Human papillomavirus infection and preterm delivery

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ABSTRACT

Background: Persistent human papillomavirus (HPV) infection causes cervical intraepithelial neoplasia (CIN). Excisional treatment of CIN has been linked to increased risk of preterm delivery (PTD). The specific mechanism behind is however unclear. Also untreated CIN has been associated with an increased risk of PTD. It is unknown whether this is attributable to the HPV infection itself or other causes.
Aims: To examine whether HPV infection, untreated CIN and/or previous treatment for CIN is associated with PTD and other adverse obstetric outcomes. To study possible causal pathways for an association, including cone length of treatment, changes in cervical microbiota and infectious complications.
Material and methods: *Paper I*; a Swedish register-based study (1999-2016), studying obstetric outcomes in women with normal cervical cytology (NCC) (n=338,109), abnormal cytology (n=11,727) or positive HPV test (n=2,550) - in conjunction with pregnancy, previously treated women (n=23,185), and women with CIN2+ diagnosed after pregnancy (n=33,760). *Paper II;* a register-based study from western Sweden (2008-2016), comparing obstetric outcomes in women with NCC (n=42,398), women with CIN during pregnancy (n=1,380) and previously treated women (n=3,250) including a subgroup with cone length measured at treatment (n=2,408). *Paper III;* a prospective observational study in Sweden/Norway (n=950) comparing obstetric outcomes in women with or without HPV infection detected in urine at mid-pregnancy and

at delivery. *Paper IV;* a prospective observational study in Norway with culture and PCR of cervical microbiota in women with CIN (n=89) before and six and 12 months after LEEP and also compared to women with NCC (n=100).
Results: *Paper I*; HPV infection (HPVtest) compared to NCC was associated with PTD (aOR 1.2, 95%CI 1.0-1.4), and preterm prelabor rupture of membranes (pPROM) (aOR 1.5, 1.2-2.0), but treated women had higher risk compared to women with HPV infection; PTD (aOR 1.7, 1.4-2.0), pPROM (aOR 1.6, 1.2-2.0). Treatment but also HPV infection were associated with increased risk of neonatal mortality and PROM at term and treatment also with chorioamnionitis and neonatal sepsis. *Paper II;* Treatment was associated with an increased risk of PTD (aOR 1.6, 1.2-2.1), pPROM (aOR 2.7, 1.7-4.5), and PROM at term compared to women with CIN, and risks increased with cone length. Small treatments (≤10 mm) were also associated with increased risk for PTD and pPROM. *Paper III;* Women positive for high-risk-HPV genotypes at mid-pregnancy had a higher frequency of PTD compared to those negative for high-risk-HPV, but comparisons were non-significant. *Paper IV;* Treatment resulted in a reduction of non-*Lactobacillus* bacterial species. More types of bacterial species were detected in women planned for LEEP than in women with NCC.
Conclusion: Women with HPV infection have increased risk of PTD, pPROM and neonatal mortality. Excisional treatment for CIN, also minor excisions, increases the risks for PTD and pPROM further compared to having untreated CIN/HPV infection. The risks increase with cone length. Previous treatment is also associated with increased risk of PROM at term and maternal and neonatal infectious complications. Treatment appears not to result in a more diverse or dysbiotic cervical microbiota while CIN is associated with increased bacterial diversity.
Keywords: CIN, HPV, LEEP, pPROM, PROM, PTD
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SAMMANFATTNING PÅ SVENSKA

Bakgrund; Behandling av cellförändringar på livmodertappen har kopplats till en ökad risk för förlossning innan fullgången tid, förtidsbörd. Förtidsbörd är i sin tur den viktigaste orsaken till sjuklighet och död hos nyfödda barn. Cellförändringar orsakas av humant papillomvirus (HPV). Detta sexuellt överförbara virus är vanligt förekommande hos kvinnor i barnafödande ålder men de flesta som smittas lyckas göra sig av med viruset inom två år. Om infektionen kvarstår kan cellförändringar utvecklas med risk för progress till livmoderhalscancer. Därför behandlas idag höggradiga cellförändringar på livmodertappen genom att man skär bort dem, oftast med en elektrod-slynga. Mekanismerna bakom varför denna behandling ökar risken för förtidsbörd är inte känd. Det finns också forskning som tyder på att kvinnor med obehandlade cellförändringar/HPV infektion har en ökad risk för förtidsbörd.

Syftet med avhandlingen var dels att fördjupa kunskaperna om varför behandling av cellförändringar ökar risken för att föda för tidigt. Därutöver syftade avhandlingen till att besvara om det finns ett samband mellan obehandlad HPV infektion och/eller cellförändringar och förtidsbörd.

Metod; Vi genomförde två register-baserade studier i Sverige och två prospektiva kohortstudier i Norge/Sverige respektive Norge.

Resultat/Konklusion; Sammanfattningsvis så fann vi att HPV-infektion under graviditet samt cellförändringar under graviditet var kopplade till ökad risk för förtidsbörd samt även till för tidig vattenavgång och neonatal dödlighet men risken var större hos kvinnor som genomgått excisions-behandling av cellförändringar och ökade med excisionens djup. Tidigare behandling var också associerad med en ökad risk för infektion i livmodern samt allvarlig infektion hos barnet efter förlossningen. Infektion i livmodern är också en viktig orsak till förtidsbörd och skulle kunna vara en mekanism bakom den ökade risken för förtidsbörd efter behandling. Sammansättningen av bakterier i livmodertappen försämrades dock inte efter excisionsbehandling.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Wiik J, Nilsson S, Kärrberg C, Strander B, Jacobsson B, Sengpiel V. Associations of treated and untreated human papillomavirus infection with preterm delivery and neonatal mortality: A Swedish population-based study. PLoS Med. 2021;18(5):e1003641.

