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Associations of Semen Quality with Seminal Non-essential Heavy Metals in Males from the Canary Islands

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Abstract

Semen quality and levels of non-essential metals such as strontium (Sr), aluminum (Al), lead (Pb), nickel (Ni), and vanadium (V) were measured. Metals were determined by ICP-OES (inductively coupled plasma – optical emission spectrometry) in semen samples from 102 men who were recruited in a Reproduction Unit in the Canary Islands. The presence of each metal was as follows: Sr: 56.9%, Al: 73.5%, Pb: 45.1%, Ni: 15.7%, and V: 79.4% of the samples. No significant differences were found in the relationship between the spermiogram, the sperm motility, and the concentration of spermatozoa levels of non-essential metals. It is noteworthy that Ni levels tend to be lower in patients with oligozoospermia ($t\,(46.4) = 1.84$; $p = 0.070$). Between lifestyle and non-essential metals, there was a significant relationship between the level of occupational exposure to metals and Ni ($\chi^2(2) = 13.91$; $p = 0.001$). We did not find significant differences in non-essential seminal metal content and smoking status but, there were differences between drinkers and the concentration of V in semen ($t\,(100) = -1.99$; $p = 0.050$). The occupational exposure to metals and place of residence have effects on Al and V levels in semen. Regarding obesity, significant differences were found in Pb levels ($t\,(18.0) = 2.34$; $p = 0.031$). Obese patients have a lower Pb level, and the percentage of progressive sperm motility was lower in obese men ($t\,(98) = 2.14$; $p = 0.035$). The detection of metals in semen opens a new field in the study of male infertility with the possibility of performing treatments aimed at correcting these possible anomalies.

Keywords Semen · Non-essential metal · Metal exposure · Male infertility · Body mass index

Introduction

The quality of human semen has been declining in the last few decades and some of the possible explanations include stress; lifestyle factors; toxic habits such as alcohol intake, tobacco, and drug abuse; and also exposure to chemicals such as pesticides and metals [1, 2]. Therefore, the study of exposure to toxic metals is interesting due to its adverse effects on human health such as degenerative diseases, endocrine disruption, and infertility [2]. Several metals have essential biological functions, but others are not necessary for living organisms and their presence can increase the formation of reactive oxygen species (ROS), decrease glutathione and other antioxidant levels, and increase lipid peroxidation of cell membranes [3], inducing DNA damage and spermatozoa apoptosis [3, 4].

Human spermatozoa are especially vulnerable to oxidative stress (OS), and when there is excessive sperm production of ROS in semen, leading to OS, a peroxidation of polyunsaturated fatty acids occurs in their plasme membrane. When the sperm cell membrane is damaged, this affects its motility and the ability to fertilize the oocyte [5, 6]. Humans are exposed to non-essential metals such as strontium (Sr), aluminum (Al), lead (Pb), nickel (Ni), and vanadium (V), which can accumulate in the male reproductive organs affecting the fertility and sperm parameters [7–12].

The accumulation of Sr can occur by air, by food, water, or accidentally ingesting soil. Alterations in bone development have been observed in children who consume or drink high levels of Sr, especially if their diet is low in calcium [7, 8]. Al is the third most abundant element in the world; it is present in

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food and in many pharmaceutical and skin products [9]. Giaccio et al. [10] measured semen volume, pH, sperm concentration and motility, total sperm count, and sperm Al levels in 600 infertile men. No correlation was found with decreased fertility.

Other publications showed higher levels of Al concentration although without significant differences between the azoospermic, oligozoospermic, and normozoospermic groups [11].

Pb is an important environmentally toxic metal that can cause reproductive harm and can accumulate in the male reproductive organs causing infertility [12]. Quintanilla-Vega et al. [13] showed that Pb can compete with Zn in human P2 protamine, which protects sperm DNA during spermatogenesis, affecting the integrity of sperm chromatin and thereby reducing the ability to fertilize the oocyte and causing DNA damage, resulting in abnormal spermatogenesis [11, 14]. Telisman et al. [15] noted that it has an adverse effect on motility, DNA integrity, and morphology, particularly at the sperm head [16].

