CHAPTER 9

Summary & Samenvatting
SUMMARY

Genital human papillomavirus (HPV) infections are responsible for a range of benign, premalignant and malignant diseases in both women and men. This thesis highlights two distinct topics in HPV research. The first part describes clinical studies in search of the most efficient way to detect cervical (pre)cancer among women who are infected with HPV. The second part of this thesis deals with male genital HPV infections, especially the presence of HPV in semen, in a more exploratory fashion. Chapter 1 provides a general introduction on human papillomaviruses, genital HPV infections in both women and men, the presence of HPV in semen, sexual transmission of HPV, cervical cancer, HPV-mediated cervical carcinogenesis and the prevention of cervical cancer.

PART ONE: How to improve the triage of high-risk HPV-positive women

The most important consequence of persistent infection with a high-risk HPV (hrHPV) type is cervical cancer, the fourth most common cancer in women worldwide. Cytology-based screening programs, organized to identify and treat women with premalignant cervical disease, have drastically reduced the incidence of and mortality from cervical cancer. Nevertheless, cervical cancer affects over half a million new women each year, warranting continuing efforts to improve the prevention of this disease.

An important improvement in cervical cancer screening is the introduction of the hrHPV test as a primary screening tool. This adaptation, which has been firmly evidence-based, is currently being introduced in several countries. It is important to understand that the majority of hrHPV-infections is self-limiting and does not lead to the development of cancer. For this reason, HPV-based screening can only succeed in combination with a second, so-called ‘triage’ test. To prevent over-diagnosis and over-treatment, this test should specifically identify only those hrHPV-positive women who harbour a persistent transforming hrHPV infection (associated with cervical high-grade premalignant disease) and are therefore truly at risk of developing cervical cancer.

Part one of this thesis describes the clinical performance of several novel triage tests in a large cohort of hrHPV-positive women. The ability of these molecular tests to identify women with high-grade premalignant cervical disease is evaluated in comparison with the accuracy of cytology, the currently advised triage test.

To compare the clinical performance of several triage tests, we recruited a large cohort (n=508) of hrHPV-positive women among those visiting gynecology outpatient departments of six Dutch hospitals (age 18-66 years). From all hrHPV-positive women, a self-sampled cervico-vaginal lavage, a physician-taken cervical scrape and at least one colposcopy-directed cervical biopsy were obtained. The (liquid-based) self-samples and scrapes were tested for several different
triage tests. The biopsies provided a histological diagnosis serving as the gold standard. Cervical cancer, adenocarcinoma *in situ*, cervical intraepithelial neoplasia grade 3 (CIN3) and grade 2 (CIN2) were considered relevant lesions.

In Chapter 2, a comparison of three triage strategies for hrHPV-positive women is presented: E7 mRNA testing, HPV16/18 DNA genotyping and cytology. The E7 mRNA test was performed using nucleic acid sequence-based amplification (NASBA) for the detection of HPV16/18/31/33/45/52/58 mRNA in women who tested DNA-positive for the respective HPV types. The sensitivity for CIN2 or worse (≥CIN2) of the E7 mRNA test was slightly higher than that of HPV16/18 DNA genotyping (67% versus 61%), at a similar specificity (55% versus 52%). Neither sensitivity nor specificity of the E7 mRNA test differed significantly from that of cytology (sensitivity 69% versus 75%; specificity 59% versus 65%).

Chapter 3 presents a molecular alternative to regular (Pap) cytology: p16/Ki-67 dual-stained cytology. This microscopy-based technique aims to identify cervical cells that exhibit a transforming hrHPV infection. In our cohort of hrHPV-positive women, the sensitivity of p16/Ki-67 dual-stained cytology for CIN3 or worse (≥CIN3) (94%) did neither differ significantly from that of Pap cytology (88%) nor from that of Pap cytology combined with HPV16/18 genotyping (95%). However, the specificity of p16/Ki-67 dual-stained cytology for ≥CIN3 (51%) was significantly higher than that of Pap cytology (45%) and Pap cytology combined with HPV16/18 genotyping (26%). After exclusion of women who had been referred to the gynecologist because of abnormal Pap cytology, the specificity of p16/Ki-67 dual-stained cytology for ≥CIN3 (57%) remained the same, whereas that of Pap cytology (60%) increased substantially, resulting in a similar specificity of both assays in this sub-cohort. We concluded that p16/Ki-67 dual-stained cytology could serve as a suitable replacement of (repeat) Pap cytology.

