HUMAN PAPILLOMAVIRUS (HPV) IN MOTHERS BEFORE AND AFTER DELIVERY

– A THREE YEAR FOLLOW-UP STUDY

by

Marja Sarkola
(née Järvenkylä)

From the Department of Oral Pathology, Institute of Dentistry and Medicity Research Laboratory, Faculty of Medicine, University of Turku and Department of Obstetrics and Gynecology, Turku University Central Hospital, Turku Graduate School of Clinical Sciences, Turku, Finland

Supervised by

Professor Stina Syrjänen, DDS, PhD
Department of Oral Pathology and Radiology,
Institute of Dentistry, Faculty of Medicine,
University of Turku
Turku, Finland

and

Docent Seija Grénman, MD, PhD
Department of Obstetrics and Gynecology,
Faculty of Medicine, University of Turku
Turku, Finland

Reviewed by

Professor Eva Rylander, MD, PhD
Division of Obstetrics and Gynecology
Danderyd Hospital, Karolinska Institutet
Stockholm, Sweden

and

Docent Eeva Auvinen, PhD
Department of Virology
Haartman Institute, University of Helsinki
Helsinki, Finland

Dissertation opponent

Docent Pekka Nieminen, MD, PhD
Department of Obstetrics and Gynecology,
Helsinki University Central Hospital,
Espoo, Finland

To my family

ABSTRACT

Marja Sarkola
Human papillomavirus (HPV) in mothers before and after delivery – a three year follow-up study.
From the Department of Oral Pathology, Institute of Dentistry and Medicity Research Laboratory, Faculty of Medicine, University of Turku and Department of Obstetrics and Gynecology, Turku University Central Hospital, Turku Graduate School of Clinical Sciences, Turku, Finland

Human papillomavirus (HPV) infections in mothers are important to consider since pregnancy may affect the outcome of the infection and the mother may transmit HPV to the child.

This thesis is part of the 3-year Finnish Family HPV Study on HPV infection dynamics within 329 families. The presence of maternal HPV antibodies and HPV DNA in placenta, umbilical cord blood and breast milk was examined. In addition, genital and oral HR-HPV carriage was studied among mothers with one or two pregnancies.

At enrollment, seropositivity to HPV 6, 11, 16, 18 and 45 was recorded in 53 %, 21 %, 35 %, 21 % and 9 % of the mothers, respectively. Age at sexual debut, number of sexual partners, a history of genital warts and antibodies to LR/HR-HPV predicted HR/LR-HPV-seropositivity. During follow-up 27 %, 14 %, 17 %, 17 % and 7 % of the mothers seroconverted to the tested HPV-types, respectively. Decay of HPV-antibodies was rare. The mother's new pregnancy was of minor impact in the outcome of oral and cervical HR-HPV infections.

HPV-DNA was present in 4.2 % and 3.5 % of the placentas and umbilical cord blood samples, and in 4.5 % and 19.7 % of the breast milk samples collected at day 3 and month 2 postpartum, respectively. HPV-positivity in placenta/cord blood was related to a history of abnormal pap-smears or genital warts, and raised the risk of the neonate being HPV-positive at birth. The mode of delivery did not predict the HPV-status of neonate, placenta, or cord blood. HPV DNA in breast milk was associated with oral HPV status of the father, but not with HPV status of the neonate.

The results indicate that exposure to HPV is common and that part of the exposure might take place already early in life. Contrary to the common claim, pregnancy is not a risk factor for HPV.

Keywords: human papillomavirus, HPV DNA, mother, pregnancy, HPV antibodies, seroprevalence, seroconversion, seropersistence, antibody decay, breast milk, placenta, umbilical cord blood, infant

TIIVISTELMÄ

Marja Sarkola
Ihmisen papilloomavirusinfektiot ennen ja jälkeen synnytyksen – kolmen vuoden seurantatutkimus.
Suupatologian- ja radiologian osasto, Hammaslääketieteen laitos, MediCity Tutkimuslaboratorio, Lääketieteellinen tiedekunta, Turun Yliopisto, Naistenklinikka, Turun Yliopistollinen keskussairaala, Turun kliininen tutkijakoulu.

Ihmisen papilloomavirus (HPV) -infektiot ennen ja jälkeen synnytyksen ovat tärkeä tutkimuskohde; raskaus saattaa vaikuttaa HPV-infektion luonnolliseen kulkuun ja HPV voi siirtyä lapseen.

Tämä väitöskirjatyö on osa kolmivuotista seurantatutkimusta, jossa selvitetään HPV-infektioiden dynamiikkaa vanhemmissa ja heidän vastasyntyneissä lapsissaan, yhteensä 329 perheessä. Tässä työssä selvitettiin HPV-vasta-aineiden esiintyvyyttä äideissä seurannan aikana sekä raskauden vaikutusta suun ja sukuelinten HPV-infektioihin. Lisäksi tutkittiin HPV:n esiintyminen istukassa ja napaveressä sekä äidinmaitonäytteissä.

Seurannan alkaessa vasta-aineita HPV-tyypeille 6, 11, 16, 18 ja 45 todettiin 53 %:lla, 21 %:lla, 35 %:lla, 21 %:lla ja 9 %:lla odottavista äideistä. Vasta-aineiden esiintymistä ennustivat varhainen yhdyntöjen aloittamisikä, seksipartnereiden suuri määrä, aikaisemmat genitaalisyylät sekä samanaikainen seropositiivisuus suuren/pienen riskin virustyypeille. Seurannan aikana HPV-vasta-aineita kyseisille virustyypeille ilmaantui 27 %, 14 %, 17 %, 17 % ja 7 % äideistä. Vasta-aineet häviävät harvoin. Äidin uudella raskaudella ei ollut vaikutusta HPV-infektion kulkuun.

HPV-DNA:ta todettiin 4.2 % ja 3.5 % istukka- ja napaverinäytteistä, sekä 4.5 % ja 19.7 % äidinmaitonäytteistä, jotka oli kerätty kolme päivää ja synnytyksen jälkeen. Äidin aikaisemmat genitaalisyylät tai poikkeava PAPA-löydös olivat riskitekijöitä napaveren/istukan HPV-positiivisuudelle. Isän suun HPV-infektiolla oli yhteyttä puolison rintamaidon HPV-positiivisuuteen. HPV:n esiintyvyys istukassa/napaveressä lisäsi merkittävästi vastasyntyneen HPV-kantajuuden riskiä. Äidinmaidon HPV-positiivisuudella ei ollut yhteyttä vastasyntyneen HPV- kantajuuteen. Vastasyntyneen HPV-kantajuudella ei ollut yhteyttä synnytystapaan, eikä synnytystavalla istukan tai napaveren HPV-positiivisuuteen.

Tuloksemme osoittavat, että HPV-infektiot ovat hyvin yleisiä ja HPV:lle voidaan altistua jo varhaislapsuudessa. Vastoin yleistä käsitystä, raskaus ei ole HPV:n riskitekijä.

Avainsanat: ihmisen papilloomavirus, HPV DNA, HPV vasta-aineet, serokonversio, äiti, raskaus, vastasyntynyt, vertikaalinen tartunta, sukuelin, suu, istukka, napaveri, rintamaito

TABLE OF CONTENTS

ABBREVIATIONS

CC	cervical cancer
CIN	cervical intraepithelial neoplasia
DNA	deoxyribonucleic acid
E	early region of the HPV genome
EBV	Epstein-Barr virus
ELISA	enzyme-linked immunosorbent assay
GP	general primer
GST	glutathione S-transferase
GW	genital wart
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPV	human papillomavirus
HR	high-risk
HC 2	Hybrid Capture 2
IARC	International Agency for Research on Cancer
Ig	Immunoglobulin
ISH	in situ hybridization
JORPP	juvenile onset recurrent respiratory papillomatosis
L	late region of the HPV genome
LCR	long control region
LR	low-risk
MFI	median fluorescence intensity
mRNA	messenger ribonucleic acid
OC	oral contraceptive
OR	odds ratio
ORF	open reading frame
PAP smear	Papanicolaou smear
PBMC	peripheral blood mononuclear cell
PBNA	pseudovirion-based neutralization assay
PCR	polymerase chain reaction
pRB	retinoblastoma tumor suppressor protein
PVA	polyvinylalcohol
PVP	polyvinylpyrrolidone
QIC	quasi-likelihood information criterion
RIA	radioimmunoassay
RNA	ribonucleic acid
RT	room temperature
SB	Southern blot
STD	sexually transmitted disease
STI	sexually transmitted infection
TZ	transformation zone
VLP	virus like particle
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This study is based on the following publications referred to in the text by the Roman numerals I-IV.

I. Syrjänen S, Waterboer T, Sarkola M, Michael K, Rintala M, Syrjänen K, Grénman S, Pawlita M: Dynamics of human papillomavirus (HPV) serology in women followed-up for 36 months after pregnancy. J Gen Virol. 2009;90:1515-26.

II. Sarkola ME, Grénman SE, Rintala MAM, Syrjänen KJ, Syrjänen SM: Effect of second pregnancy on maternal carriage and outcome of high-risk human papillomavirus (HPV). Experience from the prospective Finnish HPV Family Study. Gynecol Obstet Invest. 2009;67:208-16.

III. Sarkola ME, Grénman SE, Rintala MAM, Syrjänen KJ, Syrjänen SM: Human papillomavirus in the placenta and umbilical cord blood. Acta Obstet Gynecol Scand. 2008;87:1181-8.

IV. Sarkola ME, Rintala MAM, Grénman SE, Syrjänen SM: Human papillomavirus DNA detected in breast milk. The Pediatric Infectious Diseases Journal 2008;27:557-8.

The original communications have been reproduced with the permission of the copyright holders.

1.   INTRODUCTION

Human papillomaviruses (HPVs) are small epitheliotropic DNA viruses that have the capability to induce a variety of proliferative lesions. Over 100 types of HPV have been identified, each type infecting preferentially either cutaneous or mucosal tissue. The sites of mucosal HPV infection include the cervix, vagina, vulva, anus, oropharynx, larynx and oral cavity, but HPVs have been detected in other epithelial sites, as well. HPV infections of the mucosa typically result in benign self-limited epithelial changes, warts, papillomas and low-grade intraepithelial lesions. However, lesions caused by high-risk (HR) HPV types may persist and progress to cancer.

Epidemiological research of HPV has traditionally focused on HR-HPV infections of the genital area of sexually active women. In these populations, the key factors associated with HPV detection are age and sexual behaviour. The prevalence of HPV peaks in the youngest age groups of women studied; 20-33 % of females aged 18-25 years carry HPV. From a clinical point of view, it is important to understand that the prevalence of HPV is higher than the prevalence of HPV-associated disease. Most HPV infections are transient, although it is unclear whether infections resolve completely or remain latent. The outcome of HPV infection is modulated by viral and host factors, which are partly unknown. The host immune responses may play a major role, likely to be modified by previous HPV infections as well as genetic, hormonal and environmental factors. The immune response to HPV has not been elucidated in detail.

Pregnancy may increase the risk and alter the natural history of HPV infection. High parity is a known risk factor for cervical cancer, but data on pregnancy/parity and HPV are controversial. HPV is transmitted from mother to child, but the routes and rates are not well known. The neonate is exposed to HPV from the infected birth canal at delivery. Prenatal exposure to HPV in utero or postnatally via breast milk or saliva may occur. The data on non-sexual transmission of HPV and on HPV infections in infants are controversial and warrants furher study.

The Finnish Family HPV Study was started in 1998 and it is the first prospective study to assess HPV dynamics at multiple anatomical sites in parents and infants. The study aims to establish HPV detection rates, trasmission routes and infection outcome within families and to contribute to the understanding of the epidemiological factors involved. Altogether, 329 pregnant women at their third trimester together with 131 of their male partners were included in the study. A follow-up for 36 months after delivery was planned, and the neonates were also enrolled. This thesis presents the dynamics of HPV antibodies to the L1 capsid protein of HPV 6, 11, 16, 18 and 45 in the mothers during the follow-up, along with the baseline HPV DNA data. The effect of pregnancy on HPV detection and infection outcome at genital and oral mucosa was assessed longitudinally. The presence of HPV DNA in the placenta, umbilical cord blood and breast milk was also studied. The findings were compared with the oral and genital HPV status of the neonates, mothers and fathers to evaluate the vertical transmission. Demographic data were analyzed to identify risk determinants of HPV infection.

2.   REVIEW OF THE LITERATURE

2.1.   HUMAN PAPILLOMAVIRUSES

The papillomavirus family (*Papillomaviridae*) is a highly diverse group of small non-enveloped DNA tumor viruses (de Villiers et al., 2004). Papillomaviruses are distributed worldwide and have been detected in a wide variety of animals as well as in humans, in which they have been most extensively studied. They typically infect cutaneous and mucosal epithelial cells and induce proliferative lesions, such as benign warts and papillomas, but lesions caused by certain types bear a high risk of malignant transformation (zur Hausen, 2002). Papillomavirus-associated diseases have affected mankind since ancient times, but the viral origin of skin and genital warts (GWs) or cervical cancer (CC) was not discovered until the 20th century (reviewed in Syrjänen and Syrjänen, 2008; zur Hausen, 2009).

2.1.1. Classification

Papillomaviruses are highly host specific and are named after the species they infect. In humans, over 100 human papillomavirus (HPV) types have been completely described (deVilliers et al., 2004). HPVs are identified by complete sequence analysis, and classified as virus types on the basis of their sequence homology within the capsid protein gene L1, which is the most conserved gene within the genome (deVilliers et al., 2004). Hence, HPV types are referred to as *genotypes*. Different genotypes are identified by numbers assigned in order of their discovery and confirmation. A new HPV type is defined as a complete genome, whose L1 gene sequence is at least 10 % dissimilar to that of any other known HPV type. Within a genotype, subtypes and variants can be distinguished, with dissimilarities within the L1 gene of 2-10 %, and less than 2 %, respectively.

The rapid increase in the number of papillomavirus types since the early 1980s due to the development of DNA sequencing techniques generated a need for a taxonomic classification within the family *Papillomaviridae* agreeing with the classification standards established by the International Committee on the Taxonomy of Viruses (de Villiers et al., 2004). Based on the nucleotide sequence comparison of L1, the evolutionary relationships between different papillomavirus types can be represented in the form of phylogenetic trees, which demonstrates three taxonomic levels, i.e., genera, species and types (deVilliers et al., 2004). The human and animal papillomaviruses currenlty known are divided into 16 different genera, which share less than 60 % nucleotide sequence identity in the L1 ORF. The genera are identified by Greek letters. Within a genus, closely related PV types form "species" that share 60-70 % L1 sequence identity and considerable biological similarity. Most HPVs infecting mucosal tissue belong to the Alpha papillomaviruses, which form 15 species (de Villiers et al., 2004). The Alpha papillomavirus contains HPV types recovered in anogenital cancers (e.g. HPV 16), in genital and laryngeal warts (HPV 6 and 11), and in common skin warts (e.g. HPV 2). To date, 15 high-risk (HR) HPV types, three probable HR and 12

low-risk (LR) HPV types have been identified based on an analysis of pooled case-control data of women in nine countries with confirmed cervical squamous cell carcinoma (**Table 1**).

Table 1. Classification of HPV types by cervical oncogenicity (adapted and modified from Munoz et al., 2003)

Risk classification	HPV type[*]
High-risk	16[9], 18[7], 31[9], 33[9], 35[9], 39[7], 45[7], 51[5], 52[9], 56[6], 58[9], 59[7], 68[7], 73[11], 82[5]
Probable high-risk	26[5], 53[6], 66[6]
Low-risk	6[10], 11[10], 40[8], 42[1], 43[8], 44[10], 54[13], 61[3], 70[7], 72[3], 81[3], cand89[3]
Undetermined risk	34[11], 57[4], 83[3]

*Species superscripted according to de Villiers et al., 2004

2.1.2. Genomic organization

The HPV virion is approximately 55 nm in diameter (Williams et al., 1961). It consists of an icosahedral capsid made up of 72 capsomers and of a closed circular double-stranded DNA genome sized almost 8kb. Despite the vast heterogeneity of the papillomaviruses, the genomic organization is remarkably similar in all types. All potential protein coding sequences (open reading frames, ORFs) are located on one strand of the double-stranded DNA and were originally divided into "early" (E) and "late" (L) genes based on their location in the genome (Howley and Lowy, 2001). Overall, the genome has a capacity to encode eight proteins: E1, E2, E4-E7 are non-structural proteins involved mainly in replication, transcription and transformation, and L1 and L2 are the structural proteins that compose the capsid (Fehrmann and Laimins, 2003). Between the L1 and E6 ORFs is a non-coding, regulatory region called the long control region (LCR) sized approximately 0.85kb. It contains binding sites for a variety of cellular and viral transcription factors required for regulation of gene expression, DNA replication and genome packaging. This region contains the highest degree of variation in the viral genome (Bernard et al., 2006).

Regulation of viral gene expression is complex and controlled by cellular and viral transcription factors (zur Hausen, 1996). The expression of papillomavirus genes is directed by viral promoters. Two major promoters reside in the genomes of HR-HPVs: the early promoter (p97 in HPV16) located within the LCR (Smotkin and Wettstein, 1986) and the late promoter (p670 in HPV16) within E7 (Grassmann et al., 1996). The promoters are regulated over long distances by enhancers and clusters of binding sites for cellular transcription factors (Cripe et al., 1987; Gloss et al., 1987; Swift et al., 1987; Chong et al., 1991; Dollard et al., 1993). Although the genomic organization is well conserved among the papillomaviruses, there are significant differences in the regulation of gene expression and in the functions of specific gene products (Bernard, 2002).

2.2.   HPV INFECTIONS

2.2.1.   Target cells and tissues

2.2.1.1. Anatomical distribution

HPVs are mostly detected in the skin and in the anogenital tract, but infect also the oral, oropharyngeal, laryngeal and sinonasal mucosa. Other sites of infection include the urinary tract, conjunctiva of the eye, esophagus and, possibly, bronchial mucosa (Syrjänen and Syrjänen, 2000). There are a number of individual reports of the presence of HPV DNA in other epithelia, e.g. benign and malignant lesions of the breast (Di Lonardo et al., 1992; de Villiers et al., 2005; Akil et al., 2008; de León et al., 2009), the prostate (McNicol and Dodd, 1990; Carozzi et al., 2004), the endometrium and the ovaries (Lai et al., 1992; O'Leary et al., 1998; Fedrizzi et al., 2009), but the reports are contradictory and the precise significance of these findings is uncertain (Bratthauer et al., 1992; Effert et al., 1992; Gopalkrishna et al., 1996; de Cremoux et al., 2008).

2.2.1.2. Host cells and host epithelia

HPVs are considered to be strictly epitheliotropic; they cannot induce proliferative lesions in the underlying fibroblastic mesenchyme of the squamous epithelium, like some animal papillomaviruses do (Pfister, 1984). Cell cycle progression through the early stages of mitosis is critical for HPV infection (Pyeon et al., 2009). Full viral replication can occur only in cells committed to differentiation (Alani and Münger, 1998). Hence, HPV infection is restricted to cells that can differentiate. Stratified squamous epithelium of the mucosa undergoes constant renewal and is composed of basal, parabasal, intermediate and superficial layers of cells. The basal layer consists of a single row of cells and rests on a thin basement membrane. Active mitosis occurs in the basal cells. In addition to stratified squamous epithelium, HPVs may infect other types of epithelia, e.g. the simple columnar epithelium of the endocervix (Roberts et al., 2007).

Animal models of natural papillomavirus particles or HPV16 pseudovirus have shown that disruption of the integrity of the stratified or columnar genital epithelium is essential for HPV infection (Reuter et al., 2001; Roberts et al., 2007). It has been thought that mild abrasions or other microtrauma of the epithelium allows HPVs to penetrate the physical barrier of the upper epithelial layers and to access the receptors on the basement membrane (Culp et al., 2006). Alternatively, the process of wound healing might increase the efficiency with which HPV DNA becomes established as episomes in basal cells, because at that point the basal cells are in a hyperproliferative state (Pyeon et al., 2009). Access to the basal layer is naturally facilitated in certain areas of the body, where proliferating basal cells are in the proximity of the epithelial surface, e.g. the base of hair follicles and the junctions of two types of epithelia. The transformation zone (TZ) in the uterine cervix is a specialized junction initially covered by columnar epithelium and, through a process referred to as metaplasia, undergoes replacement by squamous epithelium.

The receptor of the papillomavirus on host cells has not been identified. Papillomavirus particles may be associated with heparan sulfate proteoglycans on the cell surface, and with a secondary receptor, such as α6 integrin and extracellular laminin 5, to facilitate uptake (Evander et al., 1997; Joyce et al., 1999; McMillan et al., 1999; Shafti-Keramat et al., 2003; Culp et al., 2006; Johnson et al., 2009). Yet, tropism of HPVs for epithelial cells does not appear to be due to any cell-type specific receptor (Howley and Lowy, 2001), since binding studies have shown that papillomaviruses can bind to a wide variety of cell types (Roden et al., 1994; Müller et al., 1995).

2.2.1.3. Tissue tropism

Typically different HPV types infect different types of epithelia of immunocompetent individuals. Historically, this tropism has formed the basis for grouping the HPVs into cutaneous and mucosal types (de Villiers, 1989). However, tissue tropism of HPVs is not as clear cut as formerly thought. Although some variations in virus entry depending on HPV type and origin of cells do exist (Mistry et al., 2008), different HPV types seem to be able to infect epithelial cells indiscriminately (Bernard, 2002; Johnson et al., 2009). Thus, the "mucosal" HPV16 pseudovirions can infect murine nongenital epidermis (Alphs et al., 2008) and the "cutaneous" HPV5 pseudovirus can infect the murine genital tract (Johnson et al., 2009). Hair follicles seem to be an important endogenous reservoir of alpha-HPV in the anogenital region (Boxman et al., 1999; Poljak et al., 2009). Functional differences, like transcriptional activation, probably restrict the ability of any particular HPV type to give rise to lesions in a subset of tissues. The relative abundance of cellular transcription factors differs from one tissue to another, with some types of tissues being more permissive than others for HPV infection and associated lesions (Bernard, 2002; Mistry et al., 2007). Currently, it is not clear whether there are subtle differences in tropism between different mucosal species (Castle et al., 2006 and 2007).

2.2.2. HPV life cycle

In squamous epithelium, the productive cycle of HPV is intimately linked to differentiation factors expressed within various layers of the host epithelial cell (Stubenrauch and Laimins, 1999). For this reason, three-dimensional organotypic or raft cultures, instead of monolayer tissue cultures of dividing cells, need to be used for studying the life cycle of HPV (Chow and Broker, 1997). It is not yet fully understood, and only very recently has efficient virus propagation in vitro been introduced (Wang HK et al., 2009). Since the HPV genome does not encode a DNA polymerase of its own, the virus is dependent on the host cell's replication machinery to produce viral progeny (Alani and Münger, 1998). The life cycle of the papillomavirus is shown in **Figure 1**.

Initially, HPV infects undifferentiated proliferative basal cells, which are capable of dividing (Howley and Lowy, 2001). Once inside the host cell, viral DNA localizes into the nucleus and establishes itself as an episome with a low copy number (some 10-200 copies per cell). At this stage, the viral proteins E1, E2, E6 and E7 transcribed from the early promoter are expressed at a low level (Doorbar, 2007). After cell division, the

daughter cells retaining the viral genome are pushed towards the suprabasal regions and begin to differentiate. This triggers a coordinated transcriptional cascade of the viral genome. Viral proteins, mainly E6 and E7, may disturb the normal terminal differentiation by stimulating cellular proliferation and DNA synthesis by interfering with and inhibiting several cell cycle regulators in order to allow high-level amplification of the viral genome to at least 1000 copies/cell (Münger and Howley, 2002). The interactions of viral E6 and E7 proteins with p53 (Werness et al., 1990) and pRB (Dyson et al., 1989, Münger et al., 1989) are the best characterized interactions so far. The late differentiation-dependent promoter is activated towards the epithelial surface, and the levels of viral proteins necessary for productive replication (E1, E2, E4 and E5) increase. After the onset of genome amplification, the capsid proteins L1 and L2 accumulate in mature epithelial cells. The assembly of infectious virions takes place in terminally differentiated cells of the upper epithelial layers and the virions are shed to the environment, as the cells are lost through desquamation.
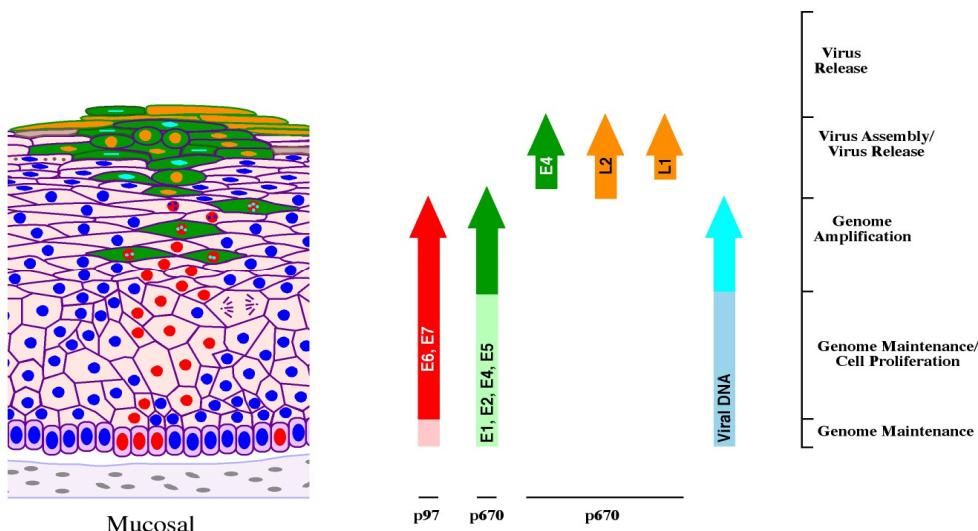
Figure 1. Papillomavirus life cycle. Adapted from John Doorbar with permission.

2.2.3. Manifestations

By presentation, HPV infections may be divided into three categories: latent, sub-clinical and clinical, but no diagnostic criteria have thus far been established for these categories. The nomenclature used in HPV research awaits clarification by studies on the life cycle of the virus, but before this becomes possible, the problem of culturing HPV in vitro needs to be solved. Most HPV infections cause no detectable clinical changes, i.e. the viral genome is present in normal epithelia, while colposcopy, cytology and histology are normal (Syrjänen S et al., 1990). This condition is often referred to as a *latent infection*, i.e. viral DNA is detectable but there is no evidence of ongoing viral replication. HPV latency is a puzzling phenomen for which no mechanism or viral gene expression pattern has been established. Latent infection may

persist for weeks to years or decades and may ultimately resolve, or become reactivated to produce clinically apparent lesions (Pfister, 1984). Clinically, strong evidence for a true state of latency comes from studies on immunosuppressed patients and children with laryngeal papillomatosis. In these situations HPV-associated lesions continue to recur in apparently normal tissue in the absence of any obvious external re-infection (Stubenrauch and Laimins, 1999; Broker et al., 2001). HPV infection is often considered *subclinical* if histology shows minor epihelial changes that are not consistent with typical clinical HPV lesion. Visible lesions that are readily apparent with usual diagnostic methods, i.e. visual examination, cytology and histology, constitute *clinical infections* (Syrjänen and Syrjänen, 2000). Clinical manifestations of HPV include anogenital warts, recurrent respiratory papillomatosis, cervical intraepithelial neoplasias and certain cancers, e.g. cervical, anal, vulvar, vaginal and penile cancer and some head and neck cancers (Muñoz et al., 2006).

2.2.4. Outcome of HPV infection

Longitudinal follow-up studies on HPV infections have shown that the HPV status often fluctuates, and single point measurements of HPV DNA are of limited value for the assessment of an individual's HPV status (Schneider et al., 1992). Genital HPV infections may have different outcomes. The duration of HR-HPV infection before the development of precancerous lesions may last only a few months (Woodman et al., 2001; Winer et al., 2005), whereas some women may never develop lesions while taking years to clear the virus (Schlecht et al., 2003). Clinical lesions result from the expression of HPV genes in maturing epithelial cells. HPV lesions are characterized by increased cellular proliferation (acanthosis), koilocytosis and abnormal keratinization (dyskeratosis) (Koss and Durfee, 1956; Meisels et al., 1976; Purola and Savia, 1977). It is thought that a period of productive infection is eventually followed by lesion regression and viral clearance or by maintenance of viral genomes as latent episomes in cells of basal layer (Doorbar, 2007). However, in a minority of cases the infection persists and may progress towards cancer (Schiffman et al., 2007). The virus-induced epithelial abnormalities can generally be divided into two categories based on their progression potential into productive, self-limited infections and potentially progressive precancerous lesions (Richart, 1973). The true precursor lesions of cervical cancer (high-grade lesions) have a more extensive proliferative phase, with the productive stages of the virus life cycle being supported only poorly (Middleton et al., 2003). Both HR- and LR-HPV types cause low-grade lesions of the uterine cervix, while most high-grade, carcinoma *in situ* and invasive cancers of the uterine cervix are caused by the HR types.

