THE ASSOCIATION BETWEEN CIRCUMCISION AND HUMAN PAPILLOMAVIRUS INFECTION IN MALES AND THEIR FEMALE SEXUAL PARTNERS

SAMANTHA B. SHAPIRO

DEPARTMENT OF EPIDEMIOLOGY, BIOSTATISTICS AND OCCUPATIONAL HEALTH

MCGILL UNIVERSITY

AUGUST 2021

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Epidemiology

ABSTRACT

Background: Human papillomavirus (HPV) is the most common sexually transmitted agent. Infections are most often transient; however, persistent infection with high-risk HPV types is implicated in multiple cancers, including cervical, vaginal, vulvar, penile, anal, and oropharyngeal. Male circumcision (MC) has been demonstrated to be protective against human immunodeficiency virus acquisition, genital ulcer disease, urinary tract infections, and invasive penile cancer. An estimated 30–39% of males worldwide are circumcised. Several studies have investigated the association between MC and HPV infection and have indicated a potential protective effect; however, estimates vary between studies and there is a paucity of data regarding the effect of MC on HPV infections in their female partners.

Objectives: The objective of this thesis is to synthesize the existing literature on the association between MC and various HPV infection outcomes in males and their female sexual partners and to conduct original research using data from the HPV Infection and Transmission among Couples through Heterosexual activity (HITCH) cohort study.

Methods: In the first manuscript, multiple databases were systematically searched for primary research articles assessing the association between MC and the prevalence, incidence, and clearance of HPV infections in males and their female sexual partners. After screening articles for inclusion and extracting relevant data, the available evidence was qualitatively synthesized. In the second manuscript, data from the HITCH cohort study was used to perform a propensity-score based analysis using multilevel mixed-effects logistic and Poisson regression to estimate the association between MC and the prevalence, transmission, and clearance of HPV infections in males and females who were between the ages of 18 and 24 and in a sexual relationship of less than six months.

Results: The qualitative synthesis of the literature shows that MC appears to have a protective effect against various HPV infection outcomes, most notably prevalence, in males and females. A particularly protective effect was observed in males for infection with high-risk HPV types at the glans and corona of the penis. The analysis of the HITCH study found that in males, MC was associated with a modest but nonsignificant decrease in prevalent HPV infections at baseline (adjusted odds ratio (OR) 0.84, 95% confidence interval (CI) 0.58–1.22) and was not associated with increased clearance of baseline infections (adjusted hazard ratio (HR) 0.84, 95% CI 0.44–

1.60). Point estimates suggested that MC may be associated with a decrease in female-to-male (adjusted HR 0.52, 95% CI 0.18–1.52), but not male-to-female (adjusted HR 1.22, 95% CI 0.44–3.44) transmission of HPV infections.

Discussion: Current evidence suggests that MC may be protective against various HPV infection outcomes. The evidence is strongest for a protective association between MC and prevalent HPV infections at distal sites of the penis. This association may be due to changes in penile keratinization or in the penile immune environment that result from removal of the foreskin. Understanding the association between male circumcision and HPV infection in males and females is important for medical decision-making among adult males and parents of infant males, especially in countries with a high burden of HPV-associated disease; however, larger couple-based studies would be needed to determine causality.

Conclusion: MC may have a modestly protective effect against various HPV outcomes in males and their female sexual partners.

RÉSUMÉ

Contexte : Le virus du papillome humain (VPH) est l'agent sexuellement transmissible le plus courant. Les infections sont le plus souvent transitoires, cependant, les infections persistantes avec les VPH à risque élevé sont impliquées dans plusieurs cancers génitaux et de l'oropharynx. La circoncision masculine (CM) a été démontré de protéger contre l'acquisition de virus de l'immunodéficience humain, les ulcères génitaux, les infections urinaires, et le cancer invasif du pénis. Il est estimé que 30–39 % des hommes dans le monde sont circoncis. Plusieurs études ont examiné l'association entre la CM et l'infection par le VPH et ont indiqué un effet protecteur potentiel. Cependant, les estimations varient considérablement d'une étude à l'autre et il existe peu de données concernant l'effet de la CM sur les infections par le VPH chez leurs partenaires féminines.

Objectifs : L'objectif de cette thèse est de synthétiser la littérature existante sur l'association entre la CM et divers résultats d'infection par le VPH chez les hommes et leurs partenaires sexuelles féminines et de mener une recherche originale en utilisant les données de l'étude de cohorte HITCH (HPV Infection and Transmission among Couples through Heterosexual activity).

Méthodes : Dans le premier manuscrit, de multiples bases de données ont été systématiquement consultées pour trouver des articles de recherche primaire évaluant l'association entre le MC et la prévalence, l'incidence et la clairance des infections par le VPH chez les hommes et leurs partenaires sexuels féminins. Après avoir vérifié l'inclusion des articles et extrait les données pertinentes, les données disponibles ont été synthétisées qualitativement. Dans le deuxième manuscrit, les données de l'étude de cohorte HITCH ont été utilisées pour effectuer une analyse basée sur le coefficient de propension avec la régression logistique et Poisson multiniveau à effets mixtes afin d'estimer l'association entre la CM et la prévalence, la transmission et l'élimination des infections par le VPH chez les hommes et les femmes âgés de 18 à 24 ans et ayant une relation sexuelle de moins de six mois.

Résultats : La synthèse qualitative de la littérature montre que la CM semble avoir un effet protecteur contre divers résultats de l'infection par le VPH, en particulier la prévalence, chez les hommes et les femmes. Un effet particulièrement protecteur a été observé chez les hommes pour l'infection par des types de VPH à haut risque au niveau du gland et de la couronne du pénis. L'analyse de l'étude HITCH a révélé que, chez les hommes, la CM était associée à une diminution

modeste mais non significative de la prévalence des infections à VPH au départ (rapport de cotes ajusté 0,84, intervalle de confiance à 95 % (IC 95 %) 0,58–1,22) et n'était pas associée à une élimination accrue des infections au départ (rapport de risques ajusté 0,84, IC 95 % 0,44–1,60). Les estimations ponctuelles suggèrent que la CM pourrait être associée à une diminution de la transmission des infections par le VPH de la femme à l'homme (rapport de risques ajusté 0,52, IC 95 % 0,18–1,52), mais pas de l'homme à la femme (rapport de risque ajusté 1,22, IC 95 % 0,44–3,44).

Discussion : Les données actuelles suggèrent que le CM pourrait avoir un effet protecteur contre diverses infections par le VPH. Les preuves sont les plus solides en ce qui concerne l'association protectrice entre la CM et la prévalence des infections à VPH dans les sites distaux du pénis. Cette association peut être due à des modifications de la kératinisation du pénis ou de l'environnement immunitaire du pénis résultant de l'ablation du prépuce. La compréhension de l'association entre la circoncision et l'infection par le VPH chez les hommes et les femmes est importante pour la prise de décision médicale chez les hommes adultes et les parents de nourrissons de sexe masculin, en particulier dans les pays où la charge de morbidité associée au VPH est élevée ; cependant, des études de couple plus importantes seraient nécessaires pour déterminer la causalité.

Conclusion : Le MC peut avoir un effet protecteur modeste contre diverses conséquences du VPH chez les hommes et leurs partenaires sexuelles féminines.

PREFACE AND AUTHOR CONTRIBUTIONS

This thesis consists of a literature review, systematic review manuscript, original research manuscript, discussion of results, and final conclusions. The contributions of each author to the two manuscripts are as follows:

For the systematic review manuscript "Association between circumcision and human papillomavirus infection in males and females: a systematic review": Samantha Shapiro and Eduardo Franco conceptualized the project. Samantha Shapiro and Cassandra Laurie conducted the methodology of the search. Samantha Shapiro analyzed its results and drafted the manuscript, which was reviewed and edited by Cassandra Laurie, Mariam El-Zein, and Eduardo Franco. Eduardo Franco and Mariam El-Zein provided supervision and guidance.

For the original research manuscript "Association between circumcision and human papillomavirus infection in males and their female sexual partners: findings from the HITCH cohort study": Eduardo Franco and Ann Burchell led the study while Pierre-Paul Tellier and François Coutlée acted as co-investigators. François Coutlée was responsible for the laboratory analysis of biological specimens for HPV DNA testing. Eduardo Franco and Michel Wissing conceptualized the analysis. Samantha Shapiro, Michel Wissing, and Farzin Khosrow-Khavar devised the methodology. Samantha Shapiro conducted the analyses. Samantha Shapiro and Michel Wissing drafted the manuscript. Michel Wissing, Farzin Khosrow-Khavar, Ann Burchell, Mariam El-Zein, Pierre-Paul Tellier, François Coutlée, and Eduardo Franco all reviewed and edited the manuscript. Eduardo Franco and Mariam El-Zein provided supervision and guidance.

ACKNOWLEDGMENTS

First and foremost, I would like to extend my utmost thanks to my thesis supervisor, Dr. Eduardo Franco. Dr. Franco not only introduced me to the field of HPV research – he introduced me to the discipline of epidemiology as a whole. I was most fortunate to secure a job as a research assistant at the Division of Cancer Epidemiology in the summer of 2017. What was supposed to be a four-month position turned into a two-year contract in a supportive and mentally stimulating workplace that shattered my perceptions of a career in research. My time as a research assistant at the DCE was what led me to pursue a Master's degree in Epidemiology, and I was truly privileged to continue in Dr. Franco's team for another two years as a graduate student. I credit Dr. Franco for my love of epidemiology, and I will be forever grateful for the time I spent under his wing.

Part of what made the DCE so exceptional was the wonderful group that worked there. One of the most special members of this team was Mariam El-Zein, the Associate Director for Research. Mariam was the heart of the DCE, an incredible mentor, and a true role model. Her door was always open for help and support, and the lunchroom was always full of laughter when she was around. Mariam exemplified hard work and dedication to her field.

Other members of the DCE to whom I'm most grateful are Drs. Farzin Khosrow-Khavar and Talía Malagón, who fielded my never-ending questions about methodology and interpretations of results. Their work and statistical expertise impressed me every day, and I consider myself most fortunate to have worked alongside the two of them, whom I credit as some of the smartest people I know. Of course, I must also mention Dr. Michel Wissing, who has been sorely missed since his departure from the DCE. While I was a research assistant, he invited me to collaborate on a small project investigating the association between circumcision and HPV – little did we know that this would balloon into an entire thesis. The foundation that he created, as well as his ideas, collaboration, and assistance, were integral for the completion of my thesis. I also give a huge thank you to the other members of the DCE, including Allita Rodrigues for her administrative support and the other students.

I would also like to thank my thesis committee member Dr. Paul Brassard for his support, my professors in EBOH for passing on their knowledge and maintaining a somewhat normal learning environment despite the challenges posed by COVID, the administrative staff at EBOH for all the assistance they provide, and the participants of the HITCH study.

Finally, I would like to thank my family, especially my parents, and my friends for all their unconditional and unwavering support over the past two years. Pursuing a degree primarily at-home through a pandemic has been a memorable (and hopefully once-in-a-lifetime) experience, and one that I would not have been able to do without their support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

ASIR	Age-standardized incidence rate
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
HITCH	HPV Infection and Transmission among Couples through Heterosexual Activity
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HR	Hazard ratio
HR-HPV	High-risk HPV
IPTW	Inverse probability of treatment weighting
LMIC	Low- and middle-income country
LR-HPV	Low-risk HPV
MC	Male circumcision
MSM	Males who have sex with males
M+F+	Male positive, female positive
M+F-	Male positive, female negative
M-F+	Male negative, female positive
M-F-	Male negative, female negative
O:E	Observed:expected
OR	Odds ratio
PLHIV	People living with HIV
RCS	Region of common support
RCT	Randomized controlled trial
STI	Sexually transmitted infection
WHO	World Health Organization

CHAPTER 1. INTRODUCTION

1.1. RATIONALE

Human papillomavirus (HPV) is the most prevalent sexually transmitted agent worldwide.[1,2] Persistent infection with high-risk HPV (HR-HPV) is a necessary cause of cervical cancer and is associated with penile, anal, vaginal, vulvar, and head and neck cancers,[3-5] while infection with low-risk HPV (LR-HPV) is associated with genital warts.[6] The burden of HPV-associated cancer varies worldwide, but is greatest in low- and middle-income countries (LMICs).[7,8] In 2018, almost 90% of the approximately 311,000 global cervical cancer deaths occurred in LMICs.[9] Many of these countries have not implemented programs for HPV vaccination or do not have a vaccine commercially available and do not have screening programs of high coverage and quality.[10]

Male circumcision (MC) has been demonstrated to be protective against a variety of sexually transmitted infections, including HIV, herpes simplex type 2, trichomoniasis, chancroid, and syphilis.[11-13] Several randomized controlled trials have been conducted to evaluate the association of MC with HIV acquisition: these trials have also included analyses of HPV as secondary endpoints. Most studies of the relationship between MC and HPV infections in males have been cross-sectional analyses, and few studies have evaluated the risk of HPV infection in the female partners of circumcised and uncircumcised males. Previous randomized controlled trials,[14,15] observational studies, systematic reviews,[16,17] and meta-analyses[18-20] have indicated that MC is protective against a variety of HPV infection outcomes in males and their female sexual partners. However, there have been multiple recently published studies on the topic, requiring an update to the existing literature. In this systematic review, we present the growing evidence that suggests that MC may protect against HPV infections in males, and that this protection may be conferred to their female sexual partners.

1.2. OBJECTIVES

The objective of this project is to examine the association between male circumcision (MC) and the prevalence, incidence, and clearance of genital HPV infections in males and their female sexual partners. To do so, I conducted a systematic review and used data from the 'HPV Infection

and Transmission among Couples through Heterosexual Activity' (HITCH) cohort study to perform my own analysis.

CHAPTER 2. LITERATURE REVIEW

2.1. HUMAN PAPILLOMAVIRUS

2.1.1. BIOLOGY AND ETIOLOGY

2.1.1.1. VIRAL STRUCTURE AND GENOME

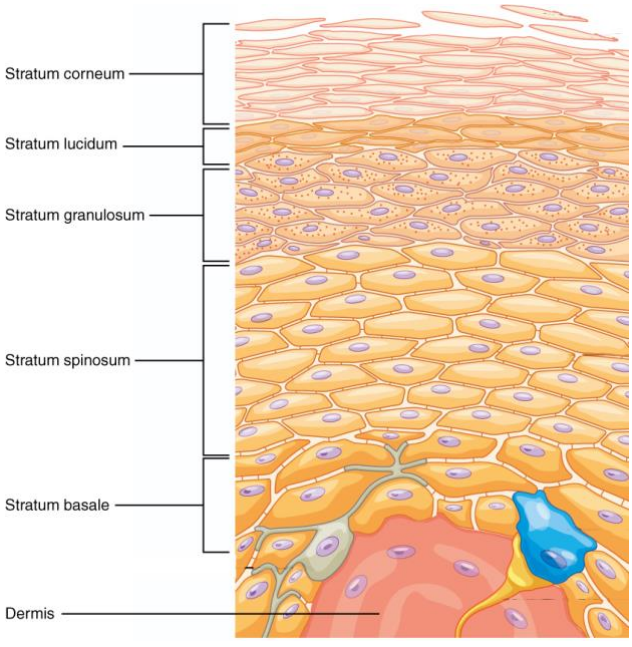
Human papillomaviruses (HPV) are non-enveloped, double-stranded DNA viruses between 52 and 55 nm in diameter.[6,21] The viral particle consists of a single double-stranded DNA approximately 8,000 base pairs long bound to histones and surrounded by a 72-part protein capsid.[6] The capsid is composed of two structural proteins, L1 and L2, both of which are encoded by the viral genome.[6]

The viral genome also encodes proteins E1, E2, E4, E5, E6, and E7, which are involved in replication. E1 and E2 recognize the origin of replication, with the latter being the main transcriptional regulator, and E6 and E7 target negative regulators of the cell cycle as well as maintaining the viral episome.[6]

2.1.1.2. NATURAL HISTORY AND MECHANISM OF INFECTION

HPV is primarily transmitted through skin-to-skin contact, mainly during sexual activity, and exclusively establishes itself in cutaneous and mucosal stratified epithelium.[6,21,22] The layers of the epithelium are illustrated in Figure 2-1. It is thought that the virus enters the epithelium through lesions and microlesions during sexual acts, infecting the basal epithelial cells (cells of the stratum basale) and begins to replicate. Upon replication, one daughter cell remains in the basal layer while the other migrates to the more superficial spinous layer (stratum spinosum) and partially differentiates. The viral DNA proliferates in the granular layer (stratum granulosum), and L1 and L2 are assembled in the upper layers of the epithelium.

Figure 2-1: Layers of the stratified epithelium

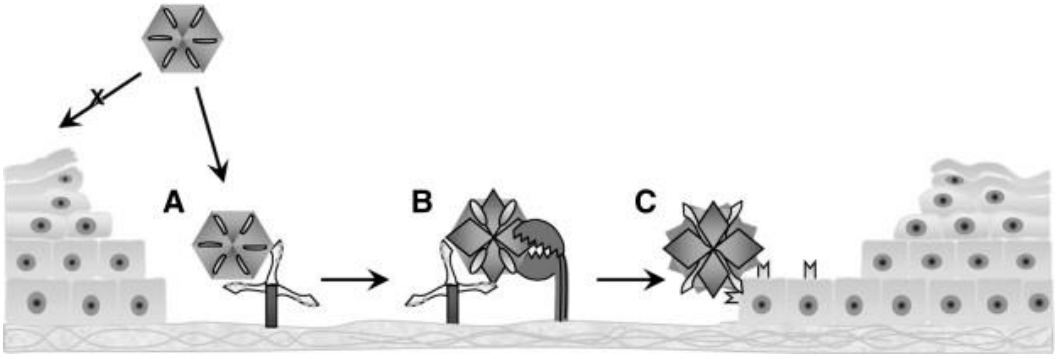


Adapted from OpenStax under a Creative Commons license[23]

The viral particle is assembled in the stratum corneum. Infected cells are sloughed off from the top of the epithelium, thereby allowing the virus to infect new individuals.[21] The length of time between initial infection and viral release is unknown, but must be at least three weeks based on the physiology of epithelial proliferation.[24]

Figure 2-2 illustrates the mechanism of HPV infection. When an HPV virion enters the body through a microabrasion, it reaches the basal layer, where it binds to a heparan sulfate proteoglycan receptor. Binding induces a conformational change in the virion that exposes the previously hidden L2 protein. L2 is cleaved by the protease furin or the proprotein convertase PC5/6 – this cleavage exposes a binding site on L1, which binds to an unknown secondary receptor on the epithelial cell surface.[22]

Figure 2-2: Mechanism of HPV infection



Reprinted with permission from Elsevier[22]

Once HPV infects the cell, the E6 protein binds to the p53 tumour suppressor gene product to prematurely degrade p53, and the E7 protein binds to the retinoblastoma tumour suppressor protein and inhibits its function. The lack of inhibitory feedback leads to cellular proliferation, which can lead to a variety of changes ranging from benign hyperplasia to invasive carcinoma.[25]

The incubation period can range from weeks to months for warts and months to years for carcinomas.[25] The required steps for carcinogenesis include HPV infection, persistence of the infection, progression to precancerous lesions, then tissue invasion. The first three steps are reversible through clearance of the HPV infection by the immune system and precancer regression; it is not uncommon for this reversal to occur.[6]

2.1.1.3. Classification of HPV types and phylogenetic subgenera

Human papillomaviruses are a part of the family *Papillomaviridae*, which contains various genera including *Alphapapillomavirus*.[26] Strains (more often referred to as genotypes or types) in

the *Alphapapillomavirus* genus cause mucosal and cutaneous lesions in humans; *Betapapillomavirus*, *Gammapapillomavirus*, *Mupapillomavirus* and *Nupapillomavirus* types cause only cutaneous lesions.[6] HPV types in the *Alphapapillomavirus* genus are consequently the ones of interest in studies of anogenital disease as they are the main infectors of mucosal tissue.

Phylogenetic analyses by Schiffman et al. group the *Alphapapillomavirus* types into three primary subgenera: subgenus 1 contains the low-risk types (LR-HPV) that are associated with genital warts, subgenus 2 contains the high-risk types (HR-HPV) associated with squamous cell carcinoma, and subgenus 3 contains types involved in commensal infections.[27,28]

Usage of the term HPV will hereon refer to types in the *Alphapapillomavirus* genus unless otherwise specified.

2.1.2. HPV-ASSOCIATED DISEASES

Most HPV infections are "transient, asymptomatic, or subclinical",[25] but persistent infections or infections in immunosuppressed individuals can lead to clinical manifestations. About 70% of HPV infections spontaneously resolve within one year and 90% in two, with the remainder becoming persistent.[29]

2.1.2.1. NON-MALIGNANT LESIONS

LR-HPV infections can cause condyloma acuminata, more often referred to as genital warts.[6] These are benign fibro-epithelial tumours that grow in the anogenital region, i.e., the vulva, vagina, ectocervix, penis, perineum, and anus.[30,31] In males, they are more often found in those that are uncircumcised and are usually located at the glans, coronal sulcus, and inner foreskin of the penis.[32] They often manifest as fleshy grouped papules but can proliferate in cauliflower-like plaques.[33] Over 90% of condyloma acuminata are caused by the LR-HPV types 6 and 11.[33] Other non-malignant lesions associated with alphapapillomaviruses include Bowenoid papulosis and Bushcke-Löwenstein tumours in the anogenital region, as well as oral papillomas and laryngeal papillomatosis in the head and neck.[30]

2.1.2.2. MALIGNANCIES

The International Agency for Research on Cancer classifies HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as group 1 carcinogens, i.e., agents that are carcinogenic to humans. Type 68 is a group 2A carcinogen, i.e., probably carcinogenic to humans, while types 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97 are group 2B carcinogens, i.e., possibly carcinogenic to

humans. Finally, types 6 and 11 are classified as group 3 carcinogens, i.e., unclassifiable as to carcinogenicity in humans.[34]

Persistent HPV infections are linked to virtually all cervical cancers and almost all anal cancers.[3] They are also implicated in vaginal, vulvar, penile, and oropharyngeal cancers.[3] HPV was established as necessary cause of cervical cancer in 1999.[5] HPV DNA has been detected in 98% of cases of cervical carcinoma *in situ*, over 90% of anal cancers, over 70% of vaginal and oropharyngeal cancers, over 60% of penile cancers, over 30% of oral cancers, and in 20% of laryngeal cancers.[4]

Cervical carcinogenesis begins as mild dysplasia, which progresses to moderate dysplasia, then severe dysplasia or carcinoma *in situ*, then invasive cervical cancer.[6] A system devised in 1968 classifies three stages of cervical intraepithelial neoplasia (CIN): CIN1, involving the lower third of the cervical epithelium, CIN2, occupying two-thirds of the epithelium, and CIN3, where the entire cervical epithelium is dysplastic.[6,35] This classification has been adapted to describe anal intraepithelial neoplasia, vulvar intraepithelial neoplasia, vaginal intraepithelial neoplasia, and penile intraepithelial neoplasia.[6]

2.1.3. DESCRIPTIVE EPIDEMIOLOGY

2.1.3.1. PREVALENCE

HPV is the most common sexually transmitted infection worldwide.[1,2,36] Prior to HPV vaccine availability, the estimated lifetime probability of acquiring an HPV infection among those with at least one opposite sex partner was 84.6% for females and 91.3% for males in the United States.[37] In Canada, estimates for the prevalence of HPV among females with normal cervical cytology range from 5.2% to 30.6%; worldwide, the estimated prevalence of HPV among females with normal cytology ranges from less than 9% to more than 25%.[38] HPV infection is most common in females under 25 and prevalence generally decreases with age, though in some geographic regions, prevalence starts to increase again between ages 45 and 54.[38-40] Prevalence is postulated to be higher among younger females as they have more sexual partners and have had less time to develop immunity to HPV[39]; it may increase later in life due to reactivation of latent infections from loss of immunity or hormonal changes from menopause, or due to acquisition of new infections from new sexual partners later in life.[41] In the United States, prevalence of any HPV during 2013–2014 was estimated to be 45.2% among males and 39.9% among females aged

18–59, whereas the prevalence of high-risk HPV was 25.1% and 20.4% among males and females, respectively.[42]

2.1.3.2. PROPHYLAXIS

Vaccination against HPV is the primary prophylactic measure. Several vaccines are available to protect against the types that have the greatest disease burden: Cervarix® protects against HPV 16 and 18, Gardasil® protects against HPV 6, 11, 16, and 18, and Gardasil®9 protects against HPV 6, 11, 16, 18, 33, 35, 45, 52, and 58.[43]

