Reviewer's report

Title: Relationships between heavy metal concentrations in three different body fluids and male reproductive parameters: A pilot study.

Version: 1 Date: 9 November 2010

Reviewer: Alica Pizent

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General:
The study evaluates associations between seminal and hormonal parameters and concentration of Pb, Cd and Hg in blood and seminal plasma and whole blood in 61 men attending the three infertility clinics in Spain. No significant difference in metal concentration in 3 body fluids between 30 men with oligo-astheno-teratozoospermia and 31 control men with normozoospermia was found. Results of multivariate analysis show no significant association between serum hormone levels and heavy metal concentrations in any of the 3 body fluids, whereas a significant positive association between the percentage of immotile sperms and seminal plasma levels of Pb and Cd was found. Manuscript describes interesting and novel data.

However, the shortcomings in the study are too small number of subjects and an insufficient control of possible influence of smoking (as smoking intensity and duration, expressed as the number of cigarettes per day and/or cigarette-years, rather than a number of smokers, affects the levels of measured metals).

Major Compulsory Comments:

1. Methods: In the description of the metals measurements in body fluids on page 5, authors should provide quality control data for the metal analysis (e.g. the use of certain quality control reference samples; participation in external quality assessment scheme) to demonstrate the accuracy of their results.

2. Table 1: Reported value for blood plasma lead concentration (2.7-3.3 microg/dL) seems to be overly high, especially with regard to relatively low whole blood lead concentration (7.5-13.3 microg/dL) – sample contamination or use of EDTA plasma (it is known that EDTA may cause a release of Pb from RBC into plasma yielding a higher plasma lead concentration)?

Minor Essential Revisions:

1. Decimal symbol should be point instead of comma (pg 5, line 125; pg 6, line 139).

2. Table 4, results of multivariate analysis for T and Cd S.P.: in the column for 95% CI, ",.10.5" should be corrected.
3. Table 5, results of multivariate analysis for Morphologically Normal Sperm and Mercury B.P.: in the column for 95% CI, “-.1.3” should be corrected.

Discretionary Revisions:
1. Methods, Study population, design and semen analysis, p.4: If possible, please provide data on smoking habits of study subjects (expressed as the number of cigarettes per day and/or cigarette-years).
2. Please provide data on semen parameters (Table 1)

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.