

Critical aspects of male fertility in the assessment of exposure to lead

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Lead effects on human male fertility are postulated to occur at blood levels (B-Pb) higher than those currently determined in the workplace [ie, above 40—50 µg/dl (2—2.5 µmol/l)]. However, the first evidence of such alterations at even lower B-Pb, ranging from 20 to 50 µg/dl (1—2.5 µmol/l) has appeared in a few studies (1).

Various differently associated effects (decrease in sperm count, volume and motility, morphological alterations, endocrine effects) were reported.

Many studies have however some methodological drawbacks. The number of examined workers is generally small, and sometimes suitable reference groups are lacking. Confounding factors and effect modifiers such as age, individual habits, seasonal variation, duration of continence before semen collection, and diseases are not controlled for. The analytical methods both for lead and effect indicators are not always standardized. And, also, the occupational exposure to lead is not adequately estimated on a quantitative basis.

The problem of correctly assessing exposure to lead is enhanced by two other considerations. In modern industrial activities, preventive measures and technological improvement have reduced occupational exposure to lead, but even for these lower levels possible effects may be possible. Second, the role of other occupational factors possibly affecting reproductive ability, such as metals, solvents, and some physical agents, has been growing in recent years.

In this contribution we have focused on some of the critical aspects of lead exposure assessment when male fertility is being investigated.

Assessment of exposure and effects

The characteristics and findings of occupational studies on semen quality, endocrine alteration, and reproductive function have been described in a recent review (1).

In this report we focus on 4 articles as examples of methods adopted in evaluating exposure and effects. The “historical” study of Lancranjan et al (2) reported the reproductive ability of 150 storage battery workers divided into 4 groups with different B-Pb levels. The subjects were lead “poisoned men” [N=23, mean B-Pb 74.5 µg/dl (3.72 µmol/l)], subjects with moderate [N=42, mean B-Pb 52.8 µg/dl (2.64 µmol/l)], slight [N=35, mean B-Pb 41 µg/dl (2.05 µmol/l)] or physiological [N=50, mean B-Pb 23 µg/dl (1.15 µmol/l)] lead absorption. Sperm samples were collected only from 89 workers nonrandomized and from 50 unexposed workers. A dose-response relationship was found with increasing lead levels as regards spermatogenesis, asthenospermia, hypospermia, and teratospermia.

Alexander et al (3) evaluated 119 lead-zinc smelter workers subdivided into 4 groups [<15, 15—24, 25—39, >40 µg/dl (<0.75, 0.75—1.2, 1.25—1.95, >2.0 µmol/l)]. The most important finding was that subjects with a

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B-Pb of $>40 \mu\text{g/dl}$ ($>2.0 \mu\text{mol/l}$) had an increased risk for below-normal sperm concentration and total sperm count. No association was found between lead exposure and sperm morphology, motility, or reproductive hormones.

Ng et al (4) studied 122 factory workers with mean B-Pb values of $35.2 \mu\text{g/dl}$ ($1.76 \mu\text{mol/l}$) and 29 referents whose mean B-Pb was $8.3 \mu\text{g/dl}$ ($0.41 \mu\text{mol/l}$). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were significantly higher in the lead workers, and testosterone was significantly lower in the older workers (>40 years of age). LH and FSH showed a moderate increase in relation to the B-Pb in the range of $10\text{--}40 \mu\text{g/dl}$ ($0.5\text{--}2.0 \mu\text{mol/l}$). The group with >10 years of lead exposure showed significantly lower LH and FSH, while testosterone was significantly lower.

In the most recent paper on this topic, Robins et al (5) studied 97 battery workers whose B-Pb ranged from 28 to $93 \mu\text{g/dl}$ ($1.4\text{--}1.65 \mu\text{mol/l}$) and whose total seminal lead ranged from 1 to $87 \mu\text{g/dl}$ ($0.05\text{--}4.35 \mu\text{mol/l}$). Significant associations were found between increased abnormal morphology and higher measures of current and cumulative B-Pb, and also with duration of exposure. No association was shown between sperm density or count and any lead exposure measures or between these measures and fertility.