Health Canada approved Gardasil in 2006, Cervarix in 2010, and Gardasil 9 in 2015. Canada's National Advisory Committee on Immunization has made recommendations regarding use of the vaccines in 2007, 2012, 2015, and 2016, and 2017.[44-48] In 2007, Gardasil was recommended for use in females aged 9–13 years old and 14–26 years old.[44] In 2012, the recommendation added females aged 27–45 years old, males aged 9–26, and males who have sex with males (MSM). This update also included Cervarix as a recommended vaccine for females regardless of age.[45] The 2015 update approved a two-dose schedule (rather than the original three-dose) for immunocompetent males and females aged 9–14.[46] In 2016, after the approval of Gardasil 9, its use in a three-dose schedule was recommended for males and females aged 9 and above.[47] The 2017 update allowed for Gardasil 9 to be given as a two-dose schedule for immunocompetent males and females aged 9–14.[48] Similar recommendations have been made in countries throughout the world.[49]

Universal vaccination against HPV could prevent a notable 70–90% of HPV-related disease.[50] Unfortunately, vaccine uptake remains suboptimal in many parts of the world, especially in less developed regions, which bear the greatest burden of HPV-associated disease.[51-53] Only 55% of WHO regions have introduced HPV vaccination partially or nationwide, with an unequal global distribution: only 31% of countries in Africa and 40% of countries in Asia have introduced programs, compared to 77% of countries in Europe and 85% of countries in North and South America.[51] First-dose vaccine coverage is estimated at 50%, 27%, 4%, and 32% in high income, upper-middle income, lower-middle income, and low income countries, respectively.[51]

2.1.3.3. RISK FACTORS

There are multiple established risk factors for genital HPV infections. Among females, the risk of HPV acquisition is increased by the female's sexual behaviours (e.g., higher number of sexual partners, younger age at first sexual intercourse),[54-57] the characteristics of her male partner

(e.g., partner's non-monogamy, partner's older age),[54] and other factors not related to sexual behaviour (e.g., long-term use of oral contraceptives, smoking, having a greater number of pregnancies, having a history of chlamydia and other sexually transmitted infections (STIs), or being of Black or Hispanic ethnicity).[54-57] HPV has been less extensively studied in males, but likely has similar risk factors. The HPV Infection in Men study, a large multinational cohort study, found that lifetime number of sexual partners and history of STIs were both significant predictors of HPV prevalence in males[58]; a 2018 meta-analysis found smoking to be an additional risk factor.[59] The effect of condoms is contentious: it may be protective against infection in males[58,59] but does not seem to confer significant protection towards females.[55,60,61]

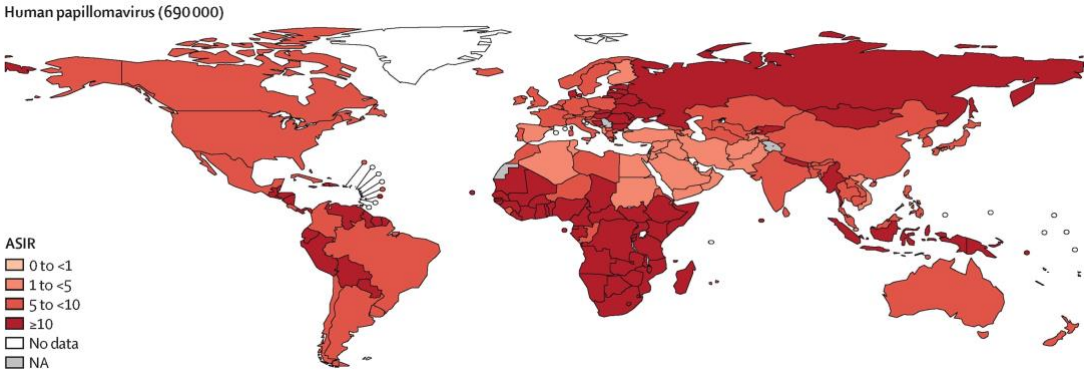
A 2016 systematic review and meta-analysis found that performance of oral sex and smoking were the primary risk factors for oral HPV infections in healthy individuals. The prevalence of any HPV, high-risk HPV, and low-risk HPV in the oral cavity was highest in MSM, then in males who have sex with females, then lowest in females.[62]

2.1.3.4. BURDEN

Anogenital warts are the most common clinical manifestation of HPV throughout the world,[8] with HPV 6 and 11 are responsible for 90% of cases.[63] The economic burden of genital warts in the United States alone in 2004 was estimated to be $200 million USD.[64] Although lesions are not dangerous, they can be associated with "severe discomfort, burning, and pruritis"[63] and decreased quality of life due to psychological distress.[65,66]

The burden of HPV-related cancers varies drastically throughout the world and is strongly associated with national income as measured by the World Bank, with lower-income countries bearing the brunt of disease.[7,8] The age-standardized incidence rate (ASIR) of cancer cases attributable to HPV per 100,000 person-years in 2018 was 6.9 in high-income and upper-middle income countries, 9.2 in lower-middle income countries, and 16.1 in low-income countries.[7] HPV was responsible for 690,000 cancer cases worldwide in 2018 and had an overall ASIR of 8.0 cancer cases per 100,000 person-years.[7] The ASIR was as low as 2.4 in Western Asia and as high as 19.3 in sub-Saharan Africa.[7] HPV is responsible for 4–5% of cancers worldwide,[50,67] ranging from an estimated 2.1% in more developed regions to 6.9% in less developed regions.[67] Figure 2-3 2-3 shows the ASIR by country in 2018.

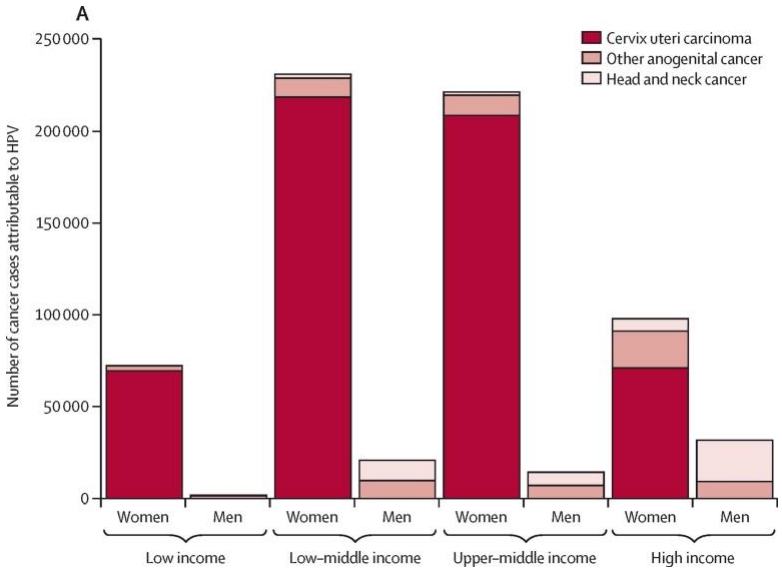
Figure 2-3: Age-standardized incidence rate of HPV-attributable cancers, 2018



Reprinted with permission from Elsevier[7]

Figure 2-4 shows the distribution of HPV-attributable cancers by sex, World Bank income group, and cancer type in 2018. In low-income countries, cervical cancer represents almost all the cancers attributable to HPV, with males having close to no cancer cases attributed to its infection. Contrarily, in high-income countries, approximately a quarter of cancers attributable to HPV infection occur in males, primarily in the form of head and neck cancers. Among HPV-attributable cancers in females in high-income countries, approximately 70% are cases of cervical cancer, 20% are other anogenital cancers, and 10% are head and neck cancers.[7] Cervical cancer is the fourth leading cause of cancer death worldwide.[50]

Figure 2-4: HPV-attributable cancers by sex, World Bank income group, and type, 2018



Reprinted with permission from Elsevier[7]

Of the estimated 7,097 cancer cases in 2015 in Canada that were caused by infections, 3,828 (53.9%) were attributed to infection HR-HPV.[68] The population attributable risk for high-

risk HPV infection in anogenital cancers was 100% for cervical cancer, 94.6% for anal cancer in females and 87.6% for anal cancer in males, 76.8% for vulvar cancers in females under 50 and 43.2% in females 50 and older, 72.2% for vaginal cancers, and 39.3% for penile cancers.[68] Infection with HPV 16 was attributed to 60.2% of oropharyngeal cancers, 12.7% of laryngeal cancers, and 8.2% of cancers of the oral cavity.[68] This translated to 1,375 cases of cervical cancer, 589 cases of anal cancer, 301 cases of vulvar cancer, 130 cases of vaginal cancer, and 81 cases of penile cancer.[68]

The World Health Organization (WHO) has called for the elimination of cervical cancer, defined by an incidence of less than 4 cases per 100,000 female person-years.[69] To meet this goal, countries should meet the 90-70-90 goal by 2030, which is to have 90% of girls fully vaccinated against HPV by age 15, 70% of females screened with an HPV DNA test by ages 35 and 45, and 90% of females with cervical precancer treated and 90% of females with invasive cervical cancer managed.[69]

2.2. CIRCUMCISION

Male circumcision (MC) is the surgical removal of some or all the foreskin of the penis. It can be performed anywhere from the neonatal period to adulthood and is one of the most common surgical procedures in the world.[70] The use of the term "circumcision" hereon refers to male circumcision.

2.2.1. PENILE ANATOMY

The penis is the male sex organ and is composed of the root, the shaft, and the glans. The root of the penis is attached to the abdominal wall. The shaft is composed of three cylindrical bodies of erectile tissue – two corpora cavernosa and one corpus spongiosum – that fill with blood upon sexual arousal. The glans is an expansion of the distal end of the corpus spongiosum and contains the external urethral orifice at the tip. The circumference of the base of the glans is referred to as the corona.[71]

The penis is enveloped in thin skin, which extends variably over the glans and folds inwards to attach to the corona via the frenulum. This folding of skin is the prepuce, or foreskin, and is composed of an inner and outer layer. The preputial sac is the space contained by the inner preputial layer and the glans.[72]

The epithelium of the penis differs over its different regions. The shaft and outer prepuce are lined by keratinized stratified squamous epithelium, whereas the inner prepuce is a mucosal surface and is thereby lined by non-keratinized stratified squamous epithelium. There is contention over the keratinization of the glans penis, but it appears to be less keratinized in uncircumcised individuals and more keratinized in circumcised individuals.[73,74]

2.2.2. HISTORY AND DETERMINANTS OF CIRCUMCISION

Circumcision was first recorded in Egyptian art dating back to 2300 BCE.[75] The word "circumcision" comes from the Latin words *circum*, meaning "around", and *cædere*, meaning "to cut".[74] It is a religious practice in Judaism, Islam, and some forms of Christianity. In Judaism, the ritual is performed on the eighth day of life; in Islam, it is often carried out on the seventh day but can be performed at any time between birth and puberty. The spread of Islam in the 7th century CE led to the adoption of circumcision in many novel regions.[75]

Ethnicity is another major determinant of circumcision. Certain ethnic groups in many countries, including but not limited to those in sub-Saharan Africa, various Pacific islands, the Philippines, and the Americas, practice non-religious circumcision. The prevalence of circumcision in many countries varies greatly by ethnic group.[75]

There are also various social determinants of circumcision, especially in regions where most males are circumcised. In cases of neonatal circumcision, motivations include the parents' desire for the newborn to have a similar appearance to the father; when circumcision is performed in late childhood or adolescence, many boys choose to be circumcised to fit in with their peers, to avoid ridicule, or simply because it is the norm. The parent or individual may also choose circumcision due to beliefs about health and hygiene, to reduce the risk of infection, or due to perceptions about how circumcision affects sexual attraction, enjoyment, and performance.[75-77]

Circumcision is also associated with socioeconomic status in several countries, particularly in those that have only recently taken up the practice. Circumcision was shown to be associated with higher socioeconomic status in the United States, United Kingdom, Australia, and Thailand. However, there was no association found between circumcision and socioeconomic status in various sub-Saharan African countries.[75]

2.2.3. DESCRIPTIVE EPIDEMIOLOGY

2.2.3.1. PREVALENCE IN CANADA

In Canada, an estimated 32% of males are estimated to be circumcised, though prevalence varies drastically by region.[75,78] In 2006 and 2007, Alberta, Ontario, and Prince Edward Island had the highest proportion of male infants that were circumcised, with a respective 44.3%, 43.7%, and 39.2%. In contrast, Nova Scotia, the Northwest Territories, and Quebec had the lowest proportions at 6.8%, 9.7%, and 12.3% respectively. No regional data was available for Yukon and Nunavut.[78] The prevalence of circumcision in Canada has been declining over the past half-century – in 1970, an estimated 48% of males were circumcised.[79]

The Canadian Paediatric Society states that "while there may be a benefit for some boys in high-risk populations and circumstances where the procedure could be considered for disease reduction or treatment, the Canadian Paediatric Society does not recommend the routine circumcision of every newborn male".[80] The Canadian Urologic Society also does not recommend routine circumcision of newborn males.[81]

2.2.3.2. Prevalence worldwide

An estimated 30–40% of males worldwide are circumcised.[75,82] Prevalence varies greatly by continental/regional grouping. Figure 2-5 shows the global prevalence of circumcision by country in 2006.

Circumcision is "almost universal" in North Africa and West Africa, less common in southern Africa, and varied in Central and East Africa. Age at circumcision varies by country, with neonatal circumcision being common in Ghana, circumcision in boyhood practiced in Burkina Faso, Zambia, and Kenya, and circumcision occurring in the late teens and twenties in South Africa and parts of the United Republic of Tanzania.[75]

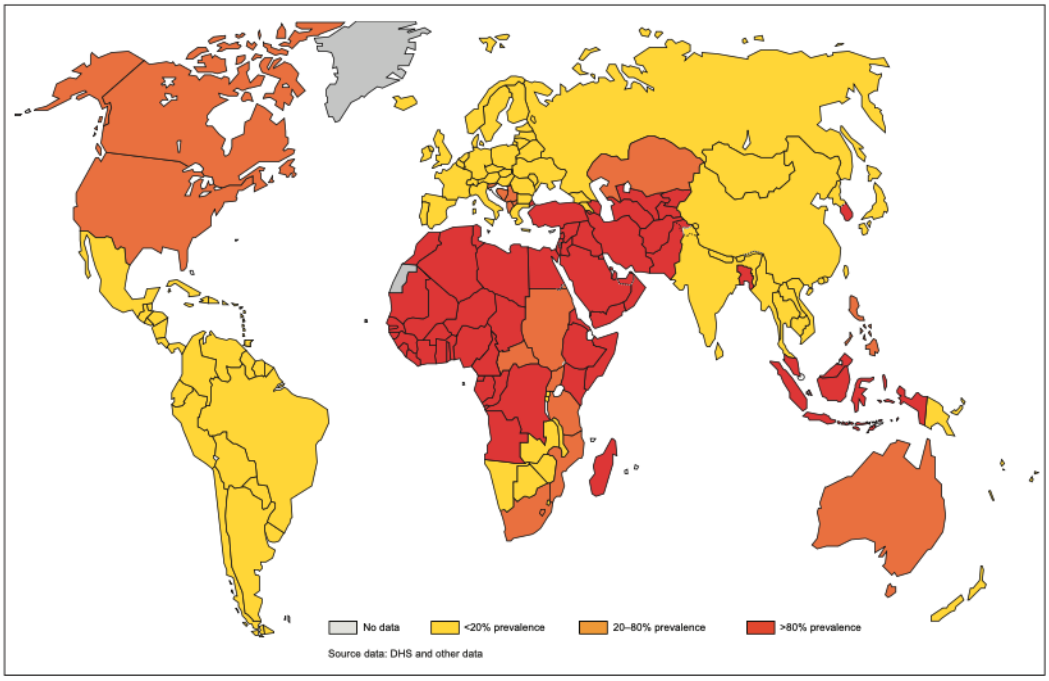
In Asia, circumcision is mostly determined by religion. In Muslim-majority countries such as Indonesia, Bangladesh, and Pakistan, circumcision is "almost universal"; there is also a substantial circumcised Muslim population in India. Circumcision is otherwise uncommon in Asia except for the Philippines, Malaysia, and the Republic of Korea, where it is culturally practiced and has a very high prevalence. Religious circumcision is most often performed in the neonatal period whereas non-religious circumcisions usually occur in late boyhood or early adolescence.[75]

Circumcision is uncommon in Central and South America, with little data available. National estimates vary, but prevalence in each country is not thought to exceed 20%.[75]

It is estimated that 100% of Muslim and Jewish males worldwide are circumcised; therefore, circumcision is almost universal in the Middle East.[75]

In North America, circumcision is more prevalent in the United States than in Canada. More than 60% of males in the United States are thought to be circumcised, with some surveys estimating a value of 76–92%. There is large regional variability in the prevalence of circumcision in Canada, as previously stated, and in the United States, where it is most common in the Midwest, slightly less common in the Northeast and the South, and rare in the West.[75,83] In Europe, circumcision is overall limited to males of the Jewish and Muslim faiths. In Oceania, circumcision has become less popular in recent decades: the estimated circumcision rate was 50% in 1974 and 17% in 2004. Most circumcisions in North America, Europe, and Oceania are performed neonatally.[75]

Figure 2-5: Global prevalence of circumcision worldwide, 2006



Reprinted with permission from the World Health Organization[75]

2.3. ASSOCIATIONS BETWEEN CIRCUMCISION AND ANOGENITAL DISEASES

Circumcision has been shown to protect against a variety of anogenital diseases and conditions in males, including but not limited to human immunodeficiency virus (HIV) and other STI acquisition from penile-vaginal sex,[36,84,85] genital ulcer disease, urinary tract infections, invasive penile cancer, and balanitis (pain and inflammation of the glans).[85] Clinical trials have demonstrated that female partners of circumcised males have reduced prevalence of genital ulcer disease, *Trichomonas vaginalis*, and bacterial vaginosis.[85] There is less evidence concerning the

relationship between circumcision and the acquisition of STIs from penile-anal sex in MSM. However, biological plausibility and data from observational studies indicate that circumcision is likely protective against the acquisition of STIs among MSM who primarily practice insertive anal sex, but not among those who primarily practice receptive anal sex.[85]

Multiple studies have demonstrated that circumcision is protective against the acquisition of HIV in males.[36,85] A 2020 meta-analysis found that in several different populations (males at high risk of HIV infection, participants in randomized controlled trials, and community cohorts), the pooled incidence proportion of HIV infections ranged from 0.29–0.56, and concluded that "voluntary medical male circumcision remains an important evidence-based intervention for control of generalized HIV epidemics".[84] There are several mechanisms by which circumcision is hypothesized to protect against HIV, including differences in keratinization and target cell density by penile site as well as changes in the local immune environment.[86-89] However, HIV and HPV have different target cells: the same mechanisms may not hold true for infection by HPV. Moreover, there is a paucity of literature examining the mechanisms by which circumcision may prevent HPV infection.

CHAPTER 3. SYSTEMATIC REVIEW

3.1. PREFACE

Several systematic reviews and meta-analyses have been performed to investigate the association between male circumcision and various STI-related outcomes, including HPV infection, in males and females. Albero et al.,[18] Larke et al.,[19] and Zhu et al.[20] conducted systematic reviews and meta-analyses in 2012, 2013, and 2017 respectively on the association between male circumcision and the prevalence, incidence, and clearance of HPV infections in males exclusively. Van Howe[90] performed a systematic review and meta-analysis in 2007 on the association between male circumcision and the prevalence of HPV infections in males: this analysis was strongly criticized by Castellsagué et al.,[91] who re-analyzed their data and found different results than did Van Howe. Grund et al.[16] and Morris et al.[17] performed systematic reviews with no associated meta-analyses in 2017 and 2019 investigating the association between male circumcision and various biomedical health outcomes, including HPV infection, in female partners.

No systematic review has previously encompassed both males and females in its study population, and no meta-analyses have been performed since 2017 for males, or ever for females. Larke et al.[19] and Albero et al.[18] addressed in their reviews the anatomical sites of the penis that were sampled and performed site-specific analyses; however, Zhu et al.'s more recent review[20] failed to acknowledge this highly relevant aspect. For this reason, I sought to perform a systematic review containing the most recent literature on the association between male circumcision and the prevalence, incidence, and clearance of HPV infections in males and females that included site-specific estimates for outcomes in males.

I will next be conducting a meta-analysis of the results presented. Following this, I will submit the manuscript to *Sexually Transmitted Infections*. The preliminary results of the systematic review were presented at the Canadian Society for Epidemiology and Biostatistics' 2021 Virtual Conference.

3.2. MANUSCRIPT 1: ASSOCIATION BETWEEN MALE CIRCUMCISION AND HUMAN PAPILLOMAVIRUS INFECTION IN MALES AND FEMALES: A SYSTEMATIC REVIEW

Samantha B. Shapiro[1], Cassandra Laurie[1], Mariam El-Zein[1], Eduardo L. Franco[1]

1 Division of Cancer Epidemiology, McGill University, 5100 Boulevard de Maisonneuve West, Suite 720, H4A 3T2, Montreal, Quebec, Canada

3.2.1. ABSTRACT

Background: Human papillomavirus (HPV) is a necessary cause of cervical cancer and is associated with anal, penile, vaginal, and vulvar cancers. Previous studies have suggested a protective effect of male circumcision (MC) on HPV infections in males and females. The purpose of this systematic review was to synthesize the available evidence on the association between MC and HPV infections in males and females.

Methods: We performed a systematic search of the MEDLINE, Embase, Scopus, Cochrane, LILACS, and ProQuest Dissertations and Theses Global databases for records published up to July 31, 2021 that assessed MC and presence of genital HPV DNA. We extracted the adjusted effect estimate when available, or the crude estimate otherwise, for the prevalence, incidence, and clearance of HPV infections in males and females, for any HPV, high-risk HPV, and low-risk HPV, and for different anatomical sampling sites.

Results: We included 31 of 604 unique publications and extracted 111 effect estimates. A protective effect of MC in males was found in most analyses of prevalent HPV infections (n=40/52) and clearance (n=17/24), and about half of analyses of incidence (n=13/23); the remainder of estimates found no association. All analyses of high-risk HPV at the distal penis found that MC had a significantly protective effect at α=0.05, as did almost all analyses from randomized controlled trials. Female partners of circumcised males may be at reduced risk for HPV infections.

Conclusions: MC may be a viable prophylactic strategy in regions with a high burden of HPV-associated disease and where the HPV vaccine is not commercially available.

3.2.2. INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide.[1,2] Most HPV infections are "transient, asymptomatic, or subclinical",[25] but those that are persistent or those in immunosuppressed individuals can lead to clinical manifestations. About 70% of HPV infections spontaneously resolve within one year and 90% in two, with the remainder becoming persistent.[29] Persistent infection with high-risk HPV (HR-HPV) is a necessary cause of cervical cancer and is associated with penile, anal, vaginal, vulvar, and head and neck cancers,[3-5] while infection with low-risk HPV (LR-HPV) is associated with genital warts.[6]

Male circumcision (MC) has been demonstrated to be protective against a variety of sexually transmitted infections, including human immunodeficiency virus (HIV), herpes simplex type 2, trichomoniasis, chancroid, and syphilis.[11-13] Several randomized controlled trials have been conducted to evaluate the association between MC and HIV acquisition: these trials have also included analyses of HPV as secondary endpoints.[14,15] Most observational studies of the relationship between MC and HPV infections in males have been cross-sectional in nature, and few studies have evaluated the risk of HPV infection in the female partners of circumcised and uncircumcised males. Previous systematic reviews[16,17] and meta-analyses[18-20] have indicated that MC protects against a variety of HPV infection outcomes in males and their female sexual partners. However, gaps in knowledge remain and there have been multiple recently published studies on the topic, requiring an update to the existing literature. In this systematic review, we synthesize the growing evidence suggestive of a protective relationship between MC and HPV infections in males, and the conferred protection to female sexual partners.