Lesion progression toward cancer

In a productive infection the expression of viral genes is closely regulated by cellular and viral factors, as the infected cells migrate toward the epithelial surface (Stoler et al., 1992; Dürst et al., 1992; Doorbar, 2007). This control sometimes fails, for reasons that are not well understood. Continued and aberrant expression of the E6 and E7 genes of HR-HPVs is associated with host cell genomic instability, which ultimately, after accumulation of mutational events, can result in malignant transformation

(Stanley et al., 2007). During this process, the viral genome often becomes integrated into the host DNA, which results in the loss of expression of many viral gene products, such as E2, which effectively "kills" the virus, i.e. the virus loses its characteristics as replicating entity (abortive infection) (Münger and Howley, 2002). Recent work implies that HPV-mediated carcinogenesis may require not only HR-HPV integration but also loss of inhibitory episomes expressing E2; this would permit natural selection of integrants with high oncogene expression (Pett et al., 2006). Alternative pathways of HPV-driven carcinogenesis occur probably as well, since some CCs contain only episomal HPV (Klaes et al., 1999).

HPV-induced cervical squamous cell carcinogenesis is a multistep process. Carcinoma develops from precursor lesions defined as cervical intraepithelial neoplasia (CIN) (Richart, 1973). The histological criteria for CIN are nuclear aneuploidy, abnormal mitotic figures and loss of normal maturation. CIN is divided into grade 1, 2 and 3, depending on the extent of aberration of cellular stratification within the epithelium (Creasman, 2007). It was originally thought that cervical carcinoma evolves from infected healthy epithelium via a continuum of longlasting, consecutive CIN lesions from 1 to 3. However, an alternative concept, that finds increasing support, is that many of the clinically relevant CIN 2/3 lesions may be rapidly induced within 2-3 years following infection (Winer et al., 2005; Muñoz et al., 2009), whereas it takes presumably another 10-12 years before invasive cervical carcinoma ensues.

Persistent infection with HR-HPV is considered to be a necessary but not sufficient condition for the development of CC (Bosch et al., 1995; Walboomers et al., 1999; zur Hausen, 2002). It has been estimated that if no therapeutic action is taken, only 3-5 % of all HPV infected women and 12-30 % of all women with CIN3 lesions ultimately develop CC (Ostör, 1993; McCredie et al., 2008). Carcinogenesis clearly requires cofactors, either endogenous or exogenous (Dürst et al., 1992). Such significant cofactors are smoking, prolonged oral contraceptive (OC) use, high parity and coinfection with HIV. Other probable cofactors include co-infection with chlamydia and immunosuppression. Genetic and immunological host factors and viral factors other than virus type, such as viral load and integration probably also play a role, but their significance is not known (Castellsaqué and Muñoz, 2003; Muñoz et al., 2006).

2.3.   HPV AND HOST IMMUNE RESPONSE

The host immune responses are thought to play a major controlling role for the outcome of HPV infections. The transient nature of most HPV infections is indicative of successful host defence. The prevalence of HPV infections and high-grade lesions is increased in individuals immunosuppressed e.g. by HIV infection or medication after organ tansplantation, which emphasizes the importance of a proper immune response to control HPV (Broker et al., 2001; De Vuyst et al., 2008; Paternoster et al., 2008). Genetic factors related to polymorphism in the HLA system may also play a role in the ability of the immune response to clear HPV infection (Wang SS et al., 2009). Immune defence is a combination of the relatively nonspecific innate immunity at the epidermal or mucosal surfaces and the antigen-specific adaptive immunity mediated by the

conventional T and B cells which recognize and eliminate foreign material. The precise details of the immune response against HPV are not clear. It is believed that neutralizing antibodies to viral capsids generated by effector B cells may be effective in preventing viral infection and spread, while innate immunity and adaptive cell mediated immunity appears important for the eventual elimination of HPV infection (Frazer, 2009).

HPV infections are slow to induce measurable immune responses and slow to clear in comparison to other pathogens. This suggests that HPVs have evolved methods to bypass immune recognition and clearance during their long coevolution with the human being. HPVs have tailored their replicative cycle to fit the differentiation program of host epithelia in a way that interaction with cells of the immune system is minimized. In addition, there is no inflammatory response to alert the immune system, as the production of virions does not cause cell lysis. According to current understanding, there is no blood-borne phase of infection, and thus the immune system outside the epithelium has little opportunity to detect the virus. It also appears that viral early proteins can directly suppress the innate and adaptive responses. As a consequence, HPV infections can persist or remain latent for long periods (Tindle, 2002; Woodworth, 2002; Stanley, 2006; Frazer, 2009; Einstein et al., 2009).

2.3.1. Measured natural immune responses to HPV infection

Past exposure of an individual to HPV can be evaluated by measuring serum antibodies. Antibodies may be generated to several HPV proteins. During natural infection HPV early proteins are expressed entirely within the epithelium, and antibody responses directed against these proteins are minimal or absent with limited clinical significance (Frazer, 2007). The abundant expression of highly immunogenic capsid proteins is restricted to differentiated epithelium, far from immune centers. The time to develop antibodies detectable in the serum varies considerably from one individual to another, but typically seroconversion after natural infection is a slow process with an estimated median time of 8 - 12 months from HPV DNA detection (Carter et al., 1996; Carter et al., 2000; Ho et al., 2004; Steele et al., 2008). Seroconversion seems to be driven by sufficient antigenic exposure, as it has been associated with either a high viral load or persistent infection (Wideroff et al., 1996; Ho et al., 2004; Wang et al., 2004). However, available data indicate that some women may never develop an antibody response even if they have persistent HPV infection (Carter et al., 2000; Ho et al., 2004; Steele et al., 2008). The underlying reasons for this phenomenon are unknown. The major isotypes of serum antibodies against HPV capsids are IgG1 and IgA (Wang et al., 2000). IgG antibodies to HPV-16 L1 are claimed to be stable over several years (Shah et al., 1997; af Geijersstam et al., 1998; Carter et al., 2000), although some investigators have disputed this contention (Ho et al., 2004) and type-specific differences have been reported (Carter et al., 2000).

Because the half life of secreted antibodies is rather short, in the range of days, their maintenance over long periods requires the continuous activity and presence of specific secretory cells (Dörner and Radbruch, 2007). These cells are generated from specific,

activated B cells, a process that can be driven by persistent antigen expression (Zinkernagel and Hengartner, 2006) or that can reflect true immunological memory (Manz et al., 2005). The generation and maintenance of humoral memory is still poorly understood. Both mucosal and systemic immune reactions contribute to humoral immunity, and they might be distinctly regulated (Mei et al., 2009). Humoral memory may provide the first line of defence against reinfections.

HPV capsid protein L1 contains multiple immunogenic epitopes, which include the main targets of the neutralizing antibodies (Christensen 1996a; b). However, the ability of HPV capsid antibodies to prevent infections after natural infection is uncertain. Antibodies to HPV capsids measured by direct ELISAs correlate well with antibodies with neutralizing capacity (Dessy et al., 2008). In animals, neutralizing antibodies confer life-long resistance to further virus challenge after natural species-specific papillomavirus infection (Ghim et al., 2000). The presence of HPV capsid antibodies in the serum may confer some protection (Ho et al., 2002), but reinfection or reemergence of homologous HPV type in the presence of circulating VLP antibody is possible and has been documented (Nakagawa et al., 2002). In larger follow-up studies seropositivity has not been associated with a statistically significantly decreased risk of re-infection with the homologous HPV type or genetically related types (Viscidi et al., 2004). The local mucosal antibody response may, consequently, be more important than the systemic response for immediate virion neutralization and for the outcome of the infection. The protective role, if any, of local IgG and IgA HPV capsid-specific antibodies, which can be demonstrated in cervical mucus after natural infection, remains unclear (Bierl et al., 2005; Passmore et al., 2007).

2.3.2.   HPV vaccines and immunity

There are two newly licensed commercial vaccines available for the prevention of HPV infections, both of which are based upon HPV L1 virus-like particles. Cervarix® by GlaxoSmithKline (London, UK) is a bivalent vaccine against HPV 16 and HPV 18. Gardasil® by Merck & Co. (NJ, USA) is a quadrivalent vaccine targeting HPV 6, 11, 16 and 18. Multiple pathways within the cellular and innate arms of the immune system are targeted upon vaccination (Garcia-Pineres et al., 2009), although the precise details of these processes are not known, as is the case with most non-replicating vaccines (Rappuoli, 2007). The main protective effectors are probably the virus neutralizing antibodies (Breitburd et al., 1995). A robust humoral response has been observed after vaccination (Fife et al., 2004; Poland et al., 2005). After three vaccine doses the measured antibody levels for targeted HPV types are 10-100 times higher than the antibody levels after natural infections. Serum antibodies against HPV-16 remain relatively high during the first years after vaccination (Harper et al., 2006; Villa et al., 2006). Vaccines are effective in protecting women against HPV lesions caused by the HPV types targeted by the vaccines (Harper, 2008; Schiller et al., 2008; Paavonen et al., 2009). The length of protection provided by vaccination is not known. The vaccines are prophylactic and do not affect the course of pre-existing infections (Hildesheim et al., 2007).

2.4. DETECTION OF HPV INFECTIONS

Papillomaviruses cannot be propagated or identified by conventional tissue culture. Available detection methods for HPV or HPV-associated lesions include 1) morphological methods assessing signs of HPV induced changes by clinical inspection or microscopy of exfoliated cells and/or biopsy samples, 2) detection of viral nucleic acids, either DNA or RNA, and 3) detection of an antibody response to HPV. Antibody detection has no clinical role in the diagnosis of HPV infections in individual patients.

2.4.1. Morphological methods

2.4.1.1. Visual examination

HPV-related macroscopic lesions can be detected by visual inspection, which is sufficient for the diagnosis of most anogenital warts. Colposopy was introduced by Hinselman (Hamburg, Germany) in 1925 but it gained popularity only in the 1960s. Visual examination of the illuminated, magnified view of the uterine cervix, especially the transformation zone, and the adjacent tissues after application of acetic acid and iodine solution enables the examining physician to detect suspicious lesions. Colposcopy aids in directing a biopsy to confirm and settle the diagnosis.

2.4.1.2. Cytology

Cytological examination of the cervix screens for abnormal cells and precancerous lesions. The technique was introduced by the Greco-American cytologist and pathologist George Papanicolaou in the 1940s, and has since then been referred to as the Pap test or the Pap smear. It involves collection of cells from the endocervix, ectocervix and vagina with a cotton swab or a small brush and Ayre's spatula, fixation of the cells on a glass slide and microscopy. Cytology is widely accepted for screening for CC. Successfully implemented screening programs have efficiently reduced the morbidity and mortality from cervical squamous cell carcinoma (Nieminen et al., 1995; Anttila A et al., 1999; van der Aa et al., 2008). Despite this encouraging experience over the years, cytological screening is far from perfect in many countries because of poor coverage, quality control and management (Kitchener et al., 2006). The Pap test has remained virtually unchanged for 50 years, although automated systems and liquid-based cytology systems have been developed and tested during the previous decade in an attempt to improve the quality (Nieminen et al., 2005; Arbyn et al., 2008). The terminology used to describe the findings of the cytological smears has undergone a series of changes during the past decades (Koss, 2000). Currently, the most widely accepted terminology is the Bethesda System, developed originally in 1988 and updated in 2001 (National Cancer Institute Workshop, 1989; Solomon et al., 2002). Cytological evaluation of the Pap smear allows the clinician to identify patients who need further diagnostic assessment. The management of an abnormal Pap smear is described in several national and international guidelines (Finnish Current Care guidelines 2006, Wright et al., 2007, Jordan et al., 2008 and 2009).

2.4.1.3. Histopathology

Histopathological examination is the golden standard to settle the diagnosis of cervical pathology. However, as for cytology, histopathology does not detect the actual presence of HPV. It is an indirect method that detects the clinical sequelae of an HPV infection, i.e. the presence of a CIN lesion or cancer. Cytological and histological features are not sensitive indicators of the presence of HPV (Bauer et al., 1991; Rozendaahl et al., 2000). In addition, morphological methods cannot distinguish HR types from LR types, and latent infections cannot be detected at all.

2.4.2.   Methods based on nucleic acid to detect HPV

2.4.2.1. Nucleic acid hybridization

Nucleic acid hybridization is a method to detect DNA or RNA by binding a probe sequence to a complementary sequence in the sample. A variety of hybridization procedures have been developed for the detection of HPV DNA or RNA. These include hybridization on filter (e.g. Southern blot, dot blot) or glass, or in tissue samples (in situ hybridization (ISH)).

2.4.2.2. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is a common and the most sensitive method for HPV detection. The target sequences can be amplified at least a million-fold by using specifically designed oligonucleotide primers and a thermostable DNA polymerase. The procedure involves repeated thermocycling including denaturation, annealing of primers and elongation of the DNA chain. Protocols employing general or consensus primers that may detect all mucosal HPV types have become the most widely used. The most frequently applied primers for these assays are the GP05+/06+ (de Roda Husman et al., 1995a) and MY09/11 (Manos et al., 1989) primers, which target the highly conserved L1 gene. Different primer sets have different analytical sensitivities and different HPV-type coverage. Other factors influencing the performance of PCR-based methods include differences in sampling methods and sample transport/storage, DNA extraction procedures, size of the PCR product, reaction conditions, performance of the DNA polymerase and the detection method of the PCR products (Castle et al., 2002; Snijders et al., 2003). Because of its superior sensitivity, PCR is very vulnerable to environmental contamination. Strong laboratory discipline and careful monitoring of the different PCR steps, i.e. preparation of PCR solutions, sample preparation and electrophoresis of PCR products in separate rooms, are needed to reduce the possibility of specimen contamination (Walboomers et al., 1997).

Variations of the basic PCR techniques have been developed. In nested PCR the initial PCR product is amplified with another set of primers annealing inside the initial PCR product. Nested PCR has higher sensitivity and specificity than conventional PCR. Thus, nested PCR is a valuable tool for the detection of HPV in samples containing a low copy number of HPV DNA or samples with limited amount of cells.

Confirmation of PCR specificity

In earlier studies HPV detection was based on the visualization of a correct sized PCR product on agarose gel. However, the specificity of the amplification needs to be confirmed by subsequent sequence-specific analysis using restriction enzyme digestion of the PCR product, direct sequencing, or hybridization with type-specific probes (Walboomers et al., 1997). Direct sequencing of the PCR product is a rapid and accurate method for HPV identification, but the method cannot detect multiple HPV infections. Hybridization is the most widely used method to confirm the specificity of the amplicons. Recently, several methods have been developed to allow large scale analyses of samples and to identify multiple types simultaneously.

2.4.2.3. Multiplex HPV genotyping

Multiplex HPV genotyping using the Luminex (xMAP) suspension array technique is a recent, high-throughput hybridization method that allows simultaneous detection and genotyping of up to 100 HPV types. The method is based on polystyrene beads with a diameter of 5.6 μm that are internally dyed with various ratios of two spectrally distinct fluorophores, creating an array of 100 different bead sets with specific absorption spectra (Earley et al., 2002). In multiplex HPV genotyping, individual oligonucleotide probes of different HPV types are coupled to different bead sets. These sets are combined to create a suspension array and, due to their unique absorption spectra, they allow up to 100 different targets to be measured simultaneously in a single reaction (multiplexing). First, biotinylated GP5+/6+ -PCR products are generated, denatured and hybridized to bead-coupled probes in 96-well plates. Then biotinylated PCR products are stained with streptavidin-phycoerythrin conjugate. The beads are analyzed in a reader which uses two laser beams to identify the bead set by the internal bead color and to quantify the reporter fluorescence on the bead. The result is expressed as the median fluorescence intensity (MFI) of at least 100 beads per set (Schmitt et al., 2006).

2.4.3. Serology

A variety of assay systems using different sources of antigens to measure serum antibodies to HPV have been developed. Since virus-like particles (VLPs) and their use in vaccination were introduced, the interest in HPV serology has increased rapidly. The VLPs contain the surface epitopes in correctly folded conformation and they are able to induce antibodies with neutralizing capacity (Kirnbauer et al., 1992; Hagensee et al., 1994; Rose et al., 1994; Breitburd et al., 1995). Conformationally dependent epitopes on intact capsids have generally been found to be type-specific, except for HPV 6 and 11 (Christensen et al., 1994 and 1996a,b). Besides VLPs, conformational and neutralizing epitopes are also displayed by homogenous capsomers formed by HPV L1 when expressed as fusion proteins with glutathione S-transferase (GST) in E.coli (Rose et al., 1998; Sehr et al., 2002; Waterboer et al., 2005; Rizk et al., 2008). Recombinant techniques have also enabled the measurement of antibodies to HPV early proteins (Gissmann, 1997)

The most common assays to detect HPV capsid antibodies are shown in **Table 2**. The wide variation in antigens and test systems has prevented reliable interpretation of data on HPV serology. Comparisons of seroprevalences for different HPV types have also been of limited value, because each laboratory has traditionally used different definitions of what is a reactive result (Ferguson et al., 2006). Assessing the sensitivity and specificity of serological assays has been more complicated for HPV infections than for other viral infections because of difficulties in finding appropriate test and reference populations (Carter and Galloway, 1997).

Table 2. Common assays for detection of HPV capsid antibodies. (Modified from Einstein et al., 2009)*

Assay	Description	Antibodies detected
ELISA	• Sensitive technique that detects antibodies bound to virus-like particles (VLPs) coated on microtiter plates either directly (direct ELISA) or with monoclonal antibodies (capture ELISA)	• Direct ELISA measures total antibody concentrations and does not distinguish neutralizing and non-neutralizing antibodies. • Single epitope-based ELISA targets a single (neutralizing) epitope
Pseudovirion-based neutralization assay	• Specific and sensitive assay based on binding of neutralizing antibodies to pseudovirions, which when neutralized are unable to infect a reporter cell line • Technically demanding but more type-specific than VLP-based ELISA	• Measures mostly neutralizing antibodies and is based on predefined epitopes considered to be important for neutralization of HPV
Luminex-based serological assays	Sensitive techniques that detect antibodies against multiple HPV types simultaneously from a single serum sample.	
• *Competitive Luminex immunoassay*	• Competition for binding of the neutralizing antibody in place of a specific fluorescent antibody	• Measures limited number of neutralizing antibodies; based on predefined epitopes considered to be important for neutralization of HPV
• *Multiplex serology*	• Based on bacterially produced in situ-purified glutathione s-transferase (GST) fusion proteins	• Measures total antibody concentrations and does not distinguish between neutralizing and non-neutralizing antibodies

*Data based on: Carter et al., 1996; Opalka et al., 2003; Pastrana et al., 2004; Dias et al., 2005; Waterboer et al., 2005; Mbulawa et al., 2008; Einstein et al., 2009; WHO HPV LabNet.

2.5.   EPIDEMIOLOGY OF HPV

HPV infections have been most extensively studied in samples taken from the uterine cervix of women who have had their sexual debut. The rate of HPV infection is apparently similar in the male genitalia (Vaccarella et al., 2006c), but the epidemiology

of these infections will not be reviewed in this work. HPV infections occur also in children (Oriel, 1992; Syrjänen and Puranen, 2000; Cason and Mant, 2005). Although the main focus of the present thesis is on maternal HPV infections, the literature on HPV infections in children will also be reviewed here since the data is important for understanding vertical transmission of HPV.

2.5.1. Genital HPV infections in women

Genital HPV infections are common among sexually active women. The lifetime risk to ever contract a clinically detectable genital HPV infection is around 80 % (Syrjänen K et al., 1990). This estimation is based on mathematical calculations of data from a mass screening study performed in Kuopio, Finland, where Pap smears were collected from women aged 22 years. The prevalence of cervical HPV infection was 3 % and the crude annual incidence 7 %. Other studies from developed countries are in overall agreement with these estimates (Koutsky, 1997). The occurrence of GWs, the clinical manifestations of HPV 6 and 11, seems to be increasing over time (Lacey et al., 2006). A recent population-based cross-sectional study of nearly 70 000 women from Denmark, Iceland, Norway and Sweden reported that 10.6 % of 18-45 year old women had ever been diagnosed with GWs and 1.3 % had experienced them within the past year. The prevalence of GWs was higher in the younger than the older birth cohorts (Kjaer et al., 2007).

Since the implementation of modern nucleic acid detection methods, HPV infections have been shown to be far more common than anticipated based on clinical manifestations. The proportion of women infected with HPV has been found to vary greatly by type of assay and sampling method and by the type of population studied. A systematic review on HPV epidemiology in the US reported that the HPV DNA prevalence ranged from 14 % to more than 90 % in different studies (Rezvina and Diclemente, 2005). The prevalence was highest among persons attending sexually transmitted disease (STD) clinics and among college students, and lowest in the general population. In Finland, the overall prevalence of HR-HPV among first year college students was 29.5 % in a study using the Hybrid Capture 2 test (HC2) (Auvinen et al., 2005) and 7.5 % in the general female population targeted by the organized screening program, i.e. among 25-65 year old women (Leinonen et al., 2008). In a large meta-analysis by the World Health Organization (WHO) the overall worldwide HPV prevalence was estimated at 10.41 % among 157 879 women with normal cytology derived from 78 published studies, but there was considerable variation in HPV prevalences by region, and, in general, the HPV prevalence was higher in the less developed countries than in the more developed countries (de Sanjosé et al., 2007).

In general, high-risk HPV types are more commonly detected than low-risk types in samples from the uterine cervix (Clifford et al., 2005; deSanjose et al., 2007; Trottier et al., 2008). Coinfections with multiple HPV types seem to be common (Cuschieri et al., 2004; Mejlhede et al., 2009). The most common HPV types in the normal cervical mucosa, in either single or multiple infections, are HPV 16, 42, 58, 31, 18, 56, 81, 35, 33, 45 and 52 according to a pooled analysis of 15 613 women aged 15-74 years

conducted by the International Agency for Research on Cancer (IARC) (Clifford et al., 2005). Variations in the distribution of the HPV type across geographical regions have been reported. However, when considering clinical lesions, the more severe the lesion, the less heterogenous is the type distribution across countries. HPV 16 and 18 apparantly account for 70 % of all CCs worldwide (Clifford et al., 2006).

2.5.1.1. *Age distribution of HPV*

The rate of detection of HPV varies greatly by age. In many developed countries, the highest rate of genital HPV infection occurs consistently among women in their teens and twenties, after sexual debut (Peto et al., 2004; Castle et al., 2005; de Sanjosé et al., 2007; Dunne et al., 2007). Several longitudinal studies on young women in their early twenties have reported a three year cumulative incidence of HPV infections of 40 % or greater (Ho et al., 1998; Moscicki et al., 2001; Woodman et al., 2001; Rodriquez et al., 2007). The incidence and prevalence of HPV infections decreases with age, although infections continue to appear throughout life (Muñoz et al., 2004; Peto et al., 2004; Castle et al., 2005; de Sanjose et al., 2007). In some countries – but not in Finland – a second minor peak in the prevalence as well as the incidence of HPV infections occurs among females aged 45-50 years (Herrero et al., 2000; Jacobs et al., 2000; Muñoz et al., 2004; Castle et al., 2005; Syrjänen et al., 2005). Among the Finnish screening population, an inverse relationship between age and high-risk HPV prevalence has recently been confirmed. A peak prevalence of HR-HPV of 24.1 % observed in the youngest cohort, women aged 25-29 years (Leinonen et al., 2008).
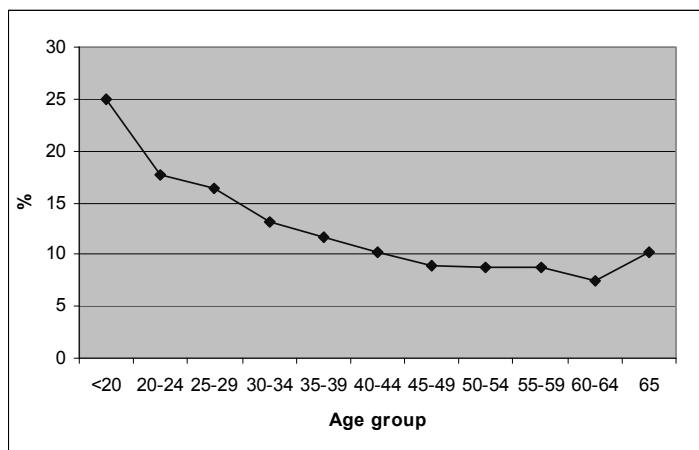
Figure 3. World-wide age-specific prevalence of HPV DNA in women with normal cervical cytology, according to the meta-analysis by de Sanjosé et al., 2007.

The peak in the prevalence of HPV after the initiation of sexual activity is followed by a peak in the incidence of cervical precancer approximately 10 years later and a peak in the prevalence of invasive cancer when the women reach 40 to 50 years of age (Schiffman and Castle, 2005). The detection of precancer lesions is delayed by their initially small size and the often low sensitivity of screening methods (Schiffman et al., 2007). In Finland, the peak incidence of CC has flattened following effective

screening, which has prevented an estimated 80 % of all CC cases (Nieminen et al., 1995).

2.5.1.2. Natural history of genital HPV

Although the incidence of HPV infections is high, most HPV infections are transient (Evander et al., 1995). Most infections (70 to 90 %) become undetectable within 1 to 2 years (Ho et al., 1998; Moscicki et al., 1998; Franco et al., 1999). The exact clearance rates show some variation across studies, as does the definition of clearance with regard to the number of consecutive negative tests required. However, clearance occurs at a relatively constant rate across all age groups (Franco et al., 1999; Syrjänen et al., 2005; Munoz et al., 2009). Prevalent infections take longer to clear than incident infections (Trottier et al., 2008).

Infections that are not cleared by the host are considered to be persistent. Although there is no consensus on the definition, these infections are strongly linked to the development of precancer lesions (Koshiol et al., 2008). Persistence is most commonly defined as detection of the same HPV type on two consecutive follow-up visits, usually 4-6 months apart (Schiffman and Kjaer, 2003). This is, however, less than the mean duration of an incident HPV infection reported in many studies, i.e. 5.1 to 15.4 months (Trottier et al., 2008). New definitions for persistence have been proposed, as for example the one which defines persistence as an infection that lasts longer than the median duration of infection (Munoz et al., 2009). Indeed, women who have HR-HPV detected for at least 1 year versus those whose infections clear are at significantly higher risk of having or developing precancerous cervical lesions (Koshiol et al., 2008). Viral DNA from a single HPV variant, from the HR and LR types, may persist for several years in the female genital tract, although this has been reported to be uncommon (in < 5 % of the cases) (Sycuro et al., 2008). HPV DNA can become undetectable and reappear later (Sycuro et al., 2008). A major unresolved question regarding the natural history of HPV is how often short-term viral clearance leads to long-term viral latency. Reinfections with the same HPV type is rare (Xi et al., 1995; Sycuro et al., 2008)

Infections by HPV 16 or other HR-HPV types persist longer than infections by the LR types (Londesborough et al., 1996; Molano et al 2003, Muñoz et al., 2004; Trottier et al., 2008). A high viral load and coinfections with several HPV types increase the duration of persistence of type-specific episodes (Ho et al., 1998; Woodman et al., 2001; Trottier et al., 2008; Munoz et al., 2009), but the results are controversial (Molano et al., 2003; Kulmala et al., 2007). Besides viral factors, the duration of persistence is modified by host-related determinants. The persistence of HPV increases significantly with age (Hildesheim et al., 1994; Castle et al., 2005). Other host-related factors include the individual's immune response, vegetable intake, condom and OC use, smoking, history of Pap smears, age at first intercourse and parity (Hogewoning et al., 2003; Molano et al., 2003; Richardson et al., 2005; Wang SS et al., 2009). However, the host-related determinants modifying the infection outcome are not yet fully understood. It is still largely unknown what transforms the virus from a passenger into a driver (Koss, 1998).

2.5.2. Oral HPV infections in women

As HPV DNA detection in the genital mucosa, the prevalence of HPV DNA in the oral mucosa varies widely by study population, sampling and detection methods. In a large meta-analysis covering 94 reports published between 1982 and 1997 and including 4680 samples from both men and women, the mean prevalence of HPV was 10.0 % in clinically healthy oral mucosa, 22.2 % in benign leukoplakia, 26.2 % in intraepithelial neoplasia, 29.5 % in verrucous carcinoma and 46.5 % in oral squamous cell carcinoma (Miller and Johnstone, 2001). The prevalence of HPV in healthy oral mucosa varied from 0 % to 60 % in the 27 data sets assessed in this meta-analysis. In the Finnish Family HPV Study, the oral HR-HPV prevalence varied from 16 % to 27 % during a mean of 26 months of follow-up of 329 women (Rintala et al., 2006). In general, the HPV detection rates in samples from the oral mucosa may be much higher in oral rinse samples (12-51 %) and oral scrapings (45 %) than in brush samples (3 %) or frank biopsies (12-23 %) (Lawton et al., 1992; Kellokoski et al., 1992b; Kreimer et al., 2004). Similarly, to emphasize the importance of the effect on detection method, the percentage of HPV DNA in buccal smears was 19 % when PGMY09/11 primers were used, but 74 % when nested PCR was used (Kay et al., 2002). Most oral HPV infections are considered to be latent and asymptomatic. The most common clinical manifestations of oral HPV include focal epithelial hyperplasias, oral papillomas and oral condylomas (Syrjänen, 2003).

