

High Exposure to Boron in Drinking Water and Sperm Parameters in Chilean Young People

Exposición Elevada a Boro en el Agua Potable y Parámetros de Esperma en Gente Joven Chilena

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SUMMARY: Our aim was to describe sperm parameters in residents from Northern Chile. We evaluated in 101 volunteers (18 and 30 years old) urinary and drinking water Boron levels using Inductively Coupled Plasma Mass Spectrometry; semen parameters were measured with standardized methods. Each individual was categorized in 3 levels of exposure: low (B levels in urine ≤ 2.94 mgL⁻¹ or tap water ≤ 3.0 mgL⁻¹), medium (urinary B between 2.95-7.4 mgL⁻¹ and B in tap water with 3.0-7.0 mgL⁻¹) and high (urinary B > 7.4 mgL⁻¹ or tap water > 7.0 mgL⁻¹). We found no significant differences among groups by pH, sperm concentration (45.1; 48.2 and 38 million/mL), motility 1th hour (38.1; 40.0 and 45.5 %) and vitality 1th hour (88.6; 88.0 and 76.9 %) respectively. Abnormal morphology was significant different (83.3; 90 and 83 %). Young men exposed to B in drinking water present sperm variations associated with the level of exposure. Most of these changes are positive at intermediate levels of B. For the highest exposures were observed negative changes in sperm morphology, concentration, motility and vitality, all relevant parameters of fertility. Beneficial effect is observed at medium exposure, like a "U curve".

KEY WORDS: Boron, Semen, Seminal Parameters, Fertility, Boron exposure, Chile

INTRODUCTION

There is growing evidence that semen parameters can be altered by different chemicals, environmental pollutants, including pesticides and metals (Giudice, 2006; Hauser, 2006; Xing *et al.*, 2008; Sifakis *et al.*, 2011; Telöken *et al.*, 2012). The main semen parameters that have an important role in male fertility and are affected with various environmental exposures are sperm concentration, motility and morphology (Esteban & Castaño, 2009; Jurewicz *et al.*, 2009).

Special interest exists with respect to Boron and its role in reproduction. Boron is an essential trace element for animals (Nielsen, 1984; Murray, 1998). Evidence states that it helps control inflammatory processes in humans and substantially participates in the absorption of calcium in bone (Usuda *et al.*, 2007). When high Boron doses of up to 80 mg/kg/day were administered to animal models, adverse effects such as anorexia, weight loss, altered bones, testicular atrophy and epididymal sperm reduction were observed,

especially in mice (Rose & Cohrssen, 2010; Korkmaz *et al.*, 2011). Either mutagenic or carcinogenic effects from exposure to this element in human normal activities have been found (Fail *et al.*, 1998; Korkmaz *et al.*, 2007a).

Population-based studies that have measured exposure to Boron and health effects at the population level are scarce and their findings have been inconclusive (Weinthal *et al.*, 2005). They are made in USA, Turkey and China, principally in workers. Studies in male Turkish population exposed to a Boron dose of 6.7 mg/day did not find significant alterations in male fertility (Korkmaz *et al.*, 2007b; Scialli *et al.*, 2010). Studies relating Boron with semen parameters finds no correlation between Boron in urine, blood and semen parameters, although there was a non-significant increase in the percentage of normal sperm morphology in workers exposed to Boron versus a control population (Robbins *et al.*, 2010). Similar observations have been reported in Turkish workers (Duydu, *et al.*, 2011).

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Other effects in human populations have been described in Chinese workers exposed to Boron concentrations from 31.3 to 125 mg/day, reporting no reproductive changes in wives of exposed workers but showing a reduction in the Y:X ratio in sperm of exposed workers (Scialli *et al.*) Similar findings were reported by Robbins who determined a decrease of the Y chromosome-bearing cells, thus causing changes in sex ratio in the offspring towards more female sex individuals (Robbins *et al.*, 2008).