3.2.3. METHODS

SEARCH STRATEGY

We performed a systematic search of the MEDLINE, Embase, Scopus, Cochrane, LILACS, and ProQuest Dissertations & Theses Global databases to identify relevant records published up to July 31, 2021. We applied no language or date restrictions. We also manually searched for potentially eligible studies that were published in previous knowledge syntheses, from books of abstracts from HPV conferences (European Research Organisation on Genital Infection and Neoplasia (EUROGIN) and International Papillomavirus (IPV)), and from the HPV World

newsletter. We applied the following search strategy, which was developed with a librarian, in MEDLINE and adapted it to the other databases:

1. Circumcision, Male/
2. (circumcis* OR uncircumcis*).ti,ab,kf.
3. 1 OR 2
4. Papillomavirus Infections/
5. papillomaviridae/ OR exp alphapapillomavirus/
6. (hpv OR papillomavir* OR papilloma vir*).ti,ab,kf.
7. 4 OR 5 OR 6
8. 3 AND 7

3.2.3.1. INCLUSION CRITERIA

We included both observational (e.g., cross-sectional, case-control, cohort) and experimental (e.g., randomized controlled trials) study designs. We excluded case reports, case series, and conference abstracts with no associated full-text article. We included both males and females of any age but excluded people living with HIV (PLHIV) from our population due to the HIV's interaction with HPV infections.[92-94] We also excluded studies that focused solely on males who have sex with males. Any study (1) whose participants had no HPV-associated genital lesions, (2) that tested for the presence of HPV DNA by a specified method in genital epithelial cells, (3) that assessed the male's (or the female's male sexual partner's) circumcision status through a specified method, and (4) that assessed the prevalence, incidence, and/or clearance of HPV infections was eligible for inclusion. Multiple publications from the same study population were eligible for inclusion if they assessed distinct outcomes.

3.2.3.2. SCREENING

S.S. and C.L. assessed the eligibility of identified records. After de-duplicating search results in EndNote, we exported records to Rayyan, independently conducted title and abstract screening, and resolved disagreements by consensus. Following title and abstract screening, we independently conducted full-text screening and again resolved disagreements by consensus. We contacted the authors of individual studies when we could not find the full-text record.

3.2.3.3. DATA EXTRACTION

S.S. and C.L. performed data extraction. We divided included records in half for independent data extraction and used a piloted standardized spreadsheet in Microsoft Excel to extract relevant information. These included study characteristics (design, year(s), country(ies), population description, number of visits if longitudinal), exposure and outcome methods (MC assessment method, genital sites sampled, frequency of genital sampling, sampling method, HPV DNA detection and genotyping method, HPV types detected and genotyped), study population results (sample size, sex, age at baseline, HPV prevalence at baseline), and outcome-related data (outcome type, i.e., prevalence, incidence, clearance; HPV risk grouping; number analyzed; number circumcised and uncircumcised; number of prevalent or incident or cleared infections; person-time at risk; effect estimate and 95% confidence interval (95% CI); and covariates adjusted for). If a study assessed multiple outcomes and/or the same outcome was considered for multiple HPV risk groupings, then we extracted the outcome-related data separately for each analysis. As well, if a study provided both site-specific and grouped-site outcome data, we extracted the grouped-site data. If multiple site groupings were provided, we extracted the grouping that contained the greatest number of relevant genital sites (urethra, glans, shaft) and the fewest irrelevant genital sites (scrotum, anus, perianal area).

We contacted the authors of original publications for missing or stratified data needed for our analyses (e.g., for HIV-negative individuals in studies that included PLHIV, to obtain HPV DNA genotyping method or MC assessment method when not explicitly stated, for missing sociodemographic data, etc.). If such data were part of the inclusion requirements but could not be provided by the author, we excluded the study.

3.2.3.4. Data analysis

For our outcomes, we defined prevalence as the presence of HPV DNA at any point in time, incidence as the presence of HPV DNA that was absent at a previously measured timepoint, and clearance as the absence of HPV DNA that was present at a previously measured timepoint. For analyses of prevalence, we calculated the conditional maximum likelihood estimate of the odds ratio and the mid-P 95% CI using OpenEpi's two by two table function[95] when we had raw data but no effect estimates. If effect estimates used circumcised males as the reference category, we took the reciprocal of the estimate and its 95% CI. For site-specific analyses, we grouped the foreskin, urethra, and glans as the distal penis and the shaft and scrotum as the proximal penis.

We classified effect estimates in males in two ways: primary vs. secondary and combined vs. site-specific. The logic for classification is presented in Table 3-1. We considered as primary estimates the effect estimates that encompassed the greatest number of genital sites sampled and secondary estimates to be those extracted from additional site-specific analyses. Combined-site estimates represented those from samples that combined proximal and distal sites of the penis, whereas site-specific estimates relate to specimens that only sampled the proximal or distal parts of the penis. Therefore, if a study sampled the glans and shaft and provided three estimates for prevalence – one at the glans or shaft, one at the glans only, and one at the shaft only – the glans or shaft estimate would be considered both a primary estimate and a combined estimate, whereas the glans-only and shaft-only estimates would each be considered secondary and site-specific estimates. Contrarily, if a study only sampled the glans, then its estimate would be considered primary and site-specific. In other words, all secondary estimates were site-specific, but not all primary estimates were from combined-site samples. Each outcome type (prevalence, incidence, and clearance) and each HPV grouping (any HPV, HR-HPV, LR-HPV) could be considered its own primary analysis. All analyses in females were considered primary.

3.2.4. RESULTS

3.2.4.1. SEARCH RESULTS

The results of our search and assessment are presented in Figure 3-1. We identified 1,368 records through systematic database searches and 10 records through manual searches. 604 unique records remained after de-duplication and underwent title and abstract screening. We excluded 498 records in this first round of screening, leaving 106 full text records for assessment. Records were excluded for reporting on an already included cohort, for participants having HPV-associated lesions, for not sampling a genital site, for missing exposure or outcome data, for not having an outcome of interest, and for not having an associated full text. We excluded eight more records for failure to obtain missing data after contacting authors. In total, we included 31 records in our qualitative synthesis.

3.2.4.2. DESCRIPTION OF INCLUDED STUDIES

Characteristics of the 31 included publications, which were published between 2002 and 2021, are presented in Table 3-2. These encompassed 24 unique study populations and reported 111 relevant effect estimates.

Of the 31 publications, 17 were cross-sectional studies,[96-112] nine were cohort studies,[113-119] and five were randomized controlled trials (RCTs).[14,15,120-122] Studies were conducted in North America (n=11),[96,99,104,111,113-119] South America (n=3),[100,106,107] Europe (n=4),[97,101,108,110] Asia (n=1),[109] Africa (n=8),[14,15,102,103,105,120-122] and intercontinentally (n=4).[98,112,123,124] MC status was self-reported or reported by a partner (n=11),[36,98-100,102,103,107-110,115,117] reported by a clinician (n=15),[96,97,101,104-106,111-114,116,118,119,123,124] or randomized and verified by a clinician (n=5).[14,15,120-122] All studies assessed for the presence of HPV DNA by PCR, 22 of which[14,15,96,97,101-103,107-109,111,113-120,122-124] genotyped for 20 or more HPV types. Samples were taken via swab (n=18),[14,15,96,98,100-103,105,110,112,116,117,120-124] textured paper and swab (n=5),[36,104,113,114,118,119] brush (n=5),[97,99,106,107,111] and brush and swab (n=2).[109,115] Samples in males were taken from multiple sites, including the urethra (n=5),[98,107,109,115,117,118] foreskin (n=14),[14,15,97,102,104-107,114,117-119,123,124] glans and/or corona (n=26),[14,15,96-98,100-102,104-107,109-111,113-121,123,124] shaft (n=18),[14,101,102,104,105,107,109-111,113-119,123,124] scrotum (n=15),[101,104,109-119,123,124] and perianal area (n=4)[101,110,112,117]; samples in females were taken from the cervix and vagina (n=4).[99,103,108,122] The primer sets used for HPV DNA typing were PGMY09/11 (n=12),[15,96,102,105,113,114,116-118,121,123,124] MY09/11 (n=8),[98,100,107,111,115,119,120,122] GP5+/6+ (n=4),[14,97,106,110] SPF10 (n=3),[101,108,109] CpI/CpIIG (n=1),[99] and type-specific and assay-specific primers (n=3).[103,104,112] HPV prevalence among all participants at baseline ranged from 8.7%[109,111] to 69.8%.[104]

Of the 111 estimates for the association between MC and various infection outcomes, two-thirds (n=75) were considered primary estimates and one-third were considered secondary (n=36); just over half (n=60) were combined and just under half (n=51) were site-specific.

For the 75 primary estimates, most (n=63) were in males. Of these 63, just over half (n=34) were for the association between MC and the prevalence of HPV infections (section 3.2.4.3); the latter half was evenly split between estimates for the association between MC and the incidence (n=15) and clearance (n=14) of HPV infections (sections 3.2.4.4 and 3.2.4.4, respectively). The remaining 12 primary estimates in females were again mostly for the association between MC and the prevalence of HPV infections (n=9); the last three primary estimates in females were for the association between MC and the incidence (n=2) and clearance (n=1) of HPV infections (section 3.2.4.6).

For the 51 site-specific estimates in males, slightly over half (n=28) were for the association between MC and the prevalence of HPV infections; the remainder were for the association between MC and the incidence (n=9) and clearance (n=14) of HPV infections (section 3.2.4.7).

3.2.4.3. STUDIES OF PREVALENCE IN MALES

The 34 primary prevalence estimates of genital HPV infections in males, reported in 20 studies (16 cross-sectional,[96-98,100-102,104-107,109,111,112,114,117] two cohort,[115,124] and two RCTs[14,15]) are presented in Table 3-3. Sample sizes ranged from 37[106] to 3,969.[124] All but three[106,107,109] effect estimates suggested a protective effect or no effect of MC. Seven out of 19 studies measuring the presence of any HPV as an outcome found a statistically significant protective association between MC and the prevalence of any HPV at the 95% confidence level.[4,15,100,103-105,108] A protective effect was also found for three of the eight studies restricting to HR-HPV[14,15,96] and four of the seven studies restricting to LR-HPV.[14,15,96,124]

3.2.4.4. STUDIES OF INCIDENCE IN MALES

As shown in Table 3-4, we extracted 15 primary estimates of HPV incidence in males coming from eight studies (three RCTs[14,120,121] and five cohort studies[115,116,118,119,123]). The smallest sample size was 210[115] and the largest was 4,033.[123] 14 estimates suggested either a protective association or no association between MC and HPV acquisition[14,115,116,118-121,123] while one suggested a harmful (albeit non-significant) association.[116] Two estimates of the protective association between MC and the incidence of any HPV infection were statistically significant,[14,115] as were three estimates of the protective association between MC and the acquisition of HR-HPV infections.[14,120,121]

3.2.4.5. STUDIES OF CLEARANCE IN MALES

Six publications (three cohort studies[113,116,123] and three RCTs[14,120,121]) ranging from 285[116] to 4,033[123] participants provided 14 primary estimates for clearance of HPV infections in males, as shown in Table 3-5. Eight of these suggested that MC was associated with increased clearance of infections[14,116,120,121]; the other six did not find evidence of an association between MC and clearance.[113,123] Seven of the eight protective point estimates were significant at α=0.05 (two for the clearance of any HPV, [14,116] four for the clearance of HR-HPV, [14,116,120,121] and one for the clearance of LR-HPV[14]).

Table 3-6 presents the results of the five studies (four cross-sectional[99,102,103,108] and one cohort of the wives of men controlled in a circumcision RCT[122]) that examined the association between MC and various HPV outcomes in females. Sample sizes ranged from 61[99] to 2,735.[108] Nine of the twelve estimates were of prevalence, and eight of these estimates found that having a circumcised male partner was associated with HPV infections in the female.[102,103,108,122] Four estimates (one of any HPV,[122] two of HR-HPV,[36,103,122] and one of LR-HPV[122]) were significant at α=0.05. The last estimate found that having a circumcised partner was a significant risk factor for HPV infection[99]; however, this was in a population of females with rheumatoid arthritis, which has been hypothesized to be a risk factor for HPV infection.[125,126] The two estimates of incidence[122] – one of HR-HPV and one of LR-HPV infections – both found that having a circumcised partner protected the female from acquisition of infections; however, only the analysis of HR-HPV acquisition was statistically significant.[122] Finally, the one estimate of clearance found that females with a circumcised partner had a significantly increased clearance rate of HR-HPV infections.[122]

3.2.4.6. SITE-SPECIFIC COMPARISONS IN MALES

Twelve publications[14,15,96-98,100,106,113,114,117,120,121] provided 51 site-specific estimates of the association between MC and the prevalence (n=28), incidence (n=9), and clearance (n=14) of HPV infections in males. These data are presented in Table 3-7, Table 3-8, and Table 3-9.

Of the 28 prevalence outcomes, 19 were analyses of the distal penis and nine were of the proximal penis. At the distal penis, 17 estimates found that MC was protective,[14,15,96,98,100,114,117] one found no association,[97] and one found MC to be a risk factor.[106] 14 of the 17 protective associations were significant at the 95% confidence level: six of nine analyses of any HPV,[14,15,96,98,114,117] three of five analyses of LR-HPV,[14,15,96] and notably, all five analyses of HR-HPV.[14,15,96,114,117] In the proximal penis, seven of the nine analyses estimated MC to be protective[14,36,114,117] while the last two found no association[14] and a harmful association.[14] Only one protective estimate, which was of the association between MC and infection with any HPV, was significant at α=0.05.[117]

Six of the nine site-specific incidence analyses were of the distal penis and three were of the proximal. In the distal penis, five of the six estimates found that MC was associated with a significantly reduced rate of incident infections,[14,120,121] including for all three analyses of HR-HPV, while the last estimate found a nonsignificant protective effect.[120] No analyses of the proximal penis found an association between MC and incidence.[14]

The 14 clearance outcomes encompassed eight analyses of the distal penis and six of the proximal penis. Each of the eight distal analyses found a significantly increased rate of clearance in circumcised males.[14,113,120,121] One analysis at the shaft found a significant increased clearance rate in circumcised males[14] while the other five analyses found no association or a non-significant protective association.[14,113]

3.2.5. DISCUSSION

MC appeared to have an overall protective effect against various HPV infection outcomes. There was stronger evidence for MC protecting against prevalent HPV infections than that for acquisition and clearance of infections in males. Protection against prevalent infections appeared to be consistent in males and females and seemed to be strongest in distal parts of the penis, i.e., the glans, coronal sulcus, and urethra. Regardless of the outcome, risk grouping, and population, MC was almost always found either to have no association with HPV infections or to be protective. Only one study, a cross-sectional analysis of females with rheumatoid arthritis,[99] found that MC was a statistically significant risk factor; however, this population was one of the least generalizable of those included in our study due to its members having an autoimmune disease. Findings from this systematic review corroborate those of previous reviews.

We included several publications that were not part of the most recently published systematic reviews on the topic: in males, we included an additional eight studies of prevalence,[14,100-102,105,106,112,124] two of incidence,[14,121] and three of clearance[14,113,121] that were not included in Zhu's 2017 review and meta-analysis[20]; in females, we added two records[99,102] that were absent in Morris' 2019 review.[17] The addition of new records did not result in different conclusions than those of previous reviews, but rather provided additional evidence for the same interpretations.

Infections with HR-HPV are of the most clinical relevance, as persistent infection with HR-HPV is a necessary cause of cervical cancer and is associated with various anogenital cancers.[3-5] All twelve effect estimates for the association between MC and HR-HPV infection at the distal penis – be it for prevalence, incidence, or clearance – found that MC had a significantly protective effect.[14,15,96,113,114,117,120,121]

When restricting records to those from RCTs, almost all primary effect estimates found a significantly protective effect of MC.[14,15,120-122] The only two primary estimates that did not find a

significant effect were for the incidence of LR-HPV[120,122]; however, these still both found that MC was protective and one of the estimates was marginally significant.

The biological mechanism by which MC is suggested to protect against HPV infections is still unclear, with theories encompassing differences in keratinization, in susceptibility to injury, and in the local immune environment of the penis.

Keratin is a fibrous structural protein that protects the skin from the external environment.[74] It was originally thought that the inner foreskin is less keratinized than the outer foreskin and the shaft, and that the glans of the uncircumcised penis is less keratinized than that of the circumcised penis.[127-129] The foreskin is retracted during sexual intercourse, exposing the inner foreskin and the glans. Therefore, HPV would be able to penetrate this less keratinized epithelium that becomes exposed during sexual intercourse in uncircumcised males, making them more susceptible to acquisition of infections. However, anatomic and histological studies have failed to find consistent results on the differences in keratinization between the glans of circumcised and uncircumcised males and between the inner and outer foreskin,[87,89] thereby making this a less likely mechanism.

The frenulum, a highly vascularized band of fibrous tissue connecting the foreskin to the glans of the penis,[73] is often removed in MC. The frenulum is particularly susceptible to trauma and abrasions during intercourse, which represents a potential route for pathogens to enter the body. Its removal could therefore confer protection by eliminating this route.[89] However, this cannot represent the sole mechanism of protection, as HPV infects the epithelium at other locations of the penis.

MC has also been postulated to change the local immune environment of the penis through changes in the microbiome and immune cell density. Removal of the foreskin eliminates the anaerobic environment of the preputial cavity.[130] In the Ugandan trial of MC,[15,120,121] investigators compared the bacterial microbiota of the coronal sulcus between the males in the control and intervention arms at baseline and at one year follow-up. At baseline, the microbiota of both groups was comparable. However, at one-year follow-up, circumcised males had a decreased total bacterial load and reduced biodiversity in their microbiota, with bacteria of twelve different taxa of anaerobic bacteria having a decreased prevalence and absolute abundance.[131] A 2017 study of 51 females showed that those who were HPV-positive were more likely to have a diverse array of facultative and strict anaerobic bacteria in their vaginal microbiome.[132] MC may therefore protect against HPV by reducing the diversity of anaerobic bacteria in the penile microbiota. The foreskin

is rich in several types of immune cells, including macrophages and Langerhans cells. Macrophages produce pro-inflammatory cytokines in response to infection as part of the innate immune response. This results in local inflammation, which can disrupt the structural integrity of the epithelium and may facilitate HPV entry.[87] Additionally, different regions of the penis have different distributions of immune cells,[88] many of which are involved in the production of specific cytokines. The presence of certain cytokines may be associated with the clearance,[133] persistence,[134] and viral load[135] of HPV infections in females, therefore, changes in the cytokine environment resulting from removal of the immune cells of the foreskin may play a role in susceptibility to HPV infection. Langerhans cells are an epithelial subtype of dendritic cells, which present antigens to other cells of the immune system, and are found with the highest density and most superficially in the foreskin and frenulum.[127] It has been demonstrated *in vivo* that Langerhans cells, unlike dendritic cells, do not display surface activation markers or secrete increased amounts of interleukin-12 after uptake of HPV virus-like particles, representing an inappropriate immune response to a pathogen.[136] MC may increase the relative abundance of other dendritic cells compared to Langerhans cells, resulting in a higher likelihood of HPV uptake by cell types capable of producing a proper immune response.

Our review had many strengths. We searched a diverse array of databases and validated our search strategy with a librarian. We did not apply study design or language restrictions and we included both males and females, multiple HPV infection-related outcomes, different HPV risk groupings, and different anatomical sampling sites. There were also several limitations of our review. We included the term "circumcision" in our search strategy and may not have captured records that measured MC and HPV infection without directly assessing their association. Only three of the 24 unique study populations included in our review[14,15,120-122] came from RCTs, which limited our ability to assess causality. We did not assess publication bias (though previous reviews in males that encompassed many of our included publications did not find evidence of publication bias[18-20]), nor did we evaluate risk of bias in the included studies or study heterogeneity. We did not consider other factors that may play a role in MC's association with HPV infection, such as method of MC, whether MC was performed before or after sexual debut, and number of sexual partners. Our next steps will be to assess risk of bias, study heterogeneity, and publication bias, and to perform a meta-analysis of the included records with multiple sensitivity analyses.

In conclusion, our systematic review showed that MC is associated with lower prevalence of HPV infections in a diverse population of males, especially at the distal penis, and that protection may be passed on to female partners. MC may be a viable preventive strategy for HPV infections, especially in regions with a high burden of HPV-associated cancers and where the HPV vaccine is not commercially available.

Acknowledgments

We would like to thank the librarian Genevieve Gore at McGill University for her assistance in developing and validating our search strategy.

Funding sources

S.B. Shapiro is funded by the Carole Epstein Fellowship, awarded by McGill University, and by the Division of Cancer Epidemiology at McGill University.

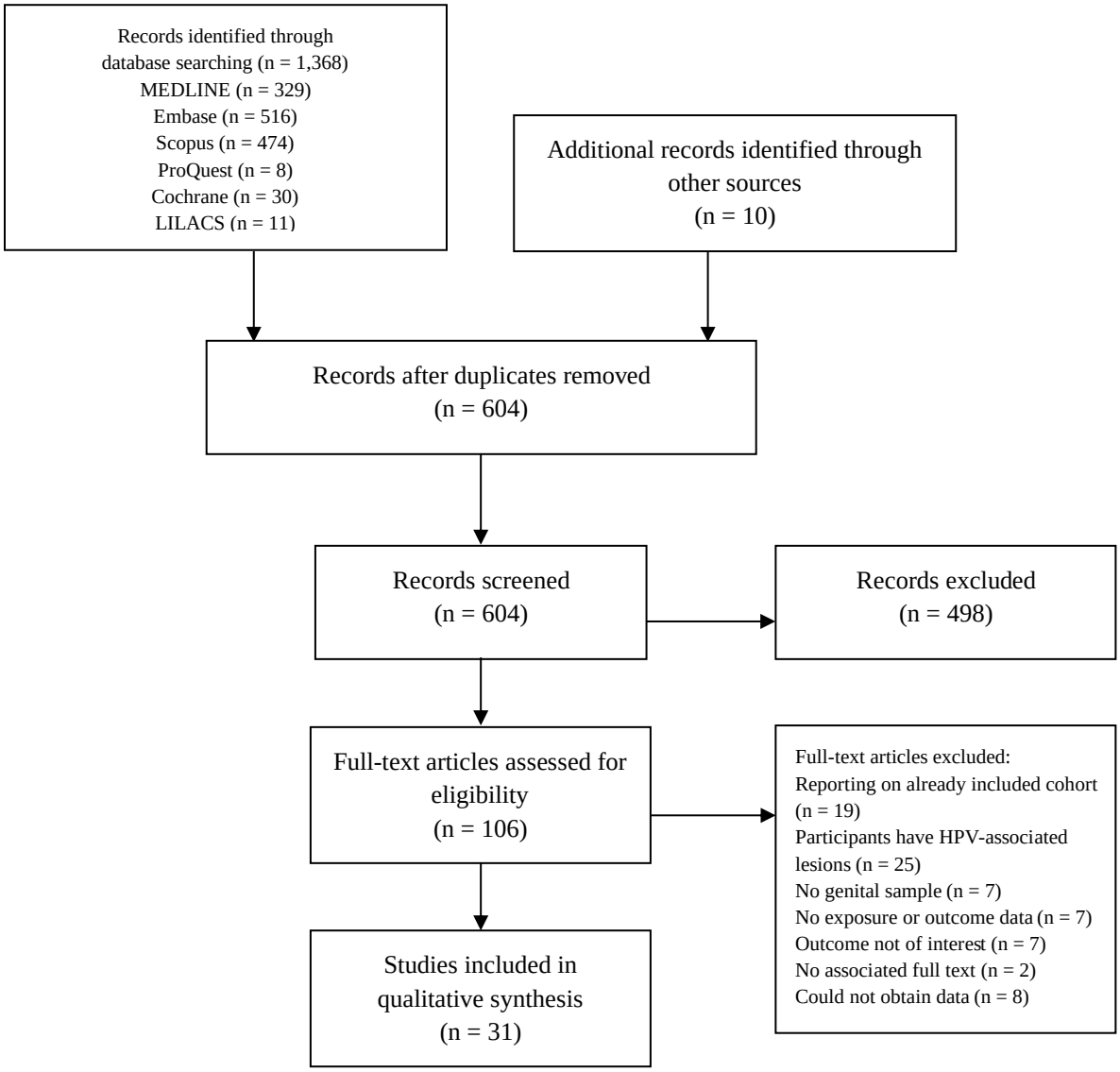
Conflicts of interest

E.L. Franco and M. El-Zein hold a patent related to the discovery "DNA methylation markers for early detection of cervical cancer", registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada (October 2018). The other authors have no relevant conflicts to disclose.

Contributions of authors

Samantha Shapiro and Eduardo Franco conceptualized the project. Samantha Shapiro and Cassandra Laurie conducted the methodology of the search. Samantha Shapiro analyzed its results and drafted the manuscript, which was reviewed and edited by Cassandra Laurie, Mariam El-Zein, and Eduardo Franco. Eduardo Franco and Mariam El-Zein provided supervision and guidance.