V is a transition metal that is ubiquitously present in the soil, water, and the atmosphere due to natural processes and human activities. It is estimated that approximately 2.3×10^6 tons of V are introduced into the environment annually. The general population is exposed through ingestion of contaminated food, drinking water, and inhalation of suspended particles [17] and is found in seminal plasma which damages sperm DNA [18].

Finally, Ni exposure in humans occurs through inhalation, dermal contact, and gastrointestinal ingestion and is known to cause nasal and lung cancers in refinery workers [19]. Some reports have suggested that it causes a significant decrease in testicular weight and attached sex organs and a reduction in the activity of steroidogenic enzymes. All of these reproductive dysfunctions are involved in a mechanism of OS [20, 21] and, therefore, in infertility, since it can affect male reproductive function by separating the flagellum from the head of the sperm [22].

The relationship between exposure to non-essential metals and seminal quality is not yet clear. For this reason, the study of metal content in semen samples and its influence on the seminal parameters is interesting. Thus, the main objective of this study was to determine the presence and concentration of non-essential metals (Sr, Al, Pb, Ni, V) in human seminal plasma and their relationship with sperm quality and toxicological effects, as well as to establish possible correlations with occupational exposure, body mass index (BMI), or lifestyle habits such as alcohol or tobacco consumption.

Material and Methods

Samples

A prospective study was performed on 102 patients who were recruited in a Reproduction Unit in Tenerife, Canary Islands. At that point, we were not aware of the male infertility factor, as part of an epidemiologic study of environmental contaminants and male reproduction. Patients were admitted to the study either by infertility of one or another, or both, which is why this group includes fertile men and a variable range of infertility problems, including female infertility [23].

The study was carried out between February and April of 2018, from patients residing in the Canary Islands.

The 102 male samples correspond to the number of patients who attended successively, for the initial evaluation during this period. The male mean age was 38.03 ± 5.70 years and ranged between 25 and 52 years, all were Caucasian and with a mean BMI of 26.41 ± 4.29 kg/m^2, 54% of the men were overweight (BMI between 25 and 29.9 kg/m^2), and 10% were obese (BMI ≥ 30.0 kg/m^2).

The approval of the Ethical Committee of the Hospital Universitario de Canarias was obtained, and all participants signed an informed consent form.

All patients were interviewed by the same professional and completed a questionnaire on current occupational and environmental exposures; place of residence; lifestyle; toxic habits such as smoking and/or alcohol consumption and drug abuse; and BMI.

Tobacco consumers were categorized into three levels: non- smoker, light smoker (less than 10 cigarettes per day (CPD)), and heavy smoker (more than 10 CPD). Light smoking has the widest set of definitions, ranging from “refusing to smoke regularly” and “denied any smoking within the past 30 days” to smoking 1 to 39 cigarettes per week and smoking 10 CPD for most authors [24]. According to these authors, we have chosen the cut-off point of 10 CPD as the limit between light and heavy consumption.

Alcohol consumers were divided into three groups: no consumption, light consumption (regular or occasional drinker during the weekend), and heavy consumption (6 or more standard drinks) [25].

Occupational exposure was classified into three categories: no exposure, intermediate, and high exposure to metals as shown in (Table 1).

All subjects had different exposure to metals, and they all lived in the Canary Islands. Of the total, 33% of men were light or heavy smokers and 66% were occasional alcohol consumers on weekends or every day. Only two of them acknowledged using drugs.

The semen samples were obtained by masturbation, and the collected samples were used for both semen analysis and metal detection. The mean sexual abstinence was 3.84 ± 6.43 days, with a range between 0 and 10 days.

The participants were categorized into two groups, according to the results of semen analysis following the World Health Organization (WHO) guidelines [26]: the normal quality semen group and the pathological semen group. The study included 41 patients with pathological spermiogram (40.2%),

Table 1 Classification of occupations according to metal exposure

Classification	Description
No exposition	Healthcare professional (medical, optician), animal assistant, businessperson (salesperson, shop assistant, sales representative, cashier), lawyer, student, bartender, police officer, teacher, unemployed, receptionist, security personal, computer specialist
Intermediate exposition	Lift technician, taxi, car or ambulance driver, courier, chef, soldier, artisan
High exposition	Factory worker, carpenter (wood, aluminum), car engineer, electrician, welder, sailor, farmer, painter, plumber, builder, garbage collector, cleaner

while the remaining 61 had a normal spermiogram (normozoospermia) (59.8%) and constituted the control group.