2.5.3. Seroepidemiology of HPV

Traditionally, most data on anti-HPV capsid antibodies have been collected from case-control studies on HPV-associated malignancy, in addition to studies on HPV (mainly HPV 16) serology among college students, STD patients and pregnant women (Carter et al., 1996; af Geijersstam et al., 1998; van Doornum et al., 1998; Hagensee et al., 1999; Kibur et al., 2000; Studentsov et al., 2003; Lehtinen et al., 2006; Clifford et al., 2007; Kaasila et al., 2009). Recently, a number of papers providing seroepidemiological data on HPV infections in general populations or placebo groups of ongoing HPV vaccination trials has appeared (Wang et al., 2004; Villa et al., 2006; Dondog et al., 2008; Michael et al., 2008; Skjeldestad et al., 2008). In these studies, the HPV 16 and HPV 18 seroprevalences have varied generally from 1.6 % to 44.4 % and from 4.2 % to 35.6 %, respectively. During pregnancy, HPV 16 and 18 antibodies have been detected in 17-28 % and 10-19 % of the pregnant women, respectively. Few studies have reported seroprevalence rates to LR-HPV types. HPV 6/11 seroprevalence rates have usually varied between 6.6 % to 12.7 % in young women, pregnant or not (Laukkanen et al., 2003; Lehtinen et al., 2006; Villa et al., 2006; Skjeldestad et al., 2008). Significantly higher seroprevalence rates were reported by Van Doornum and colleagues (1998), as 58 % and 48 % of the heterosexual adults with multiple partners had antibodies to HPV 6 and 11, respectively.

The associations between HPV antibodies and HPV DNA status have been poor; at best, only 50-60 % of women with cervical HPV16 infection have been IgG seropositive (Kirnbauer et al., 1994; Viscidi et al., 1997; Carter et al., 2000; Studentsov et al., 2003; Ho et al 2004). IgG seropositivity to HPV-16 L1 is associated with the

lifetime number of sex partners (Dillner, 1996; Wideroff et al., 1996; Viscidi et al., 1997, Silins et al., 2000; Wang et al., 2000; Stone et al., 2002; Studentsov et al., 2003; Dondog et al., 2008; Skjeldestad et al., 2008). Other significant determinants for the presence of HPV 16 antibodies in these studies have variably included, e.g. age, race/ethnicity, level of education, smoking status, use of OCs and presence of clinical HPV lesions. Seroconversion seems to be driven by sufficient antigenic exposure, as it has been associated with either a high viral load or persistent infection (Wideroff et al., 1996; Ho et al., 2004; Wang et al., 2004). When tested with the current assays, serum antibodies to HPV L1 protein are considered to be type-specific markers of past and present infection (Dillner et al., 2007). Serological studies have been valuable in demonstrating time trends is HPV epidemiology, such as the epidemic spread of HPV-16 during the last decades (Laukkanen et al., 2003).

2.5.4. Risk factors for HPV

Epidemiological studies have shown that the key risk factors for a detectable genital HPV infection include age and sexual behavior. The high incidence of HPV infections in young women might be due to maximal exposure and minimal immunity (Castle et al., 2005) and/or increased vulnerability of the adolescent cervix to HPV infection by enhanced cervical maturation (Moscicki, 2005; Hwang et al., 2009). Several studies have demonstrated strong associations between HPV detection and the number of lifetime and recent sex partners (Syrjänen et al., 1984; Ley et al., 1991; Ho et al., 1998; Peyton et al., 2001; Vaccarella et al., 2006a; Dunne et al., 2007). Also, the sexual behavior of the male partner influences a woman's risk to test positive for HPV (Karlsson et al., 1995; Burk et al., 1996; Vaccarella et al., 2006a; Winer et al., 2008).

However, young women are at high risk to test HPV positive in the uterine cervix even after only one male sex partner (Collins et al., 2002; Winer et al 2008). The 1-year cumulative incidence of HPV infection was 28.5 % and increased to almost 50 % by three years among women who were enrolled before or within three months of first intercourse and were censored at the report of a second partner (Winer et al., 2008). It is to be noted that five women (3.8 %) were excluded because they tested HPV positive even before their first intercourse. Marital status, early age at sexual debut, other sexually transmitted infections (STIs), history of GWs, ethnicity and immunosuppressive conditions are also among the risk factors for HPV (Trottier and Franco, 2006). In adolescent females OC use and smoking accelerate cervical maturation, i.e. cause increased cell proliferation, and this might lead to greater vulnerability of the cervix to HPV (Hwang et al., 2009). Maturation of the uterine cervix is also increased during pregnancy, but the effect of parity on HPV detection is unclear (Hernandez-Giron et al., 2005; Vaccarella et al., 2006b). Some studies have reported differences between the risk factor profiles for HR-HPV and LR-HPV types (Franco et al., 1995; Kjaer et al., 1997; Richardson et al., 2000), most notably the lack of correlation with lifetime measures of sexual activity and LR-HPV positivity.

2.5.5. HPV and pregnancy

In recent years, several studies employing molecular detection methods for HPV have evaluated the significance of pregnancy as a risk factor for HPV infection. The detection rates of cervical HPV during pregnancy have varied widely, from 5.2 % to 68.8 % (Arena et al., 2002). Some of the previous studies report higher HPV DNA prevalence rates in pregnant women than in non-pregnant women (Schneider et al., 1987; Fife et al., 1996; Morrison et al., 1996; Hernández-Girón et al., 2005; Bandyopadhyay et al., 2006). Also, the prevalence of HPV in the cervical mucosa has been reported to increase with increasing gestational age and to decline rapidly postpartum (Rando et al., 1989; Fife et al., 1999). However, many of the studies report no difference in the HPV DNA prevalence of pregnant women and non-pregnant women (Soares et al., 1990; de Roda Husman et al., 1995b; Chang-claude et al., 1996; Tenti et al., 1997; Chan et al., 2002) or between different gestational stages (Chang-Claude et al., 1996; Chan et al., 2002; Bandyopadhyay et al., 2006; Banura et al., 2008).

It has been claimed that pregnancy-related hormonal changes and/or immunosuppression may increase the risk of HPV infection. Increased levels of local female steroid hormones were suggested already in 1937 as a cause for the observation that genital warts grow in size during pregnancy (Wilson, 1937). The progesterone-responsive transcriptional activator element present in the genome of at least some HPV types (Gloss et al., 1987; Chan et al., 1989) might play a role in the activation of HPV replication. Pregnancy-related physiological changes such as increased local vascularity or moisture (Oriel, 1971) or enhanced squamous metaplasia at the uterine cervix (Moscicki, 2005) may also increase the risk of HPV infection. Cervical ectopy and squamous metaplasia are most marked during the second and early third trimester and also more pronounced in primigravida than in multigravida women (Jacobson et al., 2000). The immunological control of HPV infections may be naturally altered or repressed during pregnancy (Schneider et al., 1987). One study has indeed reported that the humoral response to HPV16 in pregnant women is decreased (Sethi et al., 1998).

Most of the studies thus far on the effect of pregnancy on HPV detection have been cross-sectional and potential confounding factors have seldom been described. Sexual habits are important risk factors for HPV infection but detailed data on the sexual habits of pregnant women are frequently lacking from the available literature. Longitudinal studies are scarce (Rando et al., 1989; Chang-Claude et al., 1996; Fife et al., 1999). Recent data from prospective studies using sensitive PCR-based assays that allow detection of a broad range of HPV types suggest that pregnancy is not a period of particular vulnerability to HPV infection (Minkoff et al., 2006; Banura et al., 2008), which is at variance from what might have expected based on clinical experience or the earliest molecular studies.

Data on oral HPV infections during pregnancy are scarce. There are case reports on the worsening of maternal respiratory laryngeal papillomatosis during pregnancy

(Helmrich et al., 1992; Scurry et al., 2008). A study of 574 pregnant women during their third trimester has reported a prevalence of HPV in oral cavity of 2.4 % (Smith et al., 2004). Our study group observed previously that oral HR-HPV prevalence was lowest (16 %) during pregnancy and increased steadily after delivery (Rintala et al., 2006).

2.5.6. HPV infections in children

Warts or papillomas are the most common clinical manifestations of HPV infections in childhood. Juvenile onset recurrent respiratory papillomatosis (JORRP), caused mostly by HPV types 6 and 11, is a rare pediatric disease (Mounts et al., 1982; Gissmann et al., 1983). The estimated prevalence in North-America has been reported to be 1.00-3.97 per 100,000 children aged <18 years (Armstrong et al., 2000). The age of onset is highly variable with a mean age being 4 to 5 years (Armstrong et al., 2000; Silverberg et al., 2004). Although JORRP is a rare disease, it is associated with significant morbidity with a high frequency of therapeutic surgeries (Silverberg et al., 2004).

The incidence of anogenital warts in children has steadily increased during the last decades (Oriel, 1992; Obalek et al., 1997; Syrjänen and Puranen, 2000). Since these lesions are not difficult to diagnose, this may reflect a true increase in the incidence in parallel with the current epidemic of genital HPV infections among adults. However, it is possible that increased awareness of the disease also contributes to the figures. In a study including 166 preschool children 5-6 years of age among whom the possibility of sexual abuse was excluded, the prevalence of anogenital warts was 1.8 % (Myhre et al., 2003). The mean age at presentation for anogenital warts in case series of children has ranged from 2.8 to 5.6 years (Sinal and Woods, 2005).

Subclinical and latent HPV infections occur also in the pediatric population. Using sensitive molecular detection methods for HPV, both LR-HPV DNA and HR-HPV DNA has been detected in samples from the oral cavity or external genitalia of children. HPV DNA detection rates in genital mucosa of infants have varied from 0 % (Smith et al., 1995) to 53 % (Kaye et al., 1994) and in the oral mucosa from 0 % (Watts et al., 1998) to 61 % (Puranen et al., 1997). A recent systematic review of vertical transmission in asymptomatic pregnant women, that included nine cohort studies, established a combined HPV prevalence rate of 6.5 % (95 %CI 5.0-8.0) among the 2113 infants assessed (Medeiros et al., 2005). The debate still continues whether HPV DNA positivity in infants represents true HPV infections or merely transient colonisation with maternal HPV infected cells (Dillner et al., 1999). Studies demonstrating persistent HPV DNA, active virus transcription, or HPV antibodies support the possibility of true infections in children (Kaye et al., 1996; Rice et al., 2000; Dunne et al., 2005; Marais et al., 2007)

The few studies reporting HPV detection in the genital mucosa of prepubertal children report very low HPV prevalence rates (0 % to 3 %) (Koch et al., 1997; Myhre et al., 2003). In other studies, again, some claim that HPV is common among prepubertal children, as HPV detection rates in the genital mucosa has varied from 13.6 % to 25 %

(Powell et al., 2003; Doefler et al., 2009) and in the oral mucosa from 8.7 % to 51.7 % (Puranen et al., 1996; Rice et al., 2000, Summersgill et al., 2001; Kojima et al., 2003). HPV DNA in children has generally been detected with highly sensitive PCR based methods. Recently, Doerfler and co-workers (2009) performed HPV testing with the HC2 test from the anogenital region of 110 girls aged 4-15 years who did not have any history of sexual abuse. Twenty of the girls were HPV positive (18 %); LR-HPV DNA was detected in four children (3.6 %) and HR-HPV in 15 children (13.6 %). In addition, two girls testing positive for HPV DNA had clinically apparent warts (Doerfler et al., 2009).

2.6.   TRANSMISSION OF HPV

Mucosal HPV infections are considered to be transmitted primarily sexually, usually through sexual intercourse by genital-to-genital contact, during which epithelial microabrasion and tear commonly take place and facilitate infection. Although HPV is regarded as one of the most common sexually transmitted infections (Dunne et al., 2007), its transmission dynamics are not clearly understood.

HPV is a strictly intraepithelial pathogen with no viremic phase in the course of infection. However, HPV DNA is present in the mononuclear cells of the peripheral blood (PBMCs) (Pao et al., 1991; Tseng et al., 1992). Recent evidence supports the concept of non-sexual spread of HPV. Papillomvirus DNA was detected in banked, frozen blood cells from pediatric HIV patients and in fresh blood cells from healthy donors (Bodaghi et al., 2005).

2.6.1.   Sexual transmission

The venereal nature of HPV originates from observations indicating that the behavior of GWs and even cervical neoplasia resembles that of sexually transmitted diseases. Sexual transmission as the main mode for GWs was first proposed in 1954 by Barrett and colleagues, who observed the frequent occurrence of penile warts in U.S. servicemen returning from the Far East and that many of the spouses of these men developed vulval warts, in turn (Barrett et al., 1954). Already in 1842 the Italian physician Rigoni-Stern reported that cancer of the womb was more common among married women, widows and prostitutes than in nuns, and he concluded that this type of cancer is probably related to sexual contacts. Although some of the conclusions of this specific paper have been seriously questioned later (Griffiths, 1991), altered sexual behavior altered the risk of CC in later studies (Towne et al., 1955; Taylor et al., 1959; Rotkin, 1967) and this linked CC to sexually transmitted agents. After the discovery of the HPVs as the etiologic agent of GWs and CC and the introduction of sensitive and specific HPV detection methods, epidemiological observations have corroborated the notion that HPVs are sexually transmitted.

The probability of HPV transmission through sexual contact is not known. There are no published reports based on empirical data on the transmissibility of HPV, but it is thought to be high since HPV infection is very prevalent (Burchell et al., 2006b). A study of transmissibility of GWs conducted before HPV was identified as the causal

agent observed that 64 % (62/97) of those exposed to GWs subsequently developed condylomata themselves during 9 months of observation (Oriel, 1971). In the absence of empirical data, mathematical models of HPV transmissibility based on epidemiological data on the incidence, prevalence or seroprevalence of HPV have been created. These studies suggest that HPV is more transmissible than other viral STIs, but is comparable to bacterial STIs. The median probability of HPV transmission in these models has been 40 % (range 5-100 %) per coital act (Burchell et al., 2006a) or 60-80 % per-partner male-to-female (Hughes et al., 2002; Barnabas et al., 2006). It is to be noted, that the possibility of non-sexual transmission of HPV has not been taken into account in these models. HPV transmission occurs even in moderately sexually active populations, and hence, HPV infections seem to occur without the presence of core groups or super-spreaders, the traditional concepts of applied theories in STI epidemiology and transmission (Bosch et al., 2008).

Factors affecting the rate of HPV spread in the population are insufficiently known. Although published rates of infection are similar for men and women (Vaccarella et al., 2006c), the concordance of different HPV genotypes between sex couples has been relatively poor in most studies (Hippeläinen et al., 1994; Castellsaqué et al., 1997; Franceschi et al., 2002). Viral load is likely to affect the rate of infection (Bleeker et al., 2005). The effect of condoms to protect from HPV has been a matter of debate. A meta-analysis of 20 studies suggests that using condoms may not reduce the risk of becoming HPV DNA-positive, but that it may offer protection against clinical lesions (Manhart and Koutsky, 2002). The mechanism of this protection is not yet known, but changes in viral load, reinfection probability, local immune responsiveness and cervical inflammation are probably involved (Hogewoning et al., 2003; Shew et al., 2006). Data suggest that HPV is also transmissible through nonpenetrative sexual contact (Marrazzo et al., 1998; Winer et al., 2003), but this is generally considered to be less common.

Data on the oral mucosa as a reservoir for HPV transfer to the genital area are conflicting. Studies on type specific HPV infections in the oral and genital mucosa have shown poor type concordance (Kellokoski et al., 1992a and b; Cañadas et al., 2004; Smith et al., 2004). In the study by Winer and collagues (2003) oral HPV infections among females were not clearly associated with oral sex. The Finnish Family HPV Study showed previously that persistent oral HR-HPV infection of the spouse is a significant risk factor for persistent oral HR-HPV infection. The outcome of oral HR-HPV infection of either spouse was not related to oral sex habits (Rintala et al., 2006). Recently D'Souza and collagues (2009) reported that oral sex and open-mouthed kissing are associated with the development of oral HPV infection.

2.6.2. Non-sexual transmission

The detection of HPV in virgins, infants and children suggests that sexual contact cannot be the only mode of viral transmission. Although viral transmission may occur in children through sexual abuse, there is growing evidence suggesting alternative modes of transmission (Gutman et al., 1993; Armstrong and Handley, 1997; Syrjänen

and Puranen, 2000; Sinal and Woods, 2005). Potential non-sexual transmission modes include vertical transmission from mother-to-child prenatally or at delivery, horizontal spread through auto- and heteroinoculation and indirect transmission from contaminated fomites (Gutman et al., 1993). Transmission via blood, saliva or breast milk has also been proposed as options but has been considered improbable (Cason et al., 1998).

2.6.2.1. Vertical transmission

Evidence for vertical transmission

HPV can be transmitted from mother to child from the infected birth canal. Maternal genital condyloma during pregnancy is recognized as a reservoir of HPV causing the JORRP syndrome (Quick et al., 1978; Gissmann et al., 1983). In a retrospective cohort study of Danish births between 1974 and 1993, maternal GWs during pregnancy conferred a 231-fold increased risk of JORRP in the child (Silverberg et al., 2003). However, the majority of children who developed JORRP have been born to mothers with no history of GWs during pregnancy, and these mothers might have had a sub-clinical infection. Children whose mothers had a history of GWs have been reported to develop JORRP at an earlier median age than children without such a history (4.3 vs 5.9 years) (Silverberg et al., 2004). Similarily, vertical transmission is supported by detection of anogenital warts early in childhood especially when they harbor mucosal HPV types or with a positive maternal history for GW or abnormal cervical cytology (Obalek et al., 1997; Sinclair et al., 2005; Marcoux et al., 2006).

Routes of vertical transmission

Vertical transmission is thought to result mainly from the close contact of the fetus with infected cervical and vaginal cells of the mother during birth. However, there is also data favoring intrauterine transmission, as HPV-induced lesions are occasionally present already at birth (Hajek, 1956; Tang et al., 1978; Rogo and Nyansera, 1989; Obalek et al., 1997; Marcoux et al., 2006). Also, HPV DNA positive infants have been born to HPV negative mothers (Cason et al., 1995; Mazzatenta et al., 1996; Puranen et al., 1997). Cesarean section does not completely protect newborns against HPV (Obalek et al., 1997; Puranen et al., 1997; Tseng et al., 1998; Silverberg et al., 2003). Intrauterine transmission of HPV could ascend from the maternal genital tract through microtears in fetal membranes, with blood through the placenta or even by semen at fertilization **(Figure 3)**. HPV DNA has been detected in the amniotic fluid, fetal membranes, cord blood and placental trophoblast cells (literature listed in **Appendix 1**). HPV 11, 16, 18 and 31 are able to complete their life cycle in cultured placental trophoblasts (Liu et al., 2001; You et al., 2008). Infection of trophoblasts by mucosal HPV types has been postulated to account for some spontaneous abortions (Manavi et al., 1992; Hermonat et al., 1997).

Figure 3.

Potential routes of viral infection in utero. Modified from Levy, 2007.
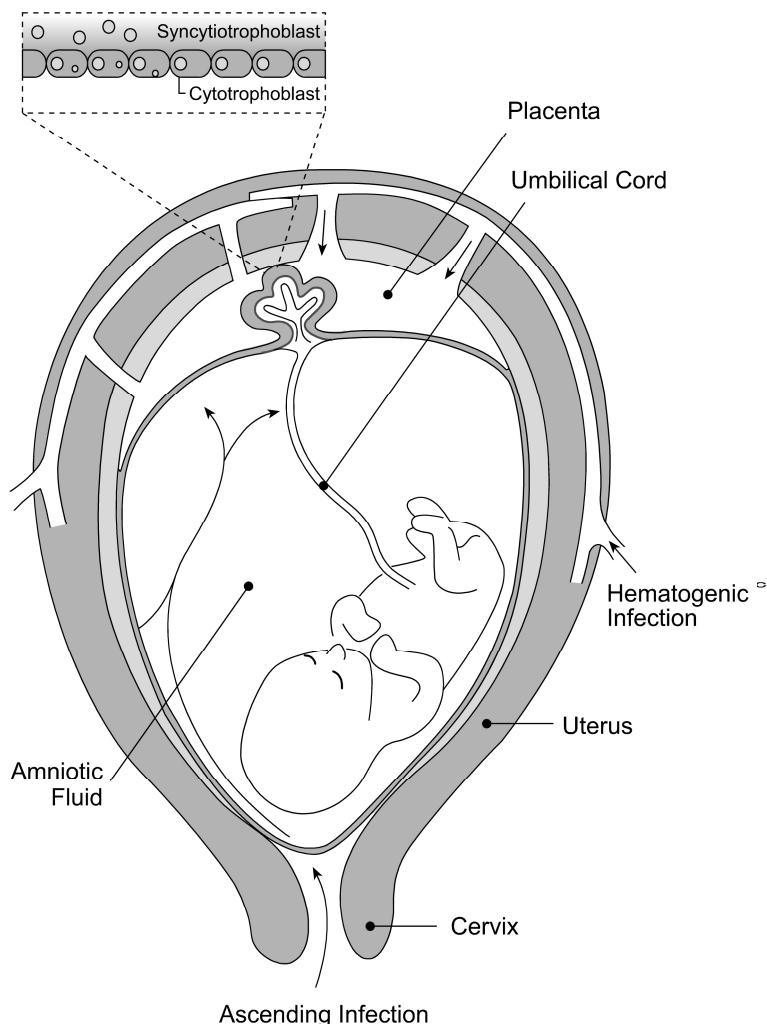
Vertical transmission could also take place postnatally through breast milk. Vertical transmission of HIV-1 or cytomegalovirus infection through lactation is well established (Dworsky et al., 1983; Kourtis et al., 2003). Also, rubella, herpes simplex, hepatitis B and C, HHV6 and Epstein-Barr virus (EBV) have been detected in human milk, but seldom infects the infant (Michie and Gilmour, 2001). Although HPV is frequently detected in the oral mucosa of infants (Syrjänen and Puranen, 2000), the possibility of HPV presence in breast milk has not been assessed.

Rates and risk factors of vertical transmission

A recent systematic review of vertical transmission from asymptomatic pregnant women showed that when mothers were HPV positive, 18.3 % of the newborns (46/252) were also HPV positive at birth (Medeiros et al., 2005). This corresponds to a 4.8 fold (95 %CI 2.1-10.9) risk of vertical transmission when compared to newborns with HPV negative mothers (16/562 = 2.8 %). Medeiros and colleagues reported a

higher risk of HPV exposure after vaginal delivery than after cesarean section (RR 1.8; 95 %CI 1.3-2.4), 27 % versus 12 %, respectively. Kaye and colleagues studied 15 pregnant women with HPV 16 infections and observed that the eight mothers who transmitted infection to their infant had significantly higher viral loads than those who did not. A previous history of abnormal cervical smear or genital warts seemed to be related with transmission of viruses to infants (Kaye et al., 1994). In the retrospective cohort study by Silverberg and colleagues (2003) seven out of every 1000 births with a maternal history of GWs during pregnancy resulted in laryngeal lesions. In women with GWs, delivery exceeding 10 hours were associated with a two-fold risk of disease. Other risk factors for vertical transmission are not known.

2.6.2.2. *Horizontal transmission*

Recent studies suggest that vertical transmission may not be the only source of HPV infection in children and that horizontal mother-to-child transmission is possible (Rintala et al., 2005b; Castellsagué et al., 2009). Horizontal transmission by close contact with an infected person or via virus-contaminated surfaces is the main mode of virus spread for skin HPVs, but for mucosal HPVs this mode is not well established. Casual digital contact is considered as a likely source of viral exposure also for mucosal HPV types. Anogenital warts in children occur as a result of hand warts in the child or relatives (Oriel, 1992; Handley et al., 1997). The proportion of cutaneous HPV types found in children's GWs is higher than in adults' GWs (15 % vs 2 %, respectively) (Fairley et al., 1995). Also, carriage of mucosal HPV DNA by hands has been demonstrated. Sonnex et al (1999) found that 27 % of subjects with genital infections had the same HPV type detected on their fingers. Furthermore, HPV 16 and 35 have been detected in skin lesions of the periungal area with presumable digital autoinoculation (Moy et al., 1989; Rüdlinger et al., 1989). Recently, we reported from the Finnish Family HPV study that persistent oral HPV infection in the child during follow-up of 26 months was associated with oral HPV in parents and hand warts in mothers, supporting HPV transmission via the saliva and hands (Rintala et al., 2005b).

3.   AIMS OF THE PRESENT STUDY

The purpose of the Finnish Family HPV Study is to evaluate HPV infection dynamics within families. The main focus of the present study is on HPV infections in mothers, who were enrolled into the study at their third trimester and followed for 36 months after delivery. The presence of HPV DNA in embryonic tissues, placenta and umbilical cord blood was also analyzed. The specific aims were:

1. to evaluate current and past HPV exposure in mothers followed for three years by assessing serum antibodies to HPV6, 11, 16, 18 and 45, and to explore the longevity of antibodies and determinants of HPV seroprevalence and seroconversion

2. to study the effects of pregnancy on genital and oral HR-HPV carriage and persistence and to evaluate the cofactors for HR-HPV carriage and persistence in a longitudinal setting among women with one or two pregnancies

3. to analyze the presence and possible impact of HPV DNA in placenta, umbilical cord blood and breast milk

4.   SUBJECTS, MATERIALS AND METHODS

4.1.   PARTICIPANTS

The Finnish Family HPV Study is a cross-sectional and prospective cohort study to study the dynamics of HPV infection in mothers, fathers and their newborn infants. It is a collaborative study between the Institute of Dentistry and the MediCity Research Laboratory, Faculty of Medicine, University of Turku and the Department of Obstetrics and Gynecology, Turku University Central Hospital. Between 1998 and 2002 a total of 329 pregnant women in their third trimester were enrolled to the study at the Maternity Unit of the Turku University Central Hospital, Turku, Finland. Fathers (n=131) and children (n=331) were also recruited to the study. The Research Ethics Committee of the Turku University Central Hospital approved the study design before initiation (#3/1998), and all parents gave written consent. The HPV status of the parents was not examined before recruitment.

The number of subjects and samples in the four original reports included in the present thesis are summarized in **Table 3**. Overall, the number of the subjects included in the different studies is based on the available material. To study the effect of a second pregnancy on HPV detection (**II**), a subset of 178 women of the whole cohort were included. First, all women from the Finnish Family HPV Study who had a new pregnancy ongoing during any of the follow-up visits scheduled at 6, 12, 24 and 36 months after delivery of the index pregnancy were identified from medical records. Only the women who were pregnant for the first time at enrollment and who completed the entire follow-up were included (**II**). There were 78 women who had a second pregnancy ongoing during any of the follow-up visits. A control group consisted of 100 women who did not have second pregnancy during the 36-month follow-up period.

Table 3. Study design: subjects, sample sites and the number of samples at different time points.

Paper	Number of subjects	Mean age (range)	Sample sites	Pre	At del	3 days	2 mo	6 mo	12 mo	24 mo	36 mo
I	290 women	25.6 (18-38)	Sera	289					284	262	259
			Pap smear	288							
			Cervical	286							
			Oral	287							
II	100 women without pregnancy at follow-up	25.0 (18-34)	Cervical	99					98	96	98
			Oral	99				98	100	96	97
	78 women with 2nd pregnancy at follow-up	25.2 (18-38)	Cervical	78					18	36	21
			Oral	78				2	17	36	21
III	315 women	25.5 (18-46)	Placenta		315						
			Pap smear	305							
			Cervical	298							
			Oral	299							
			Blood	19							
	311 infants		Cord Blood		311						
			Genital		304						
			Oral		306						
IV	223 women	25.1 (18-38)	Milk			223	183*				
			Cervical	220			213	197	196		
			Oral	218			148		196		
	87 male spouse	28.8 (19-46)	Oral	87			74	75	76		

*Previously unpublished data.

4.2.   DEMOGRAPHIC DATA AND SAMPLE COLLECTIONS

4.2.1.   Demographic data (I-IV)

Demographic data including the parents' social status, obstetric and gynecological history and risk factors for HPV infections were obtained by questionnaires filled in at the first postpartum visit (2.6 months, range 1.5-4.9 months after delivery).

4.2.2.   Genital scrapings (I-IV)

Cervico-vaginal scrapings

Cervical scrapings were taken from the mothers before delivery and at month 2, 6, 12, 24 and 36 after delivery as described earlier (Rintala et al., 2005a). The sampling brush (Cytobrush®; MedScand, Malmö, Sweden) was placed in a tube with 0.05 M phospate buffered saline (PBS) with 100 μg of gentamycin. The samples were immediately frozen at -20°C and stored at -70°C.

Genital scrapings from neonates

Genital samples were taken from the neonates at delivery with three to five gentle scrapes of a small Cytobrush® from the labia major of girls and from the preputium and scrotum of boys as described (Rintala et al., 2005a; 2005b). The brush was placed in 70 % ethanol, frozen at -20°C and stored at -70°C.

4.2.3.   Oral scrapings (I-IV)

Oral scrapings were taken from the buccal mucosa of both cheeks without touching the tongue by using a small brush (Cytobrush®). The brush was placed in a tube containing 70 % ethanol, frozen at -20°C and stored at -70°C (Rintala et al., 2005a).

4.2.4.   Pap smears (I, III)

A routine Pap smear was taken from all women at baseline and at 12, 24 and 36 months, using the conventional three-sample technique (vagina, ectocervix, endocervix) with two wooden spatulas and a Cytobrush®. The slides were fixed with a preservative (Spray-Cyte; Becton Dickinson and Company, Sparks, Md., USA). The Pap smears were analyzed according to the Bethesda system (National Cancer Institute Workshop 1989, Solomon et al 2002). The assesment included quality of the smear, infections and cytological changes and their localization with a descriptive diagnosis. The samples were also reported by Papanicolaou classification (class I-V) and degree of purity (1-3).

4.2.5.   Peripheral blood (I, III)

Blood samples were taken from the mothers at baseline, and at 12, 24 and 36 months of follow-up. Separated sera were divided into three 1 ml aliquots, and stored first at -20°C no longer than one week and than at -70°C until analysis.