Figure 3-1: Flow diagram of study inclusion for the systematic review



The flow chart depicts the process of selecting studies assessing the association between MC and various HPV infection outcomes for inclusion in our review.

Table 3-1: Effect estimate classification logic

Gender	Sites sampled in study	Sites included in analysis	Effect estimate: site classification	Effect estimate: analysis classification
Male	One site of penis	One site (proximal or distal)	Site-specific	Primary
	Multiple sites of penis	Combined sites (proximal and distal)	Combined-site	Primary
		One site (proximal or distal)	Site-specific	Secondary
Female	NA	NA	NA	Primary

Abbreviations: NA, not applicable

Table 3-2: Characteristics of included records by study design

First author (year)	Country and years conducted	Study population	Number enrolled	Circumcision assessment	Sites sampled	HPV DNA genotyping method
Randomized controlled trials						
Gray (2010)[120]	Uganda, 2003–2006	Males enrolled in the Rakai-1 trial	840	Randomized and verified by a clinician	Glans	MY09/11 PCR
Smith (2021)[14]	Kenya, 2002–2005	Males enrolled in the Kisumu circumcision trial	2,193	Randomized and verified by a clinician	Inner foreskin, glans, outer foreskin, shaft	GP5+/6+ PCR
Tobian (2009)[15]	Uganda, 2003–2007	Males enrolled in the Rakai-1 and Rakai-2 trials	3,393	Randomized and verified by a clinician	Foreskin, glans	PGMY09/11 PCR
Tobian (2012)[121]	Uganda, 2002–2009	HIV-positive and negative males* enrolled in the Rakai-1 and Rakai-2 trials	776	Randomized and verified by a clinician	Glans	PGMY09/11 PCR
Wawer (2011)[122]	Uganda, 2003–2007	Female partners of males enrolled in the Rakai-1 and Rakai-2 trials	1,245	Randomized and verified by a clinician	Vagina	MY09/11 PCR
Cohort studies						
Albero (2013)[124]	Brazil, Mexico, United States, 2005–2009	Males from the general population, universities, and organized healthcare systems	3,969	Clinical exam	Foreskin, glans, shaft, scrotum	PGMY09/11 PCR
Albero (2014)[123]	Brazil, Mexico, United States, 2005–2009	Males from the general population, universities, and organized healthcare systems	4,003	Clinical exam	Foreskin, glans, shaft, scrotum	PGMY09/11 PCR
Hernandez (2008)[114]	United States, 2004–2006	Male university students in Hawaii	379	Clinical exam	Foreskin, glans, shaft, scrotum	PGMY09/11 PCR
Hernandez (2010)[113]	United States, 2004–2006	Male university students in Hawaii	357	Clinical exam	Foreskin, glans, shaft, scrotum	PGMY09/11 PCR
Lajous (2005)[115]	Mexico, 2002–2005	Healthy military males	1,030	Self-report	Urethra, glans, shaft, scrotum	MY09/11 PCR
Lu (2009)[116]	United States, 2003–2006	Males from the general population	285	Clinical exam	Foreskin, glans, shaft, scrotum	PGMY09/11 PCR
Nielson (2009)[117]	United States, 2002–2005	Males from the general population	463	Self-report	Foreskin, urethra, glans, shaft, scrotum, perianal area, anus	PGMY09/11 PCR

Partridge (2007)[118]	United States, 2003–2006	Males university students in Washington	240	Clinical exam	Foreskin, urethra, glans, shaft, scrotum	PGMY09/11 PCR
VanBuskirk (2011)[119]	United States, 2003–2009	Male university students in Washington	477	Clinical exam	Foreskin, glans, shaft, scrotum	MY09/11 PCR
Cross-sectional studies						
Baldwin (2004)[96]	United States, 2000–2001	Males attending an STI clinic	393	Clinical exam	Glans	PGMY09/11 PCR
Bleeker (2005)[97]	Netherlands, 1995–2002	Males from a non-STI dermatology clinic and male partners of females with CIN	356	Clinical exam	Foreskin, glans	GP5+/6+ PCR
Castellsagué (2002)[98]	Spain, Colombia, Brazil, Thailand, Philippines, 1985–1993	Male partners of case females with cervical cancer and healthy control females	1,913	Self-report	Urethra, glans	MY09/11 PCR
Contreras (2008)[99]	Mexico, 2005–2006	Females with rheumatoid arthritis	61	Self-report	Cervix	CpI/CpIIG PCR
Da Rocha (2015)[100]	Brazil, 2011–2013	Males from an STI clinic, a dermatology clinic, a university, and a factory	261	Self-report	Glans	MY09/11 PCR
Hebnes (2021)[101]	Denmark, 2006–2007	Military males	2,460	Clinical exam	Preputial cavity, glans, shaft, scrotum, perineum	SPF10 PCR
Mbulawa (2009)[102]	South Africa, NR	Sexually active Black heterosexual couples*	254	Self-report	Foreskin, glans, shaft	PGMY09/11 PCR
Obiri-Yeboah (2017)[103]	Ghana, NR	Females attending an HIV or medical outpatient clinic*	170	Self-report	Cervix	RT-PCR with type-specific primers
Ogilvie (2009)[104]	Canada, NR	Heterosexual males attending an STI clinic	262	Clinical exam	Foreskin, glans, shaft, scrotum	Amplicor® primer PCR
Olesen (2019)[105]	Tanzania, 2009	Males from urban and rural areas*	1,902	Clinical exam	Foreskin, glans, shaft	PGMY09/11 PCR
Rocha (2012)[106]	Brazil, NR	Heterosexual couples in which the female HPV-related cervical lesions	43	Clinical exam	Foreskin, glans	GP5+/6+ PCR
Rombaldi (2006)[107]	Brazil, 2003–2004	Male sexual partners of females with CIN	99	Self-report	Foreskin, urethra, glans, shaft	MY09/11 PCR
Roura (2012)[108]	Spain, 2007–2008	Females attending cervical cancer screening	3,261	Partner report	Cervix	SPF10 PCR

Shin (2004)[109]	South Korea, 2002	Male university students	381	Self-report	Urethra, glans, shaft, scrotum	SPF10 PCR
Svare (2002)[110]	Denmark, 1993	Males attending an STI clinic	198	Self-report	Glans, shaft, scrotum, perianal area	GP5+/6+ PCR
Vaccarella (2006)[111]	Mexico, 2003–2004	Males requesting a vasectomy	779	Clinical exam	Glans, shaft, scrotum	MY09/11 PCR
Vardas (2011)[112]	18 countries in Africa, Asia-Pacific, Europe, Latin America, and North America, NR	Heterosexual males with 1–5 female lifetime sexual partners	3,463	Clinical exam	Penis (specific sites NR), scrotum, perianal area	RT-PCR with type-specific primers

**Only HIV-negative males were included in our summary*

Table 3-3: Studies assessing the association between MC and prevalence of HPV infection in males by HPV risk grouping

First author & year	Study design	HPV types	Age at baseline, years (range)	Circumcision prevalence (%)	HPV prevalence at baseline (%)	Number analyzed	Effect estimate: OR (95% CI)	Covariate adjustment
Any HPV								
Albero 2013	Cohort	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–70	35.9	66.7	3969	PR 0.96 (0.91–1.01)	Race, marital status, lifetime female sexual partners, female sexual partners in past 3-6 months, male anal sexual partners in the past 3 months
Vardas 2011	Cross-sectional	14 types: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	15–24	36.4	21.2	3167	0.9 (0.7–1.2)	Geographic area of residence, age, tobacco use, condom use, age at first sexual intercourse with a male partner, number of lifetime sexual partners, number of new partners in the past 6 months
Hebnes 2021	Cross-sectional	24 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 6, 11, 40, 42, 43, 44, 54, 70, 74	18–59	5.4	41.7	2331	0.7 (0.5–1.0)	Age, lifetime number of female sex partners and age at first sexual intercourse with a woman as continuous variables, time since last sexual intercourse as a categorical variable
Smith 2021	Randomized controlled trial	44 types: 6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 85, 86, 89, JC9710	18–24	50.0	50[†]	2193	**PR 0.57 (0.49–0.67)**	None
Castellsague 2002	Cross-sectional	6 types: 6, 11, 16, 18, 31, 33	NR	25.6	16	1139	**0.37 (0.16–0.85)**	Age, study location, level of education, age at first sexual intercourse, lifetime number of sexual partners, frequency of genital washing after sex
Lajous 2005	Cohort	27 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 73, 82, 6, 11, 26, 40, 42, 54, 55, 57, 66, 83, 84	16–40	10.3	44.6	925	**0.48 (0.30–0.77)**	Age, SES, lifetime number of partners
Vaccarella 2006	Cross-sectional	35 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 81, 83, 84	<25–≥45	31.7	8.7	779	**0.2 (0.1–0.4)**	Age group, lifetime number of sexual partners
Tobian 2009	Randomized controlled trial	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	15–49	44.8	62.2	520	**RR 0.70 (0.53–0.91)**	None
Nielson 2009	Cross-sectional	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–40	84.1	47.5	421	0.68 (0.36–1.27)	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
Shin 2004	Cross-sectional	25 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68/73, 6, 11, 34, 40, 42, 43, 44, 53, 54, 70, 74	18–28	88.3	8.7	368	1.8 (0.4–8.2)	Age, number of lifetime sexual partners
Baldwin 2004	Cross-sectional	27 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, 83, 6, 11, 40, 42, 53, 54, 57, 66, 84	18–70	67.4	28.2	344	**0.34 (0.20–0.57)**	Sexual frequency per month, genital warts, condom use in past 3 months, steady partner

Weaver 2004	Cross-sectional	NR	18–25	81.4	33	317	1.00 (0.53–1.93)	None
Hernandez 2008	Cross-sectional	37 types: 16, 18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 73, 82, 82/IS39, 6, 11, 40, 42, 54, 61, 70, 72, 81, 89, 55, 62, 64, 67, 69, 71, 83, 84	NR	77.8	NR	316	0.58 (0.30–1.14)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
Mbulawa 2009	Cross-sectional	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 26, 53, 66, 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 82/IS39, 83, 84, 89	20–64	93.3	43	298	0.54 (0.20–1.39)†	None
Ogilvie 2009	Cross-sectional	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	16–69	50.4	69.8	262	1.14 (0.67–1.94)†	None
Bleeker 2005	Cross-sectional	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 89	22.5–73.2	9.1	48.2	253	0.98 (0.41–2.36)†	None
Rocha 2012	Cross-sectional	7 types: 16, 18, 31, 33, 45, 6, 11	18–60	1.3	52.1	240	1.32 (0.06–28.7)†	None
Svare 2002	Cross-sectional	NR	18–≥40	12.1	45	198	**0.2 (0.06–0.6)**	Age, lifetime sex partners, sex partners in past year, genital warts
Da Rocha 2015	Cross-sectional	11 types: 16, 18, 31, 33, 35, 45, 56, 58, 6, 11, 53	18–65	3.3	16.5	182	0.52 (0.02–3.66)†	None
Rombaldi 2006	Cross-sectional	32 types: 6, 11, 13, 16, 18, 26, 31, 32, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68, 69	≤19–59	10.1	54.5	99	2.07 (0.51–10.41)†	None
High-risk HPV								
Albero 2013	Cohort	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–70	35.9	66.7	3969	PR 0.95 (0.87–1.03)	Race, marital status, lifetime female sexual partners, female sexual partners in past 3–6 months, male anal sexual partners in the past 3 months
Smith 2021	Randomized controlled trial	14 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	18–24	50.0	50†	2193	**PR 0.55 (0.46–0.67)**	None
Olesen 2019	Cross-sectional	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	14–90	91.5	17	1287	0.87 (0.53–1.47)†	None
Tobian 2009	Randomized controlled trial	14 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	15–49	44.8	62.2	520	**RR 0.65 (0.46–0.90)**	Enrollment characteristics, rates of sexual practices, symptoms of sexually transmitted infections
Nielson 2009	Cross-sectional	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–40	84.1	47.5	421	0.56 (0.29–1.11)	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
Baldwin 2004	Cross-sectional	18 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, 83	18–70	67.4	28.2	344	**0.44 (0.22–0.90)**	Sexual frequency per month, condom use in past 3 months

Hernandez 2008	Cross-sectional	19 types: 16, 18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 73, 82, 82/IS39	NR	82.6	NR	172	0.82 (0.28–2.38)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
Svare 2002	Cross-sectional	4 types: 16, 18, 31, 33	18–≥40	16.4	45	134	0.4 (0.08–1.7)	Age, lifetime sex partners
Low-risk HPV								
Albero 2013	Cohort	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–70	35.9	66.7	3969	**0.76 (0.67–0.87)**[†]	None
Smith 2021[‡]	Randomized controlled trial	22 types: 6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 89	18–24	50.6	50[†]	2193	**PR 0.59 (0.45–0.77)**	None
Tobian 2009	Randomized controlled trial	23 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	15–49	44.8	62.2	520	**RR 0.66 (0.49–0.91)**	None
Baldwin 2004	Cross-sectional	9 types: 6, 11, 40, 42, 53, 54, 57, 66, 84	18–70	67.4	28.2	344	**0.44 (0.23–0.81)**	Genital warts, condom use with last anal sex
Hernandez 2008	Cross-sectional	18 types: 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, 89	NR	80.9	NR	188	0.61 (0.25–1.47)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
Svare 2002	Cross-sectional	2 types: 6, 11	18–≥40	17.3	45	127	0.8 (0.1–4.1)	Age, number of sex partners in past year, ever had genital warts
Nielson 2009	Cross-sectional	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–40	84.1	47.5	421	0.91 (0.47–1.78)	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months

Abbreviations: NR, not reported; OR, odds ratio; PR, prevalence ratio; RR, risk ratio; CI, confidence interval

[†] *Calculation was performed by hand*

[‡] *Outcome was infection with exclusively low-risk type(s)*

Table 3-4: Studies assessing the association between MC and incidence of HPV infection in males by HPV risk grouping

First author (year)	Study design	HPV types detected	Age at baseline (range)	Circumcision prevalence (%)	HPV prevalence at baseline (%)	Number analyzed	Effect estimate: HR (95% CI)	Covariate adjustment
Any HPV								
Albero 2014	Cohort	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–70	36.4	66.8	4,033	1.08 (0.91–1.27)	Country, age, marital status, lifetime number of female sexual partners, recent number of female sexual partners, recent number of male anal sex partners, six-month visit compliance status
Smith 2021	RCT	44 types: 6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 85, 86, 89, JC9710	18–24	49.6	50[†]	1,096	**0.56 (0.45–0.70)**	None
Lu 2009	Cohort	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–44	87.7	NR	285	0.8 (0.4–1.9)	Cigarette smoking, lifetime number of sexual partners
Partridge 2007	Cohort	37 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 73, 82, 6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 69, 70, 71, 72, 81, 83, 84, 89	18–20	76.7	25.8	240	1.1 (0.6–2.0)	None
Lajous 2005	Cohort	27 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 73, 82, 6, 11, 26, 40, 42, 54, 55, 57, 66, 83, 84	16–40	16.7	44.6	210	**OR 0.48 (0.30–0.77)**	Age, SES, lifetime number of partners
High-risk HPV								
Albero 2014	Cohort	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–70	36.4	66.8	4,033	1.11 (0.94–1.31)	Country, age, marital status, lifetime number of female sexual partners, recent number of female sexual partners, and recent number of male anal sex partners
Smith 2021	RCT	14 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	18–24	50.1	50[†]	1,335	**0.58 (0.49–0.69)**	None
Tobian 2012	RCT	14 types: 6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	15–49	NR	NR	776	**IRR 0.70 (0.55–0.89)**	Age, marital status, non-marital relationships, number of sexual partners during past year, condom use past year, self-reported urethral discharge
Vanbuskirk 2011	Cohort	19 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 82/IS39	18–20	75.3	20	477	1.1 (0.8–1.4)	None
Gray 2010	RCT	14 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	15–49	46.4	38.9	448	**IRR 0.67 (0.50–0.91)**	Age, education, condom use, alcohol consumption with sex, number of sex partners

Lu 2009	Cohort	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–44	87.7	NR	285	1.7 (0.6–4.9)	Age at first sexual intercourse, lifetime number of sexual partners
Low-risk HPV								
Albero 2014	Cohort	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–70	36.4	66.8	4,033	1.11 (0.94–1.30)	Country, age, marital status, lifetime number of female sexual partners, recent number of female sexual partners, lifetime number of male anal sex partners, and six-month visit compliance status
Smith 2021‡	RCT	22 types: 6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 89	18–24	50.0†	50†	1,851	**0.61 (0.51–0.73)**	None
Gray 2010	RCT	20 types: 6, 11, 26, 40, 42, 43, 53, 54, 55, 61, 67, 70, 71, 72, 73, 81, 82, 83, 84, 108	15–49	46.4	38.9	448	IRR 0.84 (0.66–1.10)	None
Lu 2009‡	Cohort	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–44	87.7	NR	285	1.0 (0.4–2.5)	None

Abbreviations: NR, not reported; HR, hazard ratio; IRR, incidence rate ratio; CI, confidence interval

† *Calculation was performed by hand*

‡ *Outcome was infection with exclusively low-risk type(s)*

Table 3-5: Studies assessing the association between MC and clearance of HPV infections in males by HPV risk grouping

First author (year)	Study design	HPV types detected	Age at baseline (range)	Circumcision prevalence (%)	HPV prevalence at baseline (%)	Number analyzed	Effect estimate: HR (95% CI)	Covariate adjustment
Any HPV								
Albero 2014	Cohort	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–70	36.4	66.8	4,033	0.95 (0.88–1.02)	Country, age, lifetime number of female sexual partners, recent number of male anal sex partners, smoking status, HPV status at baseline, six-month visit compliance status
Smith 2021	RCT	44 types: 6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 85, 86, 89, JC9710	18–24	49.8	50	2,331	**1.98 (1.48–2.66)**	None
Hernandez 2010	Cohort	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–79	81.2	50	357	0.96 (0.71–1.32)	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
Lu 2009	Cohort	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–44	87.7	NR	285	**3.1 (1.2–8.2)**	Cigarette smoking, lifetime number of sexual partners
High-risk HPV								
Albero 2014	Cohort	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–70	36.4	66.8	4,033	0.9 (0.81–1.00)	Country, age, lifetime number of female sexual partners, lifetime number of male anal sex partners, HPV status at baseline, six-month visit compliance status
Smith 2021	RCT	14 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	18–24	48.9	50	1,239	**1.76 (1.29–2.39)**	None
Tobian 2012	RCT	14 types: 6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	15–49	NR	NR	776	**RR 1.48 (1.26–1.74)**	Age, occupation, marital status, self-reported urethral discharge, self-reported dysuria, enrollment syphilis status, HSV-2 status
Gray 2010	RCT	14 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	15–49	46.4	38.9	448	**CRR 1.39 (1.17–1.64)**	Age, education, number of sex partners, condom use
Hernandez 2010	Cohort	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–79	81.2	50	357	1.11 (0.60–2.08)	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
Lu 2009	Cohort	13 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	18–44	87.7	NR	285	**6.5 (2.1–19.7)**	Age at first sexual intercourse, lifetime number of sexual partners
Low-risk HPV								
Albero 2014	Cohort	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–70	36.4	66.8	4,033	0.98 (0.89–1.07)	Country, age, recent number of female sexual partners, recent number of male anal sex partners, smoking status, HPV status at baseline, six-month visit compliance status

Smith 2021‡	RCT	22 types: 6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 89	18–24	50†	50	1,094	**1.56 (1.21–2.00)**	None
Hernandez 2010‡	Cohort	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–79	81.2	50	357	0.87 (0.50–1.54)	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
Lu 2009‡	Cohort	24 types: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82/IS39, 83, 84, 89	18–44	87.7	NR	285	1.6 (0.7–3.7)	None

Abbreviations: NR, not reported; HR, hazard ratio; CI, confidence interval

† *Calculation was performed by hand*

‡ *Outcome was infection with exclusively low-risk type(s)*

Table 3-6: Studies assessing the association between MC and various HPV infection outcomes in females by HPV risk grouping

First author (year)	Study design	HPV types detected	Age at baseline (range)	Circumcision prevalence (%)	HPV prevalence at baseline (%)	Number analyzed	Effect estimate: OR (95% CI)	Covariate adjustment
Prevalence, any HPV								
Roura 2012	Cross-sectional	27 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 6, 11, 40, 43, 44, 54, 69/71, 70, 74)	18–65	13.5	19.3	2,735	0.8 (0.6–1.1)	Age, autonomous community, country of birth, marital status, level of education, smoking habits, lifetime number of sexual partners, history of genital warts
Wawer 2011	RCT	27 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 66, 68, 6, 11, 26, 40, 42, 53, 54, 55, 57, 73, 82, 83, 84)	15–49	52.7	55.8†	1,032	**PR 0.81 (0.72–0.92)**	None
Mbulawa 2009	Cross-sectional	37 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 26, 53, 66, 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 82/IS39, 83, 84, 89	18–65	92.1	31	202	0.42 (0.14–1.20)†	None
Contreras 2008	Cross-sectional	14+ types: 5, 6, 8, 11, 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, others	18–55	19.7	30	61	**9 (1.2–64.4)**	Age, having more than 1 sexual partner
Prevalence, high-risk HPV								
Roura 2012	Cross-sectional	18 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82	18–65	13.5	19.3	2,735	0.7 (0.5–1.0)	Age, autonomous community, country of birth, marital status, level of education, smoking habits, lifetime number of sexual partners, history of genital warts
Wawer 2011	RCT	14 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 66, 68	15–49	52.7	55.8†	1,032	**PR 0.72 (0.60–0.85)**	None
Obiri-Yeboah 2017	Cross-sectional	19 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82	18-NR	93.5	42.6	170	**RR 0.5 (0.3–0.9)**	None
Prevalence, low-risk HPV								
Roura 2012	Cross-sectional	9 types: 6, 11, 40, 43, 44, 54, 69/71, 70, 74	18–65	13.5	19.3	2,735	0.7 (0.5–1.0)	Age, autonomous community, country of birth, marital status, level of education, smoking habits, lifetime number of sexual partners, history of genital warts
Wawer 2011	RCT	13 types: 6, 11, 26, 40, 42, 53, 54, 55, 57, 73, 82, 83, 84	15–49	52.7	55.8†	1,032	**PR 0.77 (0.66–0.90)**	None
Incidence, high-risk HPV								
Wawer 2011	RCT	14 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 66, 68	15–49	52.2	55.8†	1,051	**IRR 0.77 (0.63–0.93)**	None
Incidence, low-risk HPV								
Wawer 2011	RCT	13 types: 6, 11, 26, 40, 42, 53, 54, 55, 57, 73, 82, 83, 84	15–49	52.2	55.8†	1,051	IRR 0.83 (0.69–1.00)	None
Clearance, high-risk HPV								
Wawer 2011	RCT	14 types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 66, 68	15–49	52.2	55.8†	1,051	**RR 1.12 (1.02–1.22)**	None

Abbreviations: NR, not reported; OR, odds ratio; IRR, incidence rate ratio; RR, risk ratio; CI, confidence interval
† Calculation was performed by hand

Table 3-7: Site-specific studies of the association between MC and HPV infection prevalence in males by site grouping and HPV risk grouping