Nowadays it has become relevant to study the health effects of environmental Boron exposure in populations. In this regard, Northern Chile (13° 28' S, 70° 20' W) is a region naturally rich in Boron. This region is bordered to the North by Peru, to the West by the Pacific Ocean, to the East by Bolivia and to the South by the Antofagasta Region and has a population of 175,603 inhabitants. The natural sources of water for the city are the Lluta River, which rises in the Andes and flows into the Pacific Ocean, and the wells of the Azapa Valley. These two bodies of water are the only source of drinking water for the city. The community of Arica has expressed concern about possible health damage caused by high levels of boron in their drinking water that presently exceeds the levels recommended by the World Health Organization (WHO, 1993). In response to the need to create a regulatory standard for boron in drinking water, the Ministry of Health of the Republic of Chile commissioned the project titled “Assessment of Boron Exposure in Drinking Water and its Effects on Health in the Municipality of Arica” and has two parts. The first component study tests exposure to Boron in drinking water, food and urine. High levels of this element in tap water ranged from 0.22 to 11.3 mgL⁻¹ while Boron in urine ranged from 0.45 to 17.4 mgL⁻¹ (Cortés *et al.*, 2011). Results no published on B in more frequently used foods shows values between 0.01 and 22 mgKg⁻¹; according our data mean daily boron intake was principally due by drinking water (60 %).

The second component evaluates health effects on reproductive health. This manuscript reports the results of second component focused on the effects in health in young males; we are working in the results of effects in 3000 pregnant females and their newborns. Our objective was to explore an association between urinary or tap water Boron levels and semen parameters of a group of healthy young males, volunteers and residing in Arica, Chile.

MATERIAL AND METHOD

Selection of participants. The study was conducted as part of a study funded by the Chilean Ministry of Health. The objective was to evaluate the health effects of exposure to Boron in tap water. Young men were invited to attend to assess their sperm quality. The target group was 400 young men aged 18 to 30 years, enrolled at the University of Tarapacá, Arica, Chile. A convenience sample was set at 100 subjects (25 % of the group), plus 10 % to cover possible losses. Selection criteria to enter into the study were to reside in Arica for at least 6 months, to be healthy and to have four days of sexual abstinence. We excluded subjects who reported having any andrological health problem, i.e. prior cryptorchidism and varicocele that may affect the semen analysis. A total of 101 eligible participants were entered in the study. All the participants that were invited accepted to participate. Each young man was informed of the nature of the study and asked to sign an informed consent. Those who accepted were instructed to take a morning first void urine sample and to bring a tap water sample from their houses; details of this method have been previously reported. They brought the samples to the Laboratory of Reproductive Biology of the University of Tarapacá, where they answered an epidemiological questionnaire about demographics aspects, residence, health history, occupational and reproductive history, smoking, use of alcohol, tap water and

Table I. Urinary or tap water Boron levels and general characteristics in voluntary participants, Arica, Chile.

Characteristics	Low exposure (mgL ⁻¹) B Urine ≤ 2.94 or B tap water ≤ 3.0 n=21		Medium (mgL ⁻¹) B Urine 2.95-7.4 and B tap water 3.0-7.0 n=41		High (mgL ⁻¹) B in urine 7.4- 17.4 or B tap water 7 - 11.0 n=39			Total n=101		
	n	%	n	%	n	%	n	%	P-value	
Drink alcohol (Yes)	16	76,2	34	82,9	29	74,4	79	78,2	0,63	
Drugs consumption	8	40	18	46,2	18	46,2	44	44,9	0,77	
Smoking at least 6 months	12	60	22	55	15	38,5	49	49,5	0,19	
Have children	1	25	3	37,5	2	25	6	30	0,76	
Age (mean)	21,95		22,24		22,03			22,1		0,90

other beverages (quantity and frequency), among others. The Ethics/Bioethics Committee at the University of Tarapacá and the Health Service of Coquimbo approved research protocols.

Boron exposure assessment. We assessed B exposure based on Boron levels in urine and tap water samples from the volunteers and their houses. Details about sample and analytic determination were published previously (Cortés *et al.*). In both kinds of samples Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used in certificated laboratory. According these results, we have establish that in the city of Arica there are three areas with different levels of Boron in tap water high levels ($> 7 \text{ mgL}^{-1}$) located in the north, medium levels ($3\text{-}7 \text{ mgL}^{-1}$) in the center and low levels ($< 3 \text{ mgL}^{-1}$) in the south areas of the city. Based on their highest Boron level measured in urine and tap water, volunteers were classified in high, medium or low exposure; cut points for each of these categories were determined as the 50 percentile and 75 percentile of levels of Boron in urine and tap water, shown in Table I (Cortés *et al.*).