II. Wiik J, Kärrberg C, Nilsson S, Strander B, Jacobsson B, Sengpiel V. Associations between cervical intraepithelial neoplasia during pregnancy, previous excisional treatment, cone-length and preterm delivery: a register-based study from western Sweden. BMC Medicine. 2022;20(1):61.

III. Wiik J, Værnesbranden MR, Jonassen CM, Staff AC, Carlsen KCL, Granum B, Haugen G, Hedlin G, Hilde K, Jacobsson B, Nilsson S, Nordlund B, Rangberg A, Rehbinder EM, Sengpiel V, Skjerven H, Sundet B, Söderhäll C, Vettukattil R, Sjøborg K. Maternal human papillomavirus infection during pregnancy and preterm delivery, a mother-child cohort study in Norway and Sweden. Manuscript.

IV. Wiik J, Sengpiel V, Kyrgiou M, Nilsson S, Mitra A, Tanbo T, Jonassen C M, Tone Møller Tannæs, Sjøborg K. Cervical microbiota in women with cervical intra-epithelial neoplasia, prior to and after local excisional treatment, a Norwegian cohort study. BMC Women's Health. 2019;19(1):30.

The following studies are also relevant for the subject, and were also performed during the PhD period, but are not part of the thesis:

Værnesbranden MR, Wiik J, Sjøborg K, Staff AC, Carlsen KCL, Haugen G, Hedlin G, Hilde K, Nordlund B, Nystrand CF, Rangberg A, Rehbinder EM, Rudi K, Rueegg CS, Sandberg Y, Sjelmo S, Skjerven HO, Söderhäll C, Vettukattil R, Jonassen CM. Maternal human papillomavirus infections at mid-pregnancy and delivery in a Scandinavian mother-child cohort study. Int J Infect Dis. 2021 Jul; 108:574-581. doi: 10.1016/j.ijid.2021.05.064. (1)

Jar-Allah T, Kärrberg C, Wiik J, Sengpiel V, Strander B, Holmberg E, Strandell A. Abnormal cervical cytology is associated with preterm delivery; A population based study. Acta Obstet Gynecol Scand. 2019 Jun;98(6):777-786. doi: 10.1111/aogs.13543. (2)

CONTENT

ABBREVIATIONS

AIS	Adenocarcinoma in situ
ASC-H	Atypical squamous cells-favouring high-grade lesion
ASCUS	Atypical squamous cells of uncertain significance
AOR	Adjusted odds ratio
BMI	Body mass index
BV	Bacterial vaginosis
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CST	Community state type
DNA	Deoxyribonucleic acid
GA	Gestational age
GBS	Group B Streptococcus bacteria
HGL	High grade lesion
HIV	Human immunodeficiency virus
hrHPV	High risk human papillomavirus
HSIL	High grade squamous intraepithelial lesion
IAI	Intra-amniotic inflammation
ICD	International classification of disease
IUGR	Intrauterine growth restriction
IVF	In vitro fertilization
LBC	Liquid based cervical cytology
LEEP	Loop electrosurgical excision procedure
LLETZ	Large loop excision of the transformation zone
LSIL	Low grade squamous intraepithelial lesion
MBR	The Swedish Medical Birth Register
MIAC	Microbial invasion of the amniotic cavity
NCC	Normal cervical cytology
NGS	Next generation sequencing
NKCx	The Swedish National Cervical Screening Registry
OR	Odds ratio
PCR	Polymerase chain reaction
PPROM	Preterm prelabor rupture of membranes
PROM	Prelabor rupture of membranes

PTD	Preterm delivery
PTL	Preterm labor
RNA	Ribonucleic acid
SCB	Statistics Sweden
SCC	Squamous cervical carcinoma
SCJ	Squamocolumnar junction
SGA	Small for gestational age
TLR	Toll like receptor
TVU	Transvaginal ultrasound
TZ	Transformation zone
WHO	World health organization

1 INTRODUCTION

The objective of this thesis was to examine whether human papillomavirus (HPV) infection, untreated cervical intraepithelial neoplasia (CIN) and/or previous treatment for CIN is associated with preterm delivery (PTD) and other adverse obstetric outcomes and to study possible causal pathways for an association.

Human papillomavirus infection is the most common sexually transmitted infection worldwide and the most common infection in the female genital tract. While HPV infections most often clear within two years, persistent infection with high-risk HPV (hrHPV) is causally associated with cervical cancer and precancerous lesions - CIN (3, 4). As it usually takes several years for CIN to progress to cancer it is possible to detect CIN through cervical cytology screening (5, 6) and by treatment of CIN prevent development of cervical cancer (7). Through recent implementation of vaccination against hrHPV infection in young age, before contagion of the virus, society aims to mitigate cervical cancer.

Several studies have found that pregnancies after surgical treatment of CIN are associated with an increased risk of preterm delivery (PTD), including preterm prelabor rupture of membranes (pPROM), and also with neonatal mortality (8-14). The underlying biological mechanism behind these associations remains unclear. It has been suggested that also women with untreated CIN have an increased risk for PTD (10).

