model for the effect of metal exposure on three outcome variables controlling for age, salary, education, years of marriage, tobacco and alcohol consumption, consanguinity, family history of infertility, history of reproductive illness, war exposures.

[c] Model for the effect of metal exposure on three outcome variables controlling for age, salary, education, years of marriage, tobacco and alcohol consumption, consanguinity, family history of infertility, history of reproductive illness, war *and all other metals*.

men counted in both categories). There were no significant differences between the three groups in the concentration of any of the heavy metals measured in this study, after adjusting for all other variables in the model. However, levels of arsenic, manganese, cadmium and selenium were slightly higher in fertile men, indicating a mild protective effect on sperm count in the crude analysis.

4. Discussion

In this study of male infertility in Lebanon, occupational exposure to chemical and physical agents at work (e.g., solvents, pesticides, fuel, cement) increased men's risk of infertility two-fold (OR = 2.0, $p = 0.10$), after adjusting for a variety of confounding variables (Table 4). Additionally, the data analysis showed that fertile men reporting occupational exposures (F-EO) had higher mean concentrations of lead, manganese, copper, zinc and selenium than non-exposed individuals (F-E) (Table 2). These results were significant at the $p < 0.05$ for zinc. However, concentrations of arsenic and cadmium were lower in the occupationally exposed group (F-EO) than in the non-exposed group (F-E). It is important to note that smoking is one of the principal exposure routes for both of these elements in the general population and probably in Lebanon [21–23].

When occupational and environmental exposure was stratified into fertile (F-E and F-EO) and infertile groups (INF-E and INF-EO) (Table 2), only arsenic and zinc were significantly higher among the INF-EO group when compared to F-E. With arsenic, the blood concentration of the INF-EO group was significantly lower than the unexposed group ($p < 0.10$), indicating a possible protective effect against infertility (i.e., oligospermia and asthenospermia); for the fertility outcome, a change of one interquartile range in the arsenic concentration resulted in a 39% decrease in the odds of being infertile after adjusting for confounding variables in the model.

It is important to note that the direction of this effect is the opposite to what is expected based on previous research [24–26]. For example, recent studies on arsenic exposure in many parts of the world where drinking water contains elevated levels of this metalloid indicate deleterious effects on pregnancy outcomes, because arsenic is a strong endocrine disrupter [27]. Impairment of male reproductive function by arsenic has not been extensively studied in human populations [28]. However, investigations with rats and mice report effects on male fertility at very high doses, often in the mg/L range [29,30] In this study, the blood arsenic concentrations among both cases and controls were very low (<1.5 μg/L), much lower than any dose effects reported. Thus, the observed protective effect of arsenic is conceivably spurious.

For zinc, the F-EO ($p < 0.05$), INF-E ($p < 0.05$) and INF-EO ($p < 0.10$) groups all had significantly higher concentrations of zinc than the non-exposed F-E group. However, the group with the highest concentration of zinc in the blood was the F-EO group, suggesting that elevated zinc levels may be protective against infertility, even among men with significant environmental and occupational exposures to hazardous materials. Although no effort was made to establish the prevalence of zinc deficiency in the study population, the study finding that zinc is potentially protective against male infertility is supported by a variety of studies in many parts of the world, including the Middle East, demonstrating the deleterious effects of zinc deficiency on male fertility [31–38]. Zinc plays a critical role in male reproductive function as evidenced by the fact that the prostate secretes high levels of zinc and the concentration of this metal in semen is extremely high [13,33]. The production of semen necessitates extensive cell division, and this requires large amounts of zinc, as zinc is involved extensively in nucleic acid and protein metabolism and is hence fundamental to cell differentiation and replication [39]. Zinc is essential in the production of many of the sex hormones, including testosterone and gonadotrophin-releasing hormone, and is important for the attachment of the head to tail in spermatozoa. It is also required for the production of an antibacterial compound released from the prostate gland into the semen [40]. Zinc is a cofactor for more than 300 metalloenzymes in a variety of animal species [38]. Zinc finger proteins are ubiquitous in the human genome and are involved in the genetic expression of steroid receptors [41]. There is evidence to suggestion that zinc in seminal plasma influences sperm oxygen consumption, thereby increasing fertility [42,43]. Supplementation with zinc increases daily sperm production and reduces the proportion of abnormal spermatozoa [44].

Overall, the findings of this study suggest that heavy metal concentrations in the blood of Lebanese men did not diminish their fertility in any significant way. Indeed, when categorized by fertility status (i.e., fertile, oligospermic, asthenospermic, oligoasthenospermic, azoospermic), none of the infertile groups had significantly higher or lower whole blood concentrations of heavy metals when compared to the fertile controls. This is not surprising since the blood concentrations of these metals in both cases and controls were generally below the reported effects levels for male reproductive toxicity. For instance, the adverse effects of lead on human male fertility has been postulated to occur at blood levels greater than 40 μg/dL [45–47], which is the current occupational standard in the United States and many other countries [48]. A few studies have found evidence for alterations in male fertility at lower blood lead levels (PbB), down to 20 μg/dL [49,31]. A moderate increase in luteinising hormone and follicle-stimulating hormone have been observed at PbB levels down to 10 μg/dL [50]. However, the reported thresholds of PbB associated with lead effects are well above the average for participants in this study (<10 μg/DL).

