Reviewer's report

**Title:** Brain iron accumulation in unexplained fetal and infant death victims with smoker mothers - The possible involvement of maternal methemoglobinemia

**Version:** 1  **Date:** 9 January 2011

**Reviewer:** Torben Moos

**Reviewer's report:**

This manuscript examined the distribution of histological iron in stillborn and infant death human cases. The authors hint to a possible relation between smoking in pregnancy and neuronal pathology based on the assumption that smoking could affect iron-levels on blood due to the formation of methemoglobinemia with a lower capacity for iron binding, and subsequently could the iron enter the brain and exaggerate free-radical formation. There are a number of considerations the authors need to take.

**Major concerns:**

P.3:…..denaturated products, including..: ferric iron is never a denaturated product

P. 3: Same paragraph: Could the authors refer to the possible presence of free iron in plasma and also transferrin binding ?: These parameters should be change which will strengthen the hypothesis formulated by the authors.

P.4: Protocol: The authors need to revise their protocol for the iron stain as it is apparent that eosin was used to counterstain in their illustrations that were all claimed to be Prussian blue.

P.4: The authors need a more convincing protocol to claim their labeling to be neuronal. It is mandatory to co-stain with a neuronal marker e.g. NeuN, neurofilament, Neuronal specific enolase etc. The current criteria for claiming neuronal labeling within their sections is not sufficient based on the Prussian blue staining.

P.5: The paragraph on “Perl’s Prussian Blue..” should (without a heading) be moved to the previous page and inserted into the fourth section after.."for further investigations”.

P.5: It is very hard to make anything out of the statistics as the numbers of the different cases and conditions were not adequately presented. One is left with a very biased impression of a description only of smokers cases.

P.6: It is not evident to this reviewer that iron is present in neurons! The stains (all counterstained with eosin) do display a labeling within round cell bodies. However, what is apparently overlooked by the authors is that the brain is heavily infiltrated with monocytes during development that transform into macrophages that end up as quiescent microglia. The authors may simply have overlooked that normal neurons do not contain ferric iron (please confer at least to two papers on
nonheme iron that were published in 1995 on iron in the developing brain).
The cells presented in figs like 1B, 2B, 3B, 4B, and definitively 6A and 6B by all
means look like macrophages! By contrast the eosine+ cells in 5B look like
neurons based on their content of peripheral processes which are absent in
virtually any other slide. It is very strange that the authors can report on neuronal
labeling and have that in 4A eosine+ cells of a cortical layer completely without
labeling.

A general phenomenon is that the authors fail to mark the area in their “A” figs
that gets highlighted in “b”
The Discussion is not so relevant to evaluate as the interpretation of the Results
is questionable.

Minor:
p.3: ANS ?: Please abbreviate explain: Autonomous nervous system ?
p.5: line 1: histopathological not anatom..

The section on the "blood-brain barrier" needs another explanation: The BBB is a
capillary phoenomenon. The current slide shows presumeably a larger vein.

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

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

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

**Declaration of competing interests:**

No competing interests.