Treatment of the Samples

Semen parameters such as sperm concentration and motility were analyzed and carried out using a Makler® counting chamber (Irvine Scientific, CA). The study of seminal parameters should be performed following the WHO guidelines [26].

The metal detection was implemented in each seminal sample and the seminal volume had to be at least 0.8 ml. The semen samples were digested with the Multiwave GO Digestion System (Anton Paar). Of each semen sample, 0.5 ml was introduced into teflon reactors with 2 ml of hydrogen peroxide (H_2O_2) and 4 ml of nitric acid (HNO_3). The temperature–time program used for the microwave digestion was as follows: 10 min at 70 °C; 20 min at 180 °C; and 15 min at 50 °C.

The result of the digestion was poured into a flask to a total volume of 10 ml with distilled water. The solutions were transferred to polyethylene vials and stored in the absence of light and at a cool temperature until the analysis, and the determination of metals was performed. Seminal fluid has been weighed and refers to milligrams per kilogram [27]. The metal content (Sr, Al, Pb, Ni, V) was determined using inductively coupled plasma optical emission spectrometry (ICP-OES), a reference technique for the determination of metals with high sensitivity and reproducibility of the results [28].

The instrumental conditions of the ICP-OES were gas flow (nebulization gas flow and auxiliary gas flow), 0.5 l/min; approximate radio frequency power, 1150 W; and pump flow (stabilization flow and analysis flow), 50 rpm. The metal concentration was calculated by extrapolation of the absorbance read in previously calibrated curves made from standard solutions of different concentrations for each element. The instrumental limits of detection and quantification were estimated based on the instrumental response of the devices. Specifically, it was decided by analysis of 15 blanks under reproducibility conditions [29].

The detection and quantification limits for each level of seminal metal are shown in Table 2. Regarding the data obtained, the dilution factors of the sample have been taken into account; it should be noted that these calculations are not detailed in the article as they are not of interest.

Data Analysis

The statistical analysis was performed with SPSS vs. 21 (IBM, 2012). To study the possible relationships between concentrations of different metals, correlation coefficients were calculated. To study if there is a relationship between categorical variables (spermiogram, concentration, motility, occupational exposure to metals, residence, tobacco, alcohol, and BMI) and metal levels or percent of sperm motility and concentration, ANOVAs (more than two levels) and t test (dichotomous) were performed. Chi-square tests of variable independence were performed to study the relationship between categorical variables and presence/absence of metals. A value of $p \leq 0.05$ was considered significant.

Results

The results showed that 59.8% of the patients in our study had normozoospermic semen, while 40.2% were pathological. Furthermore, within the pathological group, 1% had azoospermia, 5.9% cryptozoospermia, 6.9% severe oligoasthenozoospermia, 2.9% oligoasthenozoospermia, 12.7% oligozoospermia, and 7.8% astenozoospermia. The percentage of patients with the presence of each metal was as follows: Sr: 56.9%, Al: 73.5%, Pb:

Table 2 Detection (DL) and quantification limit (QL) of metals studied

Metal/wave-length	DL (mg/kg)	QL (mg/kg)
Al (167.0 nm)	0.033	0.100
Ni (231.6 nm)	0.006	0.025
Pb (220.0 nm)	0.003	0.008
Sr (407.7 nm)	0.006	0.025
V (310.2 nm)	0.008	0.042

45.1%, Ni: 15.7%, and V: 79.4%. The analysis of association (Pearson's correlation) between metal levels in semen samples shows a positive correlation between Al and Ni ($r = .458$; $p = 0.000$), Al and Pb ($r = .218$; $p = 0.028$), and Pb and Sr ($r = .334$; $p = 0.001$). A negative correlation between Al and Sr was observed ($r = -.272$; $p = 0.006$).

When the relationship between spermiogram, sperm motility, and concentration with metal levels is analyzed, no significant differences were found. It is noteworthy that Ni levels tend to be lower in patients with oligozoospermia (t (46.4) = 1.84; $p = 0.070$) (Table 3).