4.2.6. Placental and umbilical cord blood samples (III)

Placental and umbilical cord blood samples were taken immediately after delivery in the delivery room. Two representative samples covering all tissue layers were taken from the central part of the maternal side of the placenta: one sample was immediately frozen and kept at -70°C until DNA extraction and HPV testing, the other was fixed in neutral 10 % formalin and processed in to paraffin blocks for routine histology and ISH. Umbilical venous cord blood was obtained while the placenta was still *in situ* using vacuum EDTA-tubes and frozen at -70°C immediately.

4.2.7. Breast milk (IV)

Mothers collected the milk samples manually three days postpartum at the hospital into a 3 ml plastic container. This procedure was done apart from feeding the baby. The container was sealed by a midwife trained for the project and the samples were immediately frozen and stored at -70 ºC. Two months postpartum breast milk was collected at home.

4.3. DNA ISOLATION

DNA was extracted from genital and oral scrapings with the high salt method of Miller and coauthors (1988) as described earlier (Rintala et al., 2005a). Samples were lysed in lysis buffer (10mM Tris, 400 mM NaCl, 100 mM EDTA, 1 % SDS) and digested overnight at 37°C with proteinase K (10 μg/ml). After digestion, proteins were precipitated with saturated NaCl and ethanol. The DNA was dissolved in 50μl water, mixed for 15-30 minutes and stored at -20°C.

A piece of frozen placenta was first minced with a cutter on ice on a sterile petri dish before collection to lysis buffer. DNA was then extracted with the high-salt method.

From milk and blood samples, DNA was extracted using the high pure PCR template preparation kit (Roche Diagnostics GmbH, Penzberg, Germany), according to the manufacturer's instructions. The samples were first centrifuged for 5 min at 12000 rpm to pellet the cells, after which the cells were lysed with proteinase K in Tissue Lysis Buffer at 55°C for 1 hour. The lysate was then applied to a filter tube and passaged through a glass fleece by centrifugation. Residual impurities were removed by a series of rapid wash-and-spin steps.

4.4. DETECTION OF HPV DNA

4.4.1. Nested PCR

Extracted DNA was used for HPV testing by nested PCR using MY09/MY11 and GP05+/GP06+ (Anttila M et al., 1999). Cervical samples were tested by conventional GP05+/GP06+ PCR. PCR was done in a 50 μl reaction mixture using AmpliTaq Gold DNA polymerase (Perkin Elmer, NJ, USA). Amplification was started by an initial denaturation at 95°C for 10 minutes. For the first primer set 30 cycles of denaturation

at 95°C for 30 seconds, annealing at 55°C for 55 seconds, and elongation at 72°C for 60 seconds were used. For the second primer set 40 cycles of denaturation at 95°C for 60 seconds, annealing at 40°C for 60 seconds, and extension at 72°C for 90 seconds were done. The sensitivity of the PCR method was approximately 20 copies of HPV.

Confirmation of PCR

PCR products were run in 2.0 % agar gels (DNA agar, MBI, Derventway Delta, Canada), transferred to a nylon membrane (GeneScreen Plus; PerkinElmer, Boston, MA, USA) and hybridized with a digoxigenin-labeled (DIG Oligonucleotide 3´-End Labeling Kit; Roche Diagnostics GmbH, Penzberg, Germany) HR types 16, 18, 31, 33, 35, 39, 45, 51, 54, 56 and 58 HPV oligoprobe cocktail (Anttila M et al., 1999).

4.4.2. HPV genotyping

HPV genotyping of baseline cervical and oral samples and of the month 2 breast milk samples was done using the fluorescent bead array of 24 HPV types in PCR-amplified biotin-labeled samples according to Schmitt and coworkers (2006; Multimetrix, Regensburg, Germany). This method combines PCR with hybridization to fluorescence-labeled polystyrene suspension. The assay can detect the following 24 HPV types: LR-HPV 6, 11, 42, 43, 44, 70; HR-HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82; and also probable HR-HPV types 26, 53, 66, 70. The manufacturer's instructions were followed, except that the reactions were done in 50 μl instead of 100 μl. At the final step before reading in Luminex analyzer, 100 μl of blocking buffer was used. As target DNA, the PCR products from HPV testing were used which were now biotinylated by re-amplification with GP05+/biotin GP06+ primers. The median reporter fluorescence intensity (MFI) of at least 100 beads was computed for each bead set in the sample. The cutoff value was defined for each HPV probe individually as follows: 1.5 times the background MFI + 5 MFI. If the sample tested HPV16-positive, the original sample DNA was re-tested with an in-house, bead-based HPV16 genotyping assay. This re-testing was done to identify samples which might have become contaminated with HPV 16 during previous testing or re-amplification.

4.4.3. Sequencing

All HPV positive PCR samples from study III and IV (placental, umbilical cord blood and breast milk samples) were sequenced by ABI PRISM Big Dye Terminator Cycle Sequencing Kit, and automated on ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, U.S.A).

4.4.4. In situ hybridization

All placental samples positive for HPV 6 or HPV 16 were analyzed with tyramide-amplified ISH to identify the placental cells harboring HPV DNA. For ISH, 6 μm thick sections were cut from formalin-fixed, paraffin-embedded placental samples. After proteolytic treatment with proteinase K (0.5mg/ml) for 15 minutes at 37°C, the sections were hybridized with biotinylated HPV 6 and HPV 16 whole genome probes under high

stringency. Following hybridization, the sections were washed as follows: twice at 2 x SSC for 5 min at room temperature (RT), twice at 0.2 x SSC for 5 min at 40°C and then once at 2 x SSC for 5 min at RT. The samples were also hybridized with biotinylated pBR322 (negative probe control) and with hybridization mixture without any probe. Tyramide signal amplification was done with TSA ^{TM} Biotin System (Perkin Elmer, Boston MA, USA). The TSA Biotin System utilizes horseradish peroxidase to catalyze the deposition of the biotin-labeled tyramide. In the sections counterstained with Mayer's hematoxylin, the cells harboring specific HPV signals were identified. In our hands, the sensitivity of this method is approximately one to two copies of HPV per cell.

4.5. MEASURING HPV ANTIBODIES

Antibodies to the major capsid protein L1 of HPV types 6, 11, 16, 18 and 45 were analyzed by a multiplex serology method based on glutathione *S*-transferase (GST) fusion protein capture to fluorescent beads as described earlier (Waterboer et al., 2005; 2006). Briefly, bacterially expressed GST-L1-tag fusion proteins were affinity-purified in situ through binding to glutathione casein-coated fluorescence-labelled polystyrene beads. Spectrally distinct bead sets carrying different viral antigens were individually washed and subsequently mixed. Before mixing with the beads, sera were preincubated at 1:50 dilution on a shaker for 1 h at RT. Serum preincubation buffer contained PBS, 1 mg/ml casein, 2 mg/ml lysate from bacteria expressing GST-tag alone to block antibodies directed against residual bacterial proteins and GST-tag, 0.5 % polyvinylalcohol (PVA, Sigma-Aldrich), 0.8 % polyvinylpyrrolidone (PVP, Sigma-Aldrich) and 2.5 % Superchemiblock (CBS-K, Millipore, Billerica, MA, USA) to suppress nonspecific binding of antibodies to beads. Each 50 μl of the preincubated serum dilution and mixed beads (3000 per set) were then combined and incubated. Bound antibodies were detected with biotinylated goat-anti-human IgG secondary antibody (Dianova) and fluorescent detection conjugate (streptadivin-R-phycoerythrin, Molecular Probes). Reporter fluorescence of the beads was quantified with the Luminex analyzer and expressed as median fluorescence intensity (MFI) of at least 100 beads per set per serum. Antigen-specific reactivity was calculated by subtracting the serum reactivity to GST-tag without intervening viral antigen (background) from the total MFI value.

Sera were scored as positive when the antigenspecific median fluorescence intensity (MFI) values were greater than the cutoff level 200 MFI or 400 MFI (stringent) for L1 antigen of individual HPV types (Michael et al., 2008). Seroconversion was defined by two conditions: 1) at least 2-fold increase of the previous serum value, and 2) MFI value over the cut-off 200 MFI or 400 MFI (stringent). Similarly, antibody decay was defined by two conditions: 1) at least 2-fold decrease of the previous serum value, and 2) fall of the MFI value below the cut-off 200 MFI or 400 MFI (stringent).

4.6. STATISTICAL ANALYSES

Statistical analyses were performed using the SPSS$^{®}$ (SPSS, Inc., Chicago, USA) and STATA (Stata Corp., Texas, USA) software packages (SPSS for Windows, version

16.0.2 (**I-II**), version 12.0.1 (**III**), version 14.01 (**IV**) and STATA/SE 10.1 (I-II). Frequency tables were analyzed using the χ^2-test, with a likelihood ratio (LR) or Fisher's exact test for the assessment of significance levels between categorical variables. Odds ratios (OR) with 95 % confidence intervals (95 % CI) were calculated where appropriate. Differences between means of continuous variables were analyzed with non-parametric tests (Mann-Whitney, Kruskal-Wallis) or ANOVA (analysis of variance), after testing for normal distribution.

Predictors of genital and oral HR-HPV carriage (**II**) were calculated in univariate and multivariate regression models, with crude and adjusted Odds Ratios (OR) and 95 % CI, respectively. For all multivariate models, several covariates assessed at baseline, previously shown or implicated as risk factors of HPV infections in our cohort, were considered (Rintala et al., 2005a; 2006). Univariate survival analysis for the outcome measures (clearance, incident HPV) was based on the Kaplan-Meier method, where stratum-specific outcomes were compared using log-rank statistics. To adjust for covariates, Cox's proportional hazards regression model was used, including the covariates mentioned above. The validity of proportional hazards assumption was controlled by log-minus-log plots.

To analyze the influence of pregnancy and potential confounders on HR-HPV carriage and persistence over time (**II**), a generalized estimating equation (GEE) model approach was used, clustered by women-ID and stratified by oral and genital infections (Diggle et al., 1994; Hardin and Hilbe., 2003). GEE adjusts for the serial correlation within subjects due to the longitudinal nature of the data by modeling the covariance structure within subjects (Zeger and Liang, 1986). The dependent variable was binomial (presence/absence of HR-HPV), and hence the logit link function was used. The exchangeable working correlation structure with a robust variance estimator to account for within-subject correlation was selected as the best-fitted covariance pattern, using the Quasi-likelihood Information Criterion (QIC) (Hardin and Hilbe, 2003). In the prospective assessment of oral and genital HR-HPV persistence/transition, women (panel variable) who were HR-HPV-positive at a specific visit (t) were considered having persistent HPV, if their subsequent assessment (t+1) was also HR-HPV positive, and having transient HPV, if the subsequent visit was negative. In this analysis, we assumed that HPV persistence depends on time since previous sample, and therefore, a time variable was included as a covariate in these GEE models.

The GEE model approach was also used to analyze the influence of covariates on seroprevalence and seroconversion, clustered by woman-ID and stratified by the five HPV types (**I**, Diggle et al., 1994, Silins et al., 2000). The dependent variable was binomial (seropositive +/-; seroconversion +/-), and hence the logit link function was used. Because HPV seroconversion depends on time since previous sample, a time variable was also included as a covariate in these GEE models. In multivariate GEE models, we entered several covariates previously shown or implicated as risk factors of HPV infections in our cohort (Rintala et al., 2005a). All statistical tests performed were two-sided and declared significant at p-value <0.05.

5. RESULTS

5.1. HPV SEROPREVALENCE IN MOTHERS (I)

We studied the presence of serum antibodies to HPV 6, 11, 16, 18 and 45 L1 protein in 290 mothers sampled before delivery and at 12, 24 and 36 months of follow-up to examine the dynamics of HPV serology. Also, multiplex HPV genotyping of the baseline oral and genital scrapings was performed. The key demographic characteristics of the 290 mothers were as follows. Altogether, 47.2 % (128/271) of the mothers were married and 45 % cohabited; 7.7 % were single or divorced. Only seven (2.6 %) had started their sexual activity before 13 years of age, while the majority (56.8 %) had their first sexual intercourse between 14 years and 16 years of age. Approximately every fourth of the mothers reported one or two lifetime sexual partners, where as every fifth reported more than 10 partners. Over 90 % of the women had sometimes used oral contraception (OC), and nearly half of them (46.3 %) had initiated OC use between age 14 and 16. 50.2 % (135/269) were current or past smokers. History of any STD and previous GWs were reported by 20.3 % and 27.3 % of the women, respectively. Oral warts were reported only by eight women, whereas 160 women had had skin warts, with 37.5 % of the warts appearing on the hands, 38.8 % on the feet and 23.8 % on multiple sites.

At baseline, pregnant women had HPV antibodies to the five HPV types at cut-off 200 MFI as follows: HPV6, 53.3 %; HPV 11, 21.5 %; HPV 16, 34.9 %; HPV 18, 21.5 % and HPV 45, 9.0 %. When the more stringent cut-off (400 MFI) was used, the antibody profiles remained similar, but the level of prevalence of each L1 antibody dropped on average by 15-20 % (except for HPV 18). There was a slight increase in seropositivity to HPV 6, 11 and 16 at a cut-off 200 MFI at the 12 and 24 months follow-up visits, reverting back to the level of the baseline status at the 36 months visit, 53.3 %, 15.1 %, 28.6 %, 23.6 % and 7.3 %, respectively. The same trend for increased seroprevalence at the 12 and 24 months visits persisted with the more stringent cut-off, except for HPV 18.

5.1.1. Predictors of seropositivity

Overall, 16.4 % (47/286) and 17.8 % (51/287) of the pregnant women were positive for genital and oral HPV, respectively. At the same time, 34 (11.8 %) had abnormal cytology defined as Pap class II in their cervical smear, of whom 27 % (9/33) were HR-HPV-positive. HPV DNA typing (of 297/322 women) showed that collectively, HPV6, 11, 16, 18 or 45 DNA was detected in 16.1 % and 21.5 % of the baseline cervical and oral samples, respectively. HPV6 DNA was present in 2.2 %, HPV11 in 1.2 %, HPV16 in 9.3 %, HPV18 in 2.5 %, and HPV45 in 1.6 % of the cervical samples. In the oral mucosa HPV6 DNA was present in 2.4 %, HPV11 in 0.4 %, HPV16 in 21.5 %, HPV 18 in 0.7 % and HPV 45 in 0 % of the samples. The type-specific concordance between HPV DNA detection and seropositivity was poor to modest. The highest concordance was detected for HPV 16: 4.9 % and 9.0 % for genital and oral samples, respectively. Of all women who had a HPV 16 positive genital and/or oral sample, 12.1% were HPV 16 seropositive.

Altogether, HPV seropositivity was not associated with HPV DNA detection in the uterine cervix or oral cavity at baseline.

The significant demographic determinants associated with the seropositivity to LR-HPV and HR-HPV types at baseline visit were generally similar in time-dependent GEE model adjusted for other covariates. There were four independent predictors of seropositivity to LR-HPV: age at onset of sexual activity (OR 0.4, 95 % CI 0.25-0.74, P=0.002), lifetime number of sexual partners (OR 1.5, 95 % CI 1.01-2.31, P=0.047), history of GWs (OR 2.4, 95 % CI 1.13-4.94, P=0.023) and being seropositive for HR-HPV (OR 3.2, 95 % CI 1.72-5.95, P=0.0001). Seropositivity to HR-HPV types was significantly associated with the lifetime number of sexual partners (OR 1.8, 95 % CI 1.17-2.62, P=0.006), history of GWs (OR 2.2, 95 % CI 1.12-4.27, P=0.021) and being seropositive for LR-HPV (OR 3.1, 95 % CI 1.66-5.75, P=0.0001). The baseline seroprevalence of anti-HPV-antibodies was not significantly related to age, Pap smear result, marital status or smoking history.

5.2. SEROCONVERSION AND ANTIBODY DECAY

Seroconversion and antibody decay to the five HPV types was analyzed by Kaplan-Meier analysis (**I**). The results are summarized in **Table 4**. In total, 39.7 % of the mothers had seroconverted to HPV 6 and/or 11. Half of the mothers with HPV seroconversion (67/134) showed it only for one HPV type, while the other half seroconverted for multiple HPV types. In the post-hoc least significant difference tests, the time to seroconversion for HPV 18 was significantly longer than that for HPV 16 (p=0.007), the difference to seroconversion times of other HPV was nonsignificant. Cumulative seroconversion to HPV 6 was markedly more frequent than that to any other types, reaching rates that are almost twice as high as for any other HPV. Cumulative antibody decay between the five HPV types was not significantly different (log-rank, p=0.130).

Table 4. HPV seroconversion and antibody decay in mothers during median follow-up time of 37.2 months after delivery

HPV type	Seroconversion		Decay of antibodies*	
	Rate %	Mean time † (95 % CI)	Rate %	Mean time † (95 % CI)
HPV 6	26.7	20.3 (18.2 – 22.3)	2.3	34.1 (27.6 – 40.7)
HPV 11	13.9	20.8 (17.7 – 23.9)	4.0	28.9 (21.8 – 36.1)
HPV 16	17.0	18.2 (15.3 – 21.1)	5.3	32.9 (27.1 – 38.6)
HPV 18	16.8	23.8 (20.5 – 27.1)	4.5	27.2 (19.3 – 35.1)
HPV 45	6.6	20.7 (16.1 – 25.4)	1.5	35.8 (32.4 – 39.2)

*among initially seropositive women; † months calculated from baseline visit; CI = confidence interval

5.2.1. Predictors of seroconversion

The predictors of seroconversion to LR- and HR-HPV types were analyzed in a time-dependent GEE model, which adjusts the serial correlation within subjects in longitudinal data (Zeger and Liang, 1986). In the univariate GEE model, the only

significant predictor of seroconversion to LR-HPV was being unemployed versus being a student or employed. For seroconversion to HR-HPV, three variables were significant predictors in the univariate model: baseline genital HR-HPV status (HR-HPV-negative mothers were less likely to convert, P=0.031), marital status (unmarried mothers were more likely to convert, P=0.023) and lifetime number of sexual partners (linear relationship, P=0.004).

After adjustment for other covariates, the status of employment (OR 1.5, 95 % CI 1.07-2.09, P=0.019) and practice of anal sex (OR 0.5, 95 % CI 0.24-0.94, P=0.031) were the only predictors for LR-HPV seroconversion. Those who were employed were less likely to convert than those who were unemployed. Further, those who practiced anal sex seroconverted less probably than those who did not practice anal sex. For HR-HPV seroconversion, there were two independent predictors after adjustment for other covariates: smoking history (smokers less likely to convert, OR 0.6, 95 % CI 0.40-0.93, P= 0.021) and lifetime number of sexual partners. There was a linear rise in conversion frequency, from 16.2 % among mothers with 0-2 partners up to 39.3 % among mothers with >10 lifetime partners (OR 2.0, 95 %CI 1.26-3.03, P=0.003).

5.3.   EFFECT OF PREGNANCY ON HPV (II)

We wanted to study whether a second pregnancy would have affect genital and oral HR-HPV carriage and persistence. A part of the young mothers recruited into the Finnish Family HPV study was pregnant for the second time at one of the follow-up visits. The effect of a second pregnancy on HPV detection and outcome was tested in these women (n=78) against those primiparous women who did not get pregnant for the second time during the 36-month follow-up period (n=100). In addition, detection of HPV DNA was analyzed in women with a second pregnancy during, between and after the pregnancies in a longitudinal setting. Second pregnancies were recorded at different time points as follows: 2 (2.6 %) at 6-month, 18 (23.0 %) at 12-month, 37 (47.4 %) at 24-month and 21 (27.0 %) at 36-month follow-up visit. The visit during the second pregnancy was during the first trimester for 18 (23.0 %), during the second trimester for 30 (38.5 %) and during the third trimester for 30 (38.5 %) of the women.

The demographic, clinical and baseline HR HPV DNA data of the two groups with one or two pregnancies were compared by univariate analysis. The groups were similar with regard to age, mode of first delivery, history of STDs and HR-HPV prevalence in baseline cervical (20 % vs 14 %, p=0.285) and oral (19 % vs 18 %, p=0.833) samples. Of the other nearly 60 recorded variables, marital status, some sexual preferences and time of initiation of OC use were significantly different. The most significant variables were marital status (p=0.003) and the number of past sexual partners (p=0.009). Women with a second pregnancy were more often married and had fewer past sexual partners than women without second pregnancy during follow-up.

The point prevalence of cervical or oral HR-HPV at different follow-up visits among women with and without second pregnancy was statistically similar for the most part.

The only significant difference in the detection of cervical HR-HPV was shown at the 36 month visit, when women with one pregnancy had a substantially higher HPV detection rate than women with second pregnancy (19.4 vs 6.5 %; OR 3.46, 95 % CI 1.23-9.76, p = 0.015). The same was true for oral HR-HPV at the 6 month visit. Women with one pregnancy tested significantly more frequently HPV DNA positive than women with second pregnancy (30.6 vs 16.0 %; OR= 2.31, 95 % CI 1.09-4.91, p=0.024).

A second pregnancy did not exert any significant effect on either clearance or acquisition of incident cervical and oral HR-HPV infections by univariate survival analysis. In Cox's regression model, none of the following covariates predicted cervical HR-HPV clearance: age, marital status, age at first pregnancy, smoking, OC use, second pregnancy status (group of women), interval between first and second pregnancy, trimester of second pregnancy, number of partners during first pregnancy and number of past sexual partners. In a similar analysis, a second pregnancy (its trimester or interval) was not significant in predicting clearance of oral HR-HPV, and the same was true for all other covariates. Similar analyses were repeated for acquisition of incident cervical and oral HR-HPV. None of the above covariates predicted incident cervical HR-HPV infections. Increasing age was the only independent predictor of incident oral HR-HPV (OR=1.18, 95 % CI 1.04-1.33, p=0.007). Trimester of the second pregnancy was of borderline significance (p=0.052). The second trimester had the highest probability for incident oral HR-HPV (OR=3.95, 95 % CI 1.18-13.18, p=0.025).

There was no statistically significant difference in HR-HPV detection in oral or cervical mucosa between the first and second pregnancy (**Figure 4**). However, the HR-HPV detection rate in both cervical (OR 5.32, 95 % CI 1.7-16.6, p=0.002) and oral mucosa (OR 3.19, 95 % CI 1.4-7.0, p=0.005) was significantly higher during the inter-pregnancy period (= pre-second pregnancy) than during second pregnancy.
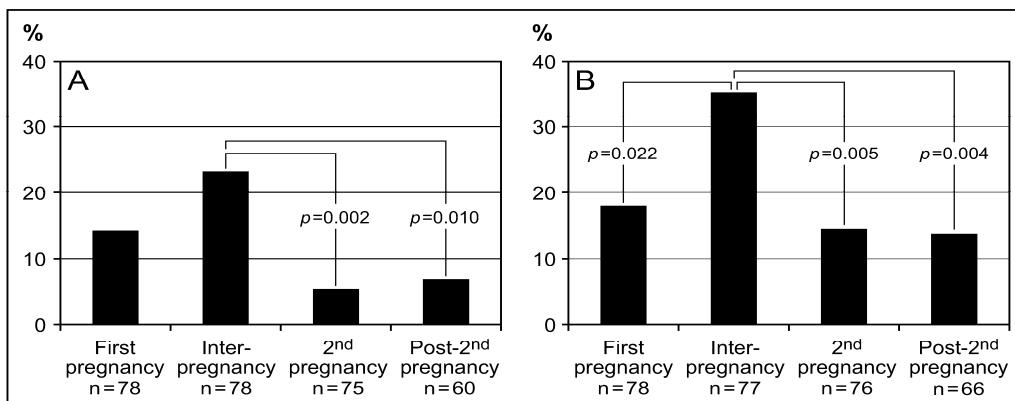

Figure 4. HR-HPV detection in **A)** cervical and **B)** oral samples during, between and after first and second pregnancy.

5.3.1. Second pregnancy covariates and HR-HPV carriage and persistence

The association of a second pregnancy and the other covariates with the detection and persistence of HR-HPV DNA in cervical and oral samples was analyzed using the GEE model approach. The GEE model approach can better adjust for covariates in this type of longitudinal study, where confounders can be multidimensional and interrelated (Diggle et al., 1994). None of the covariates were significantly associated with carriage of cervical HR-HPV. Of all covariates analyzed, the interval between the first and the second pregnancy was the only covariate that was significantly associated with detection of oral HR-HPV (p=0.001). This interval seemed to be longer among women with oral HP-HPV detected in any of their samples than that among women who remained HR-HPV negative. The second pregnancy itself, trimester or its timing (at the 6, 12, 24 or 36 month visit) were not significantly associated with oral HR-HPV carriage in these women.

None of the second pregnancy covariates were significantly associated with persistent cervical HR-HPV. In the adjusted GEE model, however, timing of the second pregnancy was significantly associated with persistence (p=0.005). Persistent HR-HPV was more likely when the second pregnancy occurred towards the end of the follow-up period, i.e., at the 24 or 36 month follow-up visits. As to persistent oral HR-HPV, there were two covariates significantly associated in the univariate GEE model: first, whether a second pregnancy took place or not (p=0.018), and second, the number of partners during the first pregnancy (p=0.002). In the adjusted GEE model, however, both variables were excluded because of colinearity with other second pregnancy-variables and the number of past sexual partners, respectively.

5.4. HPV DNA IN PLACENTA AND UMBILICAL CORD BLOOD (III)

To evaluate the possibility of transplacental transmission of HPV, we used nested PCR and tyramide-amplified ISH to analyze the presence of HPV DNA in placental samples obtained at delivery (III). The results were related to the HPV status of the neonates and their mothers and to maternal clinical and demographic variables. HPV DNA was detected by nested PCR in 4.2 % (13/306) of the placentas. Of the HPV positive samples, seven contained HPV16, five HPV6 and one HPV83 (**Table 5a**). ISH localized HPV16 and HPV6 DNA invariably in placental trophoblasts. Most positive signals were localized to the nuclei of these cells, and only weak signals were observed in the cytoplasm.

All HPV DNA positive placentas were macroscopically normal. HPV DNA positivity was not statistically significantly associated with any of the maternal demographic variables. Two of the HPV+ placental samples were obtained from caesarean sections and the remaining 11 from vaginal deliveries. Among the mothers with HPV positive placentas the mean time from rupture of the membranes to delivery was 7.6 (2.7 – 12.4) hours. This was not different from that of the mothers with HPV negative placentas (*p*=0.674). HPV-positivity of the placenta was significantly associated with Pap smear abnormality (ASCUS) at enrollment (OR=5.3, 95 % CI 1.63 – 17.35,

p=0.011). There was no statistically significant association between HPV DNA detection in placenta and in the uterine cervix (*p*=0.645) or oral mucosa (*p*=0.708) at baseline. If the placenta was HPV positive, the risk of the neonate of being a carrier of oral HPV at delivery increased 8.6-fold (*p*=0.001, 95 % CI 2.73 – 27.13). In general, the HPV DNA detection rate in neonates did not differ by mode of delivery. After vaginal birth 14.4 % (35/243) of the neonates tested positive for genital HPV and 10 % (23/242) for oral HPV. After cesarean delivery the detection rates were 18 % (12/65) and 6 % (4/64), respectively. During the follow-up period of three years placental HPV-positivity was significantly associated with genital HPV-carriage of the child at month 24 (OR=7.2, 95 % CI 1.5-34.0, p=0.026) (previously unpublished data). At other time points no significant correlations were found in follow-up.

We also analyzed umbilical cord blood samples for HPV DNA from all the neonates as well as peripheral blood samples from selected mothers. HPV DNA was present in 3.5 % (11/311) of the cord blood samples. Altogether five samples were positive for HPV 16, five for HPV 6 and one for HPV 39 (**Table 5a**). In five cases, both the placenta and the cord blood samples were positive for the same HPV-type: two for HPV 16 and three for HPV 6. As for the placentas, HPV-positivity of cord blood was significantly associated with maternal Pap smear abnormality at enrollment (OR=5.0, 95 % CI 1.39-18.18, *p*=0.024). There was no statistically significant association between HPV-detection in umbilical cord blood and HPV-positivity of the uterine cervix (*p*=0.384) or of the oral cavity (*p*=0.077) at enrollment. By univariate analysis, HPV-positivity of cord blood was significantly associated with the mother's marital status, the number of past sexual partners and a history of GWs. When these three variables and Pap smear status were entered into a multivariate regression model, a history of GWs proved to be the only independent predictor of cord blood HPV (OR=4.0, 95 % CI 1.09-14.54 [*p*=0.036)). This was not confounded by maternal age. When umbilical cord blood was HPV-positive, the risk of the neonate being a carrier of genital HPV DNA at delivery increased 4.0-fold (*p*=0.048, 95 % CI 1.08–14.83) and of oral HPV DNA 4.4-fold (*p*=0.039, 95 % CI 1.17–16.14). During the follow-up period of three years umbilical cord blood HPV-positivity was significantly associated with genital HPV-carriage of the child at month 24 (OR 5.8 95 % CI 1.3-25.8, p=0.038) (previously unpublished data). There were no statistically significant associations at the other time points. Peripheral blood samples from all mothers with HPV positive placenta or cord blood (n=19) tested negative for HPV DNA.

5.5.   HPV DNA IN BREAST MILK (IV)

We examined the presence of HPV DNA in human breast milk. First, breast milk samples taken from 223 women three days postpartum were tested for HPV DNA by nested PCR. These results were associated with the HPV status of the spouses as well as their demographic parameters in order to identify possible risk factors for breast milk HPV positivity (**IV**). During follow-up, the presence of HPV DNA in 183 breast milk samples taken two months postpartum was investigated by nested PCR and

subsequent genotyping to evaluate the persistence of HPV DNA in breast milk as well as its association with the neonate's HPV status (previously unpublished data).