Chapter 4 describes the clinical accuracy of a novel molecular triage test: *FAM19A4* methylation analysis. Hypermethylation of the promoter region of this gene has been previously associated with the presence of cervical cancer and its high-grade precursors. In the total group of hrHPV-positive women, the sensitivities for ≥CIN3 of cytology, *FAM19A4* methylation analysis, and cytology combined with HPV16/18 genotyping were 86%, 76% and 92%, respectively, with corresponding specificities of 50%, 71%, and 29%, respectively. Both sensitivity and specificity of *FAM19A4* methylation analysis were influenced by the age of the patient. In younger women (aged <30 years), the sensitivity for ≥CIN3 of *FAM19A4* methylation analysis was significantly lower than that of cytology (50% versus 87%), with a significantly higher specificity (82% versus 52%). Given the high regression rate of CIN3 in these young women, the low number of chromosomal aberrations and the short duration of HPV infection associated with these lesions it seems plausible that the methylation-negative CIN3 in this group represent regressive lesions. In women aged ≥30 years (n=287), ≥CIN3 sensitivity of *FAM19A4* methylation analysis was non-inferior to that of cytology (88% versus 86%), at a significantly higher specificity (62% versus
Based on these data, FAM19A4 methylation analysis might serve as an objective triage tool for hrHPV-positive women in this age group (screening target in the Netherlands).

An additional advantage of using a molecular triage strategy in cervical cancer screening is its applicability to self-collected specimens. The currently advised triage test among hrHPV-positive women, cytology, can only be reliably performed on physician-taken cervical scrapes. To investigate whether FAM19A4 methylation analysis on self-collected specimens could be of value, we studied its clinical performance on self-collected cervico-vaginal lavage material in comparison with paired physician-taken cervical scrapes, as is described in Chapter 5. Overall FAM19A4 methylation levels were significantly correlated between sample types, with strongest correlation in women with ≥CIN3. FAM19A4 methylation analysis in hrHPV-positive self-samples had a slightly lower sensitivity and a higher specificity for ≥CIN3 compared to paired physician-taken scrapes. In women ≥30 years, ≥CIN3 sensitivity of FAM19A4 methylation analysis was 78% in self-samples and 88% in scrapes (difference not statistically significant), with a significantly higher corresponding specificity in self-samples (73%) compared to scrapes (63%). The performance of FAM19A4 methylation analysis combined with HPV16/18 genotyping did not differ significantly between sample types and could provide a feasible triage strategy for hrHPV-positive women. In conclusion, this combination could enable fully molecular cervical cancer screening by self-sampling.

PART TWO: The prevalence and clinical relevance of HPV in semen

As genital HPV infections are predominantly sexually transmitted, men play a significant role in the distribution of the virus. HPV has been detected in several parts of the male genital tract. However, little evidence exists on the exact routes of sexual HPV transmission. Previous studies have shown that HPV can be detected in semen but the source of the HPV present is unknown and the clinical relevance of this phenomenon (in terms of fertility) has not yet been elucidated.

Chapter 6 addresses the prevalence of HPV in semen of Dutch men and its relationship with HPV infections of the penile epithelium. In an observational study, healthy male volunteers (n=213) provided one penile scrape and three semen samples each in three consecutive weeks, which were tested for HPV by two different assays (GP5+/6+-PCR and SPF10-PCR). Penoscopy was performed to detect flat penile lesions. HPV-DNA at moderate/high viral loads (i.e. GP5+/6+-PCR-positive) was detected in ≥1 semen sample(s) in 27% of participants. Most men with moderate/high viral loads in the penile scrape also had moderate/high viral loads in semen (85%). Men with a HPV-negative penile scrape were very unlikely to have moderate/high viral loads in semen (3%). The presence of HPV in semen was associated with the presence of HPV in the penile scrape also on a genotype-specific level. Having flat lesions on the penile glans was a risk
factor for HPV presence in semen. We concluded that HPV presence in semen of healthy men is common and may result from desquamation of HPV-infected epithelial cells of the penile glans.

Chapter 7 describes a study on the association between seminal HPV presence and impairment of semen quality. In a cohort of male partners of couples seeking fertility evaluation (n=430), overall HPV was detected in 15% of semen samples, including 2% that contained both high-risk HPV and low-risk (lr) HPV types, 9% with exclusively hrHPV types and 4% with exclusively lrHPV types. The presence of HPV in semen was not associated with the age of the participants, seminal pH, volume, sperm count, concentration, progressive motility or the presence of anti-sperm antibodies. In conclusion, we did not observe an association of HPV presence in semen and impairment of semen quality.

Chapter 8 concludes with a general discussion. The available literature on triage markers for hrHPV-positive women is reviewed and summarized in tables. A comparative overview of all triage markers studied in this thesis is given. Furthermore, the possible consequences of HPV presence in semen are discussed in the context of fertility screening and procedures of assisted reproduction.