Collection and analysis of semen samples. Method of sperm sampling and analysis has been described (Espinoza-Navarro *et al.*, 2010). In short, we followed international WHO recommendations (WHO, 2001; WHO, 2010). Semen samples were obtained after at least 4 days of sexual abstinence, by masturbation and ejaculation and received in a polyethylene bottle of 50 ml sterile. Samples were collected in a room outfitted to ensure the privacy of the participants.

The samples were stored at 37°C for 20 min for liquefaction and after 60 minutes volume, pH, color, odor, consistency, appearance, liquefaction, sperm count, sperm

concentration overall, sperm motility, sperm viability and morphology were analyzed. Sperm count to an aliquot of $20 \mu\text{L}$ (1:10) was used in a solution of formaldehyde and eosin yellow, using a Neubauer hemocytometer. The total sperm count was calculated as the product of seminal volume and sperm concentration. The sperm motility was determined in a direct sample of 10 L, determining the percentage of rapid spermatozoa (A), slow (B), non-progressive (C) and immobile (D). The vitality was assessed by the staining method eosin-nigrosin, determining the percentage of live sperm unstained. For sperm morphology samples were fixed and stained with the Papanicolaou method and analyzed with strict criteria (WHO, 2010). Motility, vitality and sperm morphology were evaluated in 200 sperm. Semen samples were discarded after the analysis performed.

Semen parameters were analyzed blindly from the subjects exposure, indicating the minimum and maximum values and percentages for volume, pH, sperm count, percentage of normal or abnormal shapes (morphology), type of abnormality (head, tail or cytoplasmic droplet), percentage of motility (A grade motility included very rapid sperm; B grade motility included rapid sperm) and percentage of sperm vitality. Motility and vitality were measured at hour 1 and hour 3 after collecting the semen samples.

Statistical analyses. We use descriptive statistics and after we identified whether there were significant differences between the values of each parameter according to each exposure levels defined in urine or water. We use Kruskal-Wallis test and Chi-square test to explore statistically significant differences among the three exposure groups. Data were analyzed with statistical software SPSS for Windows, version 17 (SPSS Inc, Chicago, III, 2006).

Table II. Boron exposure strata and semen characteristics in voluntary participants, Arica, Chile.

Exposure strata	Low exposure (mgL^{-1})		Medium (mgL^{-1})		High (mgL^{-1})		P-value
	B Urine ≤ 2.94 or B tap water ≤ 3.0		B Urine 2.95-7.4 and B tap water 3.0-7.0		B in urine 7.4- 17.4 or tap water 7 - 11.0		
Seminal parameters	Median	p25 - p75	Median	p25 - p75	Median	p25 - p75	
Volume (ml)	2,5	1.65 - 3.9	2.8	1.8 - 4.0	2.5	1.8 - 3.5	0.92
pH	8	7.0 - 8.0	8.1	7.5 - 8.0	8.0 ¹	7.0 - 8.0	0.079
N° sperm/mL	45,106	17.18 - 90.50	48.25	30.5 - 82.0	38.0	19.5 - 93.0	0.72
Abnormal morphology (%)	83,3	80.1 - 86.4	90.0 ²	82.0 - 92.5	83.0	79.8 - 88.9	0.010
Motility A+B 1° hour	38.1	24.5 - 65.6	40.0	25.0 - 57.5	45.5	29.1 - 58.0	0.77
Motility A+B 3° hour	7.8	0.0 - 21.0	18.0	1.3 - 27.3	8.7	0.0 - 26.1	0.44
Vitality (%) 1° hour	88.6	69.3 - 94.1	88.0	62.1 - 95.0	76.9	70.0 - 90.0	0.5
Vitality (%) 3° hour	60.0	43.5 - 80.0	65.0	43.0 - 79.8	47.4	30.9 - 66.0	0.191
Filancia (%)	26.7		46.7		26.7		0.576

P-value comparison¹ high vs medium = 0.0632 medium vs high = 0.011

Table III. Boron exposure strata and sperm defects. Voluntary participants, Arica, Chile.