HPV grouping	First author (year)	Number analyzed	Effect estimate: OR (95% CI)	Covariate adjustment
Distal penis				
Any HPV	Smith 2021	2,193	**PR 0.48 (0.41–0.56)**	None
	Castellsagué 2002	1,139	**0.37 (0.16–0.85)**	Age, study location, level of education, age at first sexual intercourse, lifetime number of sexual partners, frequency of genital washing after sex
	Tobian 2009	520	**RR 0.70 (0.53–0.91)**	None
	Nielson 2009	444	**0.44 (0.23–0.82)**	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
	Baldwin 2004	344	**0.34 (0.20–0.57)**	Sexual frequency per month, genital warts, condom use in past 3 months, steady partner
	Hernandez 2008	308	**0.51 (0.27–0.97)**	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
	Bleeker 2005	253	0.98 (0.41–2.36)	None
	Da Rocha 2015	182	0.52 (0.02–3.66)	None
	Rocha 2012	37	1.32 (0.06–28.7)	None
High-risk HPV	Smith 2021	2,193	**PR 0.46 (0.38–0.57)**	None
	Tobian 2009	520	**RR 0.65 (0.46–0.90)**	Enrollment characteristics, rates of sexual practices, symptoms of sexually transmitted infections
	Nielson 2009	444	**0.40 (0.22–0.99)**	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
	Baldwin 2004	344	**0.44 (0.22–0.90)**	Sexual frequency per month, condom use in past 3 months
	Hernandez 2008	258	**0.40 (0.18–0.90)**	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
Low-risk HPV	Smith 2021‡	2,193	**PR 0.49 (0.36–0.65)**	None
	Tobian 2009	520	**RR 0.66 (0.49–0.91)**	None
	Nielson 2009‡	444	0.62 (0.29–1.29)	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
	Baldwin 2004	344	**0.44 (0.23–0.81)**	Genital warts, condom use with last anal sex
	Hernandez 2008	280	0.52 (0.25–1.08)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
Proximal penis				
Any HPV	Smith 2021	2,193	PR 1.05 (0.84–1.31)	None
	Nielson 2009	449	**0.53 (0.28–0.99)**	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
	Hernandez 2008	334	0.63 (0.42–1.22)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
High-risk HPV	Smith 2021	2,193	PR 1.20 (0.88–1.62)	None
	Nielson 2009	449	0.50 (0.25–1.00)	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months

	Hernandez 2008	243	0.70 (0.32–1.52)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking
Low-risk HPV	Smith 2021‡	2,193	PR 0.81 (0.54–1.19)	None
	Nielson 2009‡	449	0.85 (0.40–1.80)	Date of analysis, smoking status, lifetime number of female sex partners, condom use in the past 3 months
	Hernandez 2008	292	0.59 (0.30–1.16)	Age, birthplace, race/ethnicity, education level, lifetime number of female sex partners, history of sex with men, age at initial sex, condom use, history of genital warts, history of cigarette smoking

Abbreviations: OR, odds ratio; PR, prevalence ratio; RR, risk ratio; CI, confidence interval

‡ *Outcome was infection with exclusively low-risk types*

Table 3-8: Site-specific studies of the association between MC and HPV infection incidence in males by site grouping and HPV risk grouping

HPV grouping	First author and year	Number analyzed	Effect estimate: HR (95% CI)	Covariate adjustment
Distal penis				
Any HPV	Smith 2021	1,196	**0.51 (0.43–0.61)**	None
High-risk HPV	Smith 2021	1,442	**0.48 (0.40–0.57)**	None
	Tobian 2012	776	**0.70 (0.55–0.89)**	Age, marital status, non-marital relationships, number of sexual partners during past year, condom use past year, self-reported urethral discharge
	Gray 2010	448	**0.67 (0.50–0.91)**	Age, education, condom use, alcohol consumption with sex, number of sex partners
Low-risk HPV	Smith 2021‡	1,863	**0.54 (0.44–0.65)**	None
	Gray 2010	448	0.84 (0.66–1.10)	None
Proximal penis				
Any HPV	Smith 2021	1,795	1.01 (0.87–1.17)	None
High-risk HPV	Smith 2021	1,891	0.98 (0.82–1.17)	None
Low-risk HPV	Smith 2021‡	2,048	0.86 (0.68–1.07)	None

Abbreviations: HR, hazard ratio; CI, confidence interval
‡ Outcome was infection with exclusively low-risk types

Table 3-9: Site-specific studies of the association between MC and HPV infection clearance in males by site grouping and HPV risk grouping

HPV grouping	First author and year	Number analyzed	Effect estimate: HR (95% CI)	Covariate adjustment
Distal penis				
Any HPV	Smith 2021	2,032	**1.90 (1.49–2.42)**	None
	Hernandez 2010	357	**1.69 (1.02–2.78)**	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
High-risk HPV	Smith 2021	1,051	**1.73 (1.27–2.37)**	None
	Tobian 2012	776	**RR 1.48 (1.26–1.74)**	Age, occupation, marital status, self-reported urethral discharge, self-reported dysuria, enrollment syphilis status, HSV-2 status
	Gray 2010	448	**CRR 1.39 (1.17–1.64)**	Age, education, number of sex partners, condom use
	Hernandez 2010	357	**2.78 (1.10–7.14)**	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
Low-risk HPV	Smith 2021‡	981	**1.54 (1.29–1.85)**	None
	Hernandez 2010	357	**2.00 (1.02–4.00)**	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
Proximal penis				
Any HPV	Smith 2021	624	**2.19 (1.34–3.58)**	None
	Hernandez 2010	357	0.94 (0.63–1.41)	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
High-risk HPV	Hernandez 2010	357	1.67 (0.67–4.17)	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
	Smith 2021	356	1.15 (0.59–2.25)	None
Low-risk HPV	Hernandez 2010‡	357	1.16 (0.56–2.44)	Age, race/ethnicity, birthplace, education, lifetime number of female partners, history of sex with men, condom use during prior 4 months, history of genital warts
	Smith 2021‡	268	1.31 (0.79–2.18)	None

Abbreviations: HR, hazard ratio; CRR, clearance rate ratio; RR, risk ratio; CI, confidence interval
‡ Outcome was infection with exclusively low-risk types

CHAPTER 4. ORIGINAL RESEARCH

4.1. PREFACE

Chapter 3 provided a systematic review of the evidence regarding MC's protective effect on various HPV infection outcomes. In summary, MC seems to be protective against prevalent HPV infections in males, especially at the distal end of the penis, and that protection appears to be passed on to female partners. MC may also protect against incidence and clearance of HPV infections in males, but with a weaker association.

In Chapter 4, I use data from the HPV Infection and Transmission through Heterosexual Activity cohort study to perform my own analysis of the association between MC and HPV prevalence, transmission, and clearance in university-age heterosexual couples in recently formed sexual relationships.

A condensed version of the following manuscript will be submitted to the International Journal of Epidemiology.

4.2. MANUSCRIPT 2: ASSOCIATION BETWEEN MALE CIRCUMCISION AND HUMAN PAPILLOMAVIRUS INFECTION IN MALES AND THEIR FEMALE SEXUAL PARTNERS: FINDINGS FROM THE HITCH COHORT STUDY

Samantha B. Shapiro[1], Michel D. Wissing[1], Farzin Khosrow-Khavar[1], Ann N. Burchell[2], Mariam El-Zein[1], Pierre-Paul Tellier[3], François Coutlée[1,4], Eduardo L. Franco[1]

[1] Division of Cancer Epidemiology, Department of Oncology, McGill University, 5100 Boulevard de Maisonneuve West, Suite 720, Quebec H4A 3T2, Montreal, Quebec, Canada.

[2] Department of Family and Community Medicine and MAP Centre for Urban Health Solutions, Li Ka Shing Knowledge Institute, St. Michael's Hospital, Unity Health Toronto, Toronto, Ontario, Canada.

[3] Department of Family Medicine, McGill University, Montreal, Quebec, Canada.

[4] Laboratoire de virologie moléculaire, Centre de recherche, Centre hospitalier de l'Université de Montréal (CRCHUM), et Département de Microbiologie, infectiologie et Immunologie, Université de Montréal, Montreal, Quebec, Canada.

Correspondence to:
Samantha Shapiro
Division of Cancer Epidemiology, McGill University
5100 Maisonneuve Blvd West, Suite 720
Montreal, QC, H4A 3T2, Canada
E-mail address: samantha.shapiro@mcgill.ca
Phone: +1-514-398-6032

4.2.1. ABSTRACT

Background: Previous studies that examined the association between male circumcision (MC) and various human papillomavirus (HPV) infection outcomes in males and females reported inconsistent results. We examined the effect of MC on the prevalence, transmission, and clearance of HPV infections in males and their female sexual partners using data from the HPV Infection and Transmission among Couples through Heterosexual Activity cohort study.

Methods: University-age couples in recently formed sexual relationships were enrolled (2005–2011) in a longitudinal, couple-based study conducted in Montreal, Canada. Males and their female sexual partners were scheduled for a follow-up visit at 4 months. We used multilevel mixed-effects logistic and Poisson regression adjusting for propensity score to calculate odds ratios (ORs) and rate ratios (RRs) along with 95% confidence intervals (95% CIs) for the association between MC and the baseline prevalence of HPV infections in males and females, clearance of baseline infections in males, and the association between MC and HPV transmission in males and their female sexual partners. Analyses of prevalence and transmission in males were conducted for any HPV and for high- and low-risk HPVs separately.

Results: 361 of the 413 enrolled couples were eligible for analysis and had a mean follow-up time of 163 days. MC was not associated with prevalent infections at baseline in males (adjusted OR 0.84, 95% CI 0.58–1.22) or females (adjusted OR 1.13, 95% CI 0.81–1.58), or with male-to-female transmission of infections (adjusted RR 1.10, 95% CI 0.39–3.08). There was a suggestion of a protective effect on female-to-male transmission of infections, albeit with wide confidence intervals (adjusted RR 0.50, 95% CI 0.21–1.21). MC may be associated with reduced clearance of baseline infections in males (adjusted RR 0.77, 95% CI 0.48–1.26). HPV 16 was the most commonly detected type in couples with both circumcised males and uncircumcised males.

Conclusions: We found no evidence of an association between MC and HPV infection prevalence, transmission, or clearance in both males and their female sexual partners. Our propensity score-based approach allowed us to account for many covariates while preserving estimation precision. Further couple-based studies with a longer follow-up period and separate sampling of the glans penis would be required to thoroughly investigate this association.

Keywords: HPV, human papillomavirus, circumcision

4.2.2. INTRODUCTION

Human papillomavirus (HPV) is the most prevalent sexually transmitted viral infection.[2] Persistent oncogenic HPV infections may lead to anogenital (including cervical) and head-and-neck cancers whereas non-oncogenic HPV types may cause anogenital warts.[137] An estimated 640 000 new cancer cases worldwide in 2012 were attributable to HPV.[138] Currently, vaccination against HPV protects against up to nine types (HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58) that are responsible for the greatest proportion of HPV-related morbidity and mortality.[139] Vaccination coverage is expanding globally but is still far from ideal – total population coverage is below 2%, and even in highly developed countries, vaccination coverage in adolescent females is often below 50%.[52]

The human body clears most HPV infections spontaneously[140]; however, there is no effective treatment available against persistent HPV infections. Screening for cervical cancer prevents significant HPV-related morbidity and mortality by providing an opportunity for early interventions.[141] Improved knowledge of the determinants of HPV infections may assist in developing effective interventions to prevent infections and HPV-related disease.

Several determinants of persistent HPV infections have been identified, such as high-risk sexual behavior, infection with the human immunodeficiency virus (HIV), and condom use.[29,114,142-145] Various studies have investigated the association between male circumcision (MC) and HPV infections and related disease in males and their female partners, but results were inconclusive. In a multinational retrospective study of almost 2,000 couples, penile HPV prevalence was significantly lower in circumcised males, and cervical cancer risk was reduced in females who had circumcised partners with multiple sexual partners as compared to a similar, uncircumcised population.[98] Similarly, in a randomized controlled trial conducted in Uganda, circumcised males had reduced penile HPV infections and lower viral loads.[15,120,146] As well, penile HPV infections were decreased in circumcised males in a South African study,[147] and MC resulted in a lower prevalence of HPV-related penile lesions in a Kenyan trial.[148] Contrarily, multiple cohort and cross-sectional studies found no association between MC and the prevalence,[108,109,112,117,124] incidence,[116,118,123] and clearance[116,123] of HPV infections in males.

It is of note that the results of some of the aforementioned studies may have been confounded. In observational studies, differences in baseline characteristics between uncircumcised and circumcised males and their female partners need to be evaluated in detail. For

example, males who get circumcised for religious or cultural reasons may have different sexual behavior than uncircumcised males. Randomized controlled trials such as the previously mentioned Ugandan trial did not consider the altered sexual behaviour that would result from MC of adult males.[122] Sexual behavior is likely to change after voluntary MC, not only due to pain and wound recovery directly after surgery, but also due to an individual's beliefs (e.g., decreased condom use after MC due to the belief that MC effectively protects against HIV), postoperative hygiene, and potentially due to changes in sexual satisfaction.[149-156] Hence, the observed changes in HPV prevalence and transmission after MC could be due to indirect, (often short-term) consequences of the surgery.

Using data from the HPV Infection and Transmission among Couples through Heterosexual Activity (HITCH) cohort study, we evaluated the influence of MC status on HPV prevalence, transmission, and clearance within university-age, recently formed heterosexual couples in Montreal, Canada. This prospective study collected extensive data on participants, including sexual behavior and other established risk factors for HPV transmission, allowing for detailed adjustments for potential confounders. We hypothesized that MC would result in lower HPV concordance between couples, lower prevalence and transmission in males and females, and increased clearance in males.

4.2.3. METHODS

4.2.3.1. STUDY DESIGN AND POPULATION

We used data from the HITCH cohort study, which has been previously described.[157] Briefly, the study was conducted between 2005 and 2011 in Montreal, Canada and enrolled female students aged 18–24 attending a post-secondary institution along with their male sexual partners aged 18 and older. Couples were enrolled if they had initiated sexual contact within the previous six months. Exclusion criteria for women were not having an intact uterus, having a history of cervical lesions or cervical cancer, or being pregnant or planning on becoming pregnant in the two years following study enrolment. If a couple ended their relationship and the female later had a new sexual partner, that new partner could enroll in the study if he met the inclusion criteria.

Females had a baseline visit and five follow-up visits every 4–6 months over the course of 24 months, whereas males had a baseline and one follow-up visit approximately four months later. At each visit, participants filled out an online questionnaire and provided genital samples.

Abstention from intercourse was requested for 24 hours prior to each visit to prevent contamination of genital samples by deposition.

All subjects provided written informed consent. The study was conducted in accordance with the principles and articles stipulated by the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans. Ethical approval was obtained from the institutional review boards at McGill University, Concordia University, and Centre Hospitalier de l'Université de Montréal. Ethics renewal approval is requested annually from McGill University (Study Number A09-M77-04A).

4.2.3.2. Data collection

Information on sociodemographic factors, sexual history, and sexual behaviour was collected using a self-administered web-based questionnaire for both males and females. MC status was self-reported by the female partner and/or assessed by the research nurse during the clinic visit. In case of discordant reporting, the nurse's assessment was used.

At each visit, samples from the penis (i.e., the glans up to and including the external opening of the meatus, coronal sulcus, penile shaft, and foreskin in uncircumcised males) and scrotum were collected separately. For both sites, the nurse collected epithelial cells by gently exfoliating using ultra-fine emery paper then swabbing with a saline-moistened cotton Dacron™ swab. Swabs were agitated in a vial containing Preservcyt™ medium (Hologic, Marlborough, MA, USA) then discarded. Samples were stored at 4° C until laboratory transfer.

Vaginal specimens were self-collected by female participants, who were instructed to insert a Dacron swab at least 5 cm into the vagina and to rotate the swab for three full rotations. Swabs were agitated in a vial containing Preservcyt then discarded; samples were stored at 4° C pending processing.

4.2.3.3. HPV DNA testing and typing

Vaginal and penile samples were tested by PCR using the Linear Array HPV genotyping assay (Roche Molecular Systems, Alameda, CA, USA), which amplifies a 450-bp segment in the L1 gene. This method detects the presence of 36 mucosal HPV genotypes of the Alphapapillomavirus genus (6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89).[158] A β-globin DNA sequence was co-amplified to determine sample adequacy. Samples were considered valid if they tested

positive for β-globin. Samples positive for HPV types 6, 11, 16, 18, 31, 42, and 51 were re-tested using real-time quantitative PCR with type-specific primers to measure viral load[159-161]. Males were only considered type-specific HPV-positive based on positive penile samples for a given HPV type.

We grouped HPV types into three subgenera according to Alphapapillomavirus species clusters based on oncogenicity and tissue tropism, as previously described by Schiffman et al.[27,28] Subgenus 1 includes low-risk HPV (LR-HPV) types 6, 11, 40, 42, 44 and 54; subgenus 2 includes high-risk HPV (HR-HPV) types 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73 and 82; and subgenus 3 includes commensal HPV types 61, 62, 71, 72, 81, 83, 84 and 89.

4.2.3.4. STATISTICAL ANALYSIS

We restricted analyses to 1) females and their first male sexual partner, 2) the females' first two clinic visits (since they were at a similar time point to their male partner's visit), and 3) clinic visits where the couple was in a monogamous relationship (i.e., had not ended their relationship and had not had other sexual partners).

We calculated a couple-based propensity score to to calculate the probability of MC conditional on observed baseline covariates, such that, for a given score, the distribution of covariates would be the same for couples with male partners who were circumcised or not circumcised.[162] Our logistic regression was based on 27 *a priori* variables. These included the male's and female's age at baseline, birth region (Africa, Asia, Europe/Australia/Oceania, Latin America, Middle East, North America), race (Asian, Black, Other, White), education (high school, post-secondary), lifetime smoking history, lifetime sexual partners (1–3, 4–6, 7–10, 11 or more partners), lifetime vaginal sexual partners (1–2, 3–4, 5–9, 10 or more partners), age at first vaginal sexual intercourse (≤15, 16–18, ≥19 years), lifetime history of a same-sex partner (yes/no), concurrent partner at future visit (yes/no), and partner's type-specific HPV positivity (yes/no). For females only, we included HPV vaccination status at baseline (vaccinated/unvaccinated). We also included couple-based variables such as marital status (single, common-law, married), baseline condom use (never, irregular, always), baseline frequency of sexual acts per week, baseline frequency of vaginal sex per week, and concurrent partner at future visit from either partner (yes/no). After generating a propensity score, we restricted our population to couples in the region of common support (i.e., couples with and without circumcised males whose propensity scores

had a common range) to ensure that there were MC-exposed and MC-unexposed individuals along the continuum of the propensity score to prevent positivity violation.[163]

We used descriptive statistics (means and proportions) to summarize the baseline characteristics of circumcised vs. uncircumcised males, females with a circumcised male partner vs. females with an uncircumcised male partner, and couples with a circumcised male vs. couples with an uncircumcised male, before and after restriction to the region of common support. We also assessed the balance between these groups before and after restriction using standardized differences.

For each HPV type, there were four options for positivity: male positive and female positive (concordant infection) (M+F+), male positive and female negative (discordant infection) (M+F-), male negative and female positive (discordant infection) (M-F+), and male negative and female negative (uninfected) (M-F-). We calculated the observed to expected (O:E) ratios for concordant type-specific HPV infections in couples. The ratio represents the magnitude by which infections increased (O:E greater than 1) or decreased (O:E less than 1) due to the couple's sexual relationship, as opposed to what would be expected if they were not in a relationship.

We considered three primary outcomes among males: prevalence of HPV infections at baseline, clearance of baseline infections, and female-to-male transmission of HPV infections. We also considered one primary outcome among females: male-to-female transmission of HPV infections. Secondary outcomes included prevalence of HPV infections at baseline among females as well as subgenus-specific prevalence of infections among males at baseline and female-to-male transmission. Prevalence at baseline was defined as having a type-specific HPV infection at visit 1 (enrollment visit), whereas clearance of that infection was defined as being negative for that type-specific infection at visit 2 after testing positivity at visit 1. Transmission was defined as having a type-specific, incident HPV infection at visit 2 that was absent at visit 1, but only if the partner was positive for that given HPV type at visit 1.

Analyses were mainly performed for infection with any HPV type. For analyses of HPV prevalence at baseline, we used multilevel mixed-effects logistic regression, nested at the level of the individual with robust variance estimation, to estimate the odds ratio (OR) and 95% confidence intervals (95% CI). For analyses of HPV transmission and clearance, we used multilevel mixed-effects Poisson regression again nested at the level of the individual with robust variance estimation to estimate the rate ratio (RR) and 95% CI. For analyses of HPV prevalence at baseline

among males and female-to-male HPV transmission, we conducted additional analyses for grouped HPV infections (subgenera 1 and 3 combined, subgenus 2). All analyses included adjustment for propensity score. We conducted sensitivity analyses for the primary outcomes using inverse probability of treatment weighting to create a pseudo-population and estimate the average treatment effect. Weights were generated as the propensity score for participants exposed to MC (i.e., circumcised males and their female partners) and 1 minus the propensity score for those unexposed to MC.

Finally, to ensure that differences between circumcised and uncircumcised males were not due to differences in HPV detectability, we compared the median and geometric mean viral loads in HPV-infected males. P-values for individual HPV types were calculated using Wilcoxon rank-sum tests. When HPV types were grouped (any HPV and by subgenus), p-values were calculated using the Cochran-Mantel-Haenszel test, stratifying by HPV type.

All statistical analyses were performed using Stata 17 (STATA Corp., College Station, TX).

4.2.4. RESULTS

HITCH enrolled 502 females and their 548 male partners. Six genital samples tested negative for β-globin and were excluded. Next, we limited the analyses to females and their first male partner enrolled in the study who were monogamous at baseline. The final analytical sample was restricted to the 361 couples in the score's region of common support, which encompassed values between 0.1242 and 0.8586 (Figure 4-1, Figure 4-2). Table 4-1 presents baseline characteristics of male and female participants of the 413 eligible couples and the 361 couples in the region of common support. In general, covariates were balanced between uncircumcised males and circumcised males, females with an uncircumcised male partner and females with a circumcised male partner, and couples with an uncircumcised male and couples with a circumcised male. In the restricted subset, the mean age of participants was 21.4 years. Most were born in North America, were White, had received or were receiving a post-secondary education, were single, and irregularly used condoms. Just under half of males and just under a third of females had a lifetime history of smoking 100 or more cigarettes. Uncircumcised and circumcised males and females with circumcised and uncircumcised partners were comparable in terms of their lifetime sexual partners. The mean age at first vaginal sexual intercourse was 17 years, regardless of sex and MC status. 8.7% of uncircumcised males, 6.7% of circumcised males, 11.2% of females with

uncircumcised partners, and 10.9% of females with circumcised partners had a lifetime history of having a same-sex partner. More females than males had a concurrent sexual partner at a future visit. The mean frequency of sexual acts per week was 5.0 among couples with an uncircumcised male and 5.2 among couples with a circumcised male, and the mean frequency of vaginal sex per week was 4.3 times among couples with an uncircumcised male and 4.6 times among couples with a circumcised male. The mean follow-up time was 167 days among males and 162 days among females.

Table 4-2 presents the baseline prevalence of HPV infections in males and females by MC status. 55.2% of circumcised and 52.6% of uncircumcised males had at least one HPV infection present at baseline, with respective means of 2.7 and 2.8 type-specific infections per infected male (data not presented). In total, there were 277 HPV infections detected at baseline in uncircumcised males and 257 in circumcised males. After adjusting for propensity score, circumcised males had a slightly decreased risk of HPV infection at baseline, although CI spanned the null (adjusted OR 0.84, 95% CI 0.58–1.22). No effect of MC was observed when stratifying baseline infections by subgenus. Similarly, 58.8% of females with circumcised partners and 54.1% of females with uncircumcised partners were infected with at least one type of HPV at baseline, averaging a respective 2.5 and 3.0 type-specific infections per infected female (data not presented). Females with circumcised partners had a total of 287 baseline infections whereas females with uncircumcised partners had 267. Females with circumcised male partners were at slightly increased risk of HPV infection at baseline, although non-significant (adjusted OR 1.13, 95% CI 0.81–1.58).

Table 4-3 compares the baseline prevalence of type-specific HPV infections within each couple. In couples with a circumcised male, the three most prevalent HPV types were 16, 84, and 51; in couples with an uncircumcised male, they were types 16, 84, and 89. Interestingly, HPV 16 was present in at least one partner in 25.5% of couples with a circumcised partner, compared to 15.3% in couples with an uncircumcised partner.

Our hypothesis of a higher proportion of concordantly infected couples with an uncircumcised male than couples with an uncircumcised male was not observed, as demonstrated in Table 4-4. There was a higher proportion of concordant infections in couples with an uncircumcised male for 14 HPV types (6, 18, 31, 33, 35, 42, 52, 54, 56, 58, 66, 68, 70, 81), whereas for 20 types (11, 16, 34, 39, 40, 44, 45, 51, 53, 59, 61, 62, 67, 71, 72, 73, 82, 83, 84, 89), couples

with a circumcised male had a higher proportion of concordant infections. No trends by subgenus were observed for subgenus 1 and 2: for each of these subgenera, half of its types had a higher proportion of concordant infections among couples with an uncircumcised male and half among couples with a circumcised male. Contrarily, of the eight types in subgenus 3, seven had a higher proportion of concordant infections in couples with a circumcised male and only one had a higher proportion in couples with an uncircumcised male. Expectedly, O:E ratios were all greater than one due to the direct association between sexual activity and HPV infections. For 20 HPV types (6, 16, 18, 39, 42, 44, 51, 52, 53, 56, 58, 61, 62, 68, 70, 73, 82, 83, 84, 89), the increase was greater in uncircumcised couples.