This PhD project aimed to further understand why treatment of CIN is associated with PTD, with a focus on cone length excised, infectious complications after treatment and cervical microbiota. This PhD project also aimed to study whether women with CIN have an increased risk for PTD and if this risk could be explained by the HPV infection itself. If HPV infection, as such, increases the risk for PTD, then cost-benefit of HPV vaccination programs needs to be recalculated and maybe additional genotypes of HPV should be included in the vaccine.

In the following chapters, the studied exposures; HPV, CIN and treatment of CIN as well as the major obstetric outcomes; PTD, (p)PROM, chorioamnionitis and neonatal sepsis will be briefly introduced.
Then the current knowledge regarding whether treatment of CIN, untreated CIN and/or HPV infection are associated with increased risk of PTD and the other obstetric outcomes will be presented. In addition, hypothetical mechanism behind the suggested associations will be presented.

1.1 HPV INFECTION, CIN AND CERVICAL CANCER

Human papillomavirus is a sexually transmitted small DNA virus with a peak prevalence in early adulthood (15, 16). The majority of sexually active individuals are exposed to HPV during their lifetime (15, 17). About 40 genotypes of HPV infect the cutaneous and mucosal epithelial cells of the anogenital tract (18). These HPV genotypes are classified as low-risk, possibly or probably high-risk and high-risk HPV (hrHPV), according to their association with benign or malignant lesions (19). The hrHPVs are known to cause cancer (20, 21). In the genital tract the cervix, the lowest portion of the uterus, is most vulnerable to HPV infection and HPV infection causes virtually 100% of the cervical cancers, under the influence of other co-factors (20). Low-risk HPVs are associated with anogenital warts (4). The epidemiological classification of HPV types according to consensus from the International Agency for Research on Cancer (22) is shown in Table 1.

Table 1. Classification of HPV genotypes

Classification	HPV genotypes
Low-risk	6, 11, 40, 42, 43, 44, 54, 61, 72, 81, 89
Possibly or probably high risk	26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85, 97
High risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

HPV=human papillomavirus

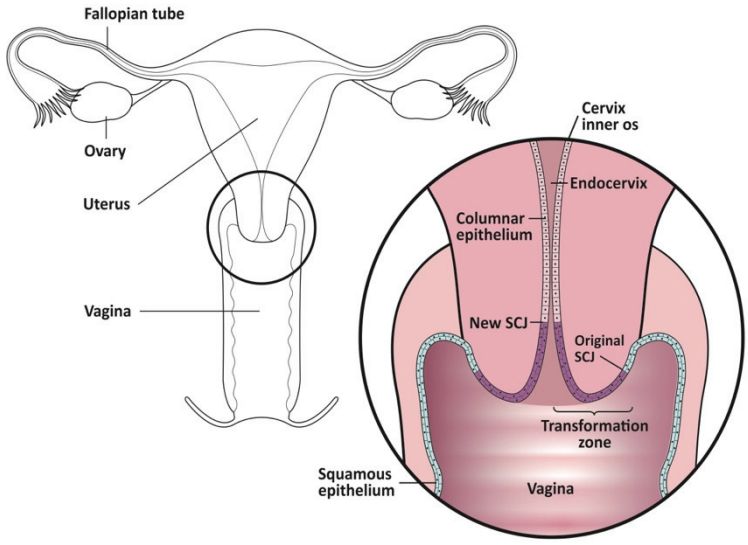
Cervical cancer is the fourth most common cancer among women worldwide (23) and squamous cell carcinoma (SCC) represent the majority of cervical cancers while adenocarcinoma is less common. (4). Worldwide, hrHPV genotypes 16 and 18 causes about 70% of all cervical cancer cases and about 52% of high-grade cervical precursor lesions (24). In 2006, two vaccines against these HPV types were introduced. Sweden has subsidized vaccination for girls (age 13-17) from 2007 and school-based HPV vaccination has been offered to girls since 2012 (ages 10-12) and to boys since 2020. The vaccine used at

present also protects against high-risk HPV 31, 33, 45, 52 and 58 and low risk HPV 6 and 11.
However at present, approximately 500 women are diagnosed with cervical cancer in Sweden yearly (25) and the incidence has actually increased in the past five years (26, 27).
In Norway, HPV vaccine was implemented in the National Vaccine Program in 2009 for girls in 7th grade and from the autumn of 2018 also for boys (28).

1.1.1 THE CERVIX AND THE TRANSFORMATION ZONE

The cylinder-shaped cervix is the lower part of the uterus. The cervical canal (endocervix) runs along its entire length, from the opening into the uterus (internal os) to the opening into the vagina (external os). The portion of cervix that protrudes into the upper vagina is called portio vaginalis (ectocervix) and is covered by multiple layers of stratified squamous epithelium. The endocervical canal is lined by a single layer of mucus-secreting columnar epithelium, enfolding into connective tissue, forming glandular-like crypts. Under the columnar epithelium there are so called "reserve cells" from which the mucosa could regenerate. The squamocolumnar junction (SCJ) is where the columnar and squamous epithelium meets (29). Due to hormonal influences at puberty and during pregnancy the cervix enlarges and the columnar epithelium everts out to the ectocervix and hence also the position of the SCJ moves out onto the ectocervix. The region between the original SCJ and the new SCJ, is named the transformation zone (TZ) (29), Figure 1.

