Recent randomized controlled trials (RCTs) conducted in Africa have confirmed the efficacy of male circumcision in preventing female-to-male transmission of human immunodeficiency virus (HIV) in high-risk populations. Does this benefit extend to other sexually transmitted infections as well, and in particular to human papillomavirus (HPV) infection? Most of the research about HPV and male circumcision has been observational in nature and was conducted in the past 10 years. These investigations are extremely varied in sampling technique, HPV detection method, and the stage of infection under study, making them extremely challenging to review. Indeed, a meta-analysis determined that there is no consistent association between male circumcision and HPV infection risk, but other investigators reached the opposite conclusion in 2 subsequent reanalyses of the data. Further obfuscating any conclusions, to my knowledge, no reviews published to date have organized the studies by the HPV infection stage they are designed to investigate (eg, acquisition vs clearance) or considered that the association might be influenced by HPV type.

In the present review, I establish a baseline of knowledge about HPV infection as it relates to circumcision status. First, I evaluate the research methods used in studies published to date. Second, strengths identified in these methods are used as article selection criteria for a more focused analysis of male circumcision as it relates to stage of HPV infection and HPV type. Finally, I evaluate the current model for the relationship between circumcision and HPV infection risk and propose future directions for research.

Overview of HPV and Circumcision Identification of Studies

Studies were identified via PubMed using the search term circumcision HPV, and the search was further expanded by examining each publication’s reference list. Articles were limited to those that explored HPV infection as it could be...
related to circumcision status of the participants. A total of 31 original studies were identified and selected for the present review.6-36

Study Design
Most investigations of HPV infection as it relates to circumcision status are observational and cross-sectional. Although cohort studies are available, such as the HPV Infection in Men studies,24,25 most of the reported data as they apply to circumcision status are derived from baseline analyses rather than follow-up of participants over time to identify new HPV infections. Four RCTs6,7,31,34 have explored male circumcision and HPV infection in men, all of which were conducted in Africa and published within the past 2 years. Most studies have been limited to reporting HPV prevalence data. Because disease prevalence is a product of both the incidence and duration of infection, it does not demonstrate whether circumcision is influencing the acquisition of HPV, the ability of the host to clear the infection, or both.

Longitudinal studies are the preferred method for identifying factors that modify HPV infection risk, because they can identify incident cases. These types of studies also allow detection of stage-specific factors that influence HPV infection, because they have the potential to follow a given individual and identify when HPV is acquired and how long the infection lasts. Unfortunately, longitudinal studies of men are underrepresented in the literature on HPV and circumcision. The effective design of these studies is hampered by a lack of knowledge about the natural history of HPV infection in men. For example, there is no consistent between-test interval that defines persistence of HPV infection in men,37 which can lead to conflicting results when studies use different intervals between HPV detection points.

Detection of HPV Infection
Until the advent of molecular methods to study HPV infection, studies were limited to clinical diagnosis of HPV lesions, most commonly genital warts. The most common HPV genotypes assayed were, by default, nononcogenic (ie, low risk). Later studies used peniscopy to detect lesions for biopsy and Southern blot analysis,38 significantly improving the quality of evidence by allowing confirmation of HPV infection and virus typing.

Polymerase chain reaction (PCR)-based methods are the current standard for investigations of HPV and circumcision. Because a biopsy of anogenital tissues is not required for PCR, an additional advantage of PCR-based methods is that they allow the detection of subclinical lesions. This aspect is important, because most subclinical lesions are due to infection with oncogenic (ie, high-risk) HPV types, which are the most important HPV types with respect to anogenital cancer risk.38 However, HPV detection by PCR only implies HPV infection; because of the extreme sensitivity of these tests for detecting HPV DNA, the tests may also detect shed cells from an infected partner or low-copy infections that will soon abort and become irrelevant.38 Serology has been used in one study,15 and does overcome these limitations, but it is generally considered an inferior method because it cannot distinguish the location of infection (eg, genital vs oral) and not every infected person generates an antibody response to HPV infection.38

Although HPV genotyping with Southern blot analysis was performed as early as 1994 among circumcision and HPV infection studies, analyses could not be performed efficiently until the introduction of PCR-based methods in 2002.11 Type-specific explorations into the subject are therefore relatively new. Various primer sets are available for the detection of HPV by genotype, but more commonly a portion of the HPV L1 gene is amplified and genotyped via reverse line blot analysis. The Digene HPV Hybrid Capture 2 test is a more recent introduction to genotyping that has not been used in any of the circumcision studies published to date. This method uses a signal amplification system to detect low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV), primarily in combination with Papanicolaou tests in women. Limitations of the test include its low specificity in differentiating between LR-HPV and HR-HPV types and its inability to distinguish specific types within these groupings.39 There is currently no clinical indication for the use of these tests in men.

