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Aluminum content of human semen: Implications for semen quality

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ABSTRACT

A deterioration of human semen quality has been observed over recent decades. A possible explanation could be an increased exposure to environmental pollutants, including aluminum. Our aim was to measure the aluminum concentration in the semen of 62 patients and to carry out a preliminary evaluation on its impact on specific semen parameters.

For each patient, semen analyses were performed according to WHO guidelines. A graphite furnace atomic absorption spectrometry method was used to determine semen aluminum concentration. A cytological analysis using an aluminum-specific fluor, lumogallion, was also performed.

The mean aluminum concentration in human semen was 339 μg/L. Patients with oligozoospermia had a statistically higher aluminum concentration than others. No significant difference was observed for other semen parameters. Cytological analysis showed the presence of aluminum in spermatozoa.

This study provided unequivocal evidence of high concentrations of aluminum in human semen and suggested possible implications for spermatogenesis and sperm count.

1. Introduction

Concerns have been raised about the deterioration of human semen parameters, especially sperm count [1]. In France, despite some regional differences, most major studies indicate a steady, nation-wide decline in sperm count since 1973 [2–5]. Most authors attribute this phenomenon to environmental factors, such as endocrine disruptors [6,7].

Another environmental factor that could potentially affect semen parameters is the metal, aluminum (Al). Human exposure to Al has increased almost exponentially over the past 125 years and has become a burgeoning problem of the 21st century [8]. Al has been detected in various biological fluids such as urine [9], cerebrospinal fluid [10], sweat [11] and semen [12]. Al is a known pro-oxidative, excitotoxic, immunogenic, pro-inflammatory and mutagenic agent [8].

Food is one source of exposure to Al through preservatives or contamination from cooking and cookware. Whilst the mean intake of Al is usually below 20 mg/day, it can be much higher depending on diet [13]. WHO recommendations for food exposure were revised in 2008 from 7 mg/kg/day to 1 mg/kg/day. Al exposure can also come from the air we breathe, which in clean areas is around 1.4 μg/day, but this value can rise up to a thousand times in polluted areas or for smokers [14]. Water contamination by Al is usually under the WHO recommendations however this value can increase significantly in cities using Al as a coagulant for water treatment [15]. Al is also found in topically applied cosmetics, especially antiperspirant. A study has highlighted the ability of Al chloride to pass through the skin in significant quantities [16] and in France, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has issued a recommendation to limit Al chloride in antiperspirant to a maximum of 2% [17]. Finally, Al can also be present in many pharmaceuticals such as antacid and buffered aspirin, which can raise Al ingestion to several grams on a daily basis. In vaccination and allergy treatment, up to a milligram of Al can be injected along with an antigen or allergen [18,19]. Due to this wide variety of sources, it is difficult to accurately evaluate Al exposure since it may vary a lot from one individual to another depending on their working environment or life habits.

Numerous studies have been carried out to evaluate the impact of Al on the male reproductive system, most of which were

