

## **Reviewer's report**

**Title:** Human papillomavirus in semen and the risk for male infertility : a systematic review and meta-analysis

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**Reviewer:** Christophe Depuydt

### **Reviewer's report:**

A similar systematic review and meta-analysis has been done before (PMID: 24365799), the incidence and clearance of genital human papillomavirus infection in men has been described in 2011 (PMID: 21367446). Selecting slightly different studies resulted in similar results.

HPV is an organism with 2 phases, the virion and the infected cell. Although it is easy to understand that viruses are organisms that pass in their ontogenetic cycle through two distinct phenotypic phases, the dissociation between viruses and virions or infection and disease remains difficult for clinicians and HPV detection (PMID: 28210481). A good example of confusing virus and virion is HPV DNA measurement. Both the infectious HPV virions (L1/L2 protein capsid with inside circular HPV DNA) as the infected cells (both dividing and non-dividing cells) contain HPV DNA. Infectious HPV virions can only be assembled in non-dividing cells. Since sperm and seminal plasma does normally not contain dividing cells the viral HPV DNA measured originates from HPV virions (infectious = never cancer). HPV virions can cause temporarily subfertility through binding the equatorial region of the sperm head and directly affect the spermatozoon's quality, but spermatozoa can also transfer infectious HPV virions into the oocyte interfering with cell division. Women with cervical cancer caused by HPV, have the HPV DNA located in the nucleus of the (clonal) dividing cells. This HPV DNA can therefore not be transferred to spermatozoa or oocytes and these women can become pregnant. Lines 85-89: the 2 HPV induced pathways seems to have been mixed up.

Lines 89-91: HPV is not a good terminology and is confusing. The virions can go wherever the Brownian movement brings them. The virions can normally only infect dividing cells, which is needed to start their lifecycle.

Line 92-93: HPV virions? This is also not the correct reference, different groups showed the presence of HPV virions on spermatozoa in 2011. The reference of Luttmer you refer to is not the correct one. Besides the study by Luttmer et al is one of the studies without control group (bias) which as a consequence fail to show an effect of HPV presence in semen and impairment of semen quality.

Line 94-100: the correct reference is (PMID: 24365799), and the inconsistency is due to the fact that HPV DNA measurement was confused/allocated to the HPV virus instead as to the HPV virions (PMID: 28210481).

Lines 120-126: It is incorrect to assume that healthy male volunteers are fertile men. It is also incorrect to assume that men seeking a fertility evaluation are infertile. It is also unfortunate that men who were diagnosed as fertile or infertile were excluded.

Low and high risk relates to the risk of developing cancer. It is not the HPV induced transformation of clonal cell populations that affect fertility, that's the other pathway. Probably all HPV types, low, intermediate and high risk can bind the head of the spermatozoa and interfere with fertility.

Lines 165-167: Men with infectious HPV virions.

Lines 168-170: HPV virions (transient HPV infections) are limited in time. Fathering a child and HPV testing afterwards introduces a bias. Confirmed fertile should mean that the sperm/semens that was used to conceive was tested for HPV. Seeking fertility evaluation doesn't make someone infertile, and not all healthy male volunteers are fertile.

Lines 202-207: different sperm fractions have different HPV prevalence, it is not clear what is meant by in spermatozoa?

Lines 208-212: PCR versus hybridization for the detection of HPV DNA makes a big difference, since numerous inhibitors present in semen inhibits PCR but has less effect on ISH.

Lines 212-215: ?  $11 < 19$ ; text and numbers in table2 are opposite. By broad spectrum PCR you mean consensus PCR's for the detection of oncogenic (high risk HPV)? Consensus PCR's detects less HPV positives compared to type specific PCR's (PMID: 17760847).

Lines 234-242: Please explain why you find a higher (not significant; CI 16.2 - 24.6%) HPV prevalence in fertility attendees compared to Laprise et al 2014 (PMID: 24365799) who finds a HPV prevalence of 16% when focusing on infertile populations (CI 10-23%).

Lines 246-253: Clonal HPV (not infectious) can cause cancer, and infectious HPV virions can cause temporal subfertility. HPV virions are the infectious messengers, especially in sperm/semens were no dividing (clonal) cells are present. It is not because HPV DNA was

detected that this HPV DNA originates from a clonal HPV transformed cell population. Actually chances are high that the HPV DNA measured originated from virions (PMID: 26890987).

Lines 259-260: is incorrect (PMID: 23932300).

Lines 262-264: the most important variation is the source of the measured HPV DNA. The virion iceberg makes it difficult to detect the sometimes very small amount of HPV DNA in cancer cells (only 1 HPV DNA copy per clonal cell).

Lines 266-272: Without proper control group/population it is indeed difficult to observe a consistent association (see PMID: 28210481 on p 219).

Lines 305-307: only three case control studies were included?

Please write in full before using the abbreviation: e.g. line 45 HPV, line 75 STI, line 209 ISH, line 284 ASAs

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

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