Human Papillomavirus DNA Detection in Sperm Using Polymerase Chain Reaction

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Objective: **To detect human papillomavirus (HPV) in semen and find if sperm washing removes HPV DNA.**

Methods: **Amplification by nested polymerase chain reaction (PCR) was used to detect viral DNA sequences in semen samples from 85 volunteers. Forty-five men had historical or clinical evidence of genital HPV infection (study group) and 40 were healthy, clinically HPV-negative semen donors.**

Results: **We detected HPV DNA in the sperm cells of 24 of 45 subjects (53%) with past or current HPV infections in contrast to three of 40 healthy subjects (8%) ($P < .001$). Overall, PCR detected HPV in 21 of 32 subjects (66%) with identifiable lesions and six of 53 (11%) without them ($P < .001$). Swim-up washings of all 27 prewash sperm cells with HPV reduced cellular HPV DNA below detectable levels in only two cases.**

Conclusion: **HPV is present in sperm cells from infected and apparently healthy subjects, and sperm washing does not eliminate the risk of HPV transmission to recipients. We suggest that HPV DNA testing should be done on the semen of prospective donors, and those with positive tests should be excluded from donation. (Obstet Gynecol 2001;97:357–60. © 2001 by The American College of Obstetricians and Gynecologists.)**

Artificial insemination using donor sperm is a well-established procedure for treating infertility in childless couples when the male partner is infertile. Serious consequences to women and offspring could arise from several bacterial and viral infections that can be transmitted in semen.[1] The risk of sexually transmitted diseases (STDs) from donor insemination has recently received greater attention largely from the pandemic of human immunodeficiency virus (HIV) infection.[1] However, despite a prevalence of 20–40% in young semen donors, human papillomavirus (HPV) infection has received little attention by donor insemination programs. The risk of men with genital warts transmitting HPV to sperm recipients is unknown, although DNA from HPV has been isolated in semen.[2–4] Recipients of donor semen might be exposed to serious long-term consequences from infection resulting from transmission of HPV because certain types of the virus, especially types 16 and 18, are well established as etiologic agents of cervical cancer.[4]

Current guidelines for screening potential semen donors do not include testing for HPV.[5–7] That oversight is partly based on the traditional assumption that sperm from donors without clinically evident HPV infection is free of the virus. Although currently we exclude men with clinically evident genital HPV from semen donation, a MEDLINE literature search from 1966 to July 2000 using the terms "human papilloma virus DNA" and "sperm" did not find any published studies that confirmed or refuted the practice. Therefore, we aimed to detect HPV in the semen of subjects with historical and clinical evidence of HPV infections and healthy subjects. We also specifically sought whether routine sperm recovery procedures that involve "swim-up" purification provided HPV-free sperm preparations when HPV was detected in preprocessing semen samples. Our hypothesis was that sperm washing procedures used for in vivo and in vitro insemination do not eliminate the virus in infected subjects. Finding HPV DNA sequences in portions of sperm cells[8] besides those found in seminal fluid, and epithelial cells in semen, supports that hypothesis.

Subjects and Methods

The study proposal, protocols, and consent form were reviewed and approved by the Institutional Review Board of the University of Saskatchewan. We studied semen samples from 85 volunteers in a longitudinal

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study. Forty-five men aged 19–43 years (median 28 years) who had prior or currently visible penile warts with or without previous local treatment formed the HPV infection group. Randomly selected controls (no HPV group) of 40 men aged 20–41 years (median 27 years) were drawn from apparently healthy semen donors without prior or current HPV infection, and who were screened by history, physical examination, and standard laboratory tests, and found acceptable according to Canadian Fertility and Andrology Society guidelines for sperm donation.[5] The evaluation of subjects for HPV infection in both groups included high-magnification, acetic acid-aided examination of the penile shaft, retraction of the foreskin, and inspection of the external meatus and scrotum using a hand lens or a colposcope. We relied on the finding of verrucopapillary external genital lesions, which are likely to be associated with most genital HPV types, as the reference standard for the clinical diagnosis of HPV infection. The duration of the study was planned to be 18 months, but it was prolonged to 2 years because of slow enrollment.