Nonetheless, some interesting patterns can be seen in the results. Men with oligospermia had consistently lower average concentrations of metals than fertile controls, with no difference in the zinc levels (Table 5). Men with oligoasthenospermia had consistently lower average concentrations of metals than fertile controls, with the exception of arsenic. Likewise, men with azoospermia had lower average concentrations of metals than fertile controls, with the exception of lead. In contrast, men with asthenospermia had consistently higher average concentrations of all metals with the exception of arsenic (Table 5). Yet, none of these differences were statistically significant and generally fall within the ±10% accuracy of the instrumental method.

Interestingly, an increase equivalent to one interquartile in the concentration of cadmium resulted in a 52% decrease in the odds of having a low sperm count (Table 6). Both examples were significant at the $p < 0.10$ level. This cadmium finding is contrary to other reports, which show that higher levels of cadmium in the blood are associated with decreases in sperm quality [24,25,51]. It should be noted that the blood cadmium levels in the study participants are very low (<1.0 μg/L), and whether such low doses can exercise a protective influence on male reproductive function through a process of hormesis remains doubtful at this time [52].

Even though the present study did not find any significant association between heavy metals in the blood and reduced sperm parameters, the effect of the metals on each of the four infertility outcomes (oligospermia, asthenospermia, oligoasthenospermia, azoospermia) strengthened after adjusting for the other variables in the model. In other words, the metals became more significant as predictors of infertility outcomes as other variables were controlled for. This would suggest that the power of the study was not great enough to detect significant differences in the concentration of metals between the various outcome categories. Although the overall sample size of the study was $n = 220$, this analysis relied on a subgroup of men (76 fertile, 74 infertile, $n = 150$), excluding those men who reported dietary vitamin supplementation, as well as those men with missing or incomplete blood and semen analysis results.

Another drawback of this study was the possibility of misclassification bias. Although the study aimed to evaluate the effects of occupational and environmental exposures on male infertility and heavy metal concentrations in blood, occupational and environmental exposure to harmful physical and chemical agents was imprecisely measured, because it was determined through self-report during interviews. Infertile cases might have been more motivated than fertile controls to recall potential exposures, a form of bias that could have resulted in misclassification of exposure status. Most research subjects, both fertile and infertile, were very clear about their work histories, and reported their work exposures with some degree of specificity. However, subjects' reports of environmental exposure to ambient pollutants were much less precise. A future study of occupational and environmental exposure would overcome these problems through sampling of environmental and workplace toxins, in order to provide objective measures of exposure status among men.

Another potential limitation relates to the study's external validity, as a clinic-based convenience sample was used rather than a population-based random sample. The study was also subject to potential selection bias, as men presenting to infertility clinics may have been more likely to report occupational exposures. Indeed, more than half of the study participants reported some occupational or environmental exposure to harmful physical or chemical agents, suggesting that selection bias was present. On the other hand, the significant level of exposure to potentially hazardous substances among men in this study could represent the realities of life in contemporary Lebanon (and other parts of the Middle East), where occupational and environmental protection measures are not firmly in place and many men work in the agricultural, manufacturing, and construction sectors.

Whole blood concentrations of heavy metals (i.e., arsenic, manganese, copper, cadmium, lead, zinc and selenium) were not significant predictors of male infertility in general or of the two major forms of male infertility (oligospermia and asthenospermia) in particular. It is possible that the metals measured in this study are working synergistically with one or more unmeasured variables related to occupational exposure. This could explain why occupational exposure appears to be a reasonably strong predictor of male infertility in the context of relatively equal heavy metal concentrations in the blood of cases and controls. Furthermore, some studies suggest that the association between certain occupations and male infertility is highly dependent on the individual's age at exposure [51,53]. Accordingly, an observed negative association could simply mean that the concentration of a specific heavy metal may not have reached its latency period for the incurred damage to men's sperm to take place. Thus, additional prospective cohort studies are needed to understand the etiology of male infertility in terms of the dose and duration of occupational exposure to heavy metals.

In conclusion, this study of occupational and environmental exposure in Lebanon has demonstrated that reported occupational exposure to harmful physical and chemical agents is associated with a two-fold increased risk of male infertility, although this risk is not directly related to heavy metal contamination. The relationship between male infertility and occupational and environmental exposures has been supported by other studies [54–60]. However, future studies are clearly needed to detangle the mixed exposures to occupational and environmental toxins, including heavy metals, and their effects on male fertility in developing-world settings such as Lebanon.

Despite Lebanon's history of war and poor environmental stewardship, Lebanese men in this study, both fertile and infertile, demonstrated blood levels of heavy metals well within the acceptable range for normal human populations. These findings are supported by recent evidence of low dietary exposure to heavy metals in the country [61]. Together, these studies suggest that exposure to toxic metals is, in fact, limited in Lebanon—a bit of good news for both fertile and infertile men in an environmentally degraded region of the world.

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