Exposure strata	Low exposure (mgL ⁻¹)		Medium (mgL ⁻¹)		High (mgL ⁻¹)		P-value
	B Urine ≤ 2.94 or B tap water ≤ 3.0		B Urine 2.95-7.4 and B tap water 3.0-7.0		B in urine 7.4- 17.4 or tap water 7 - 11.0		
Abnormality %	Median	p25 - p75	Median	p25 - p75	Median	p25 - p75	
Pointed head	30	19.2 - 34.7	23.6	15.0 - 30.95	27.5	15.0 - 37.2	0.10
Microcephalous	11.9	5.7 - 21.4	10.0	5.0 - 15.0	11.1	5.0 - 19.2	0.31
Macrocephalous	3.4	1.7 - 10.0	5.0	3.0 - 10.0	3.5	2.0 - 5.9	0.16
Bicephalous	0.0	0 - 2.1	0.0	0 - 1.9	0	0 - 2.0	0.83
Tail defect	21.3	15.2 - 29.6	25.0	18.0 - 33.2	31.1	21.4 - 36.0	0.102
Cytoplasmic droplet	5.3	3.9 - 12.5	10.0	6.0 - 15.0	7.1	5.0 - 12.5	0.044
Round cells	8.9	3.9 - 18.6	9.5	6.1 - 18.9	7.3	4.05 - 17.4	0.247

P-value comparison 3low vs medium = 0.046

RESULTS

General characteristics of the study group by exposure are shown in Table I. Participants were comparable regarding alcohol consumption, smoking, drugs use and reproductive success. There are no statistical differences among the three groups.

Boron concentrations in urine and tap water and their relation with semen parameters by level of exposure are shown in Table II. In the medium level of exposure (urinary B between 2.95-7.4 mg.L-1 and B in tap water between 3.0-7.0 mgL-1 pH shows a slightly alkaline environment, expressing the lowest percent of normal sperm morphology. Motility and vitality in the three hour has the highest values in the medium levels of exposure to boron. In the highest B exposure, sperm presented the best levels of normal morphology and motility in the first hour being statistically significant (Table II). None of other parameters reached statistical significance when comparing the exposure groups.

Changes in morphology according boron levels exposure are shown in Table III. In the medium exposure there are more immature cells, among them gonias, primary and second spermatocytes, spermatids and also white blood cells. With the highest exposure the most relevant is tail defect.

DISCUSSION

Numerous environmental contaminants have been shown as disrupters of sperm quality in male workers, affecting concentration, motility and sperm morphology (Jurewicz *et al.*; Lifeng *et al.*, 2006; Wirth *et al.*, 2007). It is

imperative to become more aware of the factors that may affect semen quality and avoid parental exposure to these hazards, which may affect human reproduction.

Volunteers evaluated here formed a homogeneous group, as they shared certain characteristics such as being healthy, living in the city of Arica for at least two years, being 18 to 30 years old and having similar lifestyle patterns (alcohol consumption or smoking, Table I). These similar conditions allowed control of these factors among the three groups evaluated. However, it should be noted that the sample size was established for convenience, so these results are not representative of the entire population and are applicable only for this group.

In Arica there was evidence of past exposures to arsenic and lead by soil. Both elements were evaluated in drinking water and urine was evaluated. Results not shown here reporting for arsenic, reported median levels of 6 µgL-1 in drinking water and 21 µgL-1; for lead the median values were 2 µgL-1 in drinking water and 3 µgL-1 in urine; there were not differences between groups.

Other factors that could have affected the quality of the studied parameters were not assessed (i.e. follicle stimulating hormone, luteinizing hormone and testosterone levels); only the survey data were used to identify patients who presented cryptorchidism, varicocele or reproductive success (Espinoza-Navarro, *et al.*).