Figure 1. The female reproductive organs, with detail of the cervix and the transformation zone. SCJ=Squamocolumnar junction. Illustration Jan Funke



A process referred to as metaplasia takes place in the TZ. An immature metaplastic squamous epithelium, derived from the sub-columnar reserve cells, forms onto the columnar epithelium. The everted columnar epithelium is thus gradually changed to immature and then mature squamous epithelium (30). The immature metaplastic squamous cells in the TZ are vulnerable to HPV infection, which can transform these cells into atypical cells, with cytoplasmic and nuclear abnormalities, and also into cancer cells (20, 29). The TZ retracts into the endocervix after pregnancy and after the menopause.

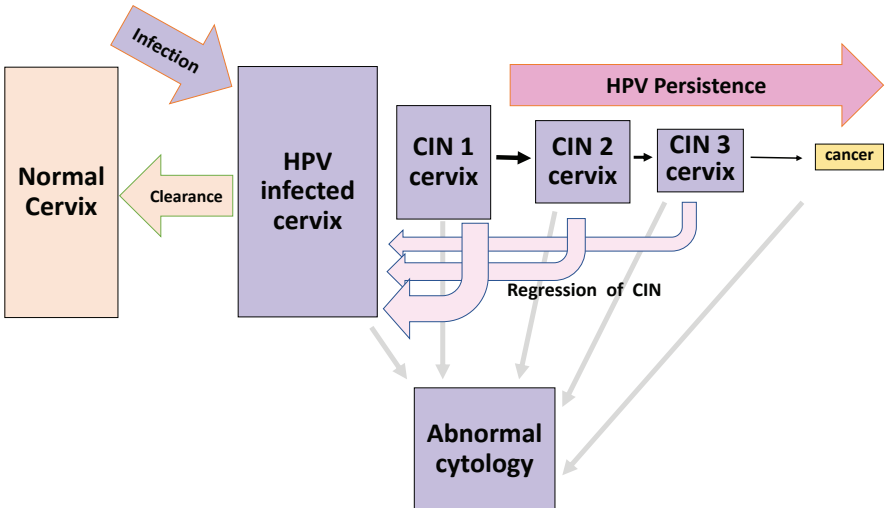
1.1.2 HPV PERSISTENCE AND CIN DEVELOPMENT

Human papillomavirus infects the basal cell layer of the epithelium in cells that divide. Here, HPV replicates little. While basal cells are pushed to the upper epithelial layers, the cells lose their ability to divide and differentiate and at the same time the virus up regulate viral gene expression and viral DNA replication. Viral release occurs at the epithelial surface because of natural turn-over of the epithelium. HPV thus effectively evades recognition by the immune system (by replication in non-dividing cells, by not causing cytolysis but only releasing viraemia at the epithelial surface and by expression of very low levels of viral proteins), and thus causes limited inflammatory response and activation of the immune system (31). Interference with the expression of Toll-like receptors (TLR) and the interferon response, key antiviral defense mechanisms, are suppressed by expression of the E6/E7 oncogenes by the hrHPVs, inhibiting the innate immune response and delaying the activation of adaptive immunity (31, 32).

Expression of oncogenes by HPV, most important E6 and E7 also leads to cell cycle progression, genomic instability, accumulation of mutations, loss of cell-growth control and eventually cancer. Precursors for SCC are CIN and classification of CIN depends on to what proportion the epithelial layer is replaced by neo-plastic cells. Low grade squamous intraepithelial neoplasia (LSIL) is called CIN1 and high grade squamous intraepithelial neoplasia (HSIL) is either moderate; CIN2, or severe; CIN3/Carcinoma in situ (CIS). The high-grade precursor for cervical adenocarcinoma is called adenocarcinoma in situ (AIS) (4, 33).

CIN can either resolve or progress to cancer. Since contagion of HPV infection often occurs in the later teens or early twenties and CIN3 diagnosis peak around 25–30 years of age the time for development of CIN3 has been calculated to be about 7–15 years (34). However, faster development of CIN2 and CIN3, within two years after incident infection, has been reported. The progression rate to cancer is about 5% for CIN2 (35) and 12-31% for CIN3 (35, 36).

Figure 2. CIN, caused by persistent HPV infection, might regress or progress to cervical cancer.
Abnormal cervical cytology can be found in women with HPV infection, CIN and cervical cancer.
CIN=cervical intraepithelial neoplasia, HPV=human papillomavirus



The majority of all women, about 90%, are able to clear a HPV infection within two years (15) while a persistent HPV infection is essential for development of cervical cancer. The immune-response leading to HPV clearance is incompletely understood (37), but the host´s innate and adaptive immune response to the infection seems to be important for clearance, evidenced by inflammation seen in regression lesions and also by the fact that women with immunodeficiencies are at increased risk for persistent HPV infection and progression to cancer (4, 38). Both acquired immunodeficiency, for example after organ transplantation or due to concomitant human immunodeficiency virus (HIV) infection, and genetic host factors related to the immune response seem to be important (38, 39).

The virus´ ability to alter local immunity in the infected cervix in such way that it presents a much more tolerant state, probably facilitates persistent hrHPV infections and progression of cervical lesions (40, 41). Persistence depends on the HPV genotype and viral load (15, 42,

43). The hrHPV genotype 16 is associated with long persistence and a faster progression to cervical lesions and cancer (15). Multiple HPV infections have also been associated with HPV persistence (42).
HPV can also establish latent infections in the basal layer of the cervix, controlled by the host's cellular immunity and not detected by HPV tests that examine cells from the upper layers (44, 45). Reactivation may occur due to immunosuppression (46).