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performed using animal models. It was shown that a 26-week oral exposure to 75 mg/kg/day of elemental Al in the form of sodium aluminum phosphate can reduce the weight of the testes in dogs [20]. Al can also induce histological anomalies such as tubular necrosis [21,22] and reductions in the thickness of seminiferous tubules [23,24] in mice and rats with various routes of exposure, chemical forms or Al concentration (intra venous Al chloride at 13 mg/kg/day for 2 weeks, intra peritoneal Al nitrate at 100 mg/kg/day for 20 days, oral Al chloride at 2.5 mg/kg/day for 6 months or *de novo* Al compounds at 6.5 mg/rat/day for 60 days). Al was also found to affect semen quality criteria depending upon the conditions being studied. For example, Yousef et al. found that after an oral administration of Al chloride at 34 mg/kg/day for 70 days in rat, all the semen parameters were altered [25]. According to them, this toxicity is likely to be due to the pro-oxidant effect of Al as co-administration of propolis, which has strong antioxidant properties, reversed the effect of Al on semen parameters. LLobet et al. showed that after intra peritoneal injection of Al nitrate at 200 mg/kg/day for 20 days in mice the epididymis sperm count was lowered while sperm mobility and morphology were not affected [22]. However Krasovskiĭ et al. and Sharma et al. found that after oral administration of Al chloride at 2.5 mg/kg/day for 6 months in mice or *de novo* Al compounds at 6.5 mg/rat/day for 60 days in rats, respectively, both the sperm count and sperm mobility were lowered [23,24]. D'Souza et al. recently showed that after a single intra peritoneal injection of Al acetate at 100 mg/kg in Swiss albino mice, the sperm count was lowered and the percentage of abnormal spermatozoa was increased [26]. In contrast, another recent study [27] found no toxic impact of Al on semen parameters after oral exposure to Al ammonium sulfate for 10 weeks at doses up to 36.3 mg/kg/day. There have been several suggestions as to the mechanisms of toxicity of Al to the male reproductive system. In particular mechanisms involving reactive oxygen species and oxidative damage [28] have been highlighted as well as endocrine disruption of testosterone production, androgen receptor expression and libido decrease [29].

Despite these findings, data on Al load in human semen are lacking as are studies pertaining to the effects of Al on semen quality. Hovatta et al. [12] measured significant amounts of Al in semen and showed effects on sperm motility and morphology but not sperm count. Dawson et al. [30] also found significant amounts of Al in seminal plasma with higher values in the lower sperm mobility group, however there have been no other recent studies on Al in human semen and its impact upon semen quality criteria. Herein we measured the Al concentration in semen from 62 individuals and compared it to available clinical data and four semen parameters: sperm count, progressive motility, vitality and morphology.

2. Patients and methods

2.1. Study population

Sixty-two male patients were recruited through the Saint-Etienne Reproductive Center (France) where they had sought medical consultation regarding their fertility. Clinical information of age, smoking habits and parenthood were also collected. Semen samples were collected by masturbation after 3–5 days of sexual abstinence.

2.2. Ethical approval

In accordance with French legislation, patients were informed about the purpose of the study and were asked for their consent. The study was approved by the ethical committee of Saint-Etienne University Hospital (France).

2.3. Semen analysis

Semen analyses were carried out according to World Health Organization guidelines [31]. Normozoospermia was evaluated on the basis of four criteria, with pathological values being defined according to WHO reference [32]: total sperm number (<39 million per ejaculate), progressive motility (<32%) and vitality (<58% live). Sperm morphology was analyzed according to the modified classification of David (<15% of normal spermatozoa) [33].

2.4. Aluminum analysis

Samples of whole semen were transported to Keele University on dry ice and thereafter maintained frozen at −20 °C until required. After thawing, samples were thoroughly mixed by vortexing and 0.5 mL volumes of whole semen were removed and added to an acid-washed digest tube. Samples were digested in a 50:50 mixture of 15.8 M HNO_3 and 30% (*w/v*) H_2O_2 using a microwave oven and an established tissue digest program [34]. Following digestion samples were made up to a total volume of 2.5 mL with ultrapure water (conductivity < 0.067 μS/cm) and transferred to Bijoux tubes for storage. The Al content of each sample was then measured by TH GFAAS (Transversely Heated Graphite Furnace Atomic Absorption Spectrometry) using an established method [34]. This method has recently been fully verified and validated against a range of available reference materials and additional quality assurance data. As part of this we have measured 174 method blanks and obtained a median Al content of 22 ng/digestion vessel. As part of our continuing pursuit of rigor in taking account of possible contamination of Al measurements we used this value to compute a method blank of 54 ng/digestion vessel (mean + 1.654 SD). This value was subtracted from all analyses of Al in semen digests. This method is applicable to all acid/peroxide digests of living tissues and we believe is the most rigorous in the terms of taking account of contamination of samples during the processes used in measurement.