The main criticism against the reviewed studies regarding the methods adopted in evaluating lead exposure concerns the fact that the quality assurance procedures for the determination of dose and effect indicators are not — or are rarely — documented, the exposure pattern (ie, steadiness, peaks of exposure, time since the beginning, and the end of lead exposure) is not always or completely investigated and described, and the assessment for chemical or physical interference, both from occupational and nonoccupational sources, is not sufficient.

Lead dose measurement

When the type of indicator for measuring lead exposure was dealt with, attention was paid mainly to B-Pb. However B-Pb reflects the exposure during the last 30–40 days, and it is inadequate to estimate the total dose of absorbed lead. B-Pb does not provide information on the lead concentration in tissues or organs, and some experimental data show that B-Pb does not appear to be correlated with testicular lead.

Other indicators provide better information about lead exposure: adjusted B-Pb (eg, $\text{B-Pb} \times \text{years of exposure}$); erythrocyte zinc protoporphyrin, which reflects longer exposure periods; indicators of body burden, such as the measurement of lead in bone or the determination of urinary lead after chelation, methods however not applicable in large-scale studies.

The list of possible indicators can be completed by the lead concentration in seminal fluid as a whole or in

its fractions, theoretically better correlated with testicular lead and therefore with histopathological alterations. However, no relationship was found between B-Pb and seminal lead with sperm quality in some studies (6), while in others only the total semen lead concentration was significantly associated with some sperm parameters, such as sperm density. Lead was determined in Leydig cells, and decreased testosterone in the serum of lead-treated rats indicated an impaired Leydig cell function. Lead was also detected in germinal cells, and therefore lead can pass through the blood-testis barrier, functionally very similar to the blood-brain barrier, and affect the germinal cells at different degrees of differentiation (spermatogonia, primary spermatocytes, spermatydes or spermatozoa). However, it is still disputable whether lead in cells or in fluids is a result of a breakdown of the blood-testis barrier or whether lead normally passes this barrier.

Finally, the prostate and seminal vesicle secretions are other possible media in which lead can be found. Another important topic is the relationship between B-Pb and element concentrations in seminal fluid. In our opinion, the determination of elements in the latter matrix appears to be justified only after clearly informative improvement has been reached.

Several investigations attempted to evaluate human sperm quality or fertility in relation to elements other than lead. The seminal concentration of elements such as zinc, calcium, magnesium, selenium, and copper was studied, especially in relation to the morphology, number and motility of spermatozoa (7) and to environmental or occupational exposure to toxic metals such as mercury. Cadmium may also affect sperm quality (8).

The determination of an element in seminal fluid and its fractions could be useful in studies of the absorption of toxic elements and possible interferences with essential elements; furthermore, it could be related to seminal impairment.

In order to evaluate the possible significance of lead and the determination of other elements in seminal fluid, we examined 18 subjects not occupationally exposed to metals (mean age 32.5 years, range 21–48) and 10 workers exposed to lead (mean age 33.1 years, range 25–51). The concentration of elements was evaluated in seminal plasma and spermatozoa by means of inductively coupled plasma mass spectrometry (ICPMS). The results are shown in table 1. Lead, barium, and bromine were found in higher amounts in sperm cells of lead-exposed workers, whereas selenium, zinc and cadmium were measured in significantly lower amounts when compared with the corresponding levels of referents. The lead, bromine, and cadmium levels were significantly higher than those of referents in the seminal plasma of lead-exposed workers, whereas selenium was significantly lower.

Table 1. Element concentrations in seminal plasma and spermatozoa in referents (N=18) and lead exposed (N=10) subjects. (ND = not detected)