Using ANOVAs (3 × 3) with occupational exposure to metals and place of residence as factors, an interaction was detected between both factors for Al (F (4,92) = 2.54; $p = 0.045$). Among patients living in metropolitan areas, there was a significant difference in Al concentration, depending on the level of occupational exposure to metals: patients with high exposure had a higher Al concentration (5.9110 ± 4.2367) than patients who have jobs with some (0.3241 ± 0.2076; $p = 0.040$) or no exposure (1.2686 ± 2.9236; $p = 0.010$); on smaller islands, patients with high metal exposure show higher Al (7.3160 ± 4.6790) than patients with mild exposure (1.3235 ± 3.0397; $p = 0.047$)) (Fig. 1).

The place of residence had an effect for V, present in 79.4% of the samples (F(2,92) = 3.44; $p = 0.036$), and patients residing on the smaller islands (<100.000 inhabitants) (0.6309 ± 0.3749) show higher levels than patients residing in Tenerife (1×10^6 inhabitants), either in metropolitan areas (0.4192 ± 0.3653; $p = 0.06$) or in the periphery (0.4023 ± 0.3557; $p = 0.05$) (Table 4).

When the presence/absence of metals is analyzed, a significant relationship was detected between the level of occupational exposure to metals and Ni ($\chi^2(2) = 13.91$; $p = 0.001$). In patients with high occupational exposure, the percentage of Ni in semen (36.7%) is higher than expected (15.8%). In addition, patients ($\chi^2(2) = 6.79$; $p = 0.033$) with high occupational exposure to metals show Sr less frequently in semen (36.7% versus 56.4% expected).

When the relationship between metals and smoking and non-smokers versus smokers (light and heavy smokers) is analyzed, no differences were observed in any of the studied metals (Table 5). According to the patients consuming alcohol (64%) or not, significant differences were found in the concentration of V in semen (t (100) = −1.99; $p = 0.050$). The mean concentration of V in semen is higher in patients who consume alcohol (0.51 ± 0.40 mg/kg) than in abstemious (0.35 ± 0.30 mg/kg) (Table 6).

When obese versus non-obese men were compared, no differences were found in non-essential metal levels (Table 7). On the other hand, significant differences in Pb levels were found (t (18.0) = 2.34; $p = 0.031$). Obese patients present a lower level of Pb (0.0071 ± 0.0745) than normozoospermic patients (0.0211 ± 0.0313). Significant differences were found (t(98) = 2.14; $p = 0.035$) in the percentage of progressive sperm motility, with obese patients having lower sperm motility (35.90 ± 20.08) than normal sperm motility patients (50.98 ± 21.24) (Table 7).

Discussion

The present study has allowed us to know the percentage of each metal present in male semen samples in our environment, the Canary Islands, and, in addition, to know the relationship between the seminal levels of non-essential metals and semen quality as well as the relationship between the lifestyle habits and metal exposure.

Among the non-essential metals, V and Al were found to be the most frequent, followed by Sr and Pb, and, to a lesser percentage, Ni.

In more than half of the patients (56.9%), the presence of Sr was detected in seminal plasma. Bachmann et al. [30], regarding sperm incubation medium, replaced calcium (Ca) with Sr and found no differences in the results of sperm capacitation. Furthermore, Sr in the blood was associated with low testosterone levels, which could affect spermatogenesis [31], but we did not find any relationship between seminal Sr levels and semen quality.

Almost all patients have Al in their semen (73.6%). At high concentration levels, Al has a negative impact on serum testosterone levels and seminal parameters in rats [32]. It appears that its toxicity may be related to oxidative stress and nitric oxide production [33]. However, in our study, no effect was

Table 3 Mean metal levels (mg/kg) as a function of spermiogram

Sperm parameters		Al	V	Ni	Sr	Pb
Spermiogram						
Norm (n= 61)	Mean	2.452	0.445	0.042	0.068	0.020
	SD	4.202	0.376	0.191	0.089	0.034
Pathol (n= 41)	Mean	2.913	0.473	0.025	0.728	0.018
	SD	4.235	0.377	0.072	0.101	0.024
*t (df)		−0.541 (100)	−0.367 (100)	0.544 (100)	−0.223 (100)	0.358 (100)
p value		0.589	0.714	0.587	0.824	0.721