2.5. Histology

2.5.1. Pre-embedding sperm cells into agar

Paraformaldehyde (PFA) was from Koch-Light Laboratories, UK and all other chemicals were from Sigma Aldrich, UK, unless otherwise stated. Sperm cells were thawed (from −20 °C) and fixed by re-suspending in fresh 4% PIPES-buffered PFA (4%, *w/v* PFA, 150 mM NaCl, 25 mM PIPES, pH 7.4) for 24 h at 4 °C. Following fixation cells were pelleted *via* centrifugation for 8 min at 8000 × *g* (these centrifuge settings were used throughout) and washed three times by re-suspension in a PIPES-based buffer (150 mM NaCl, 50 mM PIPES, pH 7.4). Cells were transferred into a BEEM® capsule (Agar Scientific, UK) into which molten agar was added to prepare 3% (*w/v*) agar cell blocks.

2.5.2. Dehydration and clearing of agar-cell blocks

Agar-cell blocks were transferred through a graded ethanol series from 30% to 100% (*v/v*) ethanol (HPLC grade) with 20 min allowed in each ethanol concentration. Agar-cell blocks were then cleared by transferring the blocks into 1.0 mL of Histo-Clear (National Diagnostics, USA) for 20 min, with one change of fresh Histo-Clear half way through.

2.5.3. Infiltration and embedding of agar-cell blocks

Cleared agar-cell blocks were infiltrated in melted paraffin at 60 °C in stainless steel histology embedding molds for 35–40 min. Once infiltrated, the agar-cell blocks were transferred into smaller

stainless steel histology embedding molds. An embedding cassette was placed over the mold and the whole assembly transferred onto ice for rapid cooling of the wax thereby minimizing imperfections in the final block. The embedded blocks were set fully at 4 °C overnight.

2.5.4. Microtomy

Paraffin-embedded agar-cell blocks were sectioned by use of a Leica RM2025 rotary microtome fitted with Leica 819 low profile microtomy blades (Leica Biosystems, GmbH, Germany) at a thickness of 1 μm. Paraffin sections were floated out on ultrapure water at 50 °C by use of a paraffin floatation bath for 30 s. Sections on glass slides were dried fully overnight at ambient temperature.

2.5.5. Lumogallion staining

Lumogallion staining was performed using methods described elsewhere [35]. Briefly, Re-hydrated cell sections were immediately placed into either 100 μM lumogallion (TCI Europe N.V. Belgium) buffered in 50 mM PIPES, pH 7.4 or the PIPES-buffer alone for auto-fluorescence analyses for 24 h. Slides were rinsed in ultrapure water for 2 min, air dried and finally mounted using ProLong® Gold Antifade Reagent with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Life Technologies, Thermo Fisher Scientific, UK).

2.5.6. Microscopy

DAPI-mounted agar-sperm cell sections were viewed using an Olympus BX50 fluorescence microscope. For DAPI imaging, a U-MWU2 fluorescence filter cube was used (bandpass excitation filter (BP): 300–385 nm, dichromatic mirror (DM): 400 nm, longpass emission filter (LP): 420 nm) and for lumogallion imaging a U-MNIB3 fluorescence filter cube was used (BP: 470–495 nm, DM: 505 nm, LP: 510 nm) (both from Olympus, UK). All images were obtained at 1000× magnification with low auto-fluorescence immersion oil (Olympus immersion oil type-F). Exposure settings for the lumogallion analyses were fixed at 1 s. Images were obtained on a ColorView III digital camera using the Cell D software package (both from Olympus, Soft Imaging Solutions, GmbH) and fluorescent channels were overlaid by use of Photoshop (Adobe Systems, Inc., USA).

2.6. Statistical analysis

Comparisons of mean Al concentration in semen and mean age between groups were performed using a Student's *t*-test. Normality of distribution was checked and logarithmic transformation was applied when needed. For comparison of qualitative data a Chi square test was used. Correlation coefficient was calculated between Al concentration in semen and either age or semen parameters. The criterion for statistical significance was $p < 0.05$.

