Reviewer's report

Title: Bulky DNA adducts in human sperm associated with semen parameters and sperm DNA fragmentation in infertile men: a cross-sectional study

Version: 1 Date: 15 July 2013

Reviewer: Bernard Robaire

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The objective of this epidemiological, uncontrolled study was to explore the potential associations between PAH-DNA adducts, standard semen analysis parameters and sperm DNA integrity, assessed by the TUNEL assay. 433 infertile men were recruited for this study; stringent exclusion criteria were used. Sperm PAH-DNA adducts was measured using immunofluorescence and FACS; the subjects were divided into quartiles and the association between PAH-DNA adducts and (i) sperm quality endpoints - including sperm concentration, total sperm count, (ii) three CASA parameters sperm motility and curvilinear velocity, and linearity – assessed by computer assisted semen analysis; and (iii) sperm DNA fragmentation (TUNEL assay) was assessed. Potential associations were explored using linear regression models on ranked categories of sperm PAH-DNA adducts levels, and P values for linear trend as well as regression coefficients were adjusted for age, body mass index, abstinence time, smoking and alcohol drinking using multivariate models.

The authors identify a significant decrease in sperm concentration, count, motility, and curvilinear velocity in the higher quartile compared to the lower one. A significant increase in TUNEL positive sperm was also observed in the higher quartile. The data suggest an inverse association between sperm PAH-DNA adducts and sperm quality, and a positive association between PAH-DNA adducts and TUNEL positive.

The study provides useful additional evidence that increased PAH-DNA adducts are associated with lower sperm quality, as measured by a number of different methods. There are a number of points the author may wish to consider:

Major Points

1. The lack of a control group renders the interpretation more limited, i.e., correlational within an infertile population. The authors should justify this approach.

2. As the authors have identified in the discussion, immunofluorescence is not the most appropriate method for PAH-DNA adducts detection; this constitutes the major weakness of the study. It is essential that the authors provide data establishing the efficiency and concordance of flow cytometry and fluorescence microscopy, to support the “good correlation” mentioned on page 9. Having used a more appropriate assay, ideally a quantitative assay (e.g., GC-MS) would certainly have made this study stronger.
3. Chromatin damage may be assessed by using a number of methods. The TUNEL assay simply reflects the number of open 3’OH end in DNA. Since the relationship is with PAH-DNA adducts, this positive correlation is intrinsically expected. Having another, or several other, markers of sperm chromatin damage would have been appropriate.

4. The rationale for the statistical methods used is poorly explained. This needs a more comprehensive discussion.

5. The lack of graphical representation of correlational analysis between PAH-DNA adducts and any of the endpoints deemed to be positively associated renders interpretation of the data difficult. Inclusion of figures that clearly demonstrate this correlation would strengthen the manuscript.

6. The discussion is overly long for the data presented in the manuscript. It should be reduced by about 30%.

Minor Point

1. Additional references are needed to better establish their reasoning (e.g. references [5-8], page 1 and [18], page 7).
2. All abbreviation must be defined the first time they are used.
3. Several of the sentences are awkward. The text would benefit from a further reading from someone fluent in scientific English.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

'I declare that I have no competing interests.