Table 4-5 presents results on female-to-male transmission, male-to-female transmission, and male clearance of HPV infections. There were 21 female-to-male transmission events among uncircumcised males over 23.3 person-years, representing 44.7% of possible events. In circumcised males, 16 transmission events occurred over 25.1 person-years, representing 25% of possible events. MC was associated with a decrease in female-to-male transmission of any type-specific HPV infections (adjusted type-specific RR 0.50, 95% CI 0.21–1.21). When stratifying by subgenus, the adjusted rate ratios were 0.81 (95% CI 0.21–3.15) for subgenus 1 and 3 infections combined and 0.36 (95% CI 0.15–0.85) for subgenus 2 infections. Male-to-female transmission events were less common overall, with 12 events occurring over 25.6 person-years in females with an uncircumcised partner (20.3% of possible transmissions) and 13 events occurring over 27.8 person-years in females with a circumcised partner (19.4% of possible transmissions). Confidence intervals were too wide to draw a meaningful conclusion (adjusted type-specific RR 1.10, 95% CI 0.39–3.08). 45 (28.0%) of baseline infections present were cleared over 77.5 person-years in uncircumcised males, compared to 40 (22.4%) baseline infections over 74.4 person-years in circumcised males. There were indications of slightly reduced clearance in circumcised males, although with low precision (adjusted type-specific RR 0.77, 95% CI 0.48–1.26).

Results from the inverse probability of treatment weighting sensitivity analysis were overall similar to those obtained by propensity score adjustment. We observed no effect of MC on prevalence among males (OR 1.02, 95% CI 0.70–1.51), a moderate protective effect on female-to-male transmission (RR 0.48, 95% CI 0.18–1.27), an inconclusive effect on male-to-female transmission (RR 0.69, 95% CI 0.13–3.58), and a moderately harmful effect on clearance in males (RR 0.56, 95% CI 0.28–1.13) (Supplementary Table 4-6).

We compared viral load in circumcised and uncircumcised males to ensure that observed effects were not due to differences in sample adequacy. There were no significant differences in viral load, indicating that epithelial cell sampling was comparable in both groups (Supplementary Table 4-7).

4.2.5. DISCUSSION

In our study of young, recently-formed heterosexual couples in Montreal, most of whom were unvaccinated, we observed no associations between MC and prevalent HPV infections in males or females, nor did we observe an association with male-to-female transmission of infections. We observed a potential mildly protective effect of MC on female-to-male transmission of infections and a potential mildly harmful effect of MC on clearance of baseline infections in males, though 95% confidence intervals for these outcomes encompassed the null.

Several analyses of over 2,000 males have failed to find a significant association between MC and prevalence of any HPV[112,124] and HR-HPV.[124] Conversely, some studies reported that MC was negatively associated with the prevalence of LR-HPV.[14,124] About half of the larger studies (1,000 or more individuals) assessing incidence and clearance did not find a significant association with MC,[116,120,121,123] but none of the studies were couple-based and therefore did not account for the sexual partner's HPV status. Our study did not find a difference between high-risk (subgenus 2) and low-risk (subgenera 1 and 3) types.

There is no clear consensus on whether MC affects HPV prevalence in females.[99,102,108,122] Only one study has assessed the association between MC and female HPV acquisition and clearance, but was not couple-based and obtained the male's MC status by partner report.[122] The authors found that MC was negatively associated with incidence and clearance of oncogenic HPV infections in 1,032 females, but was not associated with incidence of non-oncogenic infections. However, as this study was not couple based, it could not adjust for the partner's HPV positivity.

Anatomical site differences in HPV positivity pose an additional challenge for studies of HPV and MC. A 2008 study[114] of 379 males found a significant protective effect of MC on HPV prevalence at the glans, but not at the shaft or in a combined-site analysis. Similarly, a 2009 analysis[117] of 463 males found a stronger protective effect of MC towards HPV prevalence in the glans than in the shaft and did not find an association in the scrotum. A 2011 study[119] of 477 males showed that of males who acquired HPV infections, site-specific positivity varied by MC status; of circumcised HPV-positive males, about three-quarters were positive at the shaft and/or scrotum

while just under half were positive at the glans, whereas of uncircumcised HPV-positive males, about two-thirds were positive at the shaft and/or scrotum and another two-thirds were positive at the glans. MC therefore may only confer a protective effect at the glans and distal end of the penis, and this effect would not have been detected in the many studies that combined several sites in their sample, including the HITCH study.

Our study had several limitations. Self-reported sexual behaviour data may be misreported due to social desirability, leading to potential information bias. As previously mentioned, the HITCH study swabbed multiple sites of the penis and combined them in one sample, which limited our ability to assess the site-specific effect of MC. In addition, some HPV detections may have been a result of DNA deposition from sexual activity in the week prior to the visit. Male follow-up was limited to two visits and female follow-up analysis was restricted to two of the six visits in order to be able to adjust for the partner's type-specific positivity. Incident and cleared HPV infections occurred not at the time of the participants' clinic visits, but at some unknown point between the two visits; however, since we only had data at two time points, our ability to perform interval censoring was limited. We conducted Poisson regression rather than survival analysis, but this type of analysis does not consider the person-time no longer at risk after occurrence of the event, resulting in a dilution of the effect estimate.

Nevertheless, our study had numerous strengths, many regarding our statistical analysis. By restricting our analysis to couples in the region of common support, our sample was better balanced (especially in important variables such as race, lifetime (vaginal) sexual partners, and same-sex partner history), as evidenced by the reduction in standardized differences for key variables. Adjusting for propensity score minimized confounding bias while accounting for many covariates, which provided more precision for the effect estimate. Though having only one follow-up visit restricted our ability to perform survival analyses, our Poisson regression provided an excellent measure of infection incidence and clearance rates. The results of our primary analyses were supported by those using inverse probability of treatment weighting. This was a novel approach to investigating the role of MC in HPV infections. Other strengths of our study lie in its couple-based approach: there have been few couple-based HPV studies, none of which assessed in detail the impact of MC on HPV infections in both males and females.[102,122] HITCH intentionally recruited young couples in the early stages of their sexual relationships, which is the time when HPV transmission is most likely to occur.[142,164] Partner visits were at similar time

points. This allowed us to take into account in our analyses the partner's HPV status, which is the greatest predictor of HPV infection.[165]

More couple-based studies with a large sample size would be necessary to properly elucidate the effect of MC on HPV outcomes. MC is one of the most common surgeries performed worldwide[75] and may in time be shown to confer a protective effect as it does for various other STIs,[36,84,85] but it is unlikely to prevent the acquisition of HPV infection on its own. HPV-associated disease poses a significant burden worldwide, especially in low- and middle-income countries[166-169]; consequently, the determinants of transmission and alternative methods to prevent infections must be explored for disease and cancer control.

Acknowledgments

The authors wish to thank the volunteering participants. The authors would also like to thank Emilie Comète and Julie Guenoun for the processing and laboratory testing of samples, and the additional members of the HITCH Cohort Study group: Allita Rodrigues (study coordinator); Hélène Voyer and Véronique Legault (laboratory staff); Gail Kelsall, Suzanne Dumais, Natalia Morykon, and Amelia Rocamora (management of subject participation and specimen collection); Nathalie Slavtcheva (study management); Veronika Moravan (data management); Michel Roger (collaborator); and Vicky D'Anjou-Pomerleau, Jennifer Selinger, Elizabeth Montpetit-Dubrule, Jessica Sammut, Emilie Lapointe, Johanna Bleecker, and Shady Rahayel (study promotion). The authors also thank Melanie Drew (Student Health Services Clinic, Concordia University) and the staff of the Student Health Services Clinics at McGill and Concordia Universities, for their collaboration with HITCH research nurses.

Funding sources

The HITCH Cohort Study was supported by the Canadian Institutes of Health Research (grant nos. MOP-68893, CRN-83320 to E.L. Franco) and the U.S. National Institutes of Health (grant no. RO1AI073889 to E.L. Franco). Supplementary and unconditional funding support was provided by Merck-Frosst Canada Ltd., and Merck & Co. Ltd. Validation of viral load assays was supported by the Réseau sida et maladies infectieuses (SIDA/MI) du Fonds de recherche du Québec – Santé (FRQS). S.B. Shapiro is funded by the Carole Epstein Fellowship, awarded by McGill University.

Conflicts of interest

E.L. Franco and M. El-Zein hold a patent related to the discovery “DNA methylation markers for early detection of cervical cancer”, registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada (October 2018). The other authors have no relevant conflicts to disclose.

Contributions of authors

Eduardo Franco and Ann Burchell led the study while Pierre-Paul Tellier and François Coutlée acted as co-investigators. François Coutlée was responsible for the laboratory analysis of biological specimens for HPV DNA testing. Eduardo Franco and Michel Wissing conceptualized the analysis. Samantha Shapiro, Michel Wissing, and Farzin Khosrow-Khavar devised the methodology. Samantha Shapiro conducted the analyses. Samantha Shapiro and Michel Wissing drafted the manuscript. Michel Wissing, Farzin Khosrow-Khavar, Ann Burchell, Mariam El-Zein, Pierre-Paul Tellier, François Coutlée, and Eduardo Franco all reviewed and edited the manuscript. Eduardo Franco and Mariam El-Zein provided supervision and guidance.

Table 4-1: Baseline characteristics of HITCH participants by MC status

	All eligible couples			Eligible couples in region of common support		
Males	**Uncircumcised (n=218)**	**Circumcised (n=195)**	**StD (%)**	**Uncircumcised (n=196)**	**Circumcised (n=165)**	**StD (%)**
Age: mean (SD)	22.3 (3.5)	22.3 (3.7)	-1.2	22.2 (3.5)	22.1 (3.7)	-1.8
Region born: n (%)						
Africa	0 (0.0)	10 (5.1)	33.0	0 (0.0)	6 (3.6)	27.6
Asia	2 (0.9)	4 (2.1)	9.4	2 (1.0)	3 (1.8)	6.8
Europe	39 (17.9)	13 (6.7)	-34.8	28 (14.3)	13 (7.9)	-20.5
Latin America	16 (7.3)	11 (5.6)	9.2	15 (7.7)	11 (6.7)	-6.3
Middle East	3 (1.4)	20 (10.3)	38.8	2 (1.0)	4 (2.4)	10.9
North America	158 (72.5)	137 (70.3)	-3.9	149 (76.0)	128 (77.6)	5.1
Race: n (%)						
Asian	8 (3.7)	6 (3.1)	-3.3	7 (3.6)	5 (3.0)	-3.0
Black	6 (2.8)	10 (5.1)	9.9	6 (3.1)	10 (6.1)	11.9
Other	16 (7.3)	16 (8.2)	3.3	16 (8.2)	14 (8.5)	1.2
White	188 (86.2)	163 (83.6)	-6.1	167 (85.2)	136 (82.4)	-6.1
Education: n (%)						
High school or less	52 (23.9)	47 (24.1)	-0.6	46 (23.5)	38 (23.0)	-2.4
Post-secondary	166 (76.2)	147 (75.4)	-0.6	150 (76.5)	127 (77.0)	2.4
Smoker: n (%)	102 (46.8)	93 (47.7)	1.9	87 (44.4)	73 (44.2)	-0.1
Lifetime sexual partners: mean (SD)	8.8 (10.4)	9.9 (9.4)	10.6	9.0 (10.9)	9.1 (9.0)	0.5
Lifetime vaginal sexual partners: mean (SD)	7.2 (8.3)	8.1 (8.2)	10.0	7.4 (8.6)	7.5 (7.9)	1.3
Age at first vaginal sex: mean (SD)	17.2 (2.2)	17.2 (2.2)	-1.0	17.2 (2.2)	17.2 (2.2)	-3.2
Ever had a same-sex sexual partner: n (%)	20 (9.2)	11 (5.6)	-13.5	17 (8.7)	11 (6.7)	-7.5
Had a concurrent sexual partner at future visit: n (%)	15 (6.9)	21 (10.8)	13.8	14 (7.1)	16 (9.7)	9.3
Females	**Uncircumcised partner (n=218)**	**Circumcised partner (n=195)**	**StD (%)**	**Uncircumcised partner (n=196)**	**Circumcised partner (n=165)**	**StD (%)**
Age: mean (SD)	20.6 (1.8)	20.7 (1.8)	7.1	20.5 (1.7)	20.6 (1.8)	3.9
Region born: n (%)						
Africa	3 (1.4)	8 (4.1)	20.3	3 (1.5)	3 (1.8)	6.7
Asia	6 (2.8)	10 (5.1)	12.0	6 (3.1)	9 (5.5)	11.8
Europe or Oceania	26 (11.9)	15 (7.7)	-14.7	19 (9.7)	14 (8.5)	-4.4
Latin America	8 (3.7)	7 (3.6)	1.8	6 (3.1)	7 (4.2)	6.2
Middle East	3 (1.4)	10 (5.1)	21.1	2 (1.0)	3 (1.8)	6.7
North America	172 (78.9)	145 (74.4)	-12.3	160 (81.6)	129 (78.2)	-9.7
Race: n (%)						
Asian	20 (9.2)	10 (5.1)	-16.1	15 (7.7)	9 (5.5)	-9.0
Black	6 (2.8)	4 (2.1)	-4.8	5 (2.6)	4 (2.4)	-0.9

Other	12 (5.5)	19 (9.7)	15.7	12 (6.1)	14 (8.5)	8.9
White	180 (82.6)	162 (83.1)	2.0	164 (83.7)	138 (83.6)	0.1
Education: n (%)						
High school or less	33 (15.1)	26 (13.3)	-5.7	29 (14.8)	25 (15.2)	0.8
Post-secondary	184 (84.4)	169 (86.7)	7.0	166 (84.7)	140 (84.9)	0.6
Smoker: n (%)	76 (34.9)	64 (32.8)	-2.4	66 (33.7)	51 (30.9)	-5.2
Lifetime sexual partners: mean (SD)	8.2 (14.7)	9.0 (22.0)	4.1	8.1 (15.2)	9.3 (23.8)	5.8
Lifetime vaginal sexual partners: mean (SD)	5.6 (5.1)	5.8 (5.0)	-1.7	5.7 (5.0)	6.0 (5.1)	4.5
Age at first vaginal sex: mean (SD)	16.8 (2.0)	17.2 (2.1)	-6.4	16.9 (2.0)	17.1 (2.0)	10.4
Ever had a same-sex sexual partner: n (%)	25 (11.5)	22 (11.3)	19.0	22 (11.2)	18 (10.9)	-7.1
Had a concurrent sexual partner at future visit: n (%)	34 (15.6)	25 (12.8)	-8.5	30 (15.3)	22 (13.3)	-5.8
Vaccinated against HPV at baseline: n (%)	41 (18.8)	38 (19.5)	1.1	38 (19.4)	33 (20.0)	1.3

Couples	**Uncircumcised male (n=218)**	**Circumcised male (n=195)**	**StD (%)**	**Uncircumcised male (n=196)**	**Circumcised male (n=165)**	**StD (%)**
Marital status: n (%)						
Single	139 (63.8)	144 (73.9)	22.1	128 (65.3)	120 (72.7)	16.4
Common-law	76 (34.9)	48 (24.6)	-22.8	65 (33.2)	42 (25.5)	-17.3
Married	3 (1.4)	3 (1.5)	1.4	3 (1.5)	3 (1.8)	2.3
Base condom use: n (%)						
Never	35 (16.1)	42 (21.5)	14.2	33 (16.8)	35 (21.2)	11.3
Irregularly	119 (54.6)	101 (51.8)	-4.6	106 (54.1)	83 (50.3)	-6.4
Always	51 (23.4)	43 (22.1)	-4.6	45 (23.0)	39 (23.6)	0.0
Frequency of sexual acts per week: mean (SD)	5.0 (3.1)	5.5 (4.9)	12.3	5.0 (3.1)	5.2 (3.2)	5.6
Frequency of vaginal sex per week: mean (SD)	4.3 (2.3)	4.9 (4.6)	15.2	4.3 (2.4)	4.6 (2.7)	9.8
Male or female had a concurrent sexual partner at future visit: n (%)	45 (20.6)	44 (22.6)	4.7	41 (20.9)	36 (21.8)	2.3
Days since beginning of sexual relationship: mean (SD)	121.3 (69.1)	118.7 (58.6)	4.1	119.9 (54.3)	118.7 (59.0)	-2.6

Abbreviations: SD, standard deviation; StD, standardized difference

Table 4-2: Association between MC and baseline prevalence of type-specific HPV infections in males and females

Sex	HPV infections	Uncircumcised: n (%)	Circumcised: n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[†]
Males	Any	277 (3.9)	257 (4.3)	1.11 (0.79–1.55)	0.84 (0.58–1.22)
	Subgenera 1 & 3	107 (3.9)	105 (4.6)	1.14 (0.78–1.66)	0.95 (0.63–1.44)
	Subgenus 2	170 (3.9)	152 (4.2)	1.12 (0.77–1.61)	0.91 (0.62–1.35)
Females	Any	267 (3.8)	287 (4.8)	1.31 (0.96–1.78)	1.13 (0.81–1.58)

** Calculated as proportion of possible type-specific events*
[†] Adjusted for propensity score
Abbreviations: OR, odds ratio; CI, confidence interval
Subgenera 1 and 3: HPV 6, 11, 40, 42, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89
Subgenus 2: HPV 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82

Table 4-3: Baseline prevalence of type-specific infections in couples by MC

HPV type*	Subgenus	Uncircumcised				Circumcised				Proportion of couples with at least one partner positive	
		M+F+	M+F-	M-F+	M-F-	M+F+	M+F-	M-F+	M-F-	Uncircumcised	Circumcised
6	1	8	3	0	185	3	6	2	154	5.6%	6.7%
11	1	0	0	0	196	2	0	0	163	0.0%	1.2%
40	1	2	3	2	189	2	1	0	162	3.6%	1.8%
42	1	7	6	3	180	6	7	7	145	8.2%	12.1%
44	1	1	6	0	189	4	0	4	157	3.6%	4.8%
54	1	8	2	5	181	5	3	2	155	7.7%	6.1%
16	2	15	7	8	166	20	9	13	123	15.3%	25.5%
18	2	4	4	3	185	2	1	6	156	5.6%	5.5%
31	2	7	3	3	183	3	1	3	158	6.6%	4.2%
33	2	2	3	1	190	0	0	2	163	3.1%	1.2%
34	2	0	0	2	194	0	0	1	164	1.0%	0.6%
35	2	2	1	0	193	1	0	1	163	1.5%	1.2%
39	2	6	7	4	179	6	5	7	147	8.7%	10.9%
45	2	0	1	0	195	0	1	2	162	0.5%	1.8%
51	2	10	7	7	172	10	10	6	139	12.2%	15.8%
52	2	9	2	5	180	5	3	7	150	8.2%	9.1%
53	2	6	4	5	181	10	6	4	145	7.7%	12.1%
56	2	7	5	4	180	3	3	5	154	8.2%	6.7%
58	2	4	0	2	190	3	2	6	154	3.1%	6.7%
59	2	8	2	6	180	7	1	4	153	8.2%	7.3%
66	2	11	5	5	175	5	7	3	150	10.7%	9.1%
67	2	2	5	6	183	4	3	2	156	6.6%	5.5%
70	2	1	0	1	194	0	1	2	162	1.0%	1.8%
68	2	4	0	2	190	2	2	3	158	3.1%	4.2%
73	2	6	6	1	183	6	3	2	154	6.6%	6.7%
82	2	1	3	1	191	6	1	2	156	2.6%	5.5%
61	3	1	2	1	192	4	3	2	156	2.0%	5.5%
62	3	8	5	10	173	9	4	9	143	11.7%	13.3%
71	3	0	0	0	196	0	0	1	164	0.0%	0.6%
72	3	0	1	0	195	0	1	1	163	0.5%	1.2%
81	3	4	0	0	192	3	0	0	162	2.0%	1.8%
83	3	1	1	0	194	4	0	2	159	1.0%	3.6%
84	3	12	9	4	171	15	8	9	133	12.8%	19.4%
89	3	12	5	7	172	11	4	6	144	12.2%	12.7%

** No participants tested positive for HPV 26 and HPV 69*
Abbreviations: M, male; F, female

Table 4-4: Concordance of type-specific HPV infections at baseline in couples by MC

HPV type	Subgenus	Proportion of couples with concordant infections		O:E ratio of concordant infections	
		Uncircumcised	Circumcised	Uncircumcised	Circumcised
6	1	4.2%	1.8%	17.8	11.0
11	1	0.0%	1.2%	NA	82.5
40	1	1.0%	1.2%	19.6	55.0
42	1	3.7%	3.6%	10.6	5.9
44	1	0.5%	2.4%	28.0	20.6
54	1	4.2%	3.0%	12.1	14.7
16	2	7.9%	12.1%	5.8	3.4
18	2	2.1%	1.2%	14.0	13.8
31	2	3.7%	1.8%	13.7	20.6
33	2	1.0%	0.0%	26.1	NA
34	2	0.0%	0.0%	NA	NA
35	2	1.0%	0.6%	65.3	82.5
39	2	3.1%	3.6%	9.0	6.9
45	2	0.0%	0.0%	NA	0.0
51	2	5.2%	6.1%	6.8	5.2
52	2	4.7%	3.0%	11.5	8.6
53	2	3.1%	6.1%	10.7	7.4
56	2	3.7%	1.8%	10.4	10.3
58	2	2.1%	1.8%	32.7	11.0
59	2	4.2%	4.2%	11.2	13.1
66	2	5.8%	3.0%	8.4	8.6
67	2	1.0%	2.4%	7.0	15.7
68	2	2.1%	1.2%	32.7	16.5
70	2	0.5%	0.0%	98.0	0.0
73	2	3.1%	3.6%	14.0	13.8
82	2	0.5%	3.6%	24.5	17.7
61	3	0.5%	2.4%	32.7	15.7
62	3	4.2%	5.5%	6.7	6.3
71	3	0.0%	0.0%	NA	NA
72	3	0.0%	0.0%	NA	0.0
81	3	2.1%	1.8%	49.0	55.0
83	3	0.5%	2.4%	98.0	27.5
84	3	6.3%	9.1%	7.0	4.5
89	3	6.3%	6.7%	7.3	7.1

Abbreviations: O:E, observed to expected

Table 4-5: Transmission and clearance of type-specific HPV infections

Outcome	HPV infections	Uncircumcised males		Circumcised males		Crude RR (95% CI)	Adjusted RR (95% CI)†
		Events: n (%)*	Time, person-years	Events: n (%)*	Time, person-years		
Female-to-male transmission	Any	21 (44.7)	23.3	16 (25.0)	25.1	0.71 (0.37–1.36)	0.50 (0.21–1.21)
	Subgenera 1 and 3	10 (62.5)	7.0	6 (31.6)	7.1	0.59 (0.21–1.62)	0.81 (0.21–3.15)
	Subgenus 2	11 (35.5)	16.3	10 (22.2)	18.0	0.83 (0.40–1.70)	0.36 (0.15–0.85)
Male-to-female transmission	Any	12 (20.3)	25.6	13 (19.4)	27.8	1.00 (0.51–1.96)	1.10 (0.39–3.08)
Clearance of baseline infections	Any	45 (28.0)	77.5	40 (22.4)	74.4	0.93 (0.61–1.41)	0.77 (0.48–1.26)

** Calculated as proportion of possible type-specific events*

† Adjusted for propensity score

Abbreviations: RR, rate ratio; CI, confidence interval

Subgenera 1 and 3: HPV 6, 11, 40, 42, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89

Subgenus 2: HPV 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82

Supplementary Table 4-6: Inverse probability of treatment weighting sensitivity analysis

Sex	Outcome	HPV infections	Measure of association	Crude association (95% CI)	Weighted association (95% CI)
Males	**Prevalence at baseline**	Any	OR	1.11 (0.79–1.55)	1.02 (0.70–1.51)
	Female-to-male transmission	Any	RR	0.54 (0.25–1.19)	0.48 (0.18–1.27)
	Clearance of baseline infections	Any	RR	0.74 (0.41–1.31)	0.56 (0.28–1.13)
Females	**Male-to-female transmission**	Any	RR	0.74 (0.24–2.32)	0.69 (0.13–3.58)

