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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.

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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

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