Much of the perceived discordance in the HPV and circumcision literature has arisen from superficial tallies of studies without regard to the types of HPV under study. Stratifying the evidence by HPV type resolves some of this conflict. For example, of 9 studies that identified HPV infection by self-report or clinical diagnosis of genital warts,12-14,16,20,21,23,26,27 which are exclusively caused by LR-HPV infections, only 2 studies23,26 found a protective effect of circumcision. In contrast, 15 of 21 studies using molecular methods of HPV detection, which can also identify HR-HPV types, found a protective effect of circumcision.6-11,19,22,24,25,28-34,36 Among studies that included LR-HPV types in the analysis, the vast majority reported no significant difference in the prevalence of LR-HPV infection among circumcised men.6,18,22,24,25,30,32,34,35 It is therefore more appropriate to consider that the efficacy of circumcision varies with respect to HPV type and to review LR- and HR-HPV studies independently.

An additional caution is warranted when studies that assay different HPV types are considered together, because bias may be interjected into analyses of prevalence data. High-risk HPV infections are generally more persistent than LR-HPV infections.37 A difference in HPV infection prevalence would be observed if a particular group was predisposed to HR-HPV infection, even if incident infection rates did not differ between groups (Figure). Original research and meta-analyses may therefore overstate or understate disease risk when they use prevalence data for HPV infections, particularly when they do not distinguish between LR- and HR-HPV infections.

Tissue Collection for PCR-Based Methods
Detection of HPV by means of PCR
requires a small sample of cells from which to extract DNA for analysis. In contrast to cell collection from the cervix, adequate cell collection from the keratinized epithelium of the male genitalia is challenging. Cells are usually collected by rubbing with a wet cotton or Dacron swab. Preparation of the collection site with emery paper increases cell removal and reduces the incidence of incomplete specimens, as measured by internal β-globin controls.53

In circumcised men, HPV infections are most commonly detected on the penile shaft, whereas the foreskin—particularly on the inner mucosal surface—is the most common site in uncircumcised men.53 Some studies have been criticized as introducing sampling bias by sampling only specific penile sites that predispose to HPV detection in either circumcised or uncircumcised men.5 For example, in a 2004 study,33 sampling only the coronal sulcus and glans penis, prepuce (in uncircumcised men), and scrotum.41

**Analysis of Select HPV and Circumcision Studies**

With the above limitations in mind, the articles identified in this review were parsed further. Articles were limited to those that distinguished HPV infections based on type and location, which necessarily restricted inclusion to those studies employing PCR-based methods of detection. To minimize the influence of sampling bias, articles were also restricted to those that sampled multiple anogenital sites. Exceptions to these criteria were made, however, and articles were included in the analysis if they described longitudinal studies or RCTs; such studies were underrepresented in the HPV and circumcision literature but may be the most informative with regard to virus acquisition and persistence. A total of 16 articles were identified and are outlined in Table 1.

**Prevalence of HPV Infection**

As shown in Table 2, HPV type influences the association between circumcision and the prevalence of HPV infection. As explained earlier in this review and reaffirmed here, there is no consistent association of circumcision with LR-HPV infection. Only 2 studies17,31 found a statistically significant association of circumcision status with the prevalence of LR-HPV infection. By contrast, among 9 studies that reported prevalence data by HPV type7,17,19,24,25,30,32,35 5 demonstrated that circumcision is associated with a decreased prevalence of HR-HPV infections, including the 2 RCTs that explored this outcome.7,17,19,30,31 These results indicate that male circumcision does not influence the prevalence of LR-HPV infection but may be associated with a reduced prevalence of HR-HPV infection.42