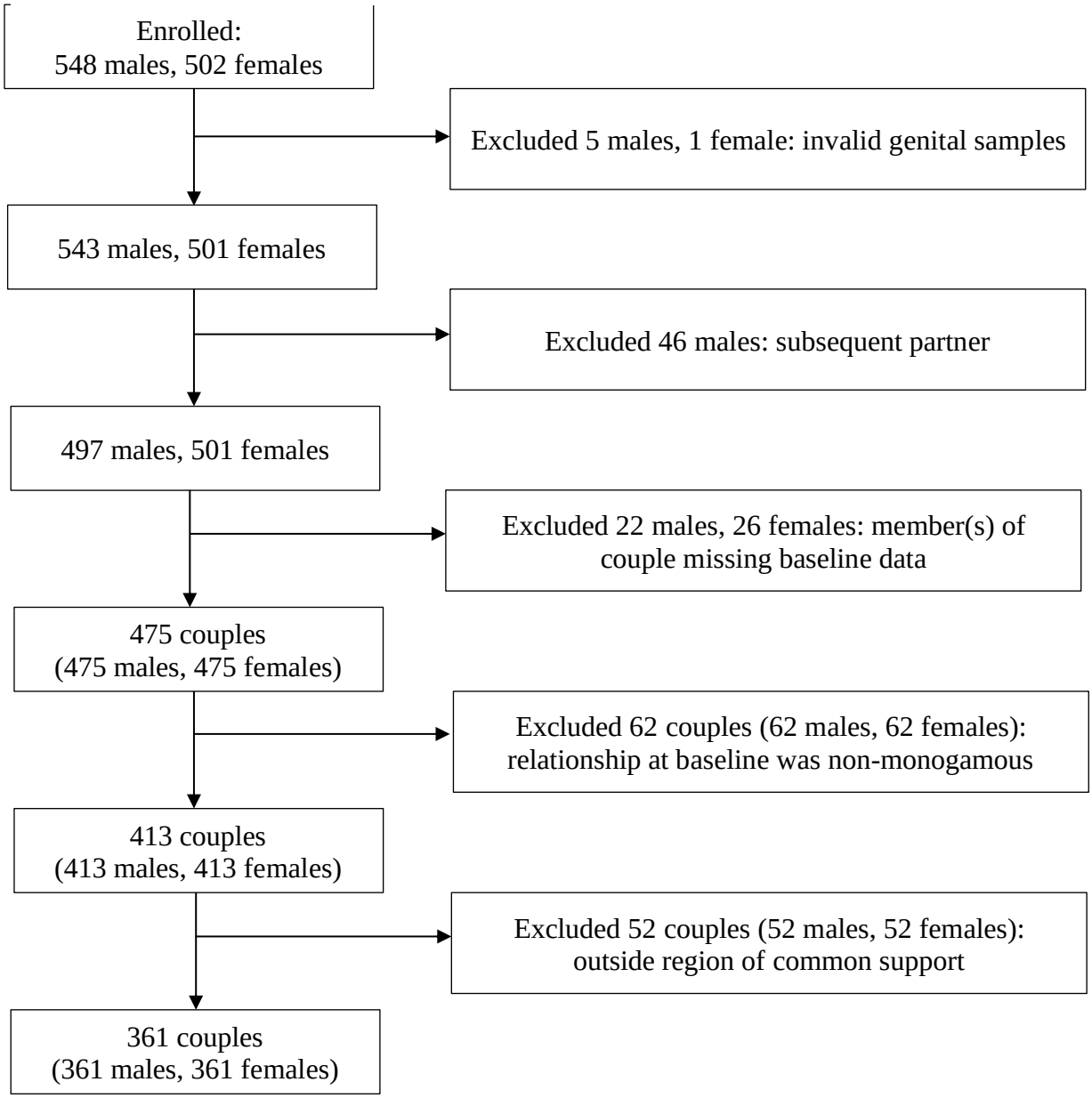
Abbreviations: OR; odds ratio, RR; rate ratio; CI, confidence interval

Supplementary Table 4-7: Viral load (copies/cell) in HPV-infected males

Subgenus	HPV type	Uncircumcised males			Circumcised males			P-value		
		n	Median (Q1-Q3)	Geometric mean (95% CI)	n	Median (Q1-Q3)	Geometric mean (95% CI)			
1	**6**	19	1.58 (0.01–81.96)	1.14 (0.13–10.02)	15	2.54 (0.04–19.99)	1.02 (0.13–7.71)	0.931		
	11	0	-	-	2	14.25 (6.32–22.2)	11.85 (0.00–34,177.37)	-	0.657	
	42	31	48.86 (3.97–378.75)	36.31 (12.61–104.55)	26	35.69 (9.34–120.26)	25.53 (10.05–64.85)	0.597		
2	**16**	38	7.92 (0.10–500.33)	7.46 (1.58–35.15)	48	17.56 (1.26–103.69)	15.32 (6.35–36.96)	0.487		0.517
	18	12	7.12 (0.05–211.77)	4.48 (0.39–51.26)	7	122.35 (0.19–135.33)	21.11 (0.81–549.72)	0.398	0.346	
	31	10	22.70 (0.28–94.14)	5.88 (0.54–63.48)	11	1.04 (0.04–175.40)	1.76 (0.10–31.29)	0.573		
	51	30	9.49 (0.29–186.96)	11.74 (2.62–52.57)	35	23.24 (2.38–403.95)	18.77 (6.19–56.92)	0.519		

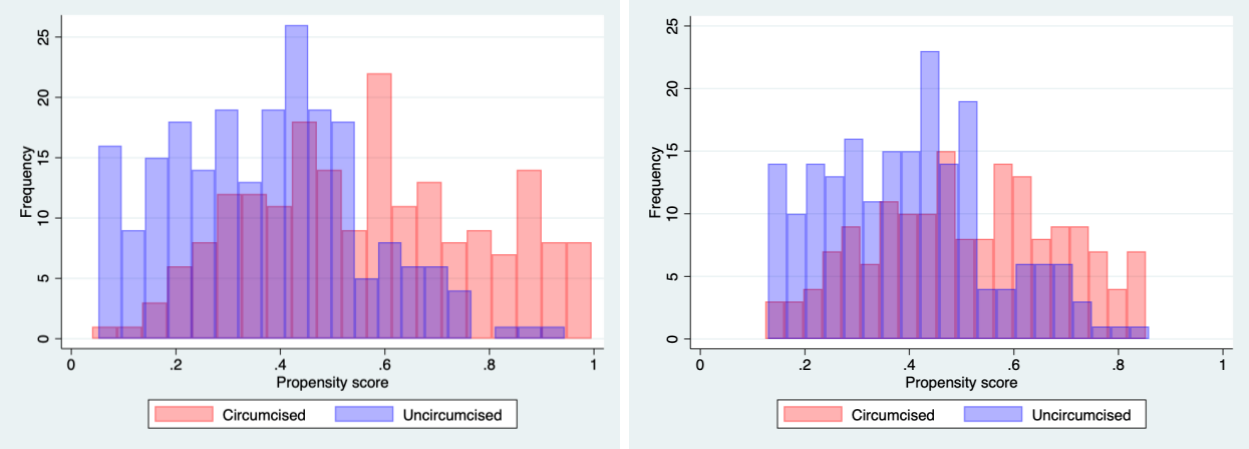
Abbreviations: Q1, 25th percentile; Q3, 75th percentile; CI, confidence interval

Figure 4-1: HITCH study participants and current analysis sample



The flow chart shows the trajectory from enrollment to analysis for the HITCH participants. Six participants were excluded because their samples tested negative for β-globin and were declared invalid. 46 males were enrolled as a second or third partner to a female participant and were excluded. 22 males and 26 females were excluded because one or both members of their couple were missing HPV data at baseline. 62 couples were excluded because their relationship was non-monogamous before they enrolled in HITCH. Of the 413 couples eligible for analysis, 52 couples were excluded because they fell outside the region of common support (Figure 4-2).

Figure 4-2: Propensity score distribution among couples



A) Before restriction to region of common support *B) After restriction to region of common support*

Figures A and B depict the frequency distribution of the propensity scores among couples with a circumcised male (red) and with an uncircumcised male (blue). In Figure A, there are some values of the propensity score that do not encompass couples with both a circumcised and uncircumcised male. These couples are referred to as being outside the region of common support. Figure B shows the distribution of the propensity score in couples with a circumcised and uncircumcised male after restriction to the propensity score. There are no values of the propensity score that do not contain couples with both circumcised and uncircumcised males.

CHAPTER 5. DISCUSSION AND CONCLUSIONS

5.1. SUMMARY OF RESULTS

This thesis explored the evidence for a protective association between MC and various HPV infection outcomes in males and their female partners.

The first manuscript of this thesis was a systematic review of the existing experimental and observational studies that assessed the relationship between MC and the prevalence, incidence, and clearance of HPV infections in both males and females. Almost all studies found either no association or a protective association between MC and infection. When separate analyses were conducted for samples taken at the distal sites of the penis, i.e., the glans, corona, and urethra, the association between MC and infection was almost always observed to be protective with 95% confidence intervals not encompassing the null.

This thesis' second manuscript was an original research paper that used data from the HITCH study. This observational study was conducted between the years 2005 and 2011 and enrolled females studying in a post-secondary institution in Montreal, Canada along with their male sexual partners. In this analysis, we investigated the association between MC and several different HPV outcomes: prevalence of baseline infections in males and in females, transmission of infections from males to females and from females to males, and clearance of baseline infections in males only. We did not find evidence of an association between the exposure and the outcome for prevalence of baseline infections in males (adjusted OR 0.84, 95% CI 0.58–1.22) or in females (adjusted OR 1.13, 95% CI 0.81–1.58), nor for male-to-female transmission of infections (adjusted RR 1.10, 95% CI 0.39–3.08). We observed that MC may be moderately protective against female-to-male transmission of infections (adjusted RR 0.50, 95% 0.21–1.21)and may be a slight risk factor for clearance of baseline infections in males (adjusted RR 0.77, 95% CI 0.48–1.26); however, 95% confidence intervals did encompass the null for these outcomes.

5.2. STRENGTHS AND LIMITATIONS

The systematic review conducted for this thesis provided an updated synthesis of the existing literature assessing the relationship between MC and HPV infections. This review was broad: we included both males and females in the study population; we encompassed prevalence, incidence, and clearance as the outcomes of interest; and we applied few restrictions on study

design and language. As a result, it provides an updated and comprehensive resource that summarizes the available evidence on the association between MC and HPV infections in males and females. Our review was limited by the quality of the available evidence: there were few experimental studies conducted, and several variables of interest (e.g., age at circumcision, method of circumcision, number of sexual partners) were not included in many records. We also did not conduct a meta-analysis for this thesis.

The original research conducted for this thesis provides evidence to the existing literature on the association between MC and HPV infections in males and females. This study was, to our knowledge, the first to use a propensity-score based approach to adjust for confounding. We obtained consistent results between our main analyses, which performed simple adjustment by propensity score, and our sensitivity analyses, where we employed inverse probability of treatment weighting. Our study population was couple based, which allowed us to account for each participant's sexual partner's HPV positivity status, and participants were recruited at early stages of their sexual relationship to best capture transmission events. However, this study was limited by its design: males were followed for only two visits over four months while females were followed for six visits over two years. Since we limited our analyses to time points where HPV data was available for both the male and female, we were only able to use data for each female's first two visits, resulting in limited power and ability to perform survival analyses. As well, genital swabs were taken from proximal and distal sites of the penis but were combined in one sample, which meant we were unable to perform site-specific analyses. Our results may have been vastly different had we been able to perform analyses limited to samples taken from the distal penis.

5.3. FUTURE DIRECTIONS

A meta-analysis of the studies included in the first manuscript will be conducted outside of the scope of this thesis. Further research using the HITCH data will look at the correlates of infection persistence and may use MC as a covariate. The biological mechanism by which MC may protect against HPV infections is still unclear. Once this mechanism is elucidated, it may aid in the formulation of more specific research questions. In the meantime, further couple-based studies, preferably in a setting where randomization would be ethical, with regular long-term follow up of both partners would be required to properly investigate this association.

5.4. CONCLUSIONS

Many countries, particularly LMICs, have a high burden of HPV-associated disease and no organized HPV immunization program.[51-53] The WHO has also recommended for gender-neutral vaccination to be temporarily halted due to vaccine shortages.[170] Further research is still required to investigate the determinants of HPV infection, transmission, and persistence to reduce the burden of its associated disease, especially in resource-limited settings. No previous studies have indicated that MC on its own would be enough to reduce the burden of HPV-associated disease. However, further research may show that its implementation alongside other preventive measures could be a viable method for infection control.

REFERENCES

1. Soudeyns C, Speybroeck N, Brisson M, Mossong J, Latsuzbaia A. HPV vaccination and sexual behaviour in healthcare seeking young women in Luxembourg. *PeerJ.* 2020;**8**:e8516.

2. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine.* 2006;**24 Suppl 1**:S1-15.

3. Brianti P, De Flammineis E, Mercuri SR. Review of HPV-related diseases and cancers. *New Microbiol.* 2017;**40**(2):80-85.

4. Saraiya M, Unger ER, Thompson TD, et al. US assessment of HPV types in cancers: implications for current and 9-valent HPV vaccines. *J Natl Cancer Inst.* 2015;**107**(6):djv086.

5. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;**189**(1):12-19.

6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human Papillomaviruses. Lyon, France: International Agency for Research on Cancer; 2007. Available from: https://www.ncbi.nlm.nih.gov/books/NBK321770/.

7. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health.* 2020;**8**(2):e180-e90.

8. Kombe Kombe AJ, Li B, Zahid A, et al. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. *Front Public Health.* 2021;**8**(1003).

9. Shinkafi-Bagudu Z. Global Partnerships for HPV Vaccine Must Look Beyond National Income. *JCO Global Oncology.* 2020(6):1746-48.

10. International Vaccine Access Center (IVAC). HPV: Vaccine Introduction: Johns Hopkins Bloomberg School of Public Health; [cited 2021 18 August]. Available from: https://view-hub.org/map/?set=current-program-type&group=vaccine-introduction&category=hpv.

11. Larke N. Male circumcision, HIV and sexually transmitted infections: a review. *Br J Nurs.* 2010;**19**(10):629-34.

12. Tobian AA, Gray RH, Quinn TC. Male circumcision for the prevention of acquisition and transmission of sexually transmitted infections: the case for neonatal circumcision. *Arch Pediatr Adolesc Med.* 2010;**164**(1):78-84.

13. Weiss HA, Thomas SL, Munabi SK, Hayes RJ. Male circumcision and risk of syphilis, chancroid, and genital herpes: a systematic review and meta-analysis. *Sex Transm Infect.* 2006;**82**(2):101-9; discussion 10.

14. Smith JS, Backes DM, Hudgens MG, et al. Male Circumcision Reduces Penile HPV Incidence and Persistence: A Randomized Controlled Trial in Kenya. *Cancer Epidemiol Biomarkers Prev.* 2021;**30**(6):1139-48.

15. Tobian AA, Serwadda D, Quinn TC, et al. Male circumcision for the prevention of HSV-2 and HPV infections and syphilis. *N Engl J Med.* 2009;**360**(13):1298-309.

16. Grund JM, Bryant TS, Jackson I, et al. Association between male circumcision and women's biomedical health outcomes: a systematic review. *Lancet Glob Health.* 2017;**5**(11):e1113-e22.

17. Morris BJ, Hankins CA, Banerjee J, et al. Does Male Circumcision Reduce Women's Risk of Sexually Transmitted Infections, Cervical Cancer, and Associated Conditions? *Front Public Health.* 2019;**7**:4.

18. Albero G, Castellsagué X, Giuliano AR, Bosch FX. Male circumcision and genital human papillomavirus: a systematic review and meta-analysis. *Sex Transm Dis.* 2012;**39**(2):104-13.

19. Larke N, Thomas SL, Dos Santos Silva I, Weiss HA. Male circumcision and human papillomavirus infection in men: a systematic review and meta-analysis. *J Infect Dis.* 2011;**204**(9):1375-90.

20. Zhu YP, Jia ZW, Dai B, et al. Relationship between circumcision and human papillomavirus infection: a systematic review and meta-analysis. *Asian J Androl.* 2017;**19**(1):125-31.

21. McMurray HR, Nguyen D, Westbrook TF, McAnce DJ. Biology of human papillomaviruses. *Int J Exp Pathol.* 2001;**82**(1):15-33.

22. Schiller JT, Day PM, Kines RC. Current understanding of the mechanism of HPV infection. *Gynecol Oncol.* 2010;**118**(1, Supplement 1):S12-S17.

23. Betts JG, Young KA, Wise JA, et al. Anatomy and Physiology. Houston, Texas: OpenStax; 2013.

24. Stanley MA. Epithelial cell responses to infection with human papillomavirus. *Clin Microbiol Rev.* 2012;**25**(2):215-22.

25. Hahn AW, Spach DH. Human Papillomavirus Infection: National STD Curriculum; 2018 [Available from: https://www.std.uw.edu/go/pathogen-based/hpv/core-concept/all#citations.

26. Schoch CL, Ciufo S, Domrachev M, et al. NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database (Oxford).* 2020;**2020**.

27. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer.* 2009;**4**:8.

28. Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology.* 2005;**337**(1):76-84.

29. Veldhuijzen NJ, Snijders PJF, Reiss P, Meijer CJLM, van de Wijgert JHHM. Factors affecting transmission of mucosal human papillomavirus. *Lancet Infect Dis.* 2010;**10**(12):862-74.

30. Cubie HA. Diseases associated with human papillomavirus infection. *Virology.* 2013;**445**(1):21-34.

31. Tyring SK, Cauda R, Baron S, Whitley RJ. Condyloma acuminatum: epidemiological, clinical and therapeutic aspects. *Eur J Epidemiol.* 1987;**3**(3):209-15.

32. Cook LS, Koutsky LA, Holmes KK. Clinical presentation of genital warts among circumcised and uncircumcised heterosexual men attending an urban STD clinic. *Genitourin Med.* 1993;**69**(4):262.

33. Clanner-Engelshofen BM, Marsela E, Engelsberger N, et al. Condylomata acuminata: A retrospective analysis on clinical characteristics and treatment options. *Heliyon.* 2020;**6**(3):e03547.

34. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological Agents. Lyon, France: International Agency for Research on Cancer; 2012. Available from: https://pubmed.ncbi.nlm.nih.gov/23189750/.

35. Comprehensive Cervical Cancer Control: A Guide to Essential Practice. Geneva, Switzerland: World Health Organization; 2014.

36. New data on male circumcision and HIV prevention: Policy and programme implications. Geneva, Switzerland: World Health Organization, UNAIDS; 2007. Report No.: 9789241595988.

37. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The Estimated Lifetime Probability of Acquiring Human Papillomavirus in the United States. *Sex Transm Dis.* 2014;**41**(11):660-64.

38. Bruni L, Albero G, Serrano B, et al. Human Papillomavirus and Related Diseases in the World. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre); 2019.

39. Argyri E, Papaspyridakos S, Tsimplaki E, et al. A cross sectional study of HPV type prevalence according to age and cytology. *BMC Infect Dis.* 2013;**13**(1):53.

40. de Sanjosé S, Diaz M, Castellsagué X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis.* 2007;**7**(7):453-9.

41. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. *Vaccine.* 2006;**24**:S52-S61.

42. McQuillin G, Kruszon-Moran D, Markowitz LE, Unger ER, Paulose-Ram R. Prevalence of HPV in Adults Aged 18–69: United States, 2011–2014. Hyattsville, MD: National Center for Health Statistics; 2017.

43. Toh ZQ, Kosasih J, Russell FM, Garland SM, Mulholland EK, Licciardi PV. Recombinant human papillomavirus nonavalent vaccine in the prevention of cancers caused by human papillomavirus. *Infect Drug Resist.* 2019;**12**:1951-67.

44. National Advisory Committee on Immunization. Statement on human papillomavirus vaccine. *Can Commun Dis Rep.* 2007;**33**.

45. National Advisory Committee on Immunization. Update on Human Papillomavirus (HPV) Vaccines. *Can Commun Dis Rep.* 2012;**38**.

46. National Advisory Committee on Immunization. Update on the recommended Human Papillomavirus (HPV) vaccine immunization schedule. Public Health Agency of Canada; 2015.

47. National Advisory Committee on Immunization. Updated Recommendations on Human Papillomavirus (HPV) Vaccines: 9-valent HPV vaccine and clarification of minimum intervals between doses in the HPV immunization schedule. Public Health Agency of Canada; 2016.

48. National Advisory Committee on Immunization. Updated Recommendations on Human Papillomavirus (HPV) Vaccines: 9-valent HPV vaccine 2-dose immunization schedule and the use of HPV vaccines in immunocompromised populations. Public Health Agency of Canada; 2017.

49. D'Addario M, Redmond S, Scott P, et al. Two-dose schedules for human papillomavirus vaccine: Systematic review and meta-analysis. *Vaccine.* 2017;**35**(22):2892-901.

50. Serrano B, Brotons M, Bosch FX, Bruni L. Epidemiology and burden of HPV-related disease. *Best Pract Res Clin Obstet Gynaecol.* 2018;**47**:14-26.

51. Bruni L, Saura-Lázaro A, Montoliu A, et al. HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010–2019. *Prev Med.* 2021;**144**:106399.

52. Bruni L, Diaz M, Barrionuevo-Rosas L, et al. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. *Lancet Glob Health.* 2016;**4**(7):e453-e63.

53. Dorji T, Nopsopon T, Tamang ST, Pongpirul K. Human papillomavirus vaccination uptake in low-and middle-income countries: a meta-analysis. *EClinicalMedicine.* 2021;**34**:100836.

54. Chelimo C, Wouldes TA, Cameron LD, Elwood JM. Risk factors for and prevention of human papillomaviruses (HPV), genital warts and cervical cancer. *J Infect.* 2013;**66**(3):207-17.

55. Shikary T, Bernstein DI, Jin Y, Zimet GD, Rosenthal SL, Kahn JA. Epidemiology and risk factors for human papillomavirus infection in a diverse sample of low-income young women. *J Clin Virol.* 2009;**46**(2):107-11.

56. Yang J, Wang W, Wang Z, et al. Prevalence, genotype distribution and risk factors of cervical HPV infection in Yangqu, China: a population-based survey of 10086 women. *Hum Vaccin Immunother*. 2020;**16**(7):1645-52.

57. Torres-Poveda K, Ruiz-Fraga I, Madrid-Marina V, Chavez M, Richardson V. High risk HPV infection prevalence and associated cofactors: a population-based study in female ISSSTE beneficiaries attending the HPV screening and early detection of cervical cancer program. *BMC Cancer*. 2019;**19**(1):1205.

58. Sichero L, Giuliano AR, Villa LL. Human Papillomavirus and Genital Disease in Men: What We Have Learned from the HIM Study. *Acta Cytol.* 2019;**63**(2):109-17.

59. Rodríguez-Álvarez MI, Gómez-Urquiza JL, Husein-El Ahmed H, Albendín-García L, Gómez-Salgado J, Cañadas-De la Fuente GA. Prevalence and Risk Factors of Human Papillomavirus in Male Patients: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health.* 2018;**15**(10).

60. Vaccarella S, Franceschi S, Herrero R, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. *Cancer Epidemiol Biomarkers Prev.* 2006;**15**(2):326-33.

61. Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta-analysis. *Sex Transm Dis.* 2002;**29**(11):725-35.

62. Shigeishi H, Sugiyama M. Risk Factors for Oral Human Papillomavirus Infection in Healthy Individuals: A Systematic Review and Meta-Analysis. *J Clin Med Res.* 2016;**8**(10):721-9.

63. Yanofsky VR, Patel RV, Goldenberg G. Genital warts: a comprehensive review. *J Clin Aesthet Dermatol.* 2012;**5**(6):25-36.

64. Markowitz LE, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* 2007;**56**(Rr-2):1-24.

65. Marra C, Ogilvie G, Gastonguay L, Colley L, Taylor D, Marra F. Patients With Genital Warts Have a Decreased Quality of Life. *Sex Transm Dis.* 2009;**36**(4):258-60.

66. Mortensen GL, Larsen HK. The quality of life of patients with genital warts: a qualitative study. *BMC Public Health.* 2010;**10**:113.

67. Forman D, de Martel C, Lacey CJ, et al. Global Burden of Human Papillomavirus and Related Diseases. *Vaccine.* 2012;**30**:F12-F23.