Semen samples were collected onsite in a private collection room adjacent to the laboratory and maintained at room temperature until complete seminal liquefaction. After liquefaction, total volume, sperm concentration, motility, and progressivity were determined using a 200× phase contrast microscope. Approximately 1 mL of each specimen was taken for HPV DNA extraction. Sperm cells in another 1-mL aliquot were separated by Percoll gradient centrifugation[9] and washed four times with sterile phosphate-buffered saline. The supernatant was removed and the sperm pellet was subjected to HPV-DNA determination.

Total DNA was purified from 100 μL of semen by proteinase K-sodium dodecyl sulfate digestion, followed by phenol-chloroform extraction and precipitation with ethanol. DNA pellets were resuspended in 25 μL of sterile distilled water and 8 μL of that suspension was tested by polymerase chain reaction (PCR). To detect a broad range of HPV genotypes simultaneously, the consensus primers MY09 and MY11 were used.[10] Those primers are targeted to a conserved region of the L1 gene found in all HPV subtypes and amplify a fragment of 448 to 454 base pairs (depending on HPV subtype). Polymerase chain reaction buffer conditions were as described by Bauer and Manos.[10] Amplification was in a Perkin-Elmer 2400 thermal cycler (Perkin-Elmer, Norwalk, CT) using denaturation at 95C for 45 seconds, primer annealing at 60C for 45 seconds, and DNA extension at 72C for 1 minute. A total of 35 cycles were used for first-round amplification. Amplification products were separated by electrophoresis in a 1.4% agarose gel and stained with ethidium bromide. A positive result was indicated by a DNA band of approximately 450 base pairs.

Specimens that were negative after the initial round of amplification cycles were subjected to a second round of amplification using a seminested set of primers. That primer pair consisted of MY09 combined with an inner primer (5′-ATG G(TC)(GA) TTT G(CT)T GG(CG) (AG)(TCA)A A(TC)C A-3′), which together amplified a fragment of 430 base pairs when the original 450 base pair amplicon was used as template. For seminested PCR, 1 μL of product from the first-round reaction was amplified using the same buffer and cycling conditions as before. A positive result in this case was indicated by a DNA band of 430 base pairs. In this study, the application of a second round of amplification with seminested primers resulted in only an additional five specimens confirmed positive for HPV DNA (data not shown). Specimens that remained negative for HPV DNA after seminested PCR were tested for possible PCR inhibitory material by doing the PCR, using primers directed against the human β-globin gene, as described by Bauer and Manos.[10] Therefore, only specimens in which a β-globin fragment was successfully amplified and which remained negative after seminested HPV PCR were considered truly negative for HPV DNA. In our study, none of the samples contained PCR inhibitory material. The primary outcome measure of this study, presence of HPV DNA, was compared between groups using χ^2 and Fisher exact tests.

Results

Among 85 participants, 27 (32%) had HPV in their semen samples. In the group of 45 subjects with past or current HPV infections, we found HPV DNA in the initial sperm samples of 24 (53%) subjects. In contrast, three of 40 (8%) apparently healthy subjects in the control group had HPV DNA in their initial sperm samples ($P < .001$). Fifty-three of 85 men had no visible genital HPV lesions (all 40 men without prior HPV infections and 13 of 45 with prior infections). When results were analyzed on the basis of identifiable genital HPV lesions, the subjects with identifiable genital HPV lesions had a higher HPV detection rate, 21 of 32 (66%), compared with those without visible genital lesions, six of 53 (11%; $P < .001$).

Polymerase chain reaction analysis of swim-up samples of all 27 prewash semen samples that originally contained HPV DNA showed HPV DNA still present in all 21 (100%) samples from men with genital lesions. Of the remaining six men who did not have visible genital lesions but had HPV in their initial sperm samples, the washing procedure reduced HPV DNA below levels detectable by PCR assays in only two (33%), including

one of the three subjects in each of the study group and control group.