3. Results

3.1. Semen quality

Thirty-three patients were characterized as normozoospermic. Twenty-nine exhibited at least one pathological sign. Among them, 12 were oligozoospermic, 14 were asthenozoospermic, 5 were necrozoospermic and 15 were teratozoospermic. For 7 patients, vitality and morphology analyses were not performed due to low sperm count (Table 1).

3.2. Patient's clinical data

The patients' mean age was 33.7 years; 28 patients were current smokers and 12 were former smokers. Fifteen patients already had

Table 1
Population characteristics depending on semen analysis.

	Patients number		Mean age (years)			Tobacco (% of smoker)			Mean aluminum concentration (μg/L)		
	Patho	Normal	Patho	Normal	*p* value	Patho	Normal	*p* value	Patho	Normal	*p* value
Number (<39 M)	12	50	33.0 (29.8–36.2)	33.9 (32.5–35.2)	0.5963 NS	58	66	0.8539 NS	**478.1** (231.6–724.7)	**305.6** (247.4–363.8)	**0.0337**
Progressive motility (<32%)	14	48	34.0 (30.8–37.1)	33.6 (32.3–34.9)	0.7959 NS	50	69	0.3216 NS	**300.7** (222.9–378.6)	**350.2** (268.3–432.0)	0.5293NS
Vitality (<58%)	5	50	29.7 (25.3–34.1)	33.9 (32.6–35.3)	0.0553 NS	60	64	0.7564 NS	**262.2** (146.0–378.4)	**349.8** (269.9–429.6)	0.4944NS
Morphology (<15%)	15	40	31.5 (39.9–33.1)	34.3 (32.7–35.9)	**0.0482**	60	65	0.9772 NS	**365.0** (251.4–478.6)	**333.1** (239.5–426.7)	0.7005NS
Global semen result	29	33	33.0 (31.4–34.6)	34.3 (32.5–36.1)	0.2842 NS	62	65	0.9844 NS	**384.7** (277.6 –491.7)	**298.9** (217.9–379.8)	0.0555NS
Total	62		33.7 (32.5–34.9)			65			**339** (274.0–404.0)		

p values point out a statistical difference between pathological and normal semen for this parameter (95% confidence interval for the mean). NS, non significant; Patho, pathological.

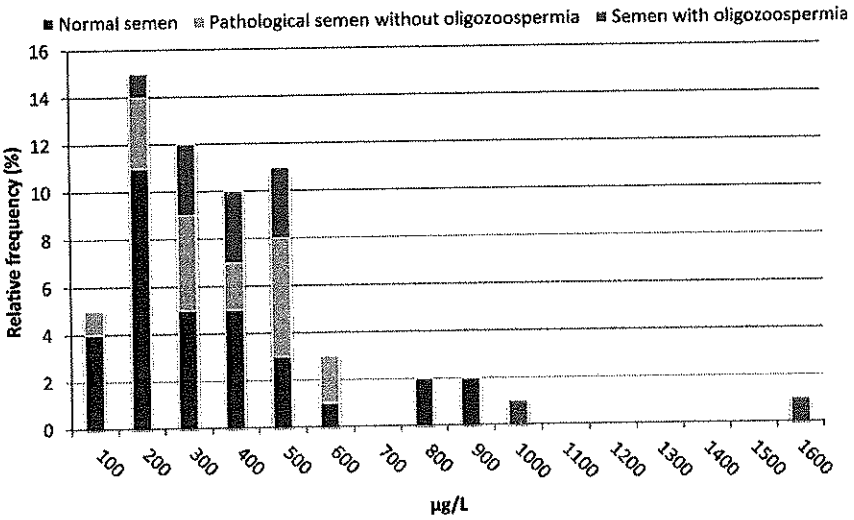

Fig. 1. Distribution of patients according to aluminum concentration in semen.

a child (one of them had two). Patient age and smoking habits were comparable between each group except that patients with teratozoospermia were slightly younger than others ($p=0.0482$). This difference is not clinically relevant however one possible explanation could be that when an infertility consultation is requested by a young couple, the problem is more likely to be non-age related (for example an anomaly in sperm parameters), as is the case with older couples reporting fertility issues.