At day 3, HR-HPV DNA was detected in 4.5 % of the samples (10/223) in the cellular compartment of breast milk. HPV 16 DNA was identified in nine of them by sequencing. One sample was inadequate for sequencing. HPV carriage in milk was not associated with genital nor oral HPV DNA status of the mother nor her demographic parameters. Instead, it was significantly associated with oral HR-HPV carriage of the spouse at 6 months (OR 3.5 95 % CI 1.7-7.5; P=0.021) and 12 mo (OR 2.9 95 % CI 1.4-5.9; P=0.022) (**IV**). At two months postpartum, HPV DNA was detected in 19.7 % of the samples (36/183) (either HPV type 16, 18, 45, 53, 56, 59, 66 or 82) (**Table 5a**). Two HPV types were found in six samples: HPV 16 and 45 in one sample, HPV 16 and 53 in two samples, HPV 16 and 59 in one sample, HPV 16 and 66 in one sample, and HPV 56 and 82 in one sample.

There were only 133 mothers with breast milk samples collected both at day 3 and at month 2 (**Table 5b**). Only five out of the ten mothers whose breast milk tested HPV positive at day 3 donated a second sample at month 2. Of these samples, four were HPV-negative, and one tested HPV-positive for the same type. Twenty-five of the cases with HPV in breast milk at month two had been HPV-negative in their first sample taken at day 3 postpartum. There was no significant association with HPV in breast milk at month 2 and the parental demographics or HPV-status, or HPV-carriage of the offspring at two months postpartum.

Table 5. a) Summary of results on HPV DNA types and detection rates in placenta, umbilical cord blood and breast milk. **b)** Breast milk HPV DNA status during follow-up.

a)

Samples	HPV+ % (n/n)	HPV Type (n)
Placenta	**4.2** (13/306)	6 (5); 16 (7); 83 (1)
Umbilical cord blood	**3.5** (11/311)	6 (5); 16 (5); 39 (1)
Breast milk 3 days postpartum	**4.5** (10/223)	16 (9)
Breast milk 2 months postpartum	**19.7** (36/183)	16 (32); 18 (1); 45 (1); 53 (3); 56 (1); 59 (1); 66 (1); 82 (1)

b)

Breast milk at day 3 (n=223) / month 2 (n=183)							
HPV status, n							
+ / NA	- / NA	NA / +	NA / -	+/+	+/-	-/-	-/+
5	85	10	40	1	4	103	25

+ = HPV positive; - = HPV negative; NA= sample not available

6.   DISCUSSION

HPV is regarded as the most common sexually transmitted disease, although not a typical one (Bosch et al., 2008). HPV can be acquired even without high risk sexual behavior (Collins et al., 2002; Winer et al., 2008), and HPV has been detected in virgins, infants and children (Syrjänen and Puranen, 2000; Winer et al., 2008). However, epidemiological research on HPV has traditionally focused on HR-HPV infections at the genital area of sexually active women. Any non-sexual modes of HPV transmission and HPV infections in infants have received much less attention. HPV infection at early ages and in non-genital epithelia may have important consequences for the outcome of the infection (Cason and Mant, 2005). Proper understanding of the epidemiology and transmission dynamics of HPV is crucial for the design and implementation of successful and costeffective prevention strategies for HPV and associated disease (Burchell et al., 2006b). Besides major public health implications in decision making policies, a better understanding of the transmission dynamics of HPV has consequences for personal health. It is well documented that testing HPV-positive imposes a psychosocial stigma on some adult women over and above an abnormal cytology result (McCaffery et al., 2006; Waller et al., 2009). Also, problems are likely to be encountered in families where a child is diagnosed with a HPV infection (Sinal and Woods, 2005). Obviously, more data and a better understanding are needed on how HPV infections are transmitted, on the epidemiological determinants involved and on infection outcome. These issues are among the aims of the Finnish Family HPV Study, which is the first prospective follow-up study of HPV infections at multiple anatomical sites within families.

6.1.   REPRESENTATIVENESS OF STUDY POPULATION

Pregnant mothers at their third trimester were enrolled to the Finnish Family HPV Study at the Maternity Unit of Turku University Hospital during the years 1998-2002 (Rintala et al., 2005a). The study population was on average of the same age as those known to have a high HPV DNA prevalence. Although current or previous HPV status of the women was not examined before recruitment, it may be assumed that women with a history of HPV infections may have been more anxious to enroll into the study. In the current cohort, 27 % of the women reported previous GWs. This is markedly more than the 10.5 % reported in the recent population-based cross-sectional study of similar aged women from Sweden, Denmark, Norway and Iceland (Kjaer et al., 2007). Every fifth of the women in the present cohort reported ever having an STD other than GWs, which is similar to the study population by Kjær et al. (2007). The reported mean number of lifetime sexual partners was about five in the current study, which is the average also in larger cohorts of young women from Nordic countries (Kjær et al., 2007; Skjeldestad et al., 2008). Practicing oral and anal sex at least occasionally was frequent among the women in our study, 80 % and 20 %, respectively. The high rate of women practicing oral sex may partly explain the high HPV DNA prevalence in the oral samples of the present study (D'Souza et al., 2009). The mean age at first full term

pregnancy of the participants in this study was 22.9 years, which is significantly lower than the average in Finland (27.6 years in 2002) (www.thl.fi).

6.2. HPV SEROPREVALENCE

Detection of HPV antibodies indicates prior or ongoing infection, but is not useful for diagnosing HPV infections (Dillner et al., 2007). The interpretation of seroepidemiology requires data on the natural history of the antibody responses, namely seroconversion and antibody decay over time, and this can only be studied in a longitudinal setting, as was done in the present study for HPV 6, 11, 16, 18, and 45 infections.

According to the literature, not more than 50-60 % of women with cervical HPV16 infection are concomitantly IgG seropositive when measured with the VLP-based ELISA assays (Kirnbauer et al., 1994; Carter et al., 2000; Studentsov et al., 2003). The concordance has been lower in studies using multiplex serology (Clifford et al., 2007; Dondog et al., 2008). In the present study, the concordance between HPV DNA status and HPV seropositivity at baseline was modest or poor, and even lower than reported previously. A poor concordance is generally accepted, since HPV DNA detection is usually a transient phenomenon (Ho et al., 1998; Moscicki et al., 1998; Franco et al., 1999), development of antibodies requires time from exposure and antibodies do not develop in every woman (Carter et al., 2000; Ho et al., 2004; Steele et al., 2008) and antibodies may persist after viral DNA has been cleared (Carter et al., 2000; Ho et al., 2004; Steele et al., 2008). Also, HPV infections have many manifestations, even without the expression of capsid proteins, against which the antibody responses are measured. Furthermore, the assessment of antibodies cannot distinguish at which site the infection occurred. Indeed, by assessing DNA positivity at any anatomical location, we could obtain a better agreement between seropositivity and DNA positivity. This suggests that also oral HPV infection contributes to the serological response.

Comparisons between absolute seroprevalence rates for the different HPV types published earlier are of limited value, because of a variety of assay systems, antigen targets and cut-off definitions have been used (Carter and Galloway, 1997; Ferguson et al., 2006). There is some evidence that the Multiplex assay used in the present study has a higher sensitivity than traditional VLP-based ELISAs (Waterboer et al., 2005; Achour et al., 2009; WHO HPV LabNet). The baseline seroprevalence for all HPV types in the present study was higher than reported previously (Studentsov et al., 2003; Lehtinen et al., 2006; Skjeldestad et al., 2008). When more stringent (400 MFI) cut-off values for the L1 antibodies was used, seroprevalence declined by 15-20 %, and in particular, the seroprevalence of HPV 16 (20 %) and HPV 18 (8 %) was in line with previous reports on pregnant women (af Geijersstam et al., 1998; Hagensee et al., 1999; Kibur et al. 2000; Kaasila et al., 2009).

There was a significantly higher seroprevalence of HPV 6 than of the other four types tested. Few other studies have reported such high prevalences. Van Doornum and

colleagues (1998) reported antibody prevalences of 58 % and 48 % to HPV 6 and 11, respectively, among HPV DNA negative heterosexual adults with multiple partners. They also reported that 4 % and 17 % of sexually inexperienced teenage girls were seropositive for HPV 6 and 11 with relatively high absorbance values, respectively. This may indicate that HPV 6 and HPV 11 immunoreactivity is, to some extent, caused by HPV exposure at non-cervical sites and before the individual becomes sexually active. Thus, the wide variation in HPV seroprevalence rates can be explained not only by different sensitivities and specificities of serological assays, but also by different study populations. The present population may be biased, since a relatively high proportion of women reported a history of GWs. However, Lehtinen and co-workers (2006) reported an increase in HPV 6 and 11 seroprevalence among 23-32-year-old women from about 10 % in 1983-1988 to 16 % in 1989-1994 in the Turku area (Lehtinen et al., 2006), from where the present study population was enrolled during 1998-2002. It is possible that the seroprevalence continues to increase further due to changes in sexual behavior. Serological studies are indeed valuable for demonstrating time trends in HPV epidemiology (Laukkanen et al., 2003).

In agreement with previous studies, the lifetime number of sexual partners proved to be an independent predictor of seropositivity to HR-HPV (Dillner et al., 1996; Wideroff et al., 1996; Carter et al., 1996; Viscidi et al., 1997; Silins et al., 2000; Wang et al., 2000; Stone et al., 2002; Studentsov et al., 2003; Clifford et al., 2007; Skjeldestad et al., 2008). Two additional independent predictors were identified in this study: being seropositive for LR-HPV, and a history of GW. Similar covariates were also predictors of seropositivity for LR-HPV, in addition to young age at onset of sexual activity. Some studies have found differences between the risk factor profiles for antibodies to HR- and LR-HPV (Silins et al., 2000). In the presetn study, essentially similar predictors of both HR and LR antibodies were identified. Interestingly, seropositivity for either the LR- or HR-HPV types also conferred an increased risk of seropositivity for the other HPV group. This has been previously reported by Silins and colleagues (2000), who also found an association with a history of GW and LR-HPV seropositivity. These data imply that the same people are exposed to many different HPV types, and that the LR-HPV types are probably the first. This would be in agreement with the detection of a higher baseline seroprevalence of HPV6 and 11 than of HPV 16 and 18 in the present cohort (**I**). Women who have seroconverted to one HPV type have a higher probability of seroconverting to other HPV types. In the current work 50 % of the seroconverted women showed seroconversion to several HPV types (**I**). Genetic or immunological predispositions may also play a role in the outcome of response to HPV, but these issues are poorly understood (Silins et al., 2000; Carter et al., 2000).

6.3.   DYNAMICS OF ANTIBODY RESPONSE TO HPV

HPV seroconversion after pregnancy was common (**I**), even more frequent than previously observed (Kibur et al., 2000). Antibody decay over three years was rare, and no significant differences were observed between the five HPV types tested.

Previous studies on HPV antibody decay have been inconclusive. While detectable HPV antibody levels have been reported to last several years and be stable over time (Shah et al., 1997; af Geijersstam et al., 1998; Villa et al., 2006), also opposite results showing an antibody decay in ~50 % of seropositive subjects within three years have been published (Ho et al., 2004). This controversy may partly be explained by different study settings. Studies reporting a higher frequency of antibody decay have been done in initially seronegative populations where antibody responses are observed after positive DNA detection (Carter et al., 2000; Wang et al., 2004). It is likely that very short-lived infections or virus exposures may not induce seroconversion to stable antibody levels (Carter et al., 2000; Ho et al., 2004). In the present study antibody decay was recorded in women who had already seroconverted to HPV. Thus, they may represent a population who has reached a stable antibody levels. Although not directly analysed in this study, there are indications that part of the HPV antibody responses are transient in nature, as the seroprevalence rates at different time points were not increased during the follow-up although seroconversion was observed to be frequent (**I**).

The independent predictors of HR-HPV seroconversion were high lifetime number of sexual partners and history of not smoking (**I**). These factors were also reported by Wang and collagues (2004) as predictors of HPV 16 seroconversion. Recently, in line with the results of this study, smoking impairs HPV 16 and 18 capsid antibody response (Wiley et al., 2006; Simen-Kapeu et al., 2008). Instead, the only predictors of LR-HPV seroconversion were status of employment and practice of anal sex. Those who participated in anal sex were less likely to convert than those who never practiced anal sex. Anal sex has been rarely recorded in HPV seroepidemiology of females, although it has been shown to be associated with incident HPV infections (Ho et al., 1998). Generally, anal sex is associated with lower rates of condom usage than vaginal intercourse, placing women practicing anal sex at high risk for STIs (Halperin, 1999). Also, antibody responses elicited by HPV at the anorectal mucosa may differ from responses elicited by HPV at other sites. Frequent anal sex may lead to continual HPV exposure and steady levels of antibodies. Thus, women who frequently practice anal sex are less likely to be identified as seroconverters in study settings like the present one.

6.4.   HPV AND PREGNANCY

Pregnancy is often implicated as a risk factor for HPV infection due to hormonal and immunological changes. However, there is no solid support for this in the literature, and reported HPV detection rates in the genital mucosa during pregnancy vary as much as 5.2 % to 68.8 % (Arena et al., 2002). In the current study the genital HPV carriage during first pregnancy was 14-20 % and during second pregnancy 5.3 %, in accordance with previous studies (de Roda Husman et al., 1995b; Tenti et al., 1997; Chan et al., 2002; Smith et al., 2004).

Women who decide to have children are likely to differ from those women who do not have children - especially with regard to sexual practices, known to be important risk

factors for HPV infection. This work showed behavioral differences even between women with only one pregnancy and those having a second pregnancy soon after first pregnancy. Marital status, number of past sexual partners, frequency of intercourse, anal sex and age at onset of oral contraception were significantly different between these two groups, which might indicate commitment to family life among women having a subsequent pregnancy soon. Although not obvious from the HPV DNA prevalence rates, the differences in HPV exposure were reflected in seroprevalence rates of the two groups at enrolment, as women with only one second pregnancy had significantly more HPV 6 and 18 antibodies when analyzed afterwards (unpublished data).

This study was not able to show that pregnancy increases HPV carriage, in line with many other studies relying on PCR based methods for HPV detection (de Roda Husman et al., 1995b; Tenti et al., 1997; Chan et al 2002). Nor could this study show that pregnancy impairs the outcome of HPV infection (**II**). Most HPV positive pregnant women had a normal cervical cytology. Furthermore, the current study could not confirm the effect of gestational age on genital or oral HR-HPV detection, contrary to Rando et al. (1989) and Morrison et al. (1996). Tenti and collaegues (1997) concluded that pregnancy as a general expression of a more conscious social and sexual behavior might even be regarded as protecting against infection. Indeed, this study found that among women with a second pregnancy within three years of the first delivery, the subsequent pregnancy may even decrease the detection of genital and oral HPV (**II**).

Although high parity is a recognized risk factor for cervical cancer (International collaboration of epidemiological studies of cervical cancer 2006), it does not seem to be a risk factor for HPV positivity (Vaccarella et al., 2006b). Some previous studies have suggested that increasing parity may even decrease the risk of HPV infections (Morrison et al., 1996; Hernandez-Giron et al., 2005; Leinonen et al., 2008). In this study, shorter birth interval between the first and the second pregnancy was significantly associated with decreased oral HR-HPV carriage in the adjusted GEE model (**II**). There are no earlier studies on the effect of a short parity interval on the risk of HPV infection. However, giving birth at intervals of less than one year has been associated with a lower risk of cervical cancer, and it has been noted that women with shorter intervals had fewer sexual partners (Hoyo et al., 2007). The biological effect of pregnancy on HPV infection is yet to be determined. Although cell-mediated immunity is suppressed during pregnancy, the suppression seems to be compensated for by an activation of innate immune responses (Veenstra van Nieuwenhoven et al., 2003). In the current study, the effect of pregnancy on HPV antibody levels was not directly analyzed. Overall, there were no significant changes in the dynamics of HPV antibodies between mothers with one or two pregnancies during follow-up (unpublished data).

The second pregnancy seemed to be of little impact on carriage and persistence of cervical and oral HR-HPV over time (**II**). Few other longitudinal studies discuss the

effect of pregnancy on the incidence and clearance of HPV infections. Minkoff and co-workers (2006) reported that the prevalence and copy number of HR and LR types among HIV infected women were not different when pregnant and non-pregnant women were compared, but the incidence of HPV was significantly lower during pregnancy than after delivery (Minkoff et al., 2006). Very recently, Banura and co-workers (2008) analyzed HPV infections among young primiparous pregnant women aged <25 years in Uganda, and found a high HPV incidence during pregnancy and after delivery. However, also clearance was frequent, leaving overall HPV prevalence unchanged during the observation period (Banura et al., 2008). Although these studies were conducted among very different study populations than the present one, the results in study **II** are in concordance, suggesting that pregnancy does not increase vulnerability to HPV infection.

6.5. EXTRAMUCOSAL HPV AND VERTICAL TRANSMISSION

The debate on HPV transmission as being sexually transmitted or sexually activated has been going on since the confirmation of the infectious nature of HPV-associated disease (Rotkin, 1967; Dillner et al., 1999; Rice et al., 1999; Cymet, 2006). Currently, several lines of evidence suggest that vertical transmission exists, but that the rates and routes have not been established (Dillner et al., 1999; Cason and Mant, 2005; Castellsagué et al., 2009). Vertical transmission might take place in utero, perinatally in the infected birth cancal or postnatally via breastfeeding. To study in more detail these options, the presence of HPV DNA were measured in placenta, umbilical cord blood and breast milk (**III, IV**).

The present series represents thus far the largest series of placenta, umbilical cord blood and breast milk specimens subjected to HPV analysis. HPV DNA was detected in the placenta and cord blood in 4.2 % and 3.5 % of the deliveries (**III**). In breast milk HPV DNA was detected in 4.5 % and 19.7 % of samples obtained three days and two months after delivery, respectively (**IV**). Previously, the reported detection rates of HPV DNA in placenta have varied from 0 % to 42.5 %, and in cord blood from 0 % to 13.5 % (Previous literature listed in **Appendix 1**). Only very recently, another paper investigating the presence of HPV DNA in secretion of mammary ductal epithelium was published (Cazzaniga et al., 2009). This study included 25 breast milk and 10 colostrums samples from lactating Japanese mothers and the detection rate of HPV DNA in these milk samples was ~8 % (3/35).

The differences in the rates of detecting intrauterine HPV in previous studies may be partly explained by different study populations. Lowest detection rates have been reported in studies which have excluded women with clinical HPV infections (Eppel et al., 2000; Worda et al., 2005). In this study a history of HPV was not an exclusion criterion. Indeed a history of GWs turned out to be an independent predictor of cord blood HPV positivity (**III**). In addition, placental and cord blood samples positive for HPV were related to Pap smear abnormalities detected before delivery. Among women with no history of GWs or abnormal cervical cytology during pregnancy the detection

rate of HPV in placenta was 2.7 % (6/225) and in cord blood 1.8 % (4/224) respectively. The varying results may also be due to sampling techniques, because only a tiny proportion of placenta is subjected to HPV analysis.A recent study by Rombaldi and co-workers (2008) demonstrated that the combined use of scraping methods and biopsies from both sides of the placental disk resulted in more frequent detection of placental HPV. In their study, HPV DNA was detected in 24.5 % of the placentas. The detection rate in biopsies from the maternal side of the central part of placenta was 2.0 % (Rombaldi et al., 2008). Similar biopsies were used in the present study and the HPV DNA prevalence was comparable (**III**).

Previous studies have implicated HPV infection of the placenta as contributing to some miscarriages (Hermonat et al., 1997; Matovina et al., 2004; Srinivas et al., 2006), genetic abnormalities of the fetus (Matovina et al., 2004) and spontaneous preterm delivery (Gomez et al., 2008). Placental dysfunction caused by HPV has been supported by studies in vitro (You et al., 2003; Gomez et al., 2008). In the present study, all HPV positive placentas were from normal pregnancies (**III**). The gestational age, history of preterm births or previous miscarriages did not differ between mothers with HPV positive and HPV negative placentas. All the HPV positive placentas appeared normal upon examination done by midwives in the delivery ward. However, examination of this kind is a rather insensitive method (Kaplan, 2008).

The origin of HPV in the placenta, umbilical cord blood or in breast milk is not known. The current consensus is that HPV multiplies locally at the site of entry on skin or mucous epithelia without viremia. However, there are reports on the presence of HPV DNA in the peripheral blood mononuclear cells (PBMCs) (Pao et al., 1991; Tseng et al., 1992). In the case of intrauterine HPV, the virus may ascend from the maternal genital tract or with blood through the placenta (Tseng et al., 1992; Armbruster-Moraes et al., 1994). Also infection by contaminated sperm cells at the time of conception is possible (Lai et al., 1996). Tseng and coworkers (1992) noted that HPV DNA in umbilical cord blood was more closely related to the status of HPV DNA in maternal PBMCs than in maternal cervicovaginal cells. The current study did not detect any HPV in maternal peripheral blood samples (**III**). Armbruster-Moraes and colleagues (1994) reported a positive correlation between the grade of cervical lesions and the presence of HPV DNA in amniotic fluid and suggested ascending infection. In the present study population, HPV detection in cord blood or placenta was associated with a maternal history of productive genital HPV infection, but not with maternal HPV DNA detection before delivery (**III**). This may be due to inadequate sampling or false negative DNA-scrapings. Also, HPV may have infected the placenta at an earlier stage of pregnancy and could have cleared from the cervix.

It seems feasible that a high viral load may result in widespread contamination of the genital tract, and this may increase the risk of an ascending intrauterine infection. It cannot be excluded that HPV was present on the endometrium already at the stage of trophoblast invasion. HPV DNA has been detected high up in the female genital tract up to the endometrium and even ovaries, but the significance of these findings is

uncertain (Lai et al., 1992; O'Leary et al., 1998; Fedrizzi et al., 2009). Furthermore, subjects with productive infections and manifest clinical lesions may also be more susceptible to persistence and spreading of HPV, maybe due to some genetic factors or altered immune responses. Rombaldi and coworkers (2008) reported a significant association between placental HPV and immunosuppressive status of the mother. Interestingly, in a very recent study by Fedrizzi and colleagues (2009), HPV DNA was 3.5 times more frequently present in the normal endometrium of smokers than non-smokers (25 % vs 7 %), although this difference was not statistically significant. In the present study (**III**), a similar trend was observed, as HPV DNA was 3 times more prevalent in the placenta of women who had ever smoked than of women with negative smoking history (6.4 % vs 2.1 %).

Epithelial cells from mammary ducts, nipple or skin could be a source of HPV, as HPV replication in primary human mammary ductal epithelial cells has been demonstrated (Ozbun et al., 2009). Also, there are reports of HPV DNA or HPV-associated lesion at these sites (Googe et al., 2000; de Villiers et al., 2005; Khan et al., 2008). It has been hypothesized that mammary epithelial cells that partly lose control in proliferation are more susceptible to persistent HPV infection (Liang and Tian, 2008). There was no significant correlation between the presence of HPV DNA in breast milk and maternal HPV DNA status or demographic parameters in the present study (**IV**). However, there was significant correlation between HPV in breast milk at day 3 and the presence of HR-HPV in oral scraping obtained from the male partner. Oral HPV infection in the father might be transmitted to epithelial cells of the breast of the female partner or vice versa. This view is supported by the study of de Villiers and coworkers (2005) suggesting a pathogenic mechanism involving HPV transfer in a retrograde fashion via the nipple, areola, lactiferous ducts and mammary sinuses.

The possibility of contamination of the placenta, umbilical cord blood and breast milk samples with HPV DNA must be taken into account. The possibility of laboratory contamination was monitored by including several negative controls in the analysis and conducting strict laboratory discipline, but there is still a possibility that the samples were contaminated during collection. However, the HPV DNA of the placental samples was localized in the trophoblasts with ISH, and this excludes surface contamination from the birth canal (**III**). Regarding breast milk samples, contamination in the hospital from the mother's hands or skin surrounding the nipple cannot be ruled out. Sonnex et al (1999) reported that 27 % of subjects with genital infections had the same HPV type on their fingers. More recently, Thomas and co-workers (2009) reported that about 25 % of sexually active young men had incident mucosal HPV DNA in fingernail tip specimens, with HPV-16 and HPV-84 being the most common types (Thomas et al., 2009). The mothers participating in the present study washed their hands but not the nipple area before giving the breast milk samples (**IV**). In the paper by Cazzaniga and colleagues (2009), also the nipple area was washed extensively, after which the samples were collected by dropping the liquid directly into sterile tubes without any contact between the skin and the sterile tubes. Still, the results

of the study by Cazzaniga et al. (2009) and the current study (**IV**) are in agreement and that HPV DNA is truly present in the breast milk proper.

The large number of mother-infant pairs analyzed in the Finnish Family HPV Study has made it possible to explore the consequences of the presence of HPV in the placenta, umbilical cord blood and breast milk with regard to the child, which is perhaps the most intriguing aspect of this study. Previously, only a limited number of mother-newborn pairs have been studied for perinatal transmission of HPV (Rombaldi et al 2008), and specifically, this is the first study to calculate the possible risk of viral transmission from mother to child when there is an HPV positive placenta or HPV positive cord blood. In agreement with previous studies, the present results suggest that HPV can pass the barrier of maternal-fetal interface (**III**), making intrauterine transmission of HPV a potential but rare route of infecting the offspring. As shown in paper **III** the presence of HPV DNA in cord blood and/or placenta increases significantly the risk of the neonate testing HPV positive for the oral or genital mucosa at birth. Also, there was a significant association between HPV in the placenta or cord blood and genital HPV carriage of the child at month 24 (previously unpublished data). In general, 15 % and 9 % of the neonates included in the study tested HPV positive for the genital and oral mucosa at delivery. The delivery mode did not have any predictive value for the neonate's HPV status. This is in line with earlier studies, showing that cesarean deliveries do not protect neonates against HPV (Medeiros et al., 2005).

Vertical transmission of other viruses, e.g. HIV and cytomegalovirus, via breast feeding is known (Dworsky et al., 1983; Kourtis et al., 2001). However, we found no association between HPV positivity in breast milk at day three or month two postpartum and the oral HPV DNA status of the neonate during the first two months. This may be explained by the low viral copy number detected in the samples by nested PCR and hybridization. It is not possible to ascertain whether infectious viral particles were present in the milk samples. Breast milk is part of the mucosal immunity with innate and adaptive defence properties that are likely to be protective. Future analyses of these antiviral properties will hopefully shed more light on the significance and infectivity of HPV in breast milk.

6.6.   STUDY STRENGTHS AND LIMITATIONS

This study is part of the ongoing Finnish Family HPV study with several aims, of which only a minority could be included in the current work. The importance of many of our findings awaits clarification from the future studies, when all the samples taken in the study have been analysed and longer follow-up data is available. However, the data presented here provides new data on HPV with many implications.

The current study suggests that previous and current exposure of mothers to HPV is common. HPV seroconversion after pregnancy is also frequent. This kind of data may help in identifying target populations for HPV prevention programs. However, it is appropriate to recall that seroepidemiological data of HPV needs to be interpreted with

caution, because there are uncertainties about the specificities and sensitivities of serological assays. The multiplex serology method used in this work to test HPV 16 antibodies has been tested and qualified (WHO HPV LabNet). The lack of HPV DNA data and demographic data of the mothers in the follow-up hindered the estimation of biologically more plausible seroconversion and antibody-decay times and the predictors of these factors. Incorporating such data in the analyses would also provide the possibility to analyze non-seroconvertors as well as any protection afforded by antibodies.

A major strength of the study on HPV and pregnancy is in its prospective design and attempt to control for potential confounding factors. However, this study has limitations. The Finnish Family HPV study was not planned to assess the effect of pregnancy on HPV but the dynamics of HPV in families. During follow-up of the whole cohort a proportion of the mothers became pregnant again, and a unique opportunity to assess the effect of a second pregnancy was provided. Because the samples were collected only at 6-12 months interval, short-lived changes in the risk of incident infections or clearance during pregnancy would not have been detected. Furthermore there is a risk that the actual effect of the second pregnancy is diluted by the ways of which the women were grouped and covariates made for the statistical analysis. Namely, grouping women into two groups according to whether or not a subsequent pregnancy occurred at some time point during the follow-up fail to adjust for the actual timing of second pregnancy, and hence, this is not the actual covariate for to reflect the effect of the "second pregnancy status". Timing of the second pregnancy and the interval between the two deliveries were shown to have some effect on HPV infection and outcome, but the significance of these findings is biased by the fact that several of the women were still pregnant at the 36-month visit. Also, the number of women in the study cohort was relatively low. However, the results (**II**) are in line with the recent literature suggesting that pregnancy is not a period of particular vulnerability to HPV infection, which is important, as HPV infections are most frequent in young women at the age of family planning.

The current study showed for the first time that the presence of HPV DNA in the placenta and/or cord blood significantly increases the risk of the neonate to test HPV positive at birth. This study represents the largest cohort of placentas and umbilical cord blood specimens subjected to HPV DNA analysis to date. Furthermore, for the first time, the presence of HPV DNA in breast milk was demonstrated with reasonable certainty. In the placenta, the PCR results could be verified by the use of ISH. Positive signals specifically localized in trophoblasts indicate the true presence of HPV in these cells and exclude the possibilty that there would be surface contamination from the maternal birth canal. Thus, these findings, together with previous reports on the presence of HPV in PBMCs, umbilical cord blood and placenta, as well as in vitro studies on HPV replication in cultured placental trophoblast support the view that HPV is not strictly a keratinocyte-specific pathogen.