Although no statistically significant differences were observed for volume, pH, concentration, motility, vitality, filament (gelatinous semen filament), when comparing increasing levels of Boron in tap water or urine, our results showed a biological effect in the group with

the medium exposure to Boron. This biological effect was evidenced by a highest percentage in sperm count (48.28 versus 45.1 or 38.0 Mml-1 for low or high exposure) and filament (46.7 versus 26.7 % both for low or high exposure) when volunteers residing in the area with levels of Boron between 3 and 7 mgL-1 and have urinary levels between 2.95 to 7.4 mgL-1 (Table II).

Regarding the morphology, was noted that in areas with medium exposure to boron, abnormal morphology was highest (90.0% versus 83.3 or 83.0% for low or high exposure), statistically significant (p value=0.01). Duydu *et al.*, did not find significant differences in sperm morphology in workers exposed to Boron in tap water which levels reached a maximum value of 9.47 mgL-1.

Abnormalities in sperm head (pointed head) and tail defect increased in the area with the highest Boron level in tap water or urine, not being statistically significant. Other authors identified no damage attributable to Boron in highly exposed Chinese workers (Scialli *et al.*; Robbins *et al.*). Our results only show significant differences by abnormalities of cytoplasmic droplet, with the highest value in the medium group (p value = 0.04).

The results observed for the group with the highest exposure represent a contribution to the discussion about the role of Boron in reproductive processes with increased normal sperm morphology, decreased sperm concentration and vitality. The medium exposure group evidenced a wider range of pH, abnormal sperm morphology and motility and vitality (3th hour). These results suggest that an optimal behavior of the seminal parameters would be expressed at average levels with excretion values of boron in urine from 2.95 to 7.38 mgL-1 or living in areas with Boron in tap water between 3 to 7 mgL-1. These findings

could be interpreted like a “U” dose-response relationship in itself. It is necessary to establish if there are compensatory effects, suggesting a beneficial effect of certain level of boron exposure on maintaining the normal sperm morphology. These results state that studies about boron contamination should continue in order to define on a scientific basis the permitted levels of boron in drinking water (Weinthal *et al.*).

CONCLUSIONS

Young men exposed to B in drinking water present variations in the sperm characteristics associated with the level of exposure. Most of these changes are positive at intermediate levels of B. But at highest exposures there were negative changes in sperm morphology, concentration, motility and vitality, all relevant parameters of fertility. Thus our study suggests a U curve for effects of B in sperm: having the best result at intermediate levels of exposure, in agreement with its micronutrient classification.

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RESUMEN: El objetivo de este trabajo fue describir los parámetros espermáticos en residentes del norte de Chile. Se evaluaron en 101 voluntarios (18 y 30 años), los niveles urinarios y de agua potable de boro, usando “Inductively Coupled Plasma Mass Spectrometry”. Los parámetros del semen se midieron con métodos estandarizados. Cada individuo se clasificó en 3 niveles de exposición: bajo (niveles B en la orina $\leq 2,94$ mgL-1 o agua potable $\leq 3,0$ mgL-1), medio (B urinario entre 2,95-7,4 mgL-1 y B en agua de beber con 3,0- 7,0 mgL-1) y alto (B urinario $>7,4$ mgL-1 o agua potable $> 7,0$ mgL-1). No se encontraron diferencias significativas entre los grupos por pH, concentración de espermatozoides (45,1; 48,2 y 38 millones/mL), motilidad a 1ª hora (38,1; 40,0 y 45,5%) y vitalidad 1ª hora (88,6; 88,0 y 76,9%) respectivamente. La morfología anormal fue significativamente diferente (83,3; 90 y 83%). Los hombres jóvenes expuestos a B en el agua potable presentan variaciones espermáticas asociadas con el nivel de exposición. La mayoría de estos cambios son positivos en niveles intermedios de B. Para las exposiciones más altas se observaron cambios negativos en la morfología, concentración, motilidad y vitalidad del esperma, parámetros relevantes de la fertilidad. Un efecto beneficioso se observa en la exposición media, como una "curva U".

PALABRAS CLAVE: Semen; Fertilidad; Exposición al boro; Jóvenes; Chile.

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