3.3. *Aluminum concentration*

The mean Al concentration was found to be 339 µg/L (CI: 274.0–404.0) with the lowest value being 65.8 µg/L and the highest being 1547.8 µg/L (Fig. 1).

Patients were divided into two groups depending on their semen analysis results, normal ($n=33$) or pathological ($n=29$). Al concentration in semen was lower for patients with normozoospermia (Table 1) however this difference was not statistically significant ($p=0.0555$). When each of the semen quality criteria (total sperm number, progressive motility, vitality and morphology) were considered separately and used to define normal and pathological groups the Al concentration in semen was found to be significantly lower for patients with oligozoospermia ($p=0.0337$). This finding was also statistically significant when comparing patients with oligozoospermia and patients with normozoospermia ($p=0.0303$). For progressive motility, vitality and morphology, no statistical difference was found between normal and pathological semen. Moreover, no significant correlation was found

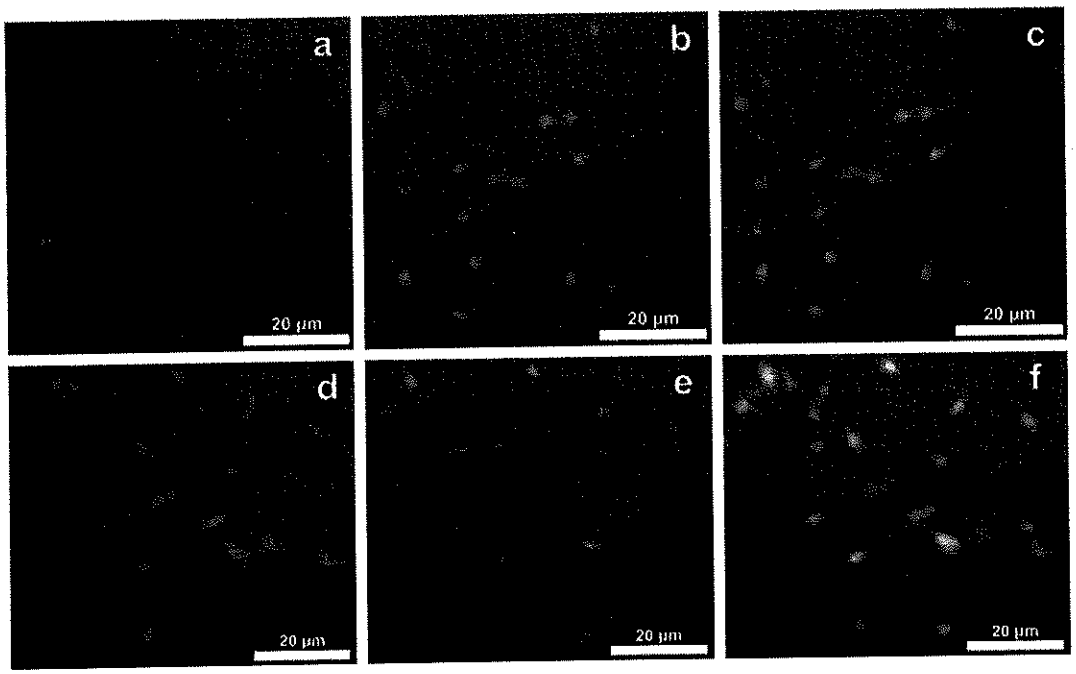

Fig. 2. Microscopic observation of 1 µm paraffin embedded agar–sperm cell sections (magnification 1000×, scale bars: 20 µm.). Control sections: (a) auto-fluorescence viewed under U-MNIB3 filter, (b) nuclei DAPI-staining viewed under U-MWU2 filter, (c) nuclei DAPI-staining and sperm auto-fluorescence overlay; Al lumogallion staining: (d) positive lumogallion staining viewed under U-MNIB3 filter, (e) nuclei DAPI-staining viewed under U-MWU2 filter, (f) nuclei DAPI-staining and positive lumogallion staining overlay.

between Al concentration in semen and either age or semen parameters.