*Student t for two independent groups

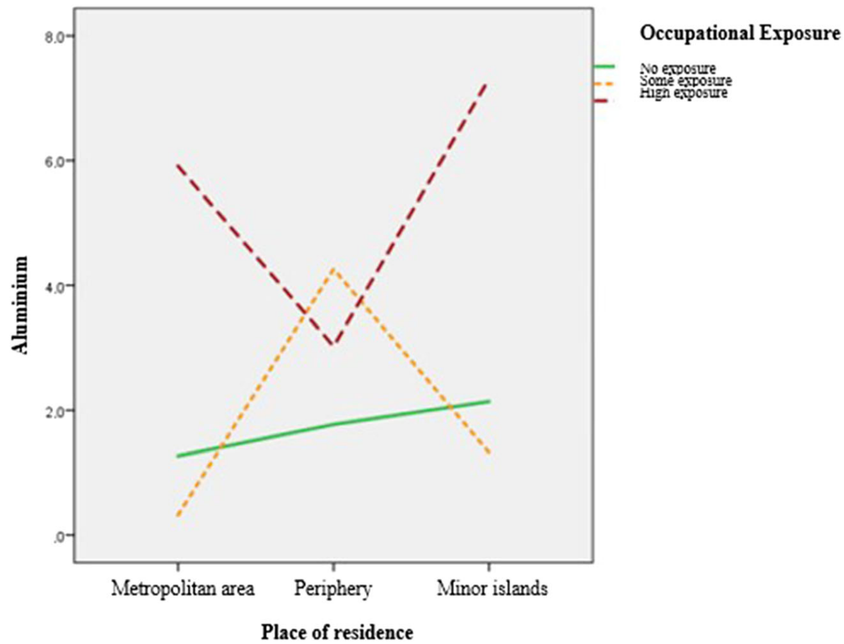

Fig. 1 Aluminum concentration (mg/kg) related to residence and occupational exposure

observed between the studied sperm parameters and Al levels as Zafar et al. [11].

Close to half of our seminal samples have Pb (41.5%). No relationship was found with sperm parameters and Pb levels in semen, unlike other authors [16], who concluded that a blood Pb level of > 40 μgr/dl produces a low semen volume and sperm count, an increase in abnormal sperm morphology, and a decrease in sperm motility [16]. Gidlow et al. [34] obtained significant effects on sperm morphology. Mendiola et al. [35] observed a significant positive association between seminal Pb and low sperm motility [36] and reported high mean Pb values in azoospermic subject (71 μg/l) followed by normozoospermic (27.95 μg/l) and oligozoospermic subjects (11.8 μg/l).

In our study, only 15.7% (16 of the 102 men) registered Ni levels in their semen sample. It is worth pointing out that in our oligozoospermic samples, there is a tendency to have lower Ni levels, but without significant differences. Bian et al. [37] refer to a decrease in Ni levels in the abnormal motility group compared to the normal control.

These authors postulate that Ni deficiency is a factor that acts by reducing sperm activity. In agreement with this, a lower Ni level was found in samples with pathological sperm concentration, as other authors [38], who had found low Ni concentration in poor sperm quality. On the other hand, an excess of Ni also impairs reproductive performance in animals due to Ni-induced OS [23]. Ni is known to interfere with the interaction of protamines with DNA by altering the functional structure of protamines, specifically P2, leading to changes in DNA structure and the appearance of oxidation products that are pro mutagens [38]. Zafar et al. [11] also found a negative correlation between Ni levels in seminal plasma and sperm concentration and motility.

Regarding V concentration, no differences were observed in any of the seminal parameters analyzed. Wang et al. [39] concluded that seminal plasma V levels were associated with an increased risk of lower sperm concentrations and sperm DNA damage as impaired altered reproductive hormones, as Zafar et al. [11], who demonstrated that V is negatively correlated with sperm concentration and motility.

The correlation between metal levels in semen samples shows a positive correlation between Al and Ni, Al and Pb, and Pb and Sr, and a negative correlation between Al and Sr. The analysis of Slivkova et al. [40] did not show a correlation between Pb and Ni, like our study, but they did not include the study of Al, V, or Sr. Other authors did not find the same correlations as us, and they believe that such trend may indicate a common source of the origin for these metals [11].

Finally, regarding the relationship of metals with lifestyle habits, we first analyzed the environmental exposure to metals; so regarding Sr, we believe that it is very important to prevent vulnerability to metals since Sr is traceable at higher levels among men with intermediate-level occupation exposure.