Several co-factors have been associated with persistence of HPV and progression to cancer. Tobacco smoking is associated with persistence of HPV (47) and is an established co-factor for cervical cancer (48). Age of first sexual intercourse and number of lifetime sexual partners (49), as well as high parity (50), is associated with cervical cancer. Low education and low socioeconomic status are associated with CIN and cancer (51), probably due to lifestyle factors and differences in screening participation. Use of oral contraceptives has been associated with both cervical cancer (52) and persistence of HPV (42). Diet and nutrition have been suggested but unproven co-factors (53).
Concurrent vaginal infections with *Chlamydia trachomatis* (54) or *Herpes Simplex type 2* (55) has also been suggested to be co-factors for progression to cervical cancer.
Recent studies indicate that the composition of the vaginal microbiota is important for acquisition and persistence of HPV as well as for the development of CIN and cervical cancer. An increased bacterial diversity and reduced levels of *Lactobacillus spp* seems to confer increased risks (56-61).

Vaginal microbiota
Vaginal *Lactobacillus spp*. normally prevent abnormal bacterial overgrowth through maintenance of a low pH (62-65), through hydrogen-peroxide production, and bacteriocin production (66-68).
A vaginal microbiota with low diversity, dominated by one or a few species of *Lactobacillus,* is associated with health in the reproductive tract. Five community state types' (CSTs) of vaginal microbiota have been described in premenopausal women through the use of 16srRNA next-generation sequencing (NGS) (69). CSTs I, II, III and V are characterized by dominance of different *Lactobacillus spp* (L crispatus, L gasseri, L iners, L jensenii) and with low species diversity. The composition of the vaginal microbiota is dynamic and the most

common transition is from CST III to IV. In contrast to the other CSTs, CST IV is devoid of *Lactobacillus spp*. and enriched with anaerobic species, like *Gardnerella vaginalis* (69). Highly diverse vaginal microbiota and lack of *Lactobacillus spp* is considered atypical or a state of dysbiosis. CST IV is associated with Bacterial Vaginosis (BV), which is characterized by a loss of the Lactobacilli species, an increased pH, and a massive increased level of other organisms, especially anaerobic gram-negative rods. The major bacteria detected are *Bacteroides species, Gardnerella vaginalis, Mobiluncus species, Mycoplasma hominis, Peptostreptococcus species, Prevotella species, Porphymonas species and Ureaplasma urealyticum* (70, 71). Vaginal wall biofilms, dominated by *Gardnerella vaginalis*, appear to play a role in the pathogenesis.

Vaginal microbiota and HPV infection

Increased vaginal pH has been associated with increased risk of HPV positivity in premenopausal women (72) and BV with higher incidence, prevalence and persistence of HPV infection and also with development of CIN (73-77). Other studies report that CST III and in particular CST IV are associated with the presence of HPV infection and development of CIN (58, 60, 78, 79). A step-wise increase in prevalence of CST IV with increasing severity of dysplasia was observed in a study from the UK (58). A longitudinal cohort study (n=87), with sampling every third month for 24 months, reported that depletion of *Lactobacillus* spp. was associated with increased risk of persistent/progressive CIN2 at 12- and 24-month follow-up, while a *Lactobacillus* spp. dominated microbiota was associated with regression (80). Another study reported that detection of *Gardnerella vaginalis, Anaerococcus vaginae,* and *L. iners* in the absence of *L. crispatus* might be a high-risk combination for development of CIN (60).

Dysbiosis is associated with high levels of vaginal proinflammatory cytokines (81, 82). To have CIN has also been associated with increased levels of proinflammatory cytokines (83), and development of HPV-induced cervical cancer is preceded by chronic inflammation (84, 85). High vaginal pH, low levels of *Lactobacillus* and genital inflammation have been linked to expression of cancer biomarkers that are associated with persistent HPV infection, development of CIN and progression to cancer (86).

It is thus hypothesized that genital dysbiosis might cause epithelial damage and thus facilitate the entry of HPV into the basal epithelial cells (56). A dysbiotic microbiota might also create a proinflammatory milieu that promotes the viral life cycle, persistence of HPV infection and development of CIN (56, 85-87).
However, causal inference remains uncertain. HPV interferes with innate immune responses and it has been suggested that alterations of the innate immune system, for example diminished activity of TLRs, might trigger a transition from a *Lactobacillus*-dominated microbiota to a BV-associated microbiota (88).
It is unknown whether these alterations seen in the microbiota are a cause or a consequence of the HPV infection/CIN and how much the findings are influenced by genetic predisposition or environmental factors (87).

1.1.3 PREVALENCE OF HPV INFECTION DURING PREGNANCY

Prevalence of HPV infection in pregnant women varies between 5% and 65% (89) across studies. It varies between countries and age groups studied (90) and results depend on detection methods used and background factors and HPV vaccination in the studied population. Previous studies comparing prevalence of HPV in pregnant and non-pregnant women have shown conflicting results, some reported higher HPV prevalence in pregnant women (91-93) and others found no difference (94-96). A meta-analyses with age matched comparisons, suggested higher HPV prevalence in pregnant women (97).