	Seminal plasma							Spermatozoa						
	Referents			Exposed				Referents			Exposed			
	Mean	SD	Range	Mean	SD	Range	P-value ^a	Mean	SD	Range	Mean	SD	Range	P-value ^a
Magnesium ^b	72	27	31—152	80	89	30—130	0.72	150	42	90—320	170	46	105—390	0.25
Calcium ^b	155	51	80—219	140	59	90—170	0.5	432	109	247—840	285	127	195—558	0.003
Zinc ^b	96	48	70—175	88	41	57—160	0.66	320	136	210—640	215	124	160—563	0.05
Bromine ^b	1.4	0.7	1—4	2.5	1.1	1—5	0.003	3.5	1.7	1—7	5.0	3.1	2—10	0.1
Rubidium ^b	2.2	1	1—5	1.7	0.8	1—4	0.18	2	0.9	1—4	1.98	1.1	1—3	0.95
Aluminium ^c	26	10	7—49	26	13	6—30	1	2395	1052	1040—3960	2320	1256	1350—4890	0.86
Manganese ^c	7	3.1	2—18	7	4	2—15	1	140	97	48—272	177	85	95—310	0.32
Nickel ^c	6	1.7	2—13	7	2	1—10	0.17	57	19	20—78	50	24	30—120	0.4
Copper ^c	77	33	36—165	85	39	40—180	0.56	690	372	345—1435	906	631	432—1560	0.26
Selenium ^c	79	25	40—135	56	21	31—99	0.02	313	107	141—545	171	0.1	95—320	0.003
Strontium ^c	44	18	19—91	55	21	22—98	0.15	235	111	78—380	220	103	150—370	0.72
Cesium ^c	2	0.9	1—4	2.4	1.4	1—5	0.36	0.7	1.0	n.d.—1.5	0.9	0.8	n.d.—2	0.59
Barium ^c	2.5	1.8	ND—5	2.8	1.6	2—7	0.66	55	31	28—107	82	57	42—136	0.11
Lead ^c	2.8	3	ND—12	30	19	10—70	0.0001	40	29	19—81	392	280	85—1060	0.0001
Chromium ^c	1.1	0.9	0.2—2	1.2	0.8	0.2—3.5	0.77	9	7.5	5—19	10	8.3	3—14	0.74
Cadmium ^c	1	0.6	ND—3	1.8	1	1—6	0.01	8	6.3	5—19	9.7	7.6	6—16	0.53
Antimony ^c	.	.	.	2.2	0.9	1—6	.	.	.	.	17	8.5	9—31	.
Tin ^c	.	.	.	3.4	1.3	1—8	.	.	.	.	48	32	20—90	.

^a Student's t-test.^b Seminal plasma values in milligrams per liter and spermatozoa values in milligrams per kilogram.^c Seminal plasma values in micrograms per liter and spermatozoa values in micrograms per kilogram.

Although larger studies are needed, especially considering the wide range of values and the different shape of the frequency distribution, the results may address the potentiality of this approach, in which the multiple element determination in different matrices may improve the knowledge of some toxicity mechanism of lead in tissues or organs critical to male fertility.

Discussion

The aim of an accurate evaluation of lead dose is to measure the metal concentration in the relevant tissue as close as possible to the "target time" (ie, the period we have to consider for estimating lead exposure). This measurement period is defined in relation to the starting time (the start of unprotected intercourse), and it could be days, weeks, or years before the target time, depending on the type of mechanisms for lead effects. For example, it could be some days in the case of effect via seminal plasma mediated by prostate or seminal vesicles, or it could be up to 12 weeks if the effects of lead on spermatogenesis, via the testis, are in question.

The measure of short-term exposure can be provided by B-Pb nearest to the target time, or a mean of the near values, for example, the values within 12 months or the mean of 2 values immediately before the closest value.

The question of the biological mechanisms of lead damage has also to be clarified. The reviewed studies showed that the effects were not always the same either

morphologically or quantitatively, neither they were always associated in the same way, although the ones on sperm count and concentration were the most frequent.

It is not yet clear whether the possible mechanism is a direct effect of lead on reproductive organs, which seems to be the most likely condition, or on the endocrine control of reproduction, or both.

If seminal lead concentration would reflect testicular lead content, human semen, which can be more easily collected, could theoretically provide a good medium for lead determination.

The identification of the most appropriate matrix (seminal plasma or sperm cells or both) seems to be important in investigations of sperm quality in relation to essential and toxic elements, since the source and the significance of the elements in the matrices may differ. In fact, the concentrations of elements in seminal plasma are influenced by several gland secretions, whereas spermatozoa, nucleated cells, may provide better information as a possible target for toxic agents, as well as for essential elements. It must be pointed out however that elemental exchange between plasma and sperm cells may be possible and, therefore, many complicate the interpretation of measurements.

Finally, the multiple determination of essential and toxic elements may be crucial when mechanisms of toxic action on male fertility are under consideration. Co-exposure or the concurrent absorption of different

elements is likely to cause metabolic interactions relevant for their toxicokinetics and toxicodynamics.

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