On the other hand, a positive relationship was found between Al concentration and occupational exposure to metals. Men with higher occupational exposure to Al have a higher concentration of Al in semen than patients with little or no contact with metals, which is consistent with other studies [41].

An increase in Pb levels related to alcohol or tobacco consumption and occupational exposure was not found, and our

Table 4 Mean metal levels (mg/kg) as functions of occupational exposure to metal and residence

Occupational metal exposure	Residence		Al	V	Ni	Sr	Pb	%Sperm motility	Sperm concentration mill/ml
No	Metropolitan area	Mean	1.2686	0.4941	0.0161	0.0690	0.0243	51.00	55.76
	(*n* =26)	SD	2.9236	0.4096	0.0608	0.0874	0.0407	22.72	69.28
	Periphery	Mean	1.7726	0.3970	0.0000	0.0693	0.0175	48.36	32.01
	(*n* =11)	SD	3.4762	0.3133	0.0000	0.0792	0.0286	29.70	31.94
	Smaller islands	Mean	2.1404	0.5259	0.0217	0.0922	0.0094	41.22	31.61
	(*n* =9)	SD	5.6327	0.4155	0.0651	0.0979	0.0152	18.46	32.64
	Total	Mean	1.5597	0.4771	0.0133	0.0736	0.0198	48.46	45.35
	(*n* =46)	SD	3.6334	0.3846	0.0536	0.0862	0.0343	23.61	56.80
Mild	Metropolitan area	Mean	0.3241	0.4109	0.0000	0.1093	0.0232	52.80	105.80
	(*n* =5)	SD	0.2076	0.2820	0.0000	0.0963	0.0319	12.76	100.74
	Periphery	Mean	4.2623	0.3705	0.1286	0.1016	0.0316	49.92	57.27
	(*n* =12)	SD	4.8210	0.3723	0.4202	0.1171	0.0360	16.93	54.25
	Smaller islands	Mean	1.3235	0.7112	0.0000	0.0317	0.0125	41.63	74.26
	(*n* =8)	SD	3.0397	0.4658	0.0000	0.0358	0.0157	27.91	89.04
	Total	Mean	2.5342	0.4876	0.0617	0.0808	0.0238	47.84	72.41
	(*n* =25)	SD	4.0440	0.4060	0.2919	0.0969	0.0301	20.15	75.50
High	metropolitan area	Mean	5.9110	0.2399	0.0509	0.0585	0.0191	55.56	59.22
	(*n* =9)	SD	4.2367	0.1947	0.0529	0.1278	0.0236	22.79	42.23
	Periphery	Mean	3.0255	0.4280	0.0415	0.0407	0.0126	48.24	43.98
	(*n* =17)	SD	4.6050	0.3877	0.0691	0.0802	0.0202	16.72	39.06
	Smaller islands	Mean	7.3160	0.7065	0.0801	0.1196	0.0257	47.00	32.50
	(*n* =4)	SD	4.6790	0.1082	0.1602	0.1403	0.0380	26.69	38.39
	Total	Mean	4.4632	0.4087	0.0495	0.0566	0.0163	50.27	47.03
	(*n* =30)	SD	4.6799	0.3404	0.0789	0.1038	0.0235	19.59	39.58
Total	Metropolitan area	Mean	2.1951	0.4265	0.0219	0.0717	0.0230	52.25	62.79
	(*n* =40)	SD	3.6569	0.3669	0.0568	0.0971	0.0359	21.40	68.97
	Periphery	Mean	3.0520	0.4023	0.0562	0.0668	0.0197	48.78	44.68
	(*n* =40)	SD	4.3894	0.3557	0.2331	0.0937	0.0284	20.55	42.57
	Smaller islands	Mean	2.8150	0.6309	0.0246	0.0744	0.0137	42.48	48.03
	(*n* =21)	SD	4.9351	0.3943	0.0800	0.0923	0.0208	22.82	62.18
	Total	Mean	2.6634	0.4594	0.0360	0.0703	0.0197	48.84	52.55
	(*n* =101)	SD	4.2135	0.3753	0.1549	0.0939	0.0303	21.46	58.36
Occupational exposure	*F(2,92)		44.16	0.06	0.63	0.04	0.21	0.16	3.37
	p value		0.002	0.938	0.537	0.962	0.809	0.850	0.039
Residence	**F(2,92)		6.46	3.44	0.40	0.10	0.24	1.08	2.16
	p value		0.666	0.036*	0.673	0.902	0.786	0.344	0.121
Interaction	***F(4,92)		2.54	1.06	1.00	1.47	0.63	0.08	0.24
	p value		0.045*	0.380	0.411	0.218	0.644	0.989	0.915