It has been suggested that increased hormonal levels during pregnancy and an altered immune response in pregnant women might increase the transcription and replication of HPV (98). Latent cervical HPV infections might be reactivated and clearance and susceptibility to new infections might be altered in pregnancy and differ between pregnancy trimesters (91, 97, 99, 100). Change in sexual behavior during pregnancy might also affect HPV prevalence.

Prevalence of HPV infection might depend on when HPV in pregnancy is studied (90, 101). One meta-analysis reported a higher prevalence in third trimester compared to second trimester (97) while another meta-analyses reported highest prevalence in first trimester (90). Most studies collected samples only at one time point. Yamasaki et al. found that multiple HPV infections were more common in early pregnancy compared with late pregnancy (102).

A few studies have examined infection during pregnancy at several time points, in the same cohort, with conflicting results. Rando et al found an increase between first and third trimester and then a decline postpartum (103). Fife et al found no difference between first and third trimester but a decline postpartum (99). Lee found highest prevalence in second trimester (104) while Domza et al found lower prevalence in third compared to first trimester (105).

In one of our earlier studies, in a cohort of pregnant Scandinavian women recruited from the general population also included in paper III, HPV (any 28 genotypes) was detected in 38% of the women at mid-pregnancy, in 28% at delivery, and genotype specific persistence was 52% (1).

1.1.4 CERVICAL CANCER SCREENING IN SWEDEN

Cervical cancer screening aim to detect and treat pre-stages of cervical cancer/CIN. Screening was initiated in Sweden in 1964 and in 1977 all counties in Sweden had implemented population-based screening programs. Since the introduction of cervical screening the incidence of cervical cancer has declined considerably, from 24 per 100,000 women in 1965 to 8 cases per 100,000 women in 2011 (26). A similar screening program exists in Norway (106).

The current Swedish guidelines (since 2015) recommend screening every third year for women aged 23-29 with liquid based cervical cytology smear (LBC) and from 30-39 with primary HPV test (107). At age 41, women are screened using both HPV test and cytology. Primary HPV screening is also used in women 50-64 every seventh year (107). A positive HPV test is triaged with cytology and a HPV reflex test can be performed on a LBC. During pregnancy, women are recommended to be tested at their first visit to the antenatal care unit (around 10-12 weeks of pregnancy) if they have not been screened the previous 2.5 years (107). Participation rates are high, more than 80% nationally, but rates vary between different healthcare regions (108). Cytology is classified and reported according to the Bethesda system (109) in Sweden as, normal, abnormal squamous cells of unspecified significance (ASCUS), Atypical squamous cells -cannot exclude high-grade squamous intraepithelial lesion (ASCH), LSIL, HSIL, SCC, atypical glandular cells (AGUS), adenocarcinoma in situ (AIS) or adenocarcinoma.
If screening results in an abnormal test; HPV with LSIL or ASCUS, any high-grade cytology or persistent HPV-infection regardless of cytological triage, a colposcopic examination, with a binocular stereoscopic microscope, is performed to determine if and where cervical biopsies should be taken to detect precancerous or cancerous lesions. If cervical biopsies are taken and classified as CIN3 or AIS, and in women above 25 years as CIN2, the women are recommended treatment. If colposcopic examination is unsatisfactory, the new SCJ and the transformation zone not visible (TZ type3) or the entire area of abnormal epithelium is not visible, treatment might also be recommended even if HSIL is not verified in prior biopsies. Age, other risk factors and screening history have to be considered.

1.1.5 CERVICAL DYSPLASIA DURING PREGNANCY

The prevalence of atypical cervical cytology during pregnancy has been reported to complicate up to 5% of pregnancies but varies between studies and was in a Danish study around 1.4% (110). An increased prevalence among pregnant women compared to non-pregnant women has been reported (111).

Women without recent negative screening test are tested early in pregnancy and analysis of the tests are prioritized. Colposcopy of test-positive women can thereby be performed early in pregnancy. The primary aim of this examination is to rule out the presence of invasive cervical cancer (107, 112, 113), but further follow up is also based on this assessment.

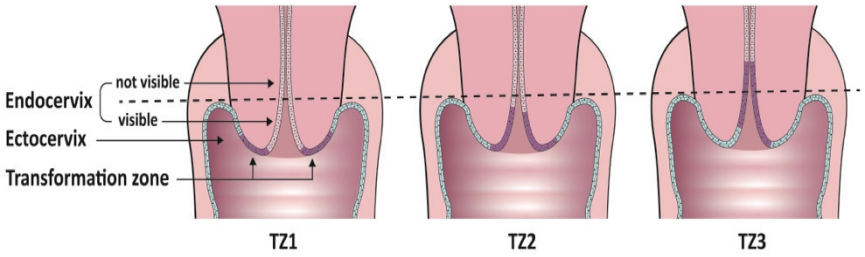
There are international variations on how atypical cervical cytology during pregnancy is managed. In Sweden colposcopy directed biopsies are recommended if scoring, according to the colposcopic scoring system Swedescore, is 5 or more. In case of high-grade findings (Swedescore 8-10) multiple biopsies or loop-biopsies are recommended (107). Loop-biopsy is preferably used if microinvasive cancer is suspected (113).
In a Swedish cohort of women who had biopsies taken during pregnancy, CIN2 or more severe findings (CIN2+), was diagnosed in around 55%, and in those with ASCUS or CIN1 in initial cytology, CIN2+ was found in about 40% of the biopsies (114).

Treatment of CIN should be postponed until after delivery if cancer is ruled out. If the woman is pregnant and diagnosed with CIN3/AIS, she is followed during the pregnancy with active surveillance. It has been shown that it is safe to manage cervical dysplasia during pregnancy conservatively and the regression rate is high after delivery, also among cases of HSIL (115). However, it requires expertise as colposcopy and management of pregnant women with CIN is demanding. In a Swedish cohort with CIN2 and CIN3 in histology during pregnancy, the rates of regression, persistence and progression were around 26%, 71%, 2% for CIN2 and 45%, 54% and 0% for CIN3 (114).

1.1.6 TREATMENT OF CIN

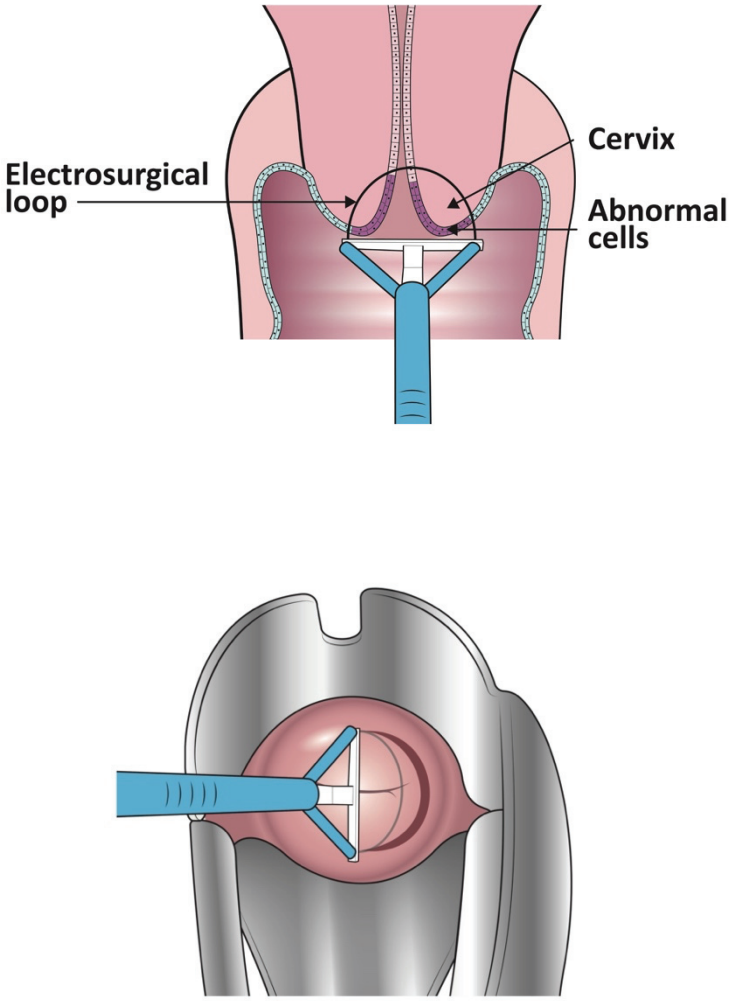
Treatment of CIN should remove or destroy the transformation zone (TZ), which is the part of the cervix where abnormal cells / dysplasia resides and cure the HPV infection. Treatment should also aim to affect healthy cervical tissue as little as possible, at least in women of childbearing age. Important factors during treatment are grade of the lesion, the extent of the lesion and the size and type of the TZ, Figure 3.

Figure 3. Tranzformation zone, types 1-3.
TZ1) TZ is completely visible on ectocervix. TZ2) TZ is completely visible but has an endocervical component. TZ3) TZ has an endocervical component and is not entirely visible. Illustration Jan Funke.



Excisional treatments are commonly preferred since this permits histological assessment of the TZ and of residual disease at the excision margins. The most commonly used treatment technique for CIN, and the dominating method used in Sweden and Norway today, is Loop Electrosurgical Excision Procedure (LEEP), also named large loop excision of the transformation zone (LLETZ). A cone shaped excision is performed, usually under local anesthesia, Figure 4. To include the crypts where disease could reside the Swedish guidelines recommends a cone length of 6-9 mm in women where the TZ is entirely visible on the portio (TZ1) and longer if the transformation zone is deeper into the cervix (TZ2-3) (107). Other types of excisional treatment include laser conization, needle or straight wire excision and cold-knife conization while ablative techniques include cold coagulation, laser ablation and cryotherapy.

Figure 4. Loop Electrosurgical Excision Procedure (LEEP) uses a wire loop heated by electric current to remove tissue and is most often performed under local anaesthesia. Illustration Jan Funke.



1.2 ADVERSE OBSTETRIC OUTCOMES

The peak age of CIN, and thus treatment of CIN, is similar as the mean age of women having their first child. A history of previous cervical treatment of CIN has unfortunately been associated with an increased risk of PTD, including pPROM, and with neonatal mortality in deliveries following treatment (8-14). It has also been suggested that previous treatment increases the risk for chorioamnionitis (116). Further pPROM and also prelabor rupture of membranes (PROM) at term are associated with increased risk for chorioamnionitis (117). Chorioamnionitis in turn confers risk for neonatal sepsis (118).

These adverse obstetric and neonatal outcomes will be briefly presented in the following section.

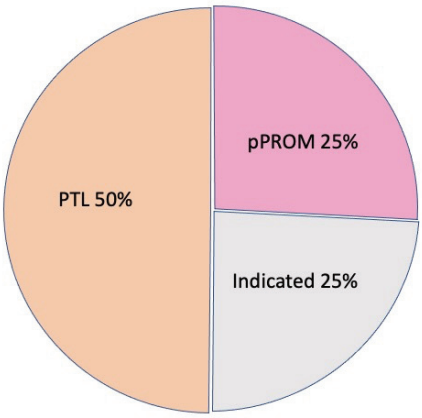
1.2.1 PRETERM DELIVERY

Definition and epidemiology
Preterm delivery is defined as birth before 37 weeks of gestation (<259 days). In Sweden, since 2008, it includes live births and stillbirths from 22 weeks. Before 2008 stillbirths were defined as deliveries from 28 weeks of gestation. Stillbirths have been reported to contribute to 5-10% of all PTDs in high income countries (119) but the frequency was only 2.7% of PTDs in a register-based Swedish study between 1991-2001. This study also found that congenital malformations were present in 4.6% and multiple gestations in 11.6% of the PTDs during the study period (120).
A study from the USA stated that around 5% of PTD occur at less than 28 weeks (extreme preterm), around 15% at 28–31 weeks (very preterm), around 20% at 32–33 weeks (moderate preterm), and 60–70% at 34–36 weeks (late preterm) (121). In Sweden, between 1991 and 2001, were 4.3% of the PTDs extreme, 10.4% very preterm and 85.3% moderate or late preterm (120). The prevalence of PTD in the Nordic countries is around 6% and among the lowest worldwide, while other countries have PTD rates up to 18% (119, 122, 123).

Preterm delivery could be iatrogen/indicated with forced early delivery, due to maternal or fetal pregnancy complications. The major part, about 70%-75%, of PTD consists of *spontaneous* PTD, starting with preterm labor and intact membranes (PTL) or pPROM (121, 124, 125) and in a few women preceded by cervical insufficiency. About 25-30% all PTDs are caused by pPROM (121, 125). The majority of women with pPROM goes into labor within days, but a minority remains undelivered for several weeks.

Consequences for the child, family and society
Preterm delivery is the main cause of mortality in children under the age of five (126). It is also the leading cause of short- and long-term morbidity, including conditions requiring neonatal intensive care and lifetime disabilities in survivors (126-128). This enormous global obstetric problem is accompanied by suffering, not only for the preterm newborn, but also for the parents, siblings and the extended family and also entails huge societal economic costs (129-131)

Figure 5. Preterm delivery could be spontaneous, starting with pPROM or PTL, or indicated due to maternal or fetal complications (125) Illustration Johanna Wiik.



pPROM=preterm prelabor rupture of membranes, PTL= preterm labor

Spontaneous PTD - a multifactorial syndrome

Spontaneous PTD is a multifactorial condition with great diversity involving several systemic changes and pathways leading to a common terminal pathway (121). Many risk factors have been associated with spontaneous PTD including *pregnancy complications* (urinary tract infection and systemic infection, placental abnormalities or abruption, polyhydramnios, pregnancy hypertension and preeclampsia, fetal growth restriction), *obstetric characteristics* (pregnancy after assisted reproduction, nulli-parity, prior PTD, multiple gestation, male fetal sex) and *maternal characteristics* (both low and high maternal age, uterus variations, chronic inflammatory disease, chronic medical conditions like hypertension and diabetes mellitus, low and high pre-pregnancy body mass index (BMI), cigarette smoking, maternal anxiety and low socio-economic and educational status (121, 128, 132, 133). Spontaneous PTD most commonly start by preterm labor in white women, but by pPROM in black women (121, 134). However, most women that deliver preterm have no major risk factors (135).

The pathway to preterm and term parturition is complex and incompletely understood but biochemical release from matured fetal organs and placenta together with timed activation in maternal decidua, myometrium and cervix is of importance (136). The corticotropin-releasing-hormone (CRH), regulating the hypothalamic-pituitary-adrenal (HPA) axis, is during pregnancy also produced in placenta and fetal membranes, and has been suggested to impact timing of delivery (137).

Parturition mechanisms at term include
-A switch in the myometrium from a quiescent to a contractile state.
-Cervical ripening with changes in the extracellular matrix leading to decreased tensile strength and increased elasticity of the cervix, herby preparing the cervix for dilation at delivery.
-Decidual/membrane activation in preparation for membrane rupture and to facilitate separation of placenta and the chorioamniotic membranes from the uterus at birth.

Spontaneous PTD is suggested to be initiated if one or more of the described parturition mechanisms are activated preterm.
This activation could be initiated either by the mother or the fetus in response to different disease processes, for example microbial induced inflammation, disruption of maternal fetal tolerance, decidual hemorrhage and ischemia due to vascular disease, deficient progesterone action, decidual senescence and also by other disease mechanisms (such as overdistention of uterus, stress, cervical disease and unknown factors) (138). Causes for PTD might thus be disease in the placenta, endometrium/decidua, myometrium and/or cervix (136).

Inflammation
A balanced feto-maternal immune-response, supported by immune regulatory mechanisms at the feto-maternal interface, ensures pregnancy maintenance and allows feto-placental growth but also remodeling of maternal tissues during pregnancy (136).

Parturition, at term and preterm, is associated with increased inflammation, activation and infiltration of immune cells, production of pro-inflammatory mediators, and decreased levels of anti-inflammatory mediators (138). Cervical ripening is an inflammatory