Discussion

The main finding of this study is that 53% of men with past or current genital HPV infections and 8% of apparently uninfected men had HPV DNA in their sperm. As more rapid and sensitive DNA tests have become available, there has been a resurgence in DNA testing to evaluate semen donors for STDs. In Canada, guidelines for screening potential sperm donors for STDs are being revised to incorporate sensitive DNA tests.[5] However, HPV transmission by donor semen has received little or no attention. Recent advances in viral diagnosis involve molecular biology techniques that identify viral genomes in cellular material from the genitourinary tract, serum, or semen.[11] Polymerase chain reaction techniques have successfully detected HPV DNA in body fluids including semen. That rapid and sensitive assay offers a unique advantage for HPV detection in potential donor semen. The assay can be semiautomated and costs approximately $6 (Canadian) per specimen for reagents only.

Our results confirm and extend the observations by Ostrow et al[2] that HPV can be identified in sperm. We also found a higher prevalence of HPV in the sperm of donors with clinically visible lesions, compared with those without them. Our results indicated that HPV can be found on sperm cells of apparently healthy subjects, which is consistent with observations that most HPV infections in men are asymptomatic.

There is a possible alternate explanation for why HPV DNA might be found in the sperm of apparently healthy subjects, as our results suggested. The exquisite sensitivity of the PCR, and especially with a nested reaction, makes the possibility of a false-positive result from carryover contamination with previously amplified DNA unable to be completely excluded.[12] However, that possibility is unlikely because our PCR reactions were done using strict laboratory techniques designed to negate contamination. As recommended by Kwok,[13] those included physically separated areas for sample processing and PCR amplicon detection, the use of aerosol sealed pipet tips, strict gloving and gowning procedures, and numerous negative controls throughout DNA extraction and PCR amplification procedures. Contamination was never detected throughout the course of laboratory investigations.

Our study might be interpreted to mean that current screening of semen donors using physical examination alone would miss about 8% of cases of HPV infection in asymptomatic individuals. Screening of semen donors for HPV is especially important because of its potential etiologic role in the development of genital neoplasia (penile and cervical). The availability of testing for this virus can influence a semen recipient's decision to accept a semen donation, especially when her safety is at stake. Screening for HPV could potentially reduce the risk of STD transmission during donor insemination.

The hypothesis that standard sperm recovery procedures that rely on migration of motile sperm away from washed epithelial cells and immotile sperm should eliminate infected epithelial cells, thereby providing virus-free sperm preparations, although biologically plausible, remains to be validated for HPV. In a study of HIV transmission in sperm, Lasheeb et al[14] and Kim and colleagues[15] showed that washing procedures reduced prewash extracellular RNA to below detectable limits in all cases. Proviral DNA in two of six prewash samples also was reduced to below detectable limits after washing. They concluded that semen washing before artificial insemination might reduce HIV transmission risk from infected men to uninfected women. Although our study was relatively small, our data are consistent with findings by Chan et al[8] that refuted the theory that sperm washing might eliminate the risk of HPV transmission to recipients. We suggest that virus transmission is not precluded by using washed sperm. However, unlike HIV, HPV is not a retrovirus, which might account for outcome differences after sperm washing. The nature of the HPV virus and the precise mechanism by which sperm cells take up exogenous HPV DNA, or by which the virus infects sperm cells, remains unknown. It is also not known whether HPV can be transmitted to fertilized oocytes then possibly to the fetus. It might be argued that the consequences of this ubiquitous viral pathogen are not as serious as those of HIV, and there have been no reports of genital warts or cervical neoplasia as a result of HPV transmission to a donor-sperm recipient. Our study was designed to find normative data on this virus in donor semen. Such a body of knowledge might make it possible to adopt techniques that ameliorate infertility and achieve pregnancies without increasing the spread of viruses.

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