Considering the patients' background, mean Al concentration in semen was not statistically different between smokers (both current and former) and non-smokers. Similarly, no statistical difference in Al concentration was found between patients who already had a child and patients who did not.

3.4. Fluorescence microscopy

Using the U-MNIB3 fluorescence filter, spermatazoa showed green autofluorescence in the absence of lumogallion (Fig. 2a), while the nuclei of spermatozoa gave characteristic blue DAPI fluorescence using the U-MWU2 filter (Fig. 2b and c). Al specificity of lumogallion has already been proven in a previous work [35]. Lumogallion fluorescence identified Al associated with unidentified structures within semen and specifically with spermatozoa as evidenced by bright orange fluorescence (Fig. 2d–f).

4. Discussion

Due to the ongoing deterioration of semen parameters in French men and the concomitant burgeoning exposure to Al, the aim of this study was to measure the amount of Al in semen and to compare this with established semen quality criteria.

The Al concentration in semen (mean 339 μg/L) was found to be high in comparison to other biological fluids. For example, mean Al concentration in serum among 44 non-exposed persons was 1.62 μg/L [36] however the data herein are in accordance with other, if limited, observations [12]. The highest value was found for a nonsmoker and was 1547.8 μg/L. Although tobacco is rich in Al and a potential source of Al to the body [14], we did not observe any relationship between smoking and semen Al concentration. Moreover, none of our patients reported any professional exposure to Al. As stated in the introduction, everyday life exposure to Al is very difficult to determine due to the wide range of exposure sources and time spent interacting with those sources [8].

Relating our findings about Al concentration to studies on animal models was found to be difficult since none of them performed Al analysis in semen. Only two studies from the same team analyzed Al concentration in blood and testis. The first study found a peak concentration of 700 μg/L in blood and 140 μg/g in testis after intra peritoneal injection of Al chloride for 2 weeks at 35 mg/kg/day of elemental Al [37]. The second study found a peak concentration of 400 μg/L in blood and 35 μg/g in testis after sub cutaneous injection of Al chloride for 2 weeks at 13 mg/kg/day [21]. In both studies, the Al concentration in blood and testis decreased to reach the concentration of controls after a few weeks.

Despite the relatively low number of individuals in this study, a significant increase in Al concentration was observed in the group who had a low sperm count. Conversely, we did not find any significant difference in Al concentration in semen between individuals with normal or pathological semen quality criteria for sperm progressive motility, vitality and morphology. When all quality criteria were taken together there was a higher though not statistically significant increase in Al in abnormal semen. This differs from the findings of Hovatta et al. [12] and Dawson et al. [30] who reported relationships between Al in semen and sperm motility but not with sperm count. However, the results herein are consistent with animal studies revealing impaired spermatogenesis and reduced sperm count after Al exposure [22,23]. We have used lumogallion and fluorescence microscopy to show for the first time that Al in semen is associated with both unidentified structures and specifically with spermatozoa. While we did not find any direct evidence that Al affected the immediate function of spermatozoa, Al is a well-known mutagen [8] and we cannot discount the possibility of more subtle effects on their viability.

In conclusion, our study revealed high concentrations of Al in the semen of French men who were under consultation for fertility issues and suggested that such might have a role to play in lower sperm count. We believe that these preliminary results justify the design of a larger cohort study to identify if human Al exposure has an impact upon male fertility.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.reprotox.2014.10.001.