*ANOVA 3 × 3 (exposure and residence) main effect occupational exposure

**ANOVA 3 × 3 (exposure and residence) main effect residence

***ANOVA 3 × 3 (exposure and residence) interaction

result could show the average exposure of the male population living in the Canary Islands.

A significant level of correlation between Ni and occupational exposure to metals was found. Patients with high occupational exposure have a significant increase in Al and Ni and less in Sr.

In addition, a statistical significance to have higher V levels was found in alcohol drinkers. It is a fact that in the present study, strict data were not collected on the type of drink or dose consumed, but in the Canary Islands, it is a very common use to consume local wine. Nevertheless, no correlation was found with sperm quality.

Table 5 Metal levels (mg/kg) as a function of smoking

A		Al	V	Ni	Sr	Pb	%Sperm motility	Sperm concentration mill/ml
Heavy (n =14)	Mean	3.5352	0.5110	0.1120	0.0320	0.0308	54.29	73.51
(>10 cig/day)	SD	4.5815	1.0398	0.3892	0.0480	0.5127	20.19	79.05
Light (n =20)	Mean	2.0535	0.4788	0.0075	0.0749	0.0153	51.30	59.59
(≤10 cig/day)	SD	3.7698	0.4263	0.0231	0.0988	0.3079	18.79	60.99
No (n =68)	Mean	2.6240	0.4381	0.0283	0.0767	0.0185	47.40	46.12
	SD	4.2696	0.3556	0.0692	0.0979	0.0237	22.52	51.65
Total (n =102)	Mean	2.6372	0.4922	0.0357	0.0702	0.0195	49.11	52.52
	SD	4.2009	0.5199	0.1542	0.0934	0.0302	21.52	58.07
*F(2,99)		0.51	0.26	2.18	1.37	1.22	0.72	1.49
p value		0.603	0.771	0.119	0.259	0.301	0.489	0.230

*One-way ANOVA for three groups of smoking behavior

Geographical variations in semen parameters can also be influenced by environmental factors such as pollution or climate [42], and this may explain the greater presence of V in the semen of patients living on the smaller islands, which could be related to the presence of this metal on the seabed near the smaller islands.

On the other hand, consumption of alcohol, cigarettes, and BMI were recorded. Excessive alcohol consumption can lead to decreased testosterone production and atrophy of the tests leading to impotence, infertility, and a reduction in male secondary sexual characteristics. In this sense, a reduction in normozoospermic sperm is reported among alcoholics [43], with a decrease in normal morphology and sperm count. But, contrary to the expected, the present study did not find a correlation between alcohol consumption and sperm parameters, but rather a notable trend was found in the group of alcohol consumers, where V levels were higher than those in the non-alcohol consumer group.

The analyses of tobacco consumption was also an objective of the present study, considering that the high concentrations of metals and other toxic elements contained in cigarettes are known as was extensively determined by Rubio et al. [44]. Although cigarette smoking is a hazardous cause of toxicity and sperm damage, we were unable to find a correlation between tobacco consumption and sperm quality as other authors did [45].

The sperm count among heavy smokers ($50 \times 10^6 \pm 20$/ml) and light smokers ($76 \times 10^6 \pm 50$/ml) was significantly lower compared to that in non-smokers ($130 \times 106 \pm 50$/ml). Among non-smokers, most patients were normozoospermic (60.3%) [45]. Gaur et al. [43] state that sperm motility deteriorated much more rapidly in heavy smokers compared to controls. But one of the main differences in the present study is that heavy smokers were those individuals who consumed 10 or more cigarettes per day as Husten et al. [24], and the research conducted by other authors [43], considered heavy smokers above 41 cigarettes per day. It is possible to think that we could find more similar findings if we compare the results by adjusting the smoking level.