**HPV Incidence and Acquisition**

Male circumcision is thought to prevent HPV infection by limiting virus access to basal keratinocytes in the more cornified epithelium of the circumcised penis.11,18,22,31 Although there is probably no difference between the keratinization of the glans penis in circumcised and uncircumcised men,43 the mucosal epithelium of the inner prepuce is unkeratinized, which is thought to make it susceptible to injury during intercourse.18,42

The relatively larger surface area provided by the foreskin could also provide more opportunity for infection.11 If this model is correct, one would expect a decreased incidence of HPV infection in circumcised men, which would reflect reduced acquisition of the virus. Very few studies have deliberately explored the effect of circumcision on virus acquisition, but this model could be tested in the longitudinal studies and several of the RCTs of male circumcision that are available.6,19,21,22,28,31,34,36

Acquisition of HPV infection in these longitudinal studies is usually defined as the detection of HPV in someone who was previously HPV negative or the detection of a different type of HPV in someone who was previously infected. The interval between detection points in these studies ranged from 2 months to 2 years, and acquisition of HPV infection was inferred from incidence data. As shown in Table 3, these studies found that circumcision status
<table>
<thead>
<tr>
<th>Study</th>
<th>Sites Sampled (Collection/Detection Method)</th>
<th>Location (Population)</th>
<th>No. of Participants (% Circumcised)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Randomized Controlled Trial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auvert et al, 20097</td>
<td>Urethra (swab/PCR)</td>
<td>Orange Farm, South Africa (males aged 18-24 years)</td>
<td>1264 (50)</td>
</tr>
<tr>
<td>Gray et al, 20104</td>
<td>Coronal sulcus and glans penis (swab/PCR)</td>
<td>Rakai, Uganda (males aged 15-49 years)</td>
<td>780 (43)</td>
</tr>
<tr>
<td>Serwadda et al, 20106</td>
<td>Coronal sulcus and glans penis (swab/PCR)</td>
<td>Rakai, Uganda (HIV-positive males aged 15-49 years)</td>
<td>210 (49)</td>
</tr>
<tr>
<td>Tobian et al, 200911</td>
<td>Coronal sulcus and preputial space (swab/PCR)</td>
<td>Rakai, Uganda (males aged 15-49 years)</td>
<td>3393 (50)</td>
</tr>
<tr>
<td><strong>Longitudinal</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hernandez et al, 20106</td>
<td>Coronal sulcus and glans penis, shaft, scrotum (textured paper, swab/PCR)</td>
<td>University of Hawaii (≥18-year-old male university students)</td>
<td>357 (81)</td>
</tr>
<tr>
<td>Lajous et al, 200519</td>
<td>Scrotum, shaft, coronal sulcus, urethral meatus* (swab/PCR)</td>
<td>Mexico City, Mexico (male soldiers aged 16-40 years)</td>
<td>336 (13.1)</td>
</tr>
<tr>
<td>Lavreys et al, 199921</td>
<td>Clinical diagnosis of genital warts (not applicable)</td>
<td>Mombasa, Kenya (HIV-seronegative trucking company employees)</td>
<td>746 (87)</td>
</tr>
<tr>
<td>Lu et al, 200922</td>
<td>Coronal sulcus and glans penis, shaft, scrotum† (swab/PCR)</td>
<td>Southern Arizona (male residents aged 18-44 years with no current or prior diagnosis of any STI)</td>
<td>285 (88)</td>
</tr>
<tr>
<td>Partridge et al, 200728</td>
<td>Glans, shaft, scrotum, urine, fingernails† (emery paper, swab, or cytobrush/PCR)</td>
<td>University of Washington in Seattle (male university students aged 18-20 years)</td>
<td>240 (76.7)</td>
</tr>
<tr>
<td><strong>Cross-Sectional</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giuliano et al, 200917</td>
<td>Coronal sulcus and glans penis, shaft, scrotum* (swab/PCR)</td>
<td>Sao Paulo, Brazil; Morelos, Mexico; University of Florida (male residents aged 18-70 years with no current or prior diagnosis of any STI)</td>
<td>988 (40.3)</td>
</tr>
<tr>
<td>Hernandez et al, 200818</td>
<td>Coronal sulcus and glans penis, shaft, scrotum, semen, urine (textured paper, swab/PCR)</td>
<td>University of Hawaii (≥18-year-old male university students)</td>
<td>351 (79)</td>
</tr>
<tr>
<td>Muller et al, 201025</td>
<td>Coronal sulcus and glans penis, shaft, perianal region if warts present* (swab/PCR)</td>
<td>Johannesburg, South Africa (≥18-year-old men attending a sexual health clinic)</td>
<td>214 (26)</td>
</tr>
<tr>
<td>Nielson et al, 200724</td>
<td>Coronal sulcus and glans penis, shaft, perianal region, urethra, anal canal, semen† (swab/PCR)</td>
<td>Tucson, AZ, and Tampa, FL (men aged 18-40 years with no previous genital warts, anogenital cancer diagnosis, or current STI)</td>
<td>463 (84)</td>
</tr>
<tr>
<td>Nielson et al, 200925</td>
<td>Coronal sulcus and glans penis, shaft, scrotum, semen, urethra, perianal region, anal canal (swab/PCR)</td>
<td>Tucson, AZ, and Tampa, FL (men aged 18-40 years with no previous genital warts, anogenital cancer diagnosis, or current STI)</td>
<td>463 (84)</td>
</tr>
<tr>
<td>Svare et al, 200220</td>
<td>Coronal sulcus and glans penis, shaft, scrotum, perianal region* (swab/PCR)</td>
<td>Copenhagen, Denmark (STD clinic male patients)</td>
<td>216 (12)</td>
</tr>
<tr>
<td>Vaccarella et al, 200622</td>
<td>Coronal sulcus and glans penis, shaft, scrotum* (cytobrush/PCR)</td>
<td>14 states in Mexico (men requesting vasectomy in public clinics)</td>
<td>779 (31.7)</td>
</tr>
</tbody>
</table>