References

[1] Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. Environ Health Perspect 1997;105:1228–32.
[2] Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med 1995;332:281–5, http://dx.doi.org/10.1056/NEJM199502023320501.
[3] De Mouzon J, Thonneau P, Spira A, Multigner L. Declining sperm count. Semen quality has declined among men born in France since 1950. Br Med J 1996;313:43–5.
[4] Geoffroy-Siraudin C, Loundou AD, Romain F, Achard V, Courbière B, Perrard M-H, et al. Decline of semen quality among 10 932 males consulting for couple infertility over a 20-year period in Marseille, France. Asian J Androl 2012;14:584–90, http://dx.doi.org/10.1038/aja.2011.173.
[5] Rolland M, Le Moal J, Wagner V, Royère D, De Mouzon J. Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. Hum Reprod Oxf Engl 2013;28:462–70, http://dx.doi.org/10.1093/humrep/des415.
[6] Perry MJ. Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. Hum Reprod Update 2008;14:233–42, http://dx.doi.org/10.1093/humupd/dmm039.
[7] Nordkap L, Joensen UN, Blomberg Jensen M, Jørgensen N. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. Mol Cell Endocrinol 2012;355:221–30, http://dx.doi.org/10.1016/j.mce.2011.05.048.
[8] Exley C. Human exposure to aluminium. Environ Sci Process Impacts 2013;15:1807, http://dx.doi.org/10.1039/c3em00374d.
[9] Davenward S, Bentham P, Wright J, Crome P, Job D, Polwart A, et al. Silicon-rich mineral water as a non-invasive test of the aluminum hypothesis in Alzheimer's disease. J Alzheimer's Dis 2013;33:423–30, http://dx.doi.org/10.3233/JAD-2012-121231.
[10] Roos PM, Vesterberg O, Syversen T, Flaten TP, Nordberg M. Metal concentrations in cerebrospinal fluid and blood plasma from patients with amyotrophic lateral sclerosis. Biol Trace Elem Res 2013;151:159–70, http://dx.doi.org/10.1007/s12011-012-9547-x.
[11] Genuis SJ, Birkholz D, Rodushkin I, Beesoon S. Blood, urine, and sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements.