7. CONLUSIONS

1. Previous and current exposure of mothers to HPV is common. Sexual activity plays a major role in eliciting the antibody response to HPV. Age at onset of sexual activity, number of sexual partners until age 20, lifetime number of sexual partners, history of genital warts and simultaneous seropositivity to HR/LR types predict the LR-HPV and HR-HPV seropositivity. HPV seroconversion after pregnancy is common, and the titers of the HPV antibodies decay only seldom over three years.

2. A second pregnancy is of minor impact on carriage and persistence of oral and cervical HR-HPV infections. This argues against the common claim that pregnancy is a risk factor for HPV infection. A second pregnancy might even decrease the rate of detection of HR-HPV in genital and oral locations, but this effect seems to be transient. Women committed to a second child soon after their first pregnancy do not seem to share many of the behavioral risk factors for HR-HPV, which are more common among those not opting for second pregnancy

3. Any given person may become exposed to HPV even early in life. A low but substantial percentage of placenta and umbilical cord blood samples contain HPV DNA at delivery. Localization of HPV in placental trophoblasts suggests that HPV truly infects the placenta. For the first time, HR-HPV DNA was also detected in breast milk. HPV DNA in breast milk was associated with the oral HPV status of the father. HPV detection in placenta and cord blood was associated with a maternal history of abnormal pap-smears and genital warts. HPV DNA in the cord blood and/or placenta increased the risk of a neonate testing positive for HPV at birth. The mode of delivery did not predict the presence of HPV DNA in the placenta or cord blood, nor the neonate's HPV status.

8.   ACKNOWLEDGEMENTS

This study was carried out in collaboration between the Department of Oral Pathology, Institute of Dentistry, the MediCity Research Laboratory, Faculty of Medicine and the Department of Obstetrics and Gynecology, Turku University Central Hospital, from 2004 to 2009. I express my thanks to Professor Risto Erkkola, Head of the Department of Obstetrics and Gynecology, Professor Sirpa Jalkanen, Head of MediCity Research Laboratory and Professor Pekka Vallittu, Head of the Institute of Dentistry, for the opportunity to work and use the facilities of these departments. I thank Docent Ulla Ekblad, Head of the Obstetrics, who has created a positive atmosphere that favors scientific research among the staff of the maternity unit.

I express my sincerest gratitude to my supervisors, Professor Stina Syrjänen and Docent Seija Grénman, talented and hard-working women who have provided me the opportunity to learn much about science. I am grateful to Stina, the principal investigator of the HUPA project, for introducing me to research by picking me up when I was a first-year medical student to work in the laboratory, and later for encouraging me to team up with this project. Stina's energy, scientific productivity and strong belief in her work, are admirable indeed. Her enthusiasm, commitment and way to make things work have been priceless to the project. I am grateful to Seija, who is an outstandingly skilful clinician and scientist combined, for her guidance and support during the years. Her organizational talent and negotiation skills have been crucial for the completion of this work. She has carefully listened to my worries and critically revised the manuscript of this thesis with great accuracy, without consideration of the time it has taken, for which I owe her my deep gratitude.

I acknowledge the valuable contribution of Professor Eva Rylander and Docent Eeva Auvinen for the thorough review of my thesis. I express my sincere gratitude to Eva who dedicated herself to reviewing this work on a tight schedule. I thank Eeva especially for her constructive criticism and detailed advice that helped me to improve my work, and for the pleasant conversations we had around the manuscript. I thank Docent Robert Paul for revising the language of this work and some of the original papers and Timo Kattelus for help with the figures.

I express my gratitude to my coworkers for their contribution to the studies. Professor Kari Syrjänen has worked hard with checking and entering the data into SPSS files and he has performed the statistical analysis and reviewed the original papers. I owe much gratitude to Dr. Marjut Rintala, PhD, who enrolled and examined the study subjects and whose studies marked much of the present work. Marjut is a great support and excellent company to travel with to congresses. I thank my coauthors from the German Cancer Research Center, Dr. Michael Pawlita, Dr. Tim Waterboer and Dr. Kristina Michael, who collaborated on the antibody analyses of serum samples. Their skill and knowledge are impressive. I direct my special thanks to Dr. Pawlita and Dr. Waterboer for the well organized and most inspiring HPV Luminex Training Course held in Heidelberg.

Lahti, November 2009

Marja Sarkola

REFERENCES

Achour M, Kahla S, Zeghal D, Kochbati L, Mongi M, Zouari F, Oueslati R. Analysis of antibody response to HPV 16 and HPV 18 antigens in Tunisian patients. Viral Immunol 2009;22:7-16.

af Geijersstam V, Kibur M, Wang Z, Koskela P, Pukkala E, Schiller J, Lehtinen M, Dillner J. Stability over time of serum antibody levels to human papillomavirus type 16. J Infect Dis 1998;177:1710-1714.

Akil N, Yasmeen A, Kassab A, Ghabreau L, Darnel AD, Al Moustafa AE. High-risk human papillomavirus infections in breast cancer in Syrian women and their association with Id-1 expression: a tissue microarray study. Br J Cancer 2008;99:404-407.

Alani RM, Münger K. Human papillomaviruses and associated malignancies. J Clin Oncol 1998;16:330-337.

Alphs HH, Gambhira R, Karanam B, Roberts JN, Jagu S, Schiller JT, Zeng W, Jackson DC, Roden RB. Protection against heterologous human papillomavirus challenge by a synthetic lipopeptide vaccine containing a broadly cross-neutralizing epitope of L2. Proc Natl Acad Sci U S A. 2008 15;105:5850-5.

Anttila A, Pukkala E, Söderman B, Kallio M, Nieminen P, Hakama M. Effect of organised screening on cervical cancer incidence and mortality in Finland, 1963-1995: recent increase in cervical cancer incidence. Int J Cancer 1999;83:59-65.

Anttila M, Syrjänen S, Ji H, Saarikoski S, Syrjänen K. Failure to demonstrate human papillomavirus DNA in epithelial ovarian cancer by general primer PCR. Gynec Oncol 1999;72:337-341.

Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J. Liquid compared with conventional cervical cytology: a systematic review and meta-analysis. Obstet Gynecol 2008;111:167-177.

Arena S, Marconi M, Ubertosi M, Frega G, Arena G, Villani C. HPV and pregnancy: diagnostic methods, transmission and evolution. Minerva Ginecol 2002;54:225-237.

Armbruster-Moraes E, Ioshimoto LM, o E, Zugaib M. Presence of human papillomavirušLea DNA in amniotic fluids of pregnant women with cervical lesions. Gynecol Oncol 1994;54:152-158.

Armstrong DK, Handley JM. Anogenital warts in prepubertal children: pathogenesis, HPV typing and management. Int J STD AIDS 1997;8:78-81.

Armstrong LR, Preston EJ, Reichert M, Phillips DL, Nisenbaum R, Todd NW, Jacobs IN, Inglis AF, Manning SC, Reeves WC. Incidence and prevalence of recurrent respiratory papillomatosis among children in Atlanta and Seattle. Clin Infect Dis 2000;31:107-109.

Auvinen E, Niemi M, Malm C, Zilliacus R, Trontti A, Fingerroos R, Lehtinen M, Paavonen B. High prevalence of HPV among female students in Finland. Scand J Infect Dis 2005;37:873-876.

Bandyopadhyay S, Chatterjee R. HPV viral load determination during pregnancy as a possible cervical cancer risk. J Exp Clin Cancer Res 2006;25:29-38.

Banura C, Franceschi S, van Doorn LJ, Arslan A, Kleter B, Wabwire-Mangen F, Mbidde EK, Quint W, Weiderpass E. Prevalence, incidence and clearance of human papillomavirus infection among young primiparous pregnant women in Kampala, Uganda. Int J Cancer 2008;123:2180-2187.

Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M, Garnett GP. Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. PLoS Med 2006;3:e138.

Barrett TJ, Silbar JD, McGinley JP. Genital warts - a venereal disease. J Am Med Assoc 1954;154:333-334.

Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, Reingold A, Manos MM. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 1991;265:472-477.

Bernard HU. Gene expression of genital human papillomaviruses and considerations on potential antiviral approaches. Antivir Ther 2002;7:219-237.

Bernard HU, Calleja-Macias IE, Dunn ST. Genome variation of human papillomavirus types: phylogenetic and medical implications. Int J Cancer 2006;118:1071-1076.

Bierl C, Karem K, Poon AC, Swan D, Tortolero-Luna G, Follen M, Wideroff L, Unger ER, Reeves WC. Correlates of cervical mucosal antibodies to human papillomavirus 16: results from a case control study. Gynecol Oncol. 2005;99:S262-268.

Bleeker MC, Hogewoning CJ, Berkhof J, Voorhorst FJ, Hesselink AT, van Diemen PM, van den Brule AJ, Snijders PJ, Meijer CJ. Concordance of specific human papillomavirus types in sex partners is more prevalent than would be expected by chance and is associated with increased viral loads. Clin Infect Dis 2005;41:612-620.

Bodaghi S, Wood LV, Roby G, Ryder C, Steinberg SM, Zheng ZM. Could human papillomaviruses be spread through blood? J Clin Microbiol 2005;43:5428-5434.

Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International

biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796-802.

Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, Muñoz N. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 2008;26 Suppl 10:K1-16.

Boxman IL, Hogewoning A, Mulder LH, Bouwes Bavinck JN, ter Schegget J. Detection of human papillomavirus types 6 and 11 in pubic and perianal hair from patients with genital warts. J Clin Microbiol 1999;37:2270-3227.

Bratthauer GL, Tavassoli FA, O'Leary TJ. Etiology of breast carcinoma: no apparent role for papillomavirus types 6/11/16/18. Pathol Res Pract 1992;188:384-386.

Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, Schiller JT, Lowy DR. Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J Virol 1995;69:3959-3963.

Broker TR, Jin G, Croom-Rivers A, Bragg SM, Richardson M, Chow LT, Vermund SH, Alvarez RD, Pappas PG, Squires KE, Hoesley CJ. Viral latency--the papillomavirus model. Dev Biol (Basel) 2001;106:443-451

Burchell AN, Richardson H, Mahmud SM, Trottier H, Tellier PP, Hanley J, Coutlée F, Franco EL. 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. 2006a163:534-43.

Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006b;S3/52-61.

Burguete T, Rabreau M, Fontanges-Darriet M, Roset E, Hager HD, Koppel A, Bischof P, Schlehofer JR. Evidence for infection of the human embryo with adeno-associated virus in pregnancy. Hum Reprod 1999;14:2396-2401.

Burk RD, Ho GY, Beardsley L, Lempa M, Peters M, Bierman R. Sexual behavior and partner characteristics are the predominant risk factors for genital human papillomavirus infection in young women. J Infect Dis 1996;174:679-689.

Cañadas MP, Bosch FX, Junquera ML, Ejarque M, Font R, Ordoñez E, de Sanjosé S. Concordance of prevalence of human papillomavirus DNA in anogenital and oral infections in a high-risk population. J Clin Microbiol 2004;42:1330-1332.

Carozzi F, Lombardi FC, Zendron P, Confortini M, Sani C, Bisanzi S, Pontenani G, Ciatto S. Association of human papillomavirus with prostate cancer: analysis of a consecutive series of prostate biopsies. Int J Biol Markers. 2004;19:257-61.

Carter JJ, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J, Kiviat N, Galloway DA. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J Infect Dis 1996;174:927-936.

Carter JJ, Galloway DA. Humoral immune response to human papillomavirus infection. Clin Dermatol 1997;15:249-259.

Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, Galloway DA. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis 2000;181:1911-1919.

Cason J, Kaye JN, Jewers RJ, Kambo PK, Bible JM, Kell B, Shergill B, Pakarian F, Raju KS, Best JM. Perinatal infection and persistence of human papillomavirus types 16 and 18 in infants. J Med Virol 1995;47:209-218.

Cason J, Rice P, Best JM. Transmission of cervical cancer-associated human papilloma viruses from mother to child. Intervirology 1998;41:213-218.

Cason J, Mant CA. High-risk mucosal human papillomavirus infections during infancy & childhood. J Clin Virol. 2005;32:S52-8. Review.

Castellsagué X, Ghaffari A, Daniel RW, Bosch FX, Muñoz N, Shah KV. Prevalence of penile human papillomavirus DNA in husbands of women with and without cervical neoplasia: a study in Spain and Colombia. J Infect Dis 1997;176:353-361.

Castellsagué X, Muñoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003;31:20-28.

Castellsagué X, Drudis T, Cañadas MP, Goncé A, Ros R, Pérez JM, Quintana MJ, Muñoz J, Albero G, de Sanjosé S, Bosch FX. Human Papillomavirus (HPV) infection in pregnant women and mother-to-child transmission of genital HPV genotypes: a prospective study in Spain. BMC Infect Dis. 2009;9:74.

Castle PE, Schiffman M, Gravitt PE, Kendall H, Fishman S, Dong H, Hildesheim A, Herrero R, Bratti MC, Sherman ME, Lorincz A, Schussler JE, Burk RD. Comparisons of HPV DNA detection by MY09/11 PCR methods. J Med Virol 2002;68:417-423.

Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, Sherman ME, Wacholder S, Tarone R, Burk RD. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis 2005;191:1808-1816.

Castle PE, Jeronimo J, Schiffman M, Herrero R, Rodríguez AC, Bratti MC, Hildesheim A, Wacholder S, Long LR, Neve L, Pfeiffer R, Burk RD. Age-related changes of the cervix influence human papillomavirus type distribution. Cancer Res 2006;66:1218-1224.

Castle PE, Rodriguez AC, Porras C, Herrero R, Schiffman M, Gonzalez P, Hildesheim A, Burk RD. A

comparison of cervical and vaginal human papillomavirus. Sex Transm Dis 2007;34:849-855.

Cazzaniga M, Gheit T, Casadio C, Khan N, Macis D, Valenti F, Miller MJ, Sylla BS, Akiba S, Bonanni B, Decensi A, Veronesi U, Tommasino M. Analysis of the presence of cutaneous and mucosal papillomavirus types in ductal lavage fluid, milk and colostrum to evaluate its role in breast carcinogenesis. Breast Cancer Res Treat 2009;114:599-605.

Chan PK, Chang AR, Tam WH, Cheung JL, Cheng AF. Prevalence and genotype distribution of cervical human papillomavirus infection: comparison between pregnant women and non-pregnant controls. J Med Virol 2002;67:583-588.

Chan WK, Klock G, Bernard HU. Progesterone and glucocorticoid response elements occur in the long control regions of several human papillomaviruses involved in anogenital neoplasia. J Virol 1989;63:3261-3269.

Chang-Claude J, Schneider A, Smith E, Blettner M, Wahrendorf J, Turek L. Longitudinal study of the effects of pregnancy and other factors on detection of HPV. Gynecol Oncol 1996;60:355-362.

Chong T, Apt D, Gloss B, Isa M, Bernard HU. The enhancer of human papillomavirus type 16: binding sites for the ubiquitous transcription factors oct-1, NFA, TEF-2, NF1, and AP-1 participate in epithelial cell-specific transcription. J Virol 1991;65:5933-5943.

Chow LT, Broker TR (1997) Small DNA tumor viruses. In Viral Pathogenesis, Nathanson N (ed), pp 267-302. Philadelphia: Lippincott-Raven

Christensen ND, Kirnbauer R, Schiller JT, Ghim SJ, Schlegel R, Jenson AB, Kreider JW. Human papillomavirus types 6 and 11 have antigenically distinct strongly immunogenic conformationally dependent neutralizing epitopes. Virology 1994;205:329-335.

Christensen ND, Dillner J, Eklund C, Carter JJ, Wipf GC, Reed CA, Cladel NM, Galloway DA. Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. Virology 1996a;223:174-184.

Christensen ND, Reed CA, Cladel NM, Hall K, Leiserowitz GS. Monoclonal antibodies to HPV-6 L1 virus-like particles identify conformational and linear neutralizing epitopes on HPV-11 in addition to type-specific epitopes on HPV-6. Virology 1996b;224:477-486.

Clifford G, Franceschi S, Diaz M, Muñoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24:S3/26-34.

Clifford GM, Gallus S, Herrero R, Muñoz N, Snijders PJ, Vaccarella S, Anh PT, Ferreccio C, Hieu NT, Matos E, Molano M, Rajkumar R, Ronco G, de Sanjosé S, Shin HR, Sukvirach S, Thomas JO, Tunsakul S, Meijer CJ, Franceschi S; IARC HPV Prevalence Surveys Study Group. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet 2005;366:991-998.

Clifford GM, Shin HR, Oh JK, Waterboer T, Ju YH, Vaccarella S, Quint W, Pawlita M, Franceschi S. Serologic response to oncogenic human papillomavirus types in male and female university students in Busan, South Korea. Cancer Epidemiol Biomarkers Prev 2007;16:1874-1879.

Collins S, Mazloomzadeh S, Winter H, Blomfield P, Bailey A, Young LS; Woodman CB. High incidence of cervical human papillomavirus infection in women during their first sexual relationship. BJOG 2002;109:96-98.

Creasman WT: Preinvasive disease of the cervix. pp. 1-36. In the Book by DiSaia PJ and Creasman WT.Clinical Gynecologic Oncology Seventh Edition. Mosby, Elsevier Inc. 2007.

Cripe TP, Haugen TH, Turk JP, Tabatabai F, Schmid PG 3rd, Dürst M, Gissmann L, Roman A, Turek LP. Transcriptional regulation of the human papillomavirus-16 E6-E7 promoter by a keratinocyte-dependent enhancer, and by viral E2 trans-activator and repressor gene products: implications for cervical carcinogenesis. EMBO J 1987;6:3745-3753.

Culp TD, Budgeon LR, Marinkovich MP, Meneguzzi G, Christensen ND. Keratinocyte-secreted laminin 5 can function as a transient receptor for human papillomaviruses by binding virions and transferring them to adjacent cells. J Virol 2006;80:8940-8950.

Cuschieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore C, Gilkisson G, McGoogan E. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. J Clin Pathol 2004;57:68-72.

Cymet TC. Sexually activated or transmitted? Questions about HPV. J Am Osteopath Assoc. 2006;106:423

de Cremoux P, Thioux M, Lebigot I, Sigal-Zafrani B, Salmon R, Sastre-Garau X; Institut Curie Breast Group. No evidence of human papillomavirus DNA sequences in invasive breast carcinoma. Breast Cancer Res Treat 2008;109:55-58.

de León DC, Montiel DP, Nemcova J, Mykyskova I, Turcios E, Villavicencio V, Cetina L, Coronel A, Hes O.Human papillomavirus (HPV) in breast tumors: prevalence in a group of Mexican patients. BMC Cancer 2009;9:26.

de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol. 1995a;76:1057-62.

de Roda Husman AM, Walboomers JM, Hopman E, Bleker OP, Helmerhorst TM, Rozendaal L, Voorhorst FJ, Meijer CJ. HPV prevalence in cytomorphologically normal cervical scrapes of pregnant women as

determined by PCR: the age-related pattern. J Med Virol 1995b;46:97-102.

de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch FX. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007;7:453-459.

de Villiers EM. Heterogeneity of the human papillomavirus group. J Virol 1989;63:4898-4903.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology 2004;324:17-27.

de Villiers E-M, Sandstrom RE, zur Hausen H, Buck CE. Presence of papillomavirus sequences in condylomatous lesions of the mamillae and in invasive carcinoma of the breast. Breast Cancer Res 2005;7:R1-11.

de Vuyst H, Lillo F, Broutet N, Smith JS. HIV, human papillomavirus, and cervical neoplasia and cancer in the era of highly active antiretroviral therapy. Eur J Cancer Prev 2008;17:545-554.

Dessy FJ, Giannini SL, Bougelet CA, Kemp TJ, David MP, Poncelet SM, Pinto LA, Wettendorff MA. Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. Hum Vaccin 2008;4:425-434.

Di Lonardo A, Venuti A, Marcante ML. Human papillomavirus in breast cancer. Breast Cancer Res Treat 1992;21:95-100.

Dias D, Van Doren J, Schlottmann S, Kelly S, Puchalski D, Ruiz W, Boerckel P, Kessler J, Antonello JM, Green T, Brown M, Smith J, Chirmule N, Barr E, Jansen KU, Esser MT. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. Clin Diagn Lab Immunol 2005;12:959-969.

Diggle PJ, Liang K-Y, Zeger SL. Analysis of longitudinal data, 1st ed. Oxford: Oxford University Press, 1994.

Dillner J, Kallings I, Brihmer C, Sikström B, Koskela P, Lehtinen M, Schiller JT, Sapp M, Mårdh PA.Seropositivities to human papillomavirus types 16, 18, or 33 capsids and to Chlamydia trachomatis are markers of sexual behavior. J Infect Dis 1996;173:1394-1398.

Dillner J, Andersson-Ellström A, Hagmar B, Schiller J. High risk genital papillomavirus infections are not spread vertically. Rev Med Virol 1999;9:23-29.

Dillner J, Arbyn M, Dillner L.Translational mini-review series on vaccines: Monitoring of human papillomavirus vaccination. Clin Exp Immunol 2007;148:199-207.

Doerfler D, Bernhaus A, Kottmel A, Sam C, Koelle D, Joura EA. Human papilloma virus infection prior to coitarche. Am J Obstet Gynecol 2009;200:487.e1-5.

Dollard SC, Broker TR, Chow LT. Regulation of the human papillomavirus type 11 E6 promoter by viral and host transcription factors in primary human keratinocytes. J Virol 1993;67:1721-1726.

Dondog B, Clifford GM, Vaccarella S, Waterboer T, Unurjargal D, Avirmed D, Enkhtuya S, Kommoss F, Wentzensen N, Snijders PJ, Meijer CJ, Franceschi S, Pawlita M. Human papillomavirus infection in Ulaanbaatar, Mongolia: a population-based study. Cancer Epidemiol Biomarkers Prev 2008;17:1731-1738.

Doorbar J. Papillomavirus life cycle organization and biomarker selection. Dis Markers 2007;23:297-313.

Dunne EF, Karem KL, Sternberg MR, Stone KM, Unger ER, Reeves WC, Markowitz LE. Seroprevalence of human papillomavirus type 16 in children. J Infect Dis. 2005;191:1817-9.

Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz LE. Prevalence of HPV infection among females in the United States. JAMA 2007;297:813-819.

D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral Sexual Behaviors Associated with Prevalent Oral Human Papillomavirus Infection. J Infect Dis. 2009;199:1263-1269.

Dürst M, Glitz D, Schneider A, zur Hausen H. Human papillomavirus type 16 (HPV 16) gene expression and DNA replication in cervical neoplasia: analysis by in situ hybridization. Virology 1992;189:132-140.

Dworsky M, Yow M, Stagno S, Pass RF, Alford C. Cytomegalovirus infection of breast milk and transmission in infancy. Pediatrics 1983;72:295-299.

Dyson N, Howley PM, Münger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989;243:934-937.

Dörner T, Radbruch A. Antibodies and B cell memory in viral immunity. Immunity 2007;27:384-392.

Earley MC, Vogt RF Jr, Shapiro HM, Mandy FF, Kellar KL, Bellisario R, Pass KA,Marti GE, Stewart CC, Hannon WH. Report from a workshop on multianalyte microsphere assays. Cytometry 2002;50:239-242.

Effert PJ, Frye RA, Neubauer A, Liu ET, Walther PJ. Human papillomavirus types 16 and 18 are not involved in human prostate carcinogenesis: analysis of archival human prostate cancer specimens by differential polymerase chain reaction. J Urol 1992;147:192-196.

Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, Halsey N, Jenkins D. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis. 2009;9:347-56. Review.

Eppel W, Worda C, Frigo P, Ulm M, Kucera E, Czerwenka K. Human papillomavirus in the cervix and placenta. Obstet Gynecol 2000;96:337-341.

Evander M, Edlund K, Gustafsson A, Jonsson M, Karlsson R, Rylander E, Wadell G. Human papillomavirus infection is transient in young women: a population-based cohort study. J Infect Dis 1995;171:1026-1030.

Evander M, Frazer IH, Payne E, Qi YM, Hengst K, McMillan NA. Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. J Virol 1997;71:2449-2456.

Fairley CK, Gay NJ, Forbes A, Abramson M, Garland SM. Hand-genital transmission of genital warts? An analysis of prevalence data. Epidemiol Infect 1995;115:169-176.

Favre M, Majewski S, De Jesus N, Malejczyk M, Orth G, Jablonska S. A possible vertical transmission of human papillomavirus genotypes associated with epidermodysplasia verruciformis. J Invest Dermatol 1998;111:333-336.

Fedrizzi EN, Villa LL, de Souza IV, Sebastião AP, Urbanetz AA, De Carvalho NS. Does human papillomavirus play a role in endometrial carcinogenesis? Int J Gynecol Pathol. 2009;28:322-7.

Fehrmann F, Laimins LA. Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene 2003;22:5201-5207.

Ferguson M, Heath A, Johnes S, Pagliusi S, Dillner J; Collaborative Study Participants. Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. Int J Cancer 2006;118:1508-1514.

Fife KH, Katz BP, Roush J, Handy VD, Brown DR, Hansell R. Cancer-associated human papillomavirus types are selectively increased in the cervix of women in the first trimester of pregnancy. Am J Obstet Gynecol 1996;174:1487-1493.

Fife KH, Katz BP, Brizendine EJ, Brown DR. Cervical human papillomavirus deoxyribonucleic acid persists throughout pregnancy and decreases in the postpartum period. Am J Obstet Gynecol 1999;180:1110-1114.

Fife KH, Wheeler CM, Koutsky LA, Barr E, Brown DR, Schiff MA, Kiviat NB, Jansen KU, Barber H, Smith JF, Tadesse A, Giacoletti K, Smith PR, Suhr G, Johnson DA. Dose-ranging studies of the safety and immunogenicity of human papillomavirus Type 11 and Type 16 virus-like particle candidate vaccines in young healthy women. Vaccine 2004;22:2943-2952.

Finnish Current Care guidelines. Diagnosis, treatment and follow-up of cytological changes in the cervix, vagina and vulva, Working group set up by the Finnish Medical Society Duodecim and the Finnish Colposcopy Association 2006.

Franceschi S, Castellsagué X, Dal Maso L, Smith JS, Plummer M, Ngelangel C, Chichareon S, Eluf-Neto J, Shah KV, Snijders PJ, Meijer CJ, Bosch FX, Muñoz N. Prevalence and determinants of human papillomavirus genital infection in men. Br J Cancer 2002;86:705-711.

Franco EL, Villa LL, Ruiz A, Costa MC. Transmission of cervical human papillomavirus infection by sexual activity: differences between low and high oncogenic risk types. J Infect Dis 1995;172:756-763.

Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Désy M, Rohan TE. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999;180:1415-1423.

Frazer I. Correlating immunity with protection for HPV infection. Int J Infect Dis 2007;11 Suppl 2:S10-16.

Frazer IH. Interaction of human papillomaviruses with the host immune system: a well evolved relationship. Virology 2009;384:410-414.

Gajewska M, Marianowski L, Wielgos M, Malejczyk M, Majewski S. The occurrence of genital types of human papillomavirus in normal pregnancy and in pregnant women with pregestational insulin dependent diabetes mellitus. Neuro Endocrinol Lett. 2005;26:766-70.

García-Piñeres AJ, Hildesheim A, Dodd L, Kemp TJ, Yang J, Fullmer B, Harro C, Lowy DR, Lempicki RA, Pinto LA. Gene expression patterns induced by HPV-16 L1 virus-like particles in leukocytes from vaccine recipients. J Immunol. 2009;182:1706-1729.

Genest DR, Sun D, Crum CP. Human papillomavirus in spontaneous abortion. Hum Pathol 1999;30:109-111.

Ghim S, Newsome J, Bell J, Sundberg JP, Schlegel R, Jenson AB. Spontaneously regressing oral papillomas induce systemic antibodies that neutralize canine oral papillomavirus. Exp Mol Pathol 2000;68:147–151.

Gissmann L, Wolnik L, Ikenberg H, Koldovsky U, Schnürch HG, zur Hausen H. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci U S A 1983;80:560-563.

Gissman L. The current role of HPV serology. Chapter 20 pp.365-371, in Book Human Papillomavirus Infections in dermatovenereology/ edited by G. Gross, G. von Krogh, 1997 by CRC Press, Inc.

Gloss B, Bernard HU, Seedorf K, Klock G. The upstream regulatory region of the human papilloma virus-16 contains an E2 protein-independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. EMBO J 1987;6:3735-3743.

Gomez LM, Ma Y, Ho C, McGrath CM, Nelson DB, Parry S. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Hum Reprod. 2008;23:709-15.

Googe PB, Chung SJ, Simmons J, King R.Giant-sized condyloma of the breast with focal acantholytic changes. J Cutan Pathol. 2000;27:319-22.

Gopalkrishna V, Singh UR, Sodhani P, Sharma JK, Hedau ST, Mandal AK, Das BC. Absence of human

papillomavirus DNA in breast cancer as revealed by polymerase chain reaction. Breast Cancer Res Treat 1996;39:197-202.

Grassmann K, Rapp B, Maschek H, Petry KU, Iftner T. Identification of a differentiation-inducible promoter in the E7 open reading frame of human papillomavirus type 16 (HPV-16) in raft cultures of a new cell line containing high copy numbers of episomal HPV-16 DNA. J Virol 1996;70:2339-2349.