One of the factors that presented a clear influence on semen quality was BMI. Niehoff et al. [46], in a prospective study not in males, but in females, found that essential metals were

Table 6 Metal levels (mg/kg) as a function of alcohol consumption

Alcohol consumption	Al	V	Ni	Sr	Pb	%Sperm motility	Sperm concentration mill/ml
No media (n =35) SD	3.0251 4.6436	0.3555 0.3007	0.0196 0.0482	0.0968 0.1230	0.0188 0.0231	48.63 21.60	52.74 51.24
Yes media (n =67) SD	2.4346 3.9719	0.5087 0.4004	0.0441 0.1870	0.0564 0.0706	0.0199 0.0334	49.36 21.64	52.41 61.71
Total media (n =102) SD	2.6372 4.2009	0.4922 0.5199	0.0357 0.1542	0.0702 0.0934	0.0195 0.0102	49.11 21.52	52.52 54.07
**t (df)* *p* value	0.67 (100) 0.503	−1.99 (100) 0.050*	−0.76 (100) 0.450	1.78 (100) 0.079	−0.18 (100) 0.860	−0.16 (100) 0.872	0.03 (100) 0.978

*Student t for two independent groups

Table 7 Metal levels (mg/kg) as a function of body mass index (BMI)

BMI	Al	V	Ni	Sr	Pb	%Sperm motility	Sperm concentration mill/ml
≥30 media (*n* =10) SD	2.9640 4.4079	0.3673 0.4250	0.0350 0.0904	0.0724 0.0745	0.0071 0.0159	35.90 20.08	23.05 27.35
<30 media (*n* =90) SD	2.5630 4.1899	0.4732 0.3705	0.03474 0.1611	0.0706 0.0963	0.0211 0.0313	50.98 21.24	56.12 60.27
Total media (*n* =100) SD	2.6031 4.1908	0.4995 0.5222	0.0347 0.1552	0.7078 0.0940	0.0197 0.0304	49.47 21.51	52.81 58.59
**t (df)* *p* value	−0.29 (98) 0.776	0.85 (98) 0.400	−0.01 (98) 0.996	−0.06 (98) 0.954	2.34 (18.0) 0.031	2.14 0.04	1.710 0.091

*Student *t* for two independent groups

jointly associated with a lower BMI, while non-essential metals were associated with a higher BMI. However, in our study, no differences were found regarding the levels of non-essential metals between both groups, but we did not find studies in men.

Non-essential metals have been shown to increase OS, which could be associated with alteration of seminal parameters and the presence of a higher BMI [3, 20, 21, 46]. This is consistent with our findings: obese men showed a significant reduction in sperm concentration and motility, with statistical differences in the percentage of progressive sperm concentration and motility, as other authors concluded [47].

Pb levels in semen were lower in obese males. Cabler et al. [47] published that most environmental toxins are fat-soluble and therefore accumulate in fat tissue and Freire et al. [27], in their study, underscore the relevance of adipose tissue as a potential target organ for the toxic metal. That confirms the importance of a healthy diet and exercise habits to maintain an adequate BMI for reproductive health.

Conclusions

The percentage of males from the Canary Islands with the presence of each of the non-essential metal was as follows: Sr:56.9%; Al: 73.5%; Pb: 45.1%; Ni: 15.7%; and V: 79.4%. No relationship was found between spermiogram, sperm motility, and concentration with metal levels, although Ni levels tend to be lower in patients with oligozoospermia.

The occupational exposure factor has a significant effect on sperm concentration. Patients with occupational exposure to metals have a lower sperm concentration. There was a significant relationship between the level of occupational exposure to metals and Ni and an increase in Al levels in the semen of workers with a high occupational exposure. In addition, occupational exposure to metals and place of residence have some effects on Al and V levels in semen.

No significant differences were found in seminal non-essential metals content or smoking status but there were differences between drinkers and V concentration in semen.

The main influencing variable found to affect sperm parameters was BMI, highlighting obesity as a detrimental factor for sperm quality. Significant differences were found in Pb levels, obese patients had a lower Pb level, and the percentage of progressive sperm motility was lower in obese men.

The detection of metals in semen opens a new field in the study of male infertility, with the possibility of performing treatments aimed at correcting these possible anomalies. Therefore, it is necessary to study the influence that other heavy metals may have on the quality of semen.

Declarations

Conflict of Interest The authors declare no competing interests.

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