Arch Environ Contam Toxicol 2011;61:344–57, http://dx.doi.org/10.1007/s00244-010-9611-5.
[12] Hovatta O, Venäläinen ER, Kuusimäki L, Heikkilä J, Hirvi T, Reima I. Aluminium, lead and cadmium concentrations in seminal plasma and spermatozoa, and semen quality in Finnish men. Hum Reprod Oxf Engl 1998;13:115–9.
[13] Bratakos SM, Lazou AE, Bratakos MS, Lazos ES. Aluminium in food and daily dietary intake estimate in Greece. Food Addit Contam B: Surveill 2012;5:33–44, http://dx.doi.org/10.1080/19393210.2012.656289.
[14] Exley C, Begum A, Woolley MP, Bloor RN. Aluminum in tobacco and cannabis and smoking-related disease. Am J Med 2006;119, http://dx.doi.org/10.1016/j.amjmed.2005.08.004, pp. 276.e9–11.
[15] WHO. Aluminium in drinking-water. Guidel Drink-Water Qual 1998:2.
[16] Pineau A, Guillard O, Favreau F, Marty M-H, Gaudin A, Vincent CM, et al. In vitro study of percutaneous absorption of aluminum from antiperspirants through human skin in the Franz™ diffusion cell. J Inorg Biochem 2012;110:21–6, http://dx.doi.org/10.1016/j.jinorgbio.2012.02.013.
[17] AFSSAPS. Évaluation du risque lié à l'utilisation de l'aluminium dans les produits cosmétiques; 2011.
[18] May JC, Progar JJ, Chin R. The aluminum content of biological products containing aluminum adjuvants: determination by atomic absorption spectrometry. J Biol Stand 1984;12:175–83.
[19] Exley C. Aluminium adjuvants and adverse events in sub-cutaneous allergy immunotherapy. Allergy Asthma Clin Immunol 2014;10:4, http://dx.doi.org/10.1186/1710-1492-10-4.
[20] Pettersen JC, Hackett DS, Zwicker GM, Sprague GL. Twenty-six week toxicity study with KASAL® (basic sodium aluminum phosphate) in beagle dogs. Environ Geochem Health 1990;12:121–3, http://dx.doi.org/10.1007/BF01734061.
[21] Guo C-H, Lu Y-F, Hsu G-SW. The influence of aluminum exposure on male reproduction and offspring in mice. Environ Toxicol Pharmacol 2005;20:135–41, http://dx.doi.org/10.1016/j.etap.2004.11.007.
[22] Llobet JM, Colomina MT, Sirvent JJ, Domingo JL, Corbella J. Reproductive toxicology of aluminum in male mice. Fundam Appl Toxicol Off J Soc Toxicol 1995;25:45–51.
[23] Krasovskiĭ GN, Vasukovich LY, Chariev OG. Experimental study of biological effects of leads and aluminum following oral administration. Environ Health Perspect 1979;30:47–51.
[24] Sharma S, Sharma RK, Sharma R, Sharma A, Rai AK, Gupta RS, et al. Synthesis and characterisation of some new aluminium derivatives of Schiff bases containing N, O and S donor atoms and the anti fertility activity of the derivative Al[SC6H4N:C(CH3)CH2COCH3]3. Bioinorg Chem Appl 2003;21:5–25, http://dx.doi.org/10.1155/S1565363303000177.
[25] Yousef MI, Salama AF. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 2009;47:1168–75, http://dx.doi.org/10.1016/j.fct.2009.02.006.
[26] D'Souza SP, Vijayalaxmi KK, Naik P. Assessment of genotoxicity of aluminium acetate in bone marrow, male germ cells and fetal liver cells of Swiss albino mice. Mutat Res Genet Toxicol Environ Mutagen 2014;766:16–22, http://dx.doi.org/10.1016/j.mrgentox.2014.02.006.
[27] Hirata-Koizumi M, Fujii S, Ono A, Hirose A, Imai T, Ogawa K, et al. Evaluation of the reproductive and developmental toxicity of aluminium ammonium sulfate in a two-generation study in rats. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 2011;49:1948–59, http://dx.doi.org/10.1016/j.fct.2011.04.035.
[28] Guo C-H, Huang C-J, Chiou Y-L, Hsu G-SW. Alteration of trace element distribution and testis ACE activity in mice with high peritoneal aluminum. Biol Trace Elem Res 2002;86:145–57, http://dx.doi.org/10.1385/BTER:86:2:145.
[29] Sun H, Hu C, Jia L, Zhu Y, Zhao H, Shao B, et al. Effects of aluminum exposure on serum sex hormones and androgen receptor expression in male rats. Biol Trace Elem Res 2011;144:1050–8, http://dx.doi.org/10.1007/s12011-011-9098-6.
[30] Dawson EB, Ritter S, Harris WA, Evans DR, Powell LC. Comparison of sperm viability with seminal plasma metal levels. Biol Trace Elem Res 1998;64:215–9, http://dx.doi.org/10.1007/BF02783337.
[31] WHO. WHO laboratory manual for the examination and processing of human semen. 5th ed; 2010.
[32] Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16:231–45, http://dx.doi.org/10.1093/humupd/dmp048.
[33] Jouannet P, Ducot B, Feneux D, Spira A. Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. Int J Androl 1988;11:379–94.
[34] House E, Esiri M, Forster G, Ince PG, Exley C. Aluminium, iron and copper in human brain tissues donated to the Medical Research Council's Cognitive Function and Ageing Study. Metallomics 2012;4:56–65, http://dx.doi.org/10.1039/c1mt00139f.
[35] Mold M, Eriksson H, Siesjö P, Darabi A, Shardlow E, Exley C. Unequivocal identification of intracellular aluminium adjuvant in a monocytic THP-1 cell line. Sci Rep 2014;4, http://dx.doi.org/10.1038/srep06287.
[36] Valkonen S, Aitio A. Analysis of aluminium in serum and urine for the biomonitoring of occupational exposure. Sci Total Environ 1997;199:103–10.
[37] Guo C-H, Huang C-J, Chen S-T, Wang Hsu G-S. Serum and testicular testosterone and nitric oxide products in aluminum-treated mice. Environ Toxicol Pharmacol 2001;10:53–60.