Griffiths M. 'Nuns, virgins, and spinsters'. Rigoni-Stern and cervical cancer revisited. Br J Obstet Gynaecol 1991;98:797-802.

Gutman LT, Herman-Giddens ME, Phelps WC. Transmission of human genital papillomavirus disease: comparison of data from adults and children. Pediatrics 1993;91:31-38.

Hagensee ME, Olson NH, Baker TS, Galloway DA. Three-dimensional structure of vaccinia virus-produced human papillomavirus type 1 capsids. J Virol 1994;68:4503-4505.

Hagensee ME, Slavinsky J 3rd, Gaffga CM, Suros J, Kissinger P, Martin DH. Seroprevalence of human papillomavirus type 16 in pregnant women. Obstet Gynecol. 1999;94:653-658.

Hajek EF. Contribution to the etiology of laryngeal papilloma in children. J Laryngol Otol 1956;70:166-168.

Halperin DT. Heterosexual anal intercourse: prevalence, cultural factors, and HIV infection and other health risks, Part I. AIDS Patient Care STDS. 1999;13:717-30.

Handley J, Hanks E, Armstrong K, Bingham A, Dinsmore W, Swann A, Evans MF, McGee JO, O'Leary J. Common association of HPV 2 with anogenital warts in prepubertal children. Pediatr Dermatol 1997;14:339-343.

Hardin J, Hilbe JM. Generalized estimating equations. (ed). Boca Raton, FL: Chapman & Hall, 2003.

Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA, Dubin G; HPV Vaccine Study group. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006;367:1247-1255.

Harper DM. Prophylactic human papillomavirus vaccines to prevent cervical cancer: review of the Phase II and III trials. Therapy 2008;5:313-324.

Helmrich G, Stubbs TM, Stoerker J. Fatal maternal laryngeal papillomatosis in pregnancy: a case report [corrected]. Am J Obstet Gynecol 1992;166:524-525.

Hermonat PL, Han L, Wendel PJ, Quirk JG, Stern S, Lowery CL, Rechtin TM. Human papillomavirus is more prevalent in first trimester spontaneously aborted products of conception compared to elective specimens. Virus Genes 1997;14:13-17.

Hernández-Girón C, Smith JS, Lorincz A, Lazcano E, Hernández-Avila M, Salmerón J. High-risk human papillomavirus detection and related risk factors among pregnant and nonpregnant women in Mexico. Sex Transm Dis 2005;32:613-618.

Herrero R, Hildesheim A, Bratti C, Sherman ME, Hutchinson M, Morales J, Balmaceda I, Greenberg MD, Alfaro M, Burk RD, Wacholder S, Plummer M, Schiffman M. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst 2000;92:464-474.

Hildesheim A, Schiffman MH, Gravitt PE, Glass AG, Greer CE, Zhang T, Scott DR, Rush BB, Lawler P, Sherman ME, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994;169:235-240.

Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, Schiller JT, Gonzalez P, Dubin G, Porras C, Jimenez SE, Lowy DR; Costa Rican HPV Vaccine Trial Group. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA 2007;298:743-753.

Hippeläinen MI, Yliskoski M, Syrjänen S, Saastamoinen J, Hippeläinen M, Saarikoski S, Syrjänen K. Low concordance of genital human papillomavirus (HPV) lesions and viral types in HPV-infected women and their male sexual partners. Sex Transm Dis 1994;21:76-82.

Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423-428.

Ho GY, Studentsov Y, Hall CB, Bierman R, Beardsley L, Lempa M, Burk RD. Risk factors for subsequent cervicovaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. J Infect Dis 2002;186:737-742.

Ho GY, Studentsov YY, Bierman R, Burk RD. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. Cancer Epidemiol Biomarkers Prev 2004;13:110-116.

Hogewoning CJ, Bleeker MC, van den Brule AJ, Voorhorst FJ, Snijders PJ, Berkhof J, Westenend PJ, Meijer CJ. Condom use promotes regression of cervical intraepithelial neoplasia and clearance of human papillomavirus: a randomized clinical trial. Int J Cancer 2003;107:811-816.

Hoyo C, Yarnall KS, Fortney J. Short birth intervals and uterine cervical cancer risk in Jamaican women. Int J Gynaecol Obstet. 2007 Jul;98(1):56-7.

Howley PM and Lowy DR. Virology, Vol. 2. Fields BN, Knipe JB, Howley PM (eds). Lippincott/The Williams & Wilkins Co: Philadelphia, PA, 2001 pp.2197-2229.

Hughes JP, Garnett GP, Koutsky LA. The theoretical population level impact of a prophylactic human papilloma virus vaccine. Epidemiology 2002;13:631–639.

Hwang LY, Ma Y, Benningfield SM, Clayton L, Hanson EN, Jay J, Jonte J, Godwin de Medina C, Moscicki AB Factors that influence the rate of epithelial maturation in the cervix in healthy young women. J Adolesc Health 2009;44:103-110.

International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical carcinoma and reproductive factors: collaborative reanalysis of individual data on 16,563 women with cervical carcinoma and 33,542 women without cervical carcinoma from 25 epidemiological studies. Int J Cancer 2006;119:1108-1124.

Jacobs MV, Walboomers JM, Snijders PJ, Voorhorst FJ, Verheijen RH, Fransen-Daalmeijer N, Meijer CJ. Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. Int J Cancer 2000;87:221-227.

Jacobson DL, Peralta L, Graham NM, Zenilman J. Histologic development of cervical ectopy: relationship to reproductive hormones. Sex Transm Dis. 2000;27:252-258.Review.

Johnson KM, Kines RC, Roberts JN, Lowy DR, Schiller JT, Day PM. Role of heparan sulfate in attachment to and infection of the murine female genital tract by human papillomavirus. J Virol 2009;83:2067-2074.

Jordan J, Arbyn M, Martin-Hirsch P, Schenck U, Baldauf JJ, Da Silva D, Anttila A, Nieminen P, Prendiville W. European guidelines for quality assurance in cervical cancer screening: recommendations for clinical management of abnormal cervical cytology, part 1. Cytopathology 2008;19:342-354.

Jordan J, Martin-Hirsch P, Arbyn M, Schenck U, Baldauf JJ, Da Silva D, Anttila A, Nieminen P, Prendiville W. European guidelines for clinical management of abnormal cervical cytology, part 2. Cytopathology 2009;20:5-16.

Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA, Jansen KU, Keller PM. The LI major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J Biol Chem 1999;274:5810-5822.

Kaasila M, Koskela P, Kirnbauer R, Pukkala E, Surcel HM, Lehtinen M. Population dynamics of serologically identified coinfections with human papillomavirus types 11, 16, 18 and 31 in fertile-aged Finnish women. Int J Cancer. 2009 [Epub ahead of print]

Kaplan CG. Gross pathology of the placenta: weight, shape, size, colour. J Clin Pathol 2008; 61: 1285-1295.

Karlsson R, Jonsson M, Edlund K, Evander M, Gustavsson A, Bodén E, Rylander E, Wadell G. Lifetime number of partners as the only independent risk factor for human papillomavirus infection: a population-based study. Sex Transm Dis 1995;22:119-127.

Kay P, Meehan K, Williamson AL. The use of nested polymerase chain reaction and restriction fragment length polymorphism for the detection and typing of mucosal human papillomaviruses in samples containing low copy numbers of viral DNA. J Virol Methods 2002;105:159-170.

Kaye JN, Cason J, Pakarian FB, Jewers RJ, Kell B, Bible J, Raju KS, Best JM. Viral load as a determinant for transmission of human papillomavirus type 16 from mother to child. J Med Virol 1994;44:415-421.

Kaye JN, Starkey WG, Kell B, Biswas C, Raju KS, Best JM, Cason J. Human papillomavirus type 16 in infants: use of DNA sequence analyses to determine the source of infection. J Gen Virol 1996;77:1139-1143.

Kellokoski J, Syrjänen S, Yliskoski M, Syrjänen K. Dot blot hybridization in detection of human papillomavirus (HPV) infections in the oral cavity of women with genital HPV infections. Oral Microbiol Immunol 1992a;7:19-23.

Kellokoski JK, Syrjänen SM, Chang F, Yliskoski M, Syrjänen KJ. Southern blot hybridization and PCR in detection of oral human papillomavirus (HPV) infections in women with genital HPV infections. J Oral Pathol Med 1992b;21:459-464.

Khan NA, Castillo A, Koriyama C, Kijima Y, Umekita Y, Ohi Y, Higashi M, Sagara Y, Yoshinaka H, Tsuji T, Natsugoe S, Douchi T, Eizuru Y, Akiba S. Human papillomavirus detected in female breast carcinomas in Japan. Br J Cancer. 2008;99:408-14.

Kibur M, af Geijerstamm V, Pukkala E, Koskela P, Luostarinen T, Paavonen J, Schiller J, Wang Z, Dillner J, Lehtinen M. Attack rates of human papillomavirus type 16 and cervical neoplasia in primiparous women and field trial designs for HPV16 vaccination. Sex Transm Infect. 2000;76(1):13-7.

Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci U S A 1992;89:12180-12184.

Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst 1994;86:494-499.

Kitchener HC, Castle PE, Cox JT. Chapter 7: Achievements and limitations of cervical cytology screening. Vaccine. 2006;24:S3/63-70.

Kjaer SK, van den Brule AJ, Bock JE, Poll PA, Engholm G, Sherman ME, Walboomers JM, Meijer CJ. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? Cancer Epidemiol Biomarkers Prev 1997;6:799-805.

Kjaer SK, Tran TN, Sparen P, Tryggvadottir L, Munk C, Dasbach E, Liaw KL, Nygård J, Nygård M. The burden of genital warts: a study of nearly 70,000

women from the general female population in the 4 Nordic countries. J Infect Dis 2007;196:1447-1454.

Klaes R, Woerner SM, Ridder R, Wentzensen N, Duerst M, Schneider A, Lotz B, Melsheimer P, von Knebel Doeberitz M. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer Res 1999;59:6132-6136.

Koch A, Hansen SV, Nielsen NM, Palefsky J, Melbye M. HPV detection in children prior to sexual debut. Int J Cancer 1997;73:621-624.

Kojima A, Maeda H, Kurahashi N, Sakagami G, Kubo K, Yoshimoto H, Kameyama Y. Human papillomaviruses in the normal oral cavity of children in Japan. Oral Oncol 2003;39:821-828.

Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol. 2008;168:123-137. Review.

Koss LG, Durfee Gr. Unusual patterns of squamous epithelium of the uterine cervix: cytologic and pathologic study of koilocytotic atypia. Ann NY Acad Sci 1956;63:1245-1261.

Koss LG. Human papillomavirus--passenger, driver, or both? Hum Pathol. 1998 Apr;29(4):309-10.

Koss LG. Evolution in cervical pathology and cytology: a historical perspective. Eur J Gynaecol Oncol 2000;21:550-554.

Kourtis AP, Butera S, Ibegbu C, Beled L, Duerr A. Breast milk and HIV-1: vector of transmission or vehicle of protection? Lancet Infect Dis 2003;3:786-793.

Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med 1997;102:3-8.

Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, Shah KV, Gillison ML. Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. J Infect Dis. 2004;189:686-698.

Kulmala SM, Shabalova IP, Petrovitchev N, Syrjänen KJ, Gyllensten UB, Johansson BC, Syrjänen SM; the New Independent States of the former Soviet Union Cohort Study Group. Type-specific persistence of high-risk human papillomavirus infections in the New Independent States of the former Soviet Union Cohort Study. Cancer Epidemiol Biomarkers Prev 2007;16:17-22.

Lacey CJ, Lowndes CM, Shah KV. Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. Vaccine 2006;24:S3/35-41.

Lai CH, Hsueh S, Lin CY, Huang MY, You GB, Chang HC, Pao CC. Human papillomavirus in benign and malignant ovarian and endometrial tissues. Int J Gynecol Pathol. 1992;11:210-5.

Lai YM, Yang FP, Pao CC. Human papillomavirus deoxyribonucleic acid and ribonucleic acid in seminal plasma and sperm cells. Fertil Steril. 1996;65:1026-30.

Laukkanen P, Koskela P, Pukkala E, Dillner J, Läärä E, Knekt P, Lehtinen M. Time trends in incidence and prevalence of human papillomavirus type 6, 11 and 16 infections in Finland. J Gen Virol. 2003;84:2105-9.

Lawton G, Thomas S, Schonrock J, Monsour F, Frazer I. Human papillomaviruses in normal oral mucosa: a comparison of methods for sample collection. J Oral Pathol Med 1992;21:265-269.

Lehtinen M, Kaasila M, Pasanen K, Patama T, Palmroth J, Laukkanen P, Pukkala E, Koskela P. Seroprevalence atlas of infections with oncogenic and non-oncogenic human papillomaviruses in Finland in the 1980s and 1990s. Int J Cancer. 2006;119:2612-9.

Leinonen M, Kotaniemi-Talonen L, Anttila A, Dyba T, Tarkkanen J, Nieminen P. Prevalence of oncogenic human papillomavirus infection in an organised screening population in Finland. Int J Cancer 2008;123:1344-1349.

Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates Nat. Rev. Immunol. 2007;7:379-390

Ley C, Bauer HM, Reingold A, Schiffman MH, Chambers JC, Tashiro CJ, Manos MM. Determinants of genital human papillomavirus infection in young women. J Natl Cancer Inst 1991;83:997-1003.

Liang W, Tian H. Hypothetic association between human papillomavirus infection and breast carcinoma. Med Hypotheses. 2008;70:305-7.

Liu Y, You H, Chiriva-Internati M, Korourian S, Lowery CL, Carey MJ, Hermonat PL. Display of complete life cycle of human papillomavirus type 16 in cultured placental trophoblasts. Virology 2001;290:99-105.

Londesborough P, Ho L, Terry G, Cuzick J, Wheeler C, Singer A. Human papillomavirus genotype as a predictor of persistence and development of high-grade lesions in women with minor cervical abnormalities. Int J Cancer 1996;69:364-368.

Manavi M, Czerwenka KF, Schurz B, Knogler W, Kubista E, Reinold E. Latent cervical virus infection as a possible cause of early abortion. Gynakol Geburtshilfliche Rundsch 1992;32:84-87.

Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta-analysis. Sex Transm Dis 2002;29:725-735.

Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. The use of polymerase chain reaction amplification for detection of genital papillomavirus. In: Furth M, Greaves M (eds.): Molecular Diagnostics of Human Cancer: Cancer Cells 1989;7:209-214.

Manz RA, Hauser AE, Hiepe F, Radbruch A. Maintenance of serum antibody levels. Annu Rev Immunol 2005;23:367-386.

Marais DJ, Sampson CC, Urban MI, Sitas F, Wiliamson AL. The seroprevalence of IgG antibodies to human papillomavirus (HPV) types HPV-16, HPV-18, and HPV-11 capsid-antigens in mothers and their children. J Med Virol. 2007;79:1370-4.

Marcoux D, Nadeau K, McCuaig C, Powell J, Oligny LL. Pediatric anogenital warts: a 7-year review of children referred to a tertiary-care hospital in Montreal, Canada. Pediatr Dermatol 2006;23:199-207.

Marrazzo JM, Koutsky LA, Stine KL, Kuypers JM, Grubert TA, Galloway DA, Kiviat NB, Handsfield HH. Genital human papillomavirus infection in women who have sex with women. J Infect Dis 1998;178:1604-1609.

Matovina M, Husnjak K, Milutin N, Ciglar S, Grce M. Possible role of bacterial and viral infections in miscarriages. Fertil Steril. 2004;81:662-9.

Maxwell GL, Carlson J, Zakarean M, Veit B, Hoshaw N. The absence of human papillomavirus in amniotic fluid. J Low Genit Tract Dis 1998;2:151-154.

Mazzatenta C, Fimiani M, Rubegni P, Andreassi L, Buffi P, Messina C. Vertical transmission of human papillomavirus in cytologically normal women. Genitourin Med 1996;72:445-446.

Mbulawa ZZ, Williamson AL, Stewart D, Passmore JA, Denny L, Allan B, Marais DJ. Association of serum and mucosal neutralizing antibodies to human papillomavirus type 16 (HPV-16) with HPV-16 infection and cervical disease. J Gen Virol 2008;89:910-914.

McCaffery K, Waller J, Nazroo J, Wardle J. Social and psychological impact of HPV testing in cervical screening: a qualitative study. Sex Transm Inf 2006;82:169-174.

McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW, Skegg DC. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol. 2008;9:425-34.

McMillan NA, Payne E, Frazer IH, Evander M. Expression of the alpha6 integrin confers papillomavirus binding upon receptor-negative B-cells. Virology 1999;261:271-279.

McNicol PJ, Dodd JG. Detection of human papillomavirus DNA in prostate gland tissue by using the polymerase chain reaction amplification assay. J Clin Microbiol 1990;28:409-412.

Medeiros LR, Ethur AB, Hilgert JB, Zanini RR, Berwanger O, Bozzetti MC, Mylius LC. Vertical transmission of the human papillomavirus: a systematic quantitative review. Cad Saude Publica 2005;21:1006-1015.

Mei HE, Yoshida T, Sime W, Hiepe F, Thiele K, Manz RA, Radbruch A, Dörner T. Blood-borne human plasma cells in steady state are derived from mucosal immune responses. Blood. 2009;113:2461-9.

Meisels A, Fortin R.Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. Acta Cytol 1976;20:505-509.

Mejlhede N, Bonde J, Fomsgaard A. High frequency of multiple HPV types in cervical specimens from Danish women. APMIS 2009;117:108-114.

Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, Bravo IG, Schlehofer J, Gärtner BC, Pawlita M. Seroprevalence of 34 human papillomavirus types in the German general population. PLoS Pathog 2008;4(6):e1000091.

Michie CA, Gilmour J. 2001 Breast feeding and the risks of viral transmission. Arch Dis Child. 2001;84:381-382.

Middleton K, Peh W, Southern S, Griffin H, Sotlar K, Nakahara T, El-Sherif A, Morris L, Seth R, Hibma M, Jenkins D, Lambert P, Coleman N, Doorbar J. Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. J Virol. 2003;77:10186-201.

Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:622-635.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.

Minkoff H, Shen X, Watts DH, Leighty R, Hershow R, Palefsky J, Tuomala R, Neu N, Zorrilla CD, Paul M, Strickler H. Relationship of pregnancy to human papillomavirus among human immunodeficiency virus-infected women. Obstet Gynecol 2006;108:953-960.

Mistry N, Simonsson M, Evander M. Transcriptional activation of the human papillomavirus type 5 and 16 long control region in cells from cutaneous and mucosal origin. Virol J 2007;4:27.

Mistry N, Wibom C, Evander M. Cutaneous and mucosal human papillomaviruses differ in net surface charge, potential impact on tropism. Virol J 2008;5:118.

Molano M, Van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, Meijer CJ, Muñoz N, Franceschi S; HPV Study Group. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am J Epidemiol 2003;158:486-494.

Morrison EA, Gammon MD, Goldberg GL, Vermund SH, Burk RD. Pregnancy and cervical infection with human papillomaviruses. Int J Gynaecol Obstet 1996;54:125-130.

Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N, Darragh TM, Brescia R, Kanowitz S, Miller SB, Stone J, Hanson E, Palefsky J. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr 1998;132:277-284.

Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, Miller S, Clayton L, Farhat S, Broering J, Darragh T, Palefsky J. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 2001;285:2995-3002.

Moscicki AB. Impact of HPV infection in adolescent populations. J Adolesc Health. 2005;37:S3-9.

Mounts P, Shah KV, Kashima H. Viral etiology of juvenile- and adult-onset squamous papilloma of the larynx. Proc Natl Acad Sci U S A 1982;79:5425-5429.

Moy RL, Eliezri YD, Nuovo GJ, Zitelli JA, Bennett RG, Silverstein S. Human papillomavirus type 16 DNA in periungual squamous cell carcinomas. JAMA 1989;261:2669-2673.

Müller M, Gissmann L, Cristiano RJ, Sun XY, Frazer IH, Jenson AB, Alonso A, Zentgraf H, Zhou J. Papillomavirus capsid binding and uptake by cells from different tissues and species. J Virol 1995;69:948-954.

Münger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. EMBO J 1989;8:4099-4105.

Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res 2002;89:213-228.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-527.

Muñoz N, Méndez F, Posso H, Molano M, van den Brule AJ, Ronderos M, Meijer C, Muñoz A; Instituto Nacional de Cancerologia HPV Study Group. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis 2004;190:2077-2087.

Muñoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006;24 S3:S1-10.

Muñoz N, Hernandez-Suarez G, Méndez F, Molano M, Posso H, Moreno V, Murillo R, Ronderos M, Meijer C, Muñoz A; Instituto Nacional de Cancerología HPV Study Group. Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br J Cancer 2009;100:1184-1190.

Myhre AK, Dalen A, Berntzen K, Bratlid D. Anogenital human papillomavirus in non-abused preschool children. Acta Paediatr 2003;92:1445-1452.

Nakagawa M, Viscidi R, Deshmukh I, Costa MD, Palefsky JM, Farhat S, Moscicki AB. Time course of humoral and cell-mediated immune responses to human papillomavirus type 16 in infected women. Clin Diagn Lab Immunol 2002;9:877-882.

National Cancer Institute Workshop. The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses. JAMA 1989;262:931-934.

Nieminen P, Kallio M, Hakama M. The effect of mass screening on incidence and mortality of squamous and adenocarcinoma of cervix uteri. Obstet Gynecol 1995;85:1017-1021.

Nieminen P, Kotaniemi L, Hakama M, Tarkkanen J, Martikainen J, Toivonen T, Ikkala J, Luostarinen T, Anttila A. A randomised public-health trial on automation-assisted screening for cervical cancer in Finland: performance with 470,000 invitations. Int J Cancer 2005;115:307-311.

Obalek S, Jabłońska S, Orth G. Anogenital warts in children. Clin Dermatol 1997;15:369-376.

O'Leary JJ, Landers RJ, Crowley M, Healy I, O'Donovan M, Healy V, Kealy WF, Hogan J, Doyle CT. Human papillomavirus and mixed epithelial tumors of the endometrium. Hum Pathol. 1998;29:383-9.

Opalka D, Lachman CE, MacMullen SA, Jansen KU, Smith JF, Chirmule N, Esser MT. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. Clin Diagn Lab Immunol 2003;10:108-115.

Oriel JD. Natural history of genital warts. Br J Vener Dis 1971;47:1-13.

Oriel JD. Sexually transmitted diseases in children: human papillomavirus infection. Genitourin Med 1992;68:80-83.

Ostör AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol. 1993 ;12:186-92. Review.

Ozbun M, Wei L, Higgins K, The 25th International Papillomavirus Conference 05/2009, abstr. P-06.66, 2009

Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira JC, Skinner SR, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G; HPV PATRICIA Study Group, Greenacre M. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. 2009 25;374:301-14.

Pao CC, Lin SS, Lin CY, Maa JS, Lai CH, Hsieh TT. Identification of human papillomavirus DNA sequences in peripheral blood mononuclear cells. Am J Clin Pathol 1991;95:540-546.

Passmore JA, Marais DJ, Sampson C, Allan B, Parker N, Milner M, Denny L, Williamson AL. Cervicovaginal, oral, and serum IgG and IgA responses to human papillomavirus type 16 in women with cervical intraepithelial neoplasia. J Med Virol 2007;79:1375-1380.

Pastrana DV, Buck CB, Pang YY, Thompson CD, Castle PE, FitzGerald PC, Krüger Kjaer S, Lowy DR, Schiller JT. Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. Virology 2004;321:205-216.

Paternoster DM, Cester M, Resente C, Pascoli I, Nanhorngue K, Marchini F, Boccagni P, Cillo U, Ribaldone R, Amoruso E, Cocca N, Cuccolo V, Bertolino M, Surico N, Stratta P. Human papilloma virus infection and cervical intraepithelial neoplasia in transplanted patients. Transplant Proc 2008;40:1877-1880.

Peto J, Gilham C, Deacon J, Taylor C, Evans C, Binns W, Haywood M, Elanko N, Coleman D, Yule R, Desai M. Cervical HPV infection and neoplasia in a large population-based prospective study: the Manchester cohort. Br J Cancer 2004 31;91:942-953.

Pett MR, Herdman MT, Palmer RD, Yeo GS, Shivji MK, Stanley MA, Coleman N. Selection of cervical keratinocytes containing integrated HPV16 associates with episome loss and an endogenous antiviral response. Proc Natl Acad Sci U S A. 2006;103:3822-3827.

Peyton CL, Gravitt PE, Hunt WC, Hundley RS, Zhao M, Apple RJ, Wheeler CM. Determinants of genital human papillomavirus detection in a US population. J Infect Dis 2001;183:1554-1564.

Pfister H. Biology and biochemistry of papillomaviruses. Rev Physiol Biochem Pharmacol 1984;99:111-181.

Poland GA, Jacobson RM, Koutsky LA, Tamms GM, Railkar R, Smith JF, Bryan JT, Cavanaugh PF Jr, Jansen KU, Barr E. Immunogenicity and reactogenicity of a novel vaccine for human papillomavirus 16: a 2-year randomized controlled clinical trial. Mayo Clin Proc 2005;80:601-610.

Poljak M, Kocjan BJ, Potocnik M, Seme K. Anogenital hairs are an important reservoir of alpha-papillomaviruses in patients with genital warts. J Infect Dis 2009;199:1270-1274.

Powell J, Strauss S, Gray J, Wojnarowska F. Genital carriage of human papilloma virus (HPV) DNA in prepubertal girls with and without vulval disease. Pediatr Dermatol 2003;20:191-194.

Puranen M, Yliskoski M, Saarikoski S, Syrjänen K, Syrjänen S. Vertical transmission of human papillomavirus from infected mothers to their newborn babies and persistence of the virus in childhood. Am J Obstet Gynecol 1996;174:694-699.

Puranen MH, Yliskoski MH, Saarikoski SV, Syrjanen KJ, Syrjanen SM. Exposure of an infant to cervical human papillomavirus infection of the mother is common. Am J Obstet Gynecol 1997;176:1039-1045.

Purola E, Savia E. Cytology of gynecologic condyloma acuminatum. Acta Cytol 1977;21:26-31.

Pyeon D, Pearce SM, Lank SM, Ahlquist P, Lambert PF. Establishment of human papillomavirus infection requires cell cycle progression. PLoS Pathog 2009;5:e1000318.

Quick CA, Faras A, Krzysek R. The etiology of laryngeal papillomatosis. Laryngoscope 1978;88:1789-1795.

Rando RF, Lindheim S, Hasty L, Sedlacek TV, Woodland M, Eder C. Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy. Am J Obstet Gynecol 1989;161:50-55.

Rappuoli R. Bridging the knowledge gaps in vaccine design. Nat Biotechnol 2007;25:1361-1366.

Reuter JD, Gomez D, Brandsma JL, Rose JK, Roberts A. Optimization of cottontail rabbit papilloma virus challenge technique. J Virol Methods 2001;98:127-134. Review.

Revzina NV, Diclemente RJ. Prevalence and incidence of human papillomavirus infection in women in the USA: a systematic review. Int J STD AIDS 2005;16:528-537.

Rice PS, Cason J, Best JM, Banatvala JE. High risk genital papillomavirus infections are spread vertically. Rev Med Virol. 1999;9:15-21.

Rice PS, Mant C, Cason J, Bible JM, Muir P, Kell B, Best JM. High prevalence of human papillomavirus type 16 infection among children. J Med Virol 2000;61:70-75.

Richardson H, Franco E, Pintos J, Bergeron J, Arella M, Tellier P. Determinants of low-risk and high-risk cervical human papillomavirus infections in Montreal University students. Sex Transm Dis 2000;27:79-86.

Richardson H, Abrahamowicz M, Tellier PP, Kelsall G, du Berger R, Ferenczy A, Coutlée F, Franco EL. Modifiable risk factors associated with clearance of type-specific cervical human papillomavirus infections in a cohort of university students. Cancer Epidemiol Biomarkers Prev. 2005 ;14:1149-1156.

Richart RM. Cervical intraepithelial neoplasia. Pathol Annu. 1973;8:301-28.

Rintala MA, Grénman SE, Puranen MH, Isolauri E, Ekblad U, Kero PO, Syrjänen SM. Transmission of high-risk human papillomavirus (HPV) between parents and infant: a prospective study of HPV in families in Finland. J Clin Microbiol 2005a;43:376-381.

Rintala MA, Grenman SE, Jarvenkyla ME, Syrjanen KJ, Syrjanen SM. High-risk types of human papillomavirus (HPV) DNA in oral and genital mucosa of infants during their first 3 years of life: experience from the Finnish HPV Family Study. Clin Infect Dis 2005b;41:1728-1733.

Rintala M, Grénman S, Puranen M, Syrjänen S. Natural history of oral papillomavirus infections in spouses: A prospective Finnish HPV family study. J Clin Virol 2006;35:89-94.

Rizk RZ, Christensen ND, Michael KM, Müller M, Sehr P, Waterboer T, Pawlita M. Reactivity pattern of 92 monoclonal antibodies with 15 human papillomavirus types. J Gen Virol. 2008;89:117-129.

Roberts JN, Buck CB, Thompson CD, Kines R, Bernardo M, Choyke PL, Lowy DR, Schiller JT. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. Nat Med 2007;13:857-861.

Roden RB, Kirnbauer R, Jenson AB, Lowy DR, Schiller JT. Interaction of papillomaviruses with the cell surface. J Virol 1994;68:7260-7266.