* The study pooled samples rather than analyzing each site independently.
† The study pooled sample data for analysis of circumcision (no site-specific statistics were reported).

Abbreviations: PCR, polymerase chain reaction; STI, sexually transmitted infection; STD, sexually transmitted disease.
had no effect on the acquisition of single HPV infection. The absence of an association holds true both for investigations that studied HPV acquisition generally and for those that conducted type-specific analyses. These observations indicate that men are equally susceptible to virus acquisition regardless of circumcision status.

Circumcision seems to protect against infection with multiple HPV types, however. With one exception, all studies exploring this association found decreased prevalence and incidence of multiple infections among circumcised men. Although the significance of multiple infections in men is unknown, multiple infections predispose to persistence of cervical HPV infections, which is believed to result from a less efficient immune response to HPV in some women. Paradoxically, the foreskin contains the highest density of Langerhans cells, CD4+ T cells, and macrophages in the penis, yet it is more susceptible to multiple infections. If circumcision modulates the immune response to HPV infection, the mechanism is unclear.

**Persistence of HPV Infection**

In contrast to its effects on virus acquisition, circumcision seems to be protective against persistent HPV infection. Five studies, including 2 RCTs, have examined the persistence of HPV infection as influenced by male circumcision. The time between HPV detection points varied from 2 months to 2 years. As shown in Table 4, almost all studies found a profound and statistically significant reduction in the persistence of HPV infection among circumcised men; the exception studied HIV-infected men—a population that would be predisposed to persistent infections. In one study of Mexican military men, the reduction in odds for persistence of HPV infection was 90% in circumcised men compared with uncircumcised men. Another study conducted in Arizona as part of the HPV Infection in Men study addressed persistence of infection by HPV type, finding 70% and 85% reductions in persistence for general and HR-HPV types, respectively. The demonstration of increased persistence of HPV infection in uncircumcised men is extremely important given that the rate of HPV acquisition seems to be uninfluenced by circumcision status. These results could explain why the prevalence of HR-HPV infection is increased among uncircumcised men in the absence of any increase in incidence: it results from a prolonged duration of infection.

**Implications and Future Directions**

The current model for the biologic plausibility of circumcision in reducing HPV infection risk is focused on virus acquisition. If circumcision status influences viral accessibility to basal keratinocytes, a statistically significant difference in HPV incidence between circumcised and uncircumcised men would be anticipated—an effect that is not observed. Rather, the key mechanism by which cir-
Male circumcision and HPV Studies Reviewed by Infection Stage and Virus Type

Table 3.

Selected Studies Exploring the Association Between Male Circumcision and Incidence of HPV Infection

<table>
<thead>
<tr>
<th>Study</th>
<th>Reported Statistics for Association of Circumcision With HPV Infection Type (95% Confidence Interval)*</th>
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<tbody>
<tr>
<td></td>
<td>Low-Risk HPV</td>
</tr>
<tr>
<td></td>
<td>(IRR, 0.78-1.52)</td>
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<td></td>
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</tr>
<tr>
<td>Gray et al, 2010‡‡§§</td>
<td>IRR, 1.09 (0.67-1.53)</td>
</tr>
<tr>
<td>Serwadda et al, 2010§</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: AHR, adjusted hazard ratio; AOR, adjusted odds ratio; HR, hazard ratio; HRR, hazard rate ratio; IRR, incidence rate ratio; NA, not available (HPV type was not investigated or statistic was not reported).

Circumcision seems to influence the prevalence of HPV infection is through promoting the clearance of infection (ie, reducing persistence).

How might circumcision status modulate viral persistence? One possibility is that the intimate association of the prepuce and glans penis promotes autoinfection. In uncircumcised men, there is frequent concordance between HPV types in the glans penis and prepuce, suggesting that infection could occur in one location and then spread sequentially, thereby prolonging HPV detection. An alternative possibility is that the uncircumcised penis has an increased susceptibility to establishment of HR-HPV infections, which generally persist longer than LR-HPV types. How- ever, studies indicate that incidence rates of HPV infection are equal across penile sites, and all sites seem equally susceptible to acquisition of LR- and HR-HPV, as reflected by equivalent incidence data from samples collected at these locations. Despite this, there is an established difference in HPV infection prevalence by penile site, and HR-HPV is more common on the inner foreskin, frenulum, and coronal sulcus. In addition, HR-HPV–positive penile intraepithelial neoplasia occurs “almost exclusively” on the inner foreskin, frenulum, and coronal sulcus. These observations indicate that persistence is influenced by anatomic site and is the most important factor influencing HPV infection prevalence in comparisons of circumcised and uncircumcised men.

Strategies for controlling the spread of HPV infection are primarily targeted at virus acquisition, but the findings summarized here indicate that promoting clearance in infected individuals may also be a worthwhile goal. To better quantify the potential role of circumcision in limiting the persistence of infection, future studies of HPV and circumcision should report disease incidence rather than prevalence data. To be most informative, longitudinal studies with frequent detection points will be required. Given the availability of molecular HPV typing, studies should also separate infections by virus type in analyses. To keep research costs reasonable and simplify analyses, it may be appropriate in many situations to limit sampling to the coronal sulcus and glans penis. Although it has been argued that sampling only the coronal sulcus and glans penis introduces sampling bias, it may be prudent to consider that these sites may be biologically biased to harbor persistent HPV infections. The almost exclusive occurrence of HPV-positive penile intraepithelial neoplasia in these sites certainly supports this possibility. Rather than introducing sampling bias, it could be argued that sampling of the coronal sulcus and glans penis yields information that is more biologically or clinically relevant.

Given that circumcision reduces the persistence and thereby the prevalence of HR-HPV infections, it could be an important adjunct to condom use, vaccination, and education in the worldwide prevention of HPV-associated malignancy. An RCT published earlier this year has demonstrated reduced prevalence, incidence, and persistence of HPV infection in the female partners of circumcised men, supporting the clinical relevance of male circumcision and HPV research findings. It is important to avoid using descriptors that exaggerate the actual
effect of circumcision, however. Some authors21-23 have described circumcision as a "surgical vaccine" against HPV (and HIV) infection—an inappropriate analogy, given that circumcision does not afford protection from HPV acquisition. Continuing to equate circumcision with vaccination promotes a false sense of protection and may unintentionally encourage behavior that increases risk of exposure to sexually transmitted infections. Circumcision should be described as only one of many factors that may collectively reduce the global burden of HPV infection.

References