68. Volesky KD, El-Zein M, Franco EL, Brenner DR, Friedenreich CM, Ruan Y. Cancers attributable to infections in Canada. *Prev Med.* 2019;**122**:109-17.

69. Global strategy to accelerate the elimination of cervical cancer as a public health problem. Geneva, Switzerland: World Health Organization; 2020.

70. American Academy of Pediatrics Task Force. Male circumcision. *Pediatrics.* 2012;**130**(3):e756-85.

71. Gilroy AM, Voll MM, Wesker K. Anatomy: An Essential Textbook. 2 ed: Thieme; 2017.

72. Standring S. Gray's Anatomy: The Anatomical Basis of Clinical Practice. Forty-first edition. ed. [Philadelphia]: Elsevier Limited; 2016.

73. Sanchez DF, Cubilla AL. Anatomy & Histology - Penis 2020 [updated 19 May 2021. Available from: https://www.pathologyoutlines.com/topic/penscrotumanat.html.

74. Mills SE. Histology for Pathologists. Philadelphia: Wolters Kluwer; 2020. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=booktext&D=books2&AN=02107294/5th_Edition
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=n&CSC=Y&PAGE=booktext&D=books&AN=02107294$&XPATH=/PG(0)&EPUB=Y

http://ovidsp.ovid.com/ovidweb.cgi?T=JS&NEWS=n&CSC=Y&PAGE=booktext&D=books&AN=02107294$&XPATH=/PG(0)
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=booktext&NEWS=N&DF=bookdb&CSC=Y&AN=02107294/5th_Edition&XPATH=/PG(0).

75. Male circumcision: global trends and determinants of prevalence, safety and acceptability. Geneva, Switzerland: World Health Organization, UNAIDS; 2007.

76. Mitchell TM. Male circumcision. *Pediatrics.* 2012;**130**(3):e756-85.

77. Mitchell TM, Beal C. Shared Decision Making for Routine Infant Circumcision: A Pilot Study. *J Perinat Educ.* 2015;**24**(3):188-200.

78. What Mothers Say: The Canadian Maternity Experiences Survey. Ottawa, Canada: Public Health Agency of Canada; 2009.

79. Fetus and Newborn Committee CPS. Neonatal circumcision revisited. *Can Med Assoc J.* 1996;**154**(6):769.

80. Sorokan ST, Finlay JC, Jefferies AL, Canadian Paediatric Society F, Newborn Committee ID, Immunization C. Newborn male circumcision. *Paediatr Child Health.* 2015;**20**(6):311-20.

81. Dave S, Afshar K, Braga LH, Anderson P. Canadian Urological Association guideline on the care of the normal foreskin and neonatal circumcision in Canadian infants (full version). *Canadian Urological Association journal = Journal de l'Association des urologues du Canada.* 2018;**12**(2):E76-E99.

82. Morris BJ, Wamai RG, Henebeng EB, et al. Estimation of country-specific and global prevalence of male circumcision. *Population health metrics.* 2016;**14**:4-4.

83. Maeda JL, Chari R, Elixhauser A. Circumcisions Performed in U.S. Community Hospitals, 2009. Rockwell, MD: Agency for Healthcare Research and Quality; 2012.

84. Farley TM, Samuelson J, Grabowski MK, Ameyan W, Gray RH, Baggaley R. Impact of male circumcision on risk of HIV infection in men in a changing epidemic context - systematic review and meta-analysis. *J Int AIDS Soc.* 2020;**23**(6):e25490-e90.

85. National Center for HIV/AIDS VH, STD, and TB Prevention (U.S.). Division of HIV/AIDS Prevention. Information for providers counseling male patients and parents regarding male circumcision and the prevention of HIV infection, STIs, and other health outcomes. 2018.

86. Anderson D, Politch JA, Pudney J. HIV infection and immune defense of the penis. *Am J Reprod Immunol.* 2011;**65**(3):220-9.

87. Prodger JL, Kaul R. The biology of how circumcision reduces HIV susceptibility: broader implications for the prevention field. *AIDS Res Ther.* 2017;**14**(1):49.

88. Sennepin A, Real F, Duvivier M, et al. The Human Penis Is a Genuine Immunological Effector Site. *Front Immunol.* 2017;**8**:1732-32.

89. Szabo R, Short RV. How does male circumcision protect against HIV infection? *BMJ (Clinical research ed).* 2000;**320**(7249):1592-94.

90. Van Howe RS. Human papillomavirus and circumcision: a meta-analysis. *J Infect.* 2007;**54**(5):490-6.

91. Castellsagué X, Albero G, Clèries R, Bosch FX. HPV and circumcision: A biased, inaccurate and misleading meta-analysis. *J Infect.* 2007;**55**(1):91-93.

92. Clifford GM, Gonçalves MA, Franceschi S. Human papillomavirus types among women infected with HIV: a meta-analysis. *AIDS.* 2006;**20**(18):2337-44.

93. Kojic EM, Cu-Uvin S, Conley L, et al. Human papillomavirus infection and cytologic abnormalities of the anus and cervix among HIV-infected women in the study to understand the natural history of HIV/AIDS in the era of effective therapy (the SUN study). *Sex Transm Dis.* 2011;**38**(4):253-9.

94. Massad LS, Xie X, Burk RD, et al. Association of cervical precancer with human papillomavirus types other than 16 among HIV co-infected women. *Am J Obstet Gynecol.* 2016;**214**(3):354.e1-6.

95. Dean A, Sullivan K, Soe M. OpenEpi: Open Source Epidemiologic Statistics for Public Health [Available from: https://www.openepi.com/TwobyTwo/TwobyTwo.htm.

96. Baldwin SB, Wallace DR, Papenfuss MR, Abrahamsen M, Vaught LC, Giuliano AR. Condom use and other factors affecting penile human papillomavirus detection in men attending a sexually transmitted disease clinic. *Sex Transm Dis.* 2004;**31**(10):601-7.

97. Bleeker MC, Hogewoning CJ, Voorhorst FJ, et al. HPV-associated flat penile lesions in men of a non-STD hospital population: less frequent and smaller in size than in male sexual partners of women with CIN. *Int J Cancer.* 2005;**113**(1):36-41.

98. Castellsagué X, Bosch FX, Muñoz N, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. *N Engl J Med.* 2002;**346**(15):1105-12.

99. Contreras WR, Fuentes H, Nava J, et al. Prevalencia y factores asociados con infecciÛn por virus del papiloma humano cervical en pacientes con artritis reumatoide. *Ginecol Obstet Mex.* 2008;**76**:9-17.

100. Da Rocha WM, Afonso LA, Dobao E, Gouvea TD, Carestiato FN, Cavalcanti SMB. Evaluation of anogenital human papillomavirus infection in asymptomatic men from Rio de Janeiro, Brazil. *Journal of Tropical Pathology.* 2015;**44**(4).

101. Hebnes JB, Munk C, Frederiksen K, Joergensen HO, Iftner T, Kjaer SK. The role of circumcision, tobacco, and alcohol use in genital human papillomavirus infection among men from Denmark. *Int J STD AIDS.* 2021:9564624211014727.

102. Mbulawa ZZ, Coetzee D, Marais DJ, et al. Genital human papillomavirus prevalence and human papillomavirus concordance in heterosexual couples are positively associated with human immunodeficiency virus coinfection. *J Infect Dis.* 2009;**199**(10):1514-24.

103. Obiri-Yeboah D, Akakpo PK, Mutocheluh M, et al. Epidemiology of cervical human papillomavirus (HPV) infection and squamous intraepithelial lesions (SIL) among a cohort of HIV-infected and uninfected Ghanaian women. *BMC Cancer.* 2017;**17**(1):688.

104. Ogilvie GS, Taylor DL, Achen M, Cook D, Krajden M. Self-collection of genital human papillomavirus specimens in heterosexual men. *Sex Transm Infect.* 2009;**85**(3):221-5.

105. Olesen TB, Munk C, Mwaiselage J, et al. Male circumcision and the risk of gonorrhoea, syphilis, HIV and human papillomavirus among men in Tanzania. *Int J STD AIDS.* 2019;**30**(14):1408-16.

106. Rocha MGdL, Faria FL, Goncalves L, Souza MdCM, Fernandes PA, Fernandes AP. Prevalence of DNA-HPV in male sexual partners of HPV-infected women and concordance of viral types in infected couples. *PLoS One.* 2012;**7**(7):e40988.

107. Rombaldi RL, Serafini EP, Villa LL, et al. Infection with human papillomaviruses of sexual partners of women having cervical intraepithelial neoplasia. *Braz J Med Biol Res.* 2006;**39**(2):177-87.

108. Roura E, Iftner T, Vidart JA, et al. Predictors of human papillomavirus infection in women undergoing routine cervical cancer screening in Spain: the CLEOPATRE study. *BMC Infect Dis.* 2012;**12**:145.

109. Shin HR, Franceschi S, Vaccarella S, et al. Prevalence and determinants of genital infection with papillomavirus, in female and male university students in Busan, South Korea. *J Infect Dis.* 2004;**190**(3):468-76.

110. Svare EI, Kjaer SK, Worm AM, Osterlind A, Meijer CJ, van den Brule AJ. Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic. *Sex Transm Infect.* 2002;**78**(3):215-8.

111. Vaccarella S, Lazcano-Ponce E, Castro-Garduno JA, et al. Prevalence and determinants of human papillomavirus infection in men attending vasectomy clinics in Mexico. *Int J Cancer.* 2006;**119**(8):1934-9.

112. Vardas E, Giuliano AR, Goldstone S, et al. External genital human papillomavirus prevalence and associated factors among heterosexual men on 5 continents. *J Infect Dis.* 2011;**203**(1):58-65.

113. Hernandez BY, Shvetsov YB, Goodman MT, et al. Reduced clearance of penile human papillomavirus infection in uncircumcised men. *J Infect Dis.* 2010;**201**(9):1340-3.

114. Hernandez BY, Wilkens LR, Zhu X, et al. Circumcision and human papillomavirus infection in men: a site-specific comparison. *J Infect Dis.* 2008;**197**(6):787-94.

115. Lajous M, Mueller N, Cruz-Valdéz A, et al. Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. *Cancer Epidemiol Biomarkers Prev.* 2005;**14**(7):1710-6.

116. Lu B, Wu Y, Nielson CM, et al. Factors associated with acquisition and clearance of human papillomavirus infection in a cohort of US men: a prospective study. *J Infect Dis.* 2009;**199**(3):362-71.

117. Nielson CM, Schiaffino MK, Dunne EF, Salemi JL, Giuliano AR. Associations between male anogenital human papillomavirus infection and circumcision by anatomic site sampled and lifetime number of female sex partners. *J Infect Dis.* 2009;**199**(1):7-13.

118. Partridge JM, Hughes JP, Feng Q, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. *J Infect Dis.* 2007;**196**(8):1128-36.

119. Vanbuskirk K, Winer RL, Hughes JP, et al. Circumcision and acquisition of human papillomavirus infection in young men. *Sex Transm Dis.* 2011;**38**(11):1074-81.

120. Gray RH, Serwadda D, Kong X, et al. Male circumcision decreases acquisition and increases clearance of high-risk human papillomavirus in HIV-negative men: a randomized trial in Rakai, Uganda. *J Infect Dis.* 2010;**201**(10):1455-62.
121. Tobian AA, Kigozi G, Gravitt PE, et al. Human papillomavirus incidence and clearance among HIV-positive and HIV-negative men in sub-Saharan Africa. *AIDS.* 2012;**26**(12):1555-65.
122. Wawer MJ, Tobian AAR, Kigozi G, et al. Effect of circumcision of HIV-negative men on transmission of human papillomavirus to HIV-negative women: a randomised trial in Rakai, Uganda. *The Lancet.* 2011;**377**(9761):209-18.
123. Albero G, Castellsagué X, Lin HY, et al. Male circumcision and the incidence and clearance of genital human papillomavirus (HPV) infection in men: the HPV Infection in men (HIM) cohort study. *BMC Infect Dis.* 2014;**14**:75.
124. Albero G, Villa LL, Lazcano-Ponce E, et al. Male circumcision and prevalence of genital human papillomavirus infection in men: a multinational study. *BMC Infect Dis.* 2013;**13**:18.
125. Kim SC, Feldman S, Moscicki A-B. Risk of human papillomavirus infection in women with rheumatic disease: cervical cancer screening and prevention. *Rheumatology.* 2018;**57**(suppl_5):v26-v33.
126. Liu Y-T, Tsou H-K, Chiou J-Y, Wang Y-H, Chou M-C, Wei JC-C. Association of human papillomavirus infection with risk for rheumatoid arthritis: a population-based cohort study. *Ann Rheum Dis.* 2019;**78**(12):1734.
127. McCoombe SG, Short RV. Potential HIV-1 target cells in the human penis. *AIDS.* 2006;**20**(11):1491-95.
128. Patterson BK, Landay A, Siegel JN, et al. Susceptibility to Human Immunodeficiency Virus-1 Infection of Human Foreskin and Cervical Tissue Grown in Explant Culture. *The American Journal of Pathology.* 2002;**161**(3):867-73.
129. Weiss HA. Male circumcision as a preventive measure against HIV and other sexually transmitted diseases. *Curr Opin Infect Dis.* 2007;**20**(1):66-72.
130. Price LB, Liu CM, Johnson KE, et al. The effects of circumcision on the penis microbiome. *PLoS One.* 2010;**5**(1):e8422.
131. Liu CM, Hungate BA, Tobian AA, et al. Male circumcision significantly reduces prevalence and load of genital anaerobic bacteria. *mBio.* 2013;**4**(2):e00076.

132. Shannon B, Yi TJ, Perusini S, et al. Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunol.* 2017;**10**(5):1310-19.
133. Scott ME, Shvetsov YB, Thompson PJ, et al. Cervical cytokines and clearance of incident human papillomavirus infection: Hawaii HPV cohort study. *Int J Cancer.* 2013;**133**(5):1187-96.
134. Kemp TJ, Hildesheim A, García-Piñeres A, et al. Elevated systemic levels of inflammatory cytokines in older women with persistent cervical human papillomavirus infection. *Cancer Epidemiol Biomarkers Prev.* 2010;**19**(8):1954-9.
135. Song SH, Lee JK, Seok OS, Saw HS. The relationship between cytokines and HPV-16, HPV-16 E6, E7, and high-risk HPV viral load in the uterine cervix. *Gynecol Oncol.* 2007;**104**(3):732-8.
136. Fausch SC, Da Silva DM, Rudolf MP, Kast WM. Human Papillomavirus Virus-Like Particles Do Not Activate Langerhans Cells: A Possible Immune Escape Mechanism Used by Human Papillomaviruses. *The Journal of Immunology.* 2002;**169**(6):3242-49.
137. Franco EL, de Sanjose S, Broker TR, et al. Human papillomavirus and cancer prevention: gaps in knowledge and prospects for research, policy, and advocacy. *Vaccine.* 2012;**30 Suppl 5**:F175-82.
138. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *The Lancet Global Health.* 2016;**4**(9):e609-e16.
139. Joura EA, Giuliano AR, Iversen OE, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *N Engl J Med.* 2015;**372**(8):711-23.
140. Skinner SR, Wheeler CM, Romanowski B, et al. Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study. *Int J Cancer.* 2016;**138**(10):2428-38.
141. Tota JE, Ramana-Kumar AV, El-Khatib Z, Franco EL. The road ahead for cervical cancer prevention and control. *Curr Oncol.* 2014;**21**(2):e255-64.
142. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol.* 2003;**157**(3):218-26.

143. Burchell AN, Richardson H, Mahmud SM, et al. Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada. *Am J Epidemiol.* 2006;**163**(6):534-43.

144. Burchell AN, Rodrigues A, Moravan V, et al. Determinants of prevalent human papillomavirus in recently formed heterosexual partnerships: a dyadic-level analysis. *J Infect Dis.* 2014;**210**(6):846-52.

145. van Rijn VM, Mooij SH, Mollers M, et al. Anal, penile, and oral high-risk HPV infections and HPV seropositivity in HIV-positive and HIV-negative men who have sex with men. *PLoS One.* 2014;**9**(3):e92208.

146. Wilson LE, Gravitt P, Tobian AA, et al. Male circumcision reduces penile high-risk human papillomavirus viral load in a randomised clinical trial in Rakai, Uganda. *Sex Transm Infect.* 2013;**89**(3):262-6.

147. Auvert B, Sobngwi-Tambekou J, Cutler E, et al. Effect of male circumcision on the prevalence of high-risk human papillomavirus in young men: results of a randomized controlled trial conducted in Orange Farm, South Africa. *J Infect Dis.* 2009;**199**(1):14-9.

148. Backes DM, Bleeker MC, Meijer CJ, et al. Male circumcision is associated with a lower prevalence of human papillomavirus-associated penile lesions among Kenyan men. *Int J Cancer.* 2012;**130**(8):1888-97.

149. Kibira SP, Sandoy IF, Daniel M, Atuyambe LM, Makumbi FE. A comparison of sexual risk behaviours and HIV seroprevalence among circumcised and uncircumcised men before and after implementation of the safe male circumcision programme in Uganda. *BMC Public Health.* 2016;**16**:7.

150. Kibira SPS, Atuyambe LM, Sandoy IF, Makumbi FE, Daniel M. "Now that you are circumcised, you cannot have first sex with your wife": post circumcision sexual behaviours and beliefs among men in Wakiso district, Uganda. *J Int AIDS Soc.* 2017;**20**(1):21498.

151. Ledikwe JH, Ramabu NM, Spees LP, et al. Early resumption of sexual activity following voluntary medical male circumcision in Botswana: A qualitative study. *PLoS One.* 2017;**12**(11):e0186831.

152. Mukama T, Ndejjo R, Musinguzi G, Musoke D. Perceptions about medical male circumcision and sexual behaviours of adults in rural Uganda: a cross sectional study. *Pan Afr Med J.* 2015;**22**:354.

153. Homfray V, Tanton C, Mitchell KR, et al. Examining the association between male circumcision and sexual function: evidence from a British probability survey. *AIDS.* 2015;**29**(11):1411-6.

154. Tian Y, Liu W, Wang JZ, Wazir R, Yue X, Wang KJ. Effects of circumcision on male sexual functions: a systematic review and meta-analysis. *Asian J Androl.* 2013;**15**(5):662-6.

155. Bronselaer GA, Schober JM, Meyer-Bahlburg HF, T'Sjoen G, Vlietinck R, Hoebeke PB. Male circumcision decreases penile sensitivity as measured in a large cohort. *BJU Int.* 2013;**111**(5):820-7.

156. Dias J, Freitas R, Amorim R, Espiridiao P, Xambre L, Ferraz L. Adult circumcision and male sexual health: a retrospective analysis. *Andrologia.* 2014;**46**(5):459-64.

157. El-Zein M, Coutlee F, Tellier PP, et al. Human Papillomavirus Infection and Transmission Among Couples Through Heterosexual Activity (HITCH) Cohort Study: Protocol Describing Design, Methods, and Research Goals. *JMIR Res Protoc.* 2019;**8**(1):e11284.

158. Coutlée F, Rouleau D, Petignat P, et al. Enhanced Detection and Typing of Human Papillomavirus (HPV) DNA in Anogenital Samples with PGMY Primers and the Linear Array HPV Genotyping Test. *J Clin Microbiol.* 2006;**44**(6):1998-2006.

159. Marks M, Gupta SB, Liaw KL, et al. Confirmation and quantitation of human papillomavirus type 52 by Roche Linear Array using HPV52-specific TaqMan E6/E7 quantitative real-time PCR. *J Virol Methods.* 2009;**156**(1-2):152-6.

160. Fontaine J, Hankins C, Money D, et al. Human papillomavirus type 16 (HPV-16) viral load and persistence of HPV-16 infection in women infected or at risk for HIV. *J Clin Virol.* 2008;**43**(3):307-12.

161. Khouadri S, Villa LL, Gagnon S, et al. Viral load of episomal and integrated forms of human papillomavirus type 33 in high-grade squamous intraepithelial lesions of the uterine cervix. *Int J Cancer.* 2007;**121**(12):2674-81.

162. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res.* 2011;**46**(3):399-424.

163. Garrido MM, Kelley AS, Paris J, et al. Methods for constructing and assessing propensity scores. *Health Serv Res.* 2014;**49**(5):1701-20.

164. Collins S, Mazloomzadeh S, Winter H, et al. High incidence of cervical human papillomavirus infection in women during their first sexual relationship. *BJOG.* 2002;**109**(1):96-8.

165. Parada R, Morales R, Giuliano AR, Cruz A, Castellsagué X, Lazcano-Ponce E. Prevalence, concordance and determinants of human papillomavirus infection among heterosexual partners in a rural region in central Mexico. *BMC Infect Dis.* 2010;**10**(1):223.

166. Hull R, Mbele M, Makhafola T, et al. Cervical cancer in low and middle-income countries. *Oncol Lett.* 2020;**20**(3):2058-74.

167. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *Int J Cancer.* 2017;**141**(4):664-70.

168. Tota JE, Chevarie-Davis M, Richardson LA, Devries M, Franco EL. Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med.* 2011;**53 Suppl 1**:S12-21.

169. de Sanjosé S, Serrano B, Tous S, et al. Burden of Human Papillomavirus (HPV)-Related Cancers Attributable to HPVs 6/11/16/18/31/33/45/52 and 58. *JNCI Cancer Spectrum.* 2019;**2**(4).

170. World Health Organization = Organisation mondiale de la S. Weekly Epidemiological Record, 2019, vol. 94, 47 [full issue]. *Weekly Epidemiological Record = Relevé épidémiologique hebdomadaire.* 2019;**94**(47):541-60.

APPENDICES

APPENDIX 1: HITCH STUDY IRB APPROVAL

McGill

Faculty of Medicine
3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

Faculté de médecine
3655, Promenade Sir William Osler
Montréal, QC, H3G 1Y6

Fax/Télécopieur: (514) 398-3595

October 8, 2004

JAN 24 2005

Dr. Eduardo Franco
Director, Division of Epidemiology
546 Pine Avenue West
Montreal, Quebec H3A 2T5

Dear Dr. Franco,

We have received correspondence in support of the research proposal AØØ-M77-04A entitled "HITCH Cohort – HPV Infection and Transmission among Couples Through Heterosexual Activity" which was reviewed by the Institutional Review Board, Faculty of Medicine at its meeting of September 13, 2004.

The responses and revisions were found to be acceptable and we are pleased to inform you that final approval for the clinical protocol (September 2004) and revised consent forms for men and women (October 1, 2004), was provided on October 8, 2004, valid until **September 2005**. The certification document *(executed)* is enclosed.

We ask you to take note that review of all research involving human subjects is required on an annual basis in accord with the date of initial review and approval (September 14 2004). Should any modification to the study or unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

C. Celeste Johnston

Celeste Johnston, DEd, RN
Co-Chair,
Institutional Review Board

cc: AØ9-M77-04A

APPENDIX 2: HITCH STUDY IRB RENEWAL

McGill | Faculty of Medicine and Health Sciences | Faculté de médecine et des sciences de la santé

3655 Sir William Osler #633
Montreal, Quebec H3G 1Y6

3655, Promenade Sir William Osler #633
Montréal (Québec) H3G 1Y6

Tél/Tel: (514) 398-3124

September 14, 2021

Dr. Eduardo L. Franco
Division of Cancer Epidemiology
5100 de Maisonneuve Blvd. W., Suite 720
Montreal QC H4A 3T2

RE: IRB Study Number A09-M77-04A
"HITCH Cohort – HPV Infection and Transmission among Couples Through Heterosexual *Activity" (NIH title: HITCH: HPV infection and transmission among couples)*

Dear Dr. Franco,

Thank you for submitting an application for Continuing Ethics Review for the above-referenced study.

The study progress report was reviewed and full Board re-approval was provided on September 13, 2021. The ethics certification renewal is valid from **September 7, 2021 to September 6, 2022.**

The Investigator is reminded of the requirement to report all IRB approved protocol and consent form modifications to the Research Ethics Offices (REOs) for the participating hospital sites. Please contact the individual hospital REOs for instructions on how to proceed. Research funds may be withheld and / or the study's data may be revoked for failing to comply with this requirement.

Should any modification or unanticipated development occur prior to the next review, please notify the IRB promptly. Regulation does not permit the implementation of study modifications prior to IRB review and approval.

Regards,

Roberta M. Palmour, PhD
Chair
Institutional Review Board

cc: Dr. Mariam El-Zein
Allita Rodrigues
A09-M77-04A