Rodriguez AC, Burk R, Herrero R, Hildesheim A, Bratti C, Sherman ME, Solomon D, Guillen D, Alfaro M, Viscidi R, Morales J, Hutchinson M, Wacholder S, Schiffman M. The natural history of human papillomavirus infection and cervical intraepithelial neoplasia among young women in the Guanacaste cohort shortly after initiation of sexual life. Sex Transm Dis 2007;34:494-502.

Rogo KO, Nyansera PN. Congenital condylomata acuminata with meconium staining of amniotic fluid and fetal hydrocephalus: case report. East Afr Med J 1989;66:411-413.

Rombaldi RL, Serafini EP, Mandelli J, Zimmermann E, Losquiavo KP. Transplacental transmission of Human Papillomavirus. Virol J 2008;5:106.

Rose RC, White WI, Li M, Suzich JA, Lane C, Garcea RL. Human papillomavirus type 11 recombinant L1 capsomeres induce virus-neutralizing antibodies. J Virol 1998;72:6151-6154.

Rotkin ID. Adolescent coitus and cervical cancer: associations of related events with increased risk. Cancer Res 1967;27:603-617.

Rozendaal L, Westerga J, van der Linden JC, Walboomers JM, Voorhorst FJ, Risse EK, Boon ME, Meijer CJ. PCR based high risk HPV testing is superior to neural network based screening for predicting incident CIN III in women with normal cytology and borderline changes. J Clin Pathol 2000;53:606-611.

Rüdlinger R, Grob R, Yu YX, Schnyder UW. Human Papillomavirus-35-Positive Bowenoid Papulosis of the Anogenital Area and Concurrent Human Papillomavirus-35-Positive Verruca With Bowenoid Dysplasia of the Periungual Area. Arch Dermatol 1989;125:655-659.

Ruffin MT 4th, Bailey JM, Roulston D, Lee DR, Tucker RA, Swan DC, Unger ER. Human papillomavirus in amniotic fluid. BMC Pregnancy Childbirth 2006;6:28.

Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 2003;31:14-19.

Schiffman M, Castle PE. The promise of global cervical-cancer prevention. N Engl J Med. 2005 17;353:2101-2104.

Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007;370:890-907.

Schiller JT, Castellsagué X, Villa LL, Hildesheim A. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. Vaccine 2008;26 Suppl 10:K53-61.

Schlecht NF, Platt RW, Duarte-Franco E, Costa MC, Sobrinho JP, Prado JC, Ferenczy A, Rohan TE, Villa LL, Franco EL. Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia. J Natl Cancer Inst 2003;95:1336-1343.

Schmitt M, Bravo IG, Snijders PJ, Gissmann L, Pawlita M, Waterboer T. Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol 2006;44:504-512.

Schneider A, Hotz M, Gissmann L. Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women. Int J Cancer 1987;40:198-201.

Schneider A, Kirchhoff T, Meinhardt G, Gissmann L. Repeated evaluation of human papillomavirus 16 status in cervical swabs of young women with a history of normal Papanicolaou smears. Obstet Gynecol 1992;79:683-688.

Scurry WC Jr, McGinn JD. Recurrent respiratory papillomatosis in pregnancy: a case of emergent airway management. Ear Nose Throat J 2008;87:E8-11.

Sehr P, Müller M, Höpfl R, Widschwendter A, Pawlita M. HPV antibody detection by ELISA with capsid protein L1 fused to glutathione S-transferase. J Virol Methods 2002;106:61-70.

Sethi S, Müller M, Schneider A, Blettner M, Smith E, Turek L, Wahrendorf J, Gissmann L, Chang-Claude J. Serologic response to the E4, E6, and E7 proteins of human papillomavirus type 16 in pregnant women. Am J Obstet Gynecol. 1998;178:360-4.

Shafti-Keramat S, Handisurya A, Kriehuber E, Meneguzzi G, Slupetzky K, Kirnbauer R. Different heparan sulfate proteoglycans serve as cellular receptors for human papillomaviruses. J Virol 2003;77:13125-13135.

Shah KV, Viscidi RP, Alberg AJ, Helzlsouer KJ, Comstock GW. Antibodies to human papillomavirus 16 and subsequent in situ or invasive cancer of the cervix. Cancer Epidemiol Biomarkers Prev 1997;6:233-237.

Shew ML, Fortenberry JD, Tu W, Juliar BE, Batteiger BE, Qadadri B, Brown DR. Association of condom use, sexual behaviors, and sexually transmitted infections with the duration of genital human papillomavirus infection among adolescent women. Arch Pediatr Adolesc Med 2006;160:151-156.

Silins I, Kallings I, Dillner J. Correlates of the spread of human papillomavirus infection. Cancer Epidemiol Biomarkers Prev. 2000;9:953-9.

Silverberg MJ, Thorsen P, Lindeberg H, Grant LA, Shah KV. Condyloma in pregnancy is strongly predictive of juvenile-onset recurrent respiratory papillomatosis. Obstet Gynecol 2003;101:645-652.

Silverberg MJ, Thorsen P, Lindeberg H, Ahdieh-Grant L, Shah KV. Clinical course of recurrent respiratory papillomatosis in Danish children. Arch Otolaryngol Head Neck Surg 2004;130:711-716.

Simen-Kapeu A, Kataja V, Yliskoski M, Syrjänen K, Dillner J, Koskela P, Paavonen J, Lehtinen M. Smoking impairs human papillomavirus (HPV) type 16 and 18 capsids antibody response following natural HPV infection. Scand J Infect Dis. 2008;40:745-51.

Sinal SH, Woods CR. Human papillomavirus infections of the genital and respiratory tracts in young children. Semin Pediatr Infect Dis 2005;16:306-316.

Sinclair KA, Woods CR, Kirse DJ, Sinal SH. Anogenital and respiratory tract human papillomavirus infections among children: age, gender, and potential transmission through sexual abuse. Pediatrics 2005;116:815-825.

Skjeldestad FE, Mehta V, Sings HL, Øvreness T, Turpin J, Su L, Boerckel P, Roberts C, Bryan J, Jansen KU, Esser MT, Liaw KL. Seroprevalence and genital DNA prevalence of HPV types 6, 11, 16 and 18 in a cohort of young Norwegian women: study design and cohort characteristics. Acta Obstet Gynecol Scand 2008;87:81-88.

Smith EM, Johnson SR, Cripe T, Perlman S, McGuinness G, Jiang D, Cripe L, Turek LP. Perinatal transmission and maternal risks of human papillomavirus infection. Cancer Detect Prev 1995;19:196-205.

Smith EM, Ritchie JM, Yankowitz J, Wang D, Turek LP, Haugen TH. HPV prevalence and concordance in the cervix and oral cavity of pregnant women. Infect Dis Obstet Gynecol 2004;12:45-56.

Smotkin D, Wettstein FO. Transcription of human papillomavirus type 16 early genes in a cervical cancer and a cancer-derived cell line and identification of the E7 protein. Proc Natl Acad Sci U S A 1986;83:4680-4684.

Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J Pathol 2003;201:1-6.

Soares VR, Nieminen P, Aho M, Vesterinen E, Vaheri A, Paavonen J. Human papillomavirus DNA in unselected pregnant and non-pregnant women. Int J STD AIDS 1990;1:276-278.

Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287:2114-2119.

Sonnex C, Strauss S, Gray JJ. Detection of human papillomavirus DNA on the fingers of patients with genital warts. Sex Transm Infect 1999;75:317-319.

Srinivas SK, Ma Y, Sammel MD, Chou D, McGrath C, Parry S, Elovitz MA. Placental inflammation and viral infection are implicated in second trimester pregnancy loss. Am J Obstet Gynecol. 2006;195:797-802.

Stanley M. Immune responses to human papillomavirus. Vaccine 2006;24:S16-22.

Stanley MA, Pett MR, Coleman N. HPV: from infection to cancer. Biochem Soc Trans 2007;35:1456-1460.

Steele J, Collins S, Wen K, Ryan G, Constandinou-Williams C, Woodman CB. Measurement of the humoral immune response following an incident human papillomavirus type 16 or 18 infection in young women by a pseudovirion-based neutralizing antibody assay. Clin Vaccine Immunol 2008;15:1387-1390.

Stoler MH, Rhodes CR, Whitbeck A, Wolinsky SM, Chow LT, Broker TR. Human papillomavirus type 16 and 18 gene expression in cervical neoplasias. Hum Pathol 1992;23:117-128.

Stone KM, Karem KL, Sternberg MR, McQuillan GM, Poon AD, Unger ER, Reeves WC. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396-1402.

Stubenrauch F, Laimins LA. Human papillomavirus life cycle: active and latent phases. Semin Cancer Biol 1999;9:379-386.

Studentsov YY, Ho GY, Marks MA, Bierman R, Burk RD. Polymer-based enzyme-linked immunosorbent assay using human papillomavirus type 16 (HPV16) virus-like particles detects HPV16 clade-specific serologic responses. J Clin Microbiol 2003;41:2827-2834.

Summersgill KF, Smith EM, Levy BT, Allen JM, Haugen TH, Turek LP. Human papillomavirus in the oral cavities of children and adolescents. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:62-69.

Swift FV, Bhat K, Younghusband HB, Hamada H. Characterization of a cell type-specific enhancer found in the human papilloma virus type 18 genome. EMBO J 1987;6:1339-1344.

Sycuro LK, Xi LF, Hughes JP, Feng Q, Winer RL, Lee SK, O'Reilly S, Kiviat NB, Koutsky LA. Persistence of genital human papillomavirus infection in a long-term follow-up study of female university students. J Infect Dis 2008;198:971-978.

Syrjänen K, Väyrynen M, Castrén O, Yliskoski M, Mäntyjärvi R, Pyrhönen S, Saarikoski S. Sexual behaviour of women with human papillomavirus (HPV) lesions of the uterine cervix. Br J Vener Dis. 1984;60:243-8.

Syrjänen K, Hakama M, Saarikoski S, Väyrynen M, Yliskoski M, Syrjänen S, Kataja V, Castrén O. Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. Sex Transm Dis 1990;17:15-19.

Syrjänen K, Syrjänen S. Papillomavirus infections in human pathology. Wiley, London-Paris, pp. 1-615, 2000.

Syrjänen S, Saastamoinen J, Chang FJ, Ji HX, Syrjänen K. Colposcopy, punch biopsy, in situ DNA hybridization, and the polymerase chain reaction in searching for genital human papillomavirus (HPV) infections in women with normal PAP smears. J Med Virol. 1990;31:259-66.

Syrjänen S, Puranen M. Human papillomavirus infections in children: the potential role of maternal transmission. Crit Rev Oral Biol Med 2000;11:259-274.

Syrjänen S. Human papillomavirus infections and oral tumors. Med Microbiol Immunol. 2003;192:123-8.

Syrjänen S, Shabalova I, Petrovichev N, Podistov J, Ivanchenko O, Zakharenko S, Nerovjna R, Kljukina L, Branovskaja M, Juschenko A, Tosi P, Syrjänen K; NIS Cohort Study Group. Age-specific incidence and clearance of high-risk human papillomavirus infections in women in the former Soviet Union. Int J STD AIDS 2005;16:217-223.

Syrjänen S, Syrjänen K. The history of papillomavirus research. Cent Eur J Public Health 2008;16:S7-13.

Tang CK, Shermeta DW, Wood C. Congenital condylomata acuminata. Am J Obstet Gynecol 1978;131:912-913.

Taylor RS, Carroll BE, Lloyd JW. Mortality among women in 3 Catholic religious orders with special reference to cancer. Cancer 1959;12:1207-1225.

Tenti P, Zappatore R, Migliora P, Spinillo A, Maccarini U, De Benedittis M, Vesentini N, Marchitelli G, Silini E, Carnevali L. Latent human papillomavirus infection in pregnant women at term: a case-control study. J Infect Dis 1997;176:277-280.

Thomas M, Fenq Q, Partriadge J, Winer R, Stern M, Lee S, O'Reilly S, Kiviat N, Koutsky La, the 25th International Papillomavirus Conference 2009 abstr. P-27.13.

Tindle RW. Immune evasion in human papillomavirus-associated cervical cancer. Nat Rev Cancer 2002;2:59-65.

Towne JE. Carcinoma of the cervix in nulliparous and celibate women. Am J Obstet Gynecol 1955;69:606-613.

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

Trottier H, Mahmud S, Prado JC, Sobrinho JS, Costa MC, Rohan TE, Villa LL, Franco EL. Type-specific duration of human papillomavirus infection:

implications for human papillomavirus screening and vaccination. J Infect Dis 2008;197:1436-1447.

Tseng CJ, Lin CY, Wang RL, Chen LJ, Chang YL, Hsieh TT, Pao CC. Possible transplacental transmission of human papillomaviruses. Am J Obstet Gynecol 1992;166:35-40.

Tseng CJ, Liang CC, Soong YK, Pao CC. Perinatal transmission of human papillomavirus in infants: relationship between infection rate and mode of delivery. Obstet Gynecol 1998;91:92-96.

Vaccarella S, Franceschi S, Herrero R, Muñoz N, Snijders PJ, Clifford GM, Smith JS, Lazcano-Ponce E, Sukvirach S, Shin HR, de Sanjosé S, Molano M, Matos E, Ferreccio C, Anh PT, Thomas JO, Meijer CJ; IARC HPV Prevalence Surveys Study Group. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006a;15:326-333.

Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas JO, Hoang Anh PT, Ferreccio C, Matos E, Posso H, de Sanjosé S, Shin HR, Sukvirach S, Lazcano-Ponce E, Ronco G, Rajkumar R, Qiao YL, Muñoz N, Franceschi S. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006b;15:2148-2153.

Vaccarella S, Lazcano-Ponce E, Castro-Garduño JA, Cruz-Valdez A, Díaz V, Schiavon R, Hernández P, Kornegay JR, Hernández-Avila M, Franceschi S. Prevalence and determinants of human papillomavirus infection in men attending vasectomy clinics in Mexico. Int J Cancer 2006c;119:1934-1939.

van der Aa MA, Pukkala E, Coebergh JW, Anttila A, Siesling S. Mass screening programmes and trends in cervical cancer in Finland and the Netherlands. Int J Cancer 2008;122:1854-1858.

van Doornum G, Prins M, Andersson-Ellström A, Dillner J. Immunoglobulin A, G, and M responses to L1 and L2 capsids of human papillomavirus types 6, 11, 16, 18, and 33 L1 after newly acquired infection. Sex Transm Infect. 1998;74:354-60.

Veenstra van Nieuwenhoven AL, Heineman MJ, Faas MM. The immunology of successful pregnancy. Hum Reprod Update. 2003;9:347-57.

Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, Brown DR, Ferenczy A, Harper DM, Koutsky LA, Kurman RJ, Lehtinen M, Malm C, Olsson SE,Ronnett BM, Skjeldestad FE, Steinwall M, Stoler MH, Wheeler CM, Taddeo FJ, Yu J, Lupinacci L, Railkar R, Marchese R, Esser MT, Bryan J, Jansen KU, Sings HL, Tamms GM, Saah AJ, Barr E. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine 2006;24:5571-5583.

Viscidi RP, Kotloff KL, Clayman B, Russ K, Shapiro S, Shah KV. Prevalence of antibodies to human

papillomavirus (HPV) type 16 virus-like particles in relation to cervical HPV infection among college women. Clin Diagn Lab Immunol 1997;4:122-126.

Viscidi RP, Schiffman M, Hildesheim A, Herrero R, Castle PE, Bratti MC, Rodriguez AC, Sherman ME, Wang S, Clayman B, Burk RD. Seroreactivity to human papillomavirus (HPV) types 16, 18, or 31 and risk of subsequent HPV infection: results from a population-based study in Costa Rica. Cancer Epidemiol Biomarkers Prev 2004;13:324-327.

Walboomers JMM, Jacobs MV, vaan Oostveen JW, van den Brule AJC, Snijders PJF, Meijer CJLM: Detection of genital human papillomavirus infections and possible clinical implications pp.341-364 in Book Human Papillomavirus Infections in Dermatovenereology, 1997 by CRC Press, Inc.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-19.

Waller J, Marlow LA, Wardle J. Anticipated shame and worry following an abnormal Pap test result: The impact of information about HPV. Prev Med. 2009;48:415-9.

Wang HK, Duffy AA, Broker TR, Chow LT. Robust production and passaging of infectious HPV in squamous epithelium of primary human keratinocytes. Genes Dev 2009;23:181-194.

Wang SS, Schiffman M, Herrero R, Carreon J, Hildesheim A, Rodriguez AC, Bratti MC, Sherman ME, Morales J, Guillen D, Alfaro M, Clayman B, Burk RD, Viscidi RP. Determinants of human papillomavirus 16 serological conversion and persistence in a population-based cohort of 10 000 women in Costa Rica. Br J Cancer. 2004;91:1269-74.

Wang SS, Bratti MC, Rodríguez AC, Herrero R, Burk RD, Porras C, González P, Sherman ME, Wacholder S, Lan ZE, Schiffman M, Chanock SJ, Hildesheim A. Common variants in immune and DNA repair genes and risk for human papillomavirus persistence and progression to cervical cancer. J Infect Dis. 2009;199:20-30.

Wang X, Zhu Q, Rao H. Maternal-fetal transmission of human papillomavirus. Chin Med J 1998;111:726-727.

Wang ZH, Kjellberg L, Abdalla H, Wiklund F, Eklund C, Knekt P, Lehtinen M, Kallings I, Lenner P, Hallmans G, Mählck CG, Wadell G, Schiller J, Dillner J. Type specificity and significance of different isotypes of serum antibodies to human papillomavirus capsids. J Infect Dis 2000;181:456-462.

Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, Templin MF, Pawlita M. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin Chem 2005;51:1845-1853.

Waterboer T, Sehr P, Pawlita M. Suppression of non-specific binding in serological Luminex assays. J Immunol Methods 2006;309:200-204.

Watts DH, Koutsky LA, Holmes KK, Goldman D, Kuypers J, Kiviat NB, Galloway DA. Low risk of perinatal transmission of human papillomavirus: results from a prospective cohort study. Am J Obstet Gynecol 1998;178:365-373.

Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 1990;248:76-79.

WHO HPV LabNet
http://www.who.int/biologicals/areas/human_papillom avirus/WHO_HPV_LabNet/en/index.html

Wideroff L, Schiffman MH, Hoover R, Tarone RE, Nonnenmacher B, Hubbert N, Kirnbauer R, Greer CE, Lorincz AT, Manos MM, Glass AG, Scott DR, Sherman ME, Buckland J, Lowy D, Schiller J. Epidemiologic determinants of seroreactivity to human papillomavirus (HPV) type 16 virus-like particles in cervical HPV-16 DNA-positive and-negative women. J Infect Dis. 1996;174(5):937-943.

Wiley DJ, Wiesmeier E, Masongsong E, Gylys KH, Koutsky LA, Ferris DG, Barr E, Yu Rao J; Proof of Principle Study Investigative Group. Smokers at higher risk for undetected antibody for oncogenic human papillomavirus type 16 infection. Cancer Epidemiol Biomarkers Prev. 2006;15:915-20.

Williams MG, Howatson AF, Almeida JD. Morphological characterization of the virus of the human common wart (verruca vulgaris). Nature 1961;189:895-897.

Wilson JF. Genital warts. J roy Army med. Cps, 1937;68:227-316.

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;157:218-226.

Winer RL, Kiviat NB, Hughes JP, Adam DE, Lee SK, Kuypers JM, Koutsky LA. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis 2005;191:731-738.

Winer RL, Feng Q, Hughes JP, O'Reilly S, Kiviat NB, Koutsky LA. Risk of female human papillomavirus acquisition associated with first male sex partner. J Infect Dis 2008;197:279-282.

Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason TP, Young LS. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001;357:1831-1836.

Woodworth CD. HPV innate immunity. Front Biosci 2002;7:d2058-2071.

Worda C, Huber A, Hudelist G, Schatten C, Leipold H, Czerwenka K, Eppel W. Prevalence of cervical and intrauterine human papillomavirus infection in the third trimester in asymptomatic women. J Soc Gynecol Investig 2005;12:440-444.

Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D; 2006 American Society for Colposcopy and Cervical Pathology-sponsored Consensus Conference. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 2007;197:346-355.

Xi LF, Demers GW, Koutsky LA, Kiviat NB, Kuypers J, Watts DH, Holmes KK, Galloway DA Analysis of human papillomavirus type 16 variants indicates establishment of persistent infection. J Infect Dis. 1995;172:747-755.

You H, Liu Y, Agrawal N, Prasad CK, Chiriva-Internati M, Lowery CL, Kay HH, Hermonat PL. Infection, replication, and cytopathology of human papillomavirus type 31 in trophoblasts. Virology. 2003;316:281-9.

You H, Liu Y, Agrawal N, Prasad CK, Edwards JL, Osborne AF, Korourian S, Lowery CL, Hermonat PL.

Multiple human papillomavirus types replicate in 3A trophoblasts. Placenta 2008;29:30-38.

Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986;42:121-130.

Zinkernagel RM, Hengartner H. Protective 'immunity' by pre-existent neutralizing antibody titers and preactivated T cells but not by so-called 'immunological memory'. Immunol Rev 2006;211:310-319.

zur Hausen H. Papillomavirus infections – a major cause of human cancers. Biochimica et Biophysica Acta 1996;1288:F55-F78.

zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342-350.

zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 2009;384:260-265.

Appendix 1. HPV DNA in placenta, umbilical cord blood, amniotic fluid and in aborted material

HPV in placental tissue

Study	Number of subjects	Inclusion criteria	Exclusion criteria	Mean age, years (range)	Method	Sites (sampling)	Number of HPV DNA +	HPV types (n)
Rombaldi et al., 2008	49 preg HPV+	History of HPV infection, macroscopic HPV lesion or cytological abnormality		24.6 ± 7.7 (14–41)	Multiplex PCR: (PGMY09/11), RFLP; NMPCR	Placenta (2 swabs & 2 biopsies after complete delivery)	12/49 (**24.5 %**)	6/11(n=6) 16 (n=3) 18 (n=2) 42 (n=1) 52 (n=1) 58 (n=1)
	14 preg HPV-						0/13 (**0 %**)	
Gomez et al., 2008	Total 108 preg		STDs during pregnancy		PCR, membrane hybridizations (HPV 6/11/16/18), sequencing	At delivery, Extravillous region	29/108 (**26.9 %**)	6/11 (~45 %) 16/18 (~55 %)
	Cases: (n=48)	Delivery<w37, Severe pre-eclampsia		25.9+7.2			8/48 (**16.7 %**)	
	(n=30)	Spontaneus preterm		28.1+6.5			15/30 (**50.0%**)	
	Controls: (n=30)	Normal delivery at term		26.3+6.6			6/30 (**20 %**)	
Worda et al., 2005	153 preg	Cesarean delivery	Macroscopic HPV lesion, history of conization or LSIL/HSIL	30.5 (18–45)	HC2	Cervix (swab)	56/153 (36.6 %)	LR (n=22) HR (n=45)
						Placenta (swab, central part, maternal side)	8/153 (**5.2 %**)	LR (n=1) HR (n=8)
Eppel et al., 2000	226 preg	CVS or placental biopsy with clinical indications	Macroscopic HPV lesion or cytological abnormality	29.5 (15.3–46.2)	HC2	Cervix (n=179) (swab)	44/179 (24.6 %)	
						Transabdominal puncture of placental villi/ chorionic tissue	0/147 (**0 %**)	
Favre et al., 1998	An EV patient known to be infected with HPV19, HPV24, HPV36, HPV5, HPV8, HPV3 undergoing cesarean section			39	Nested PCR HPV3,5,8,24,36 Sequencing	3 fragments of placenta during cesarean	+ (3/3)	5, 8, 24, 3
Wang et al., 1998	73 preg and NBs				PCR and endonuclease method (HPV16, 18, 35)	Cervical cells during pregnancy	26/73 (35.6 %)	16 (n=18) 18 (n=7) 35 (n=1)
						Fetal membranes at delivery	31/73 (**42.5 %**)	

HPV in umbilical cord blood

Study	Number of subjects	Inclusion criteria	Exclusion criteria	Mean age, years (range)	Method	Sites (sampling)	Number of HPV DNA +	HPV types (n)
Rombaldi et al., 2008	49 preg HPV+ 14 preg HPV-	(see above)		24.6 ± 7.7 (14–41)	Multiplex PCR: (PGMY09/11), RFLP; NMPCR	Arterial umbilical cord blood blood	3/49 (**6.1 %**) 0/14 (**0 %**)	6/11(n=1) 18(n=1) 52 (n=1)
Worda et al., 2005	153 preg	(see above)	(see above)	30.5 (18–45)	The PCR Human Papillomavirus Typing Set	Venous umbilical cord blood	0/153 (**0 %**)	
Gajewska et al., 2005	15 preg and NBs	IDDM dgn before preg		(22–32)	PCR (HPV6/11,16)	Umbilical cord blood	2/15 (**13.3 %**)	16 (n=1) 6/11 (n=1)
	30 preg and NBs	normal preg		(18–38)		Umbilical cord blood	3/30 (**10 %**)	16 (n=2) 6/11 (n=1)
Tseng et al, 1992	52 mother-NB pairs	-	-	28.5	Nested PCR (HPV16,18), restriction endonuclease analysis, Southern blot DNA hybridization	Cervicovaginal lavage during pregnancy	7/52 (13.5 %)	16 (n=6) 18 (n=1)
						Maternal PBMCs during pregnancy	10/52 (19.2 %)	16 (n=9) 18 (n=2)
						Cord blood at delivery	7/52 (**13.5 %**)	16 (n=7)

HPV in amniotic fluid

Study	Number of subjects	Inclusion criteria	Exclusion criteria	Mean age, years (range)	Method	Sites (sampling)	Number of HPV DNA +	HPV types (n)
Ruffin et al., 2006	142 preg	Amniocentesis with clinical indications, intact membranes	-	35.2 (23–55)	Nested PCR (PGMY09/11, GP5+/6+)	Residual amniotic fluid and amniotic cell culture pellets retrieved from the cytogenetics laboratory	0/142 (**0 %**)	-
Worda et al., 2005	153 preg	(see above)	(see above)	30.5 (18–45)	The PCR Human Papillomavirus Typing Set	Amniotic fluid directly from amniotic cavity prior to ROM	0/153 (**0 %**)	
Burguete et al., 1999	238 preg	Transabdominal amniocentesis with clinical indications	-	(18–46)	PCR (GP1/GP2 + HPV16/18/33 anticontamination primers)	Amniotic fluid	25/208 (**12 %**)	18 (n=13) 16 (n=9) x (n=3)
Maxwell et al., 1998	53 preg	Transabdominal amniocentesis for genetic counseling or obstetrical reasons	ROM	25.3 ± 6.45	PCR MY09/MY11	Cervical-vaginal swabs (n=42)	10/42 (23.8%)	
						Amniotic fluid	0/52 (**0 %**)	

Study	Number of subjects	Inclusion criteria	Exclusion criteria	Mean age, years (range)	Method	Sites (sampling)	Number of HPV DNA +	HPV types (n)
Favre et al., 1998	(see above)				Nested PCR Sequencing	Amniotic fluid (syringe) prior to ROM	+	5, 8, 24, 36, 3
Armbuster-Moraes et al., 1994	37 preg	Transabdominal amniocentesis with clinical indications, Cervical histology or DNA HPV +			PCR (GP1/GP2 + HPV16/18/33 anticontamination primers), membrane hybridizations	Amniotic fluid	24/37 (**65 %**)	16 (n=13) 18 (n=5)
HPV in aborted material								
Srinivas et al., 2006		Cases: patients with spontaneus II trimester loss (n=101)	Multifetal pregnancies, intrauterine fetal death		Nested PCR (type specific primers for HPV6,11,16,18)	Placental region near the basal plate	48/84 (**57 %**)	
		Controls: patients with induction of labor (during II trimester) for fetal or maternal indications (n=19)					5/16 (**31 %**)	
Matovina et al., 2004	108	Miscarriages, Normal cervix	Chronic or hereditary diseases	32 (18-46) gest.age 10 (4-19)	PCR (MY09/MY11, and HPV16,18 specific primers)	Chorionic villi from aborted material (curettage)	8/108 (**7.4 %**)	HPV16 (n=6), HPV18 (n=6)
Genest et al., 1999	30	First trimester spontaneous abortions	-	-	PCR, RFLP	Placental tissue	0/30 (**0 %**)	
Hermonat et al., 1997		Cases: I trimester spontaneus abortions (n=25)			PCR (pU-1M& U-31B/pU-2R broad spectrum primers targeting E6/E7), Dot Blot Hybridization Analysis	Enriched chorionic villus material obtained from aborted material	15/25 (**60 %**)	
		Controls: I trimester elective abortions (n=15)					3/15 (**20 %**)	

NMPCR=Nested multiplex PCR; PE=pre-eclampsia; DM=diabetes mellitus; GW=genital wart; PROM= premature rupture of membranes; HIV= human immunodeficiency virus; HBV= hepatitis B virus; HCV= hepatitis C virus; LSIL= low-grade squamous intraepithelial lesion; HSIL= high-grade squamous intraepithelial lesion; IUGR= intrauterine growth restriction; HELLP= hemolysis, elevated liver enzymes, and low platelets; HC2=Hybrid Capture II technology HPV DNA test, Digene; The PCR Human Papillomavirus Typing Set (TaKaRa, TaKaRa Shuzo Co Ltd, Biomedical Group, Otsu, Shiga, Japan); CVS=chorionic villus sampling; ROM= rupture of membranes; EV= Epidermodysplasia Verruciformis; NB= newborn