Reviewer's report

Title: Human papillomavirus is found in peripheral blood CD20+ and CD56+ cells during HPV-16 semen infection.

Version: 1 Date: 10 November 2013

Reviewer: Alyssa Cornall

Reviewer's report:

Comments

The available published data supporting a negative effect of HPV on male fertility (by the current author and others) does not appear to be strong at this stage. Furthermore, the Conclusions (Discussion) section of this manuscript pays little attention to the issue of HPV and male infertility, instead referring to a wide-range of rare congenital diseases that may have some link to horizontal transmission of HPV, as well as a fleeting mention of the possibility of blood-borne HPV leading to widespread somatic cancers. The significance of this reported finding of HPV DNA and protein in leukocytes of both semen and blood are diffusely reported. The conclusions/discussion should be more tightly focussed and the potential significance of the findings in the field of male infertility more clearly stated.

Major Compulsory Revisions

1. In the background, it should be made clear that few, if any, of the previous studies on HPV DNA in blood have shown HPV inside blood cells; most or all of the studies have merely shown the present of HPV DNA in blood, or on the outside surface of cells.

2. In the absence of evidence of HPV DNA in the nucleus, or mRNA expression, the presence of infection cannot be proven. Please adjust the descriptions of your findings to say "detection", "positivity" etc as appropriate, rather than infection unless there is enough evidence to prove infection.

3. Results Analysis of semen round cells Line 23: Please state whether these cells are CD45 positive or negative. Remove the phrase "not myeloid mononuclear leukocytes (17)."

4. Background Line 29: It should be made clear that the metastatic cells referenced are not blood cells, as is inferred from previous sentences.

5. Results page 8 Analysis of peripheral blood leukocytes: The absence of HPV DNA in the nucleus of these cells would indicate that they are not infected; please replace the word infected in lines 1 and 5.

6. Conclusions Page 9 Line 9: The authors have not proven that the CD56+ cells are NK cells, as a number of different immune cells express CD56. Either provide
more evidence of the identity of these cells or amend the statement.

7. Conclusions Page 9 Line 18 and 24: Reference 29 is used inappropriately as there is no mention of HPV in the cited article. Please amend to the correct reference or omit.

8. Conclusions Page 9 Line 25: Replace "understood to be the primary HPV receptor" with "theorised to potentially be the primary HPV receptor" and cite appropriate reference(s). A secondary receptor is thought to also be necessary for HPV infection; the authors should comment on this in the context of their results.

9. Conclusions Page 9 Line 26: Heparan-sulfate proteoglycans are expressed by many cell types and the attachment of HPV to HSG's is non-specific. Therefore this statement does not lend weight to the authors' argument and is misleading. Please amend.

10. What were the specific CD receptor expression patterns of HPV DNA or HPV protein-positive cells? CD4+/-, CD8+/-, CD20+/-, CD45+/-, CD56+/-? Please make clear so that the reader can decide for themselves which cell types these were.

Minor Essential Revisions

11. Background Line 6: HPV has been associated with most male *anal* cancers.

12. Background Line 9: replace "sperm parameters" with "sperm motility"

13. Background Line 9: recent data disputes the role of HPV in poor DNA integrity in sperm; remove "and DNA fragmentation".


No increased sperm DNA fragmentation index in semen containing human papillomavirus or herpesvirus.

Kaspersen MD, Bungum M, Fedder J, Bonde J, Larsen PB, J Ingerslev H, Höllsberg P.

14. Background Line 24-25: The study in reference 12 used Bovine Papillomavirus, therefore on Line 24 use of "HPV-DNA" is not appropriate and should instead say "papillomavirus DNA".

15. Results Analysis of semen round cells Line 10-11: Please state cell counts as x 10^3 rather than x 10^6.

16. Results Analysis of semen round cells Line 26: Cell surface markers should be described as being found "on" x% of cells rather than "in" x% of cells; please adjust elsewhere as necessary.
Discretionary Revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**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:**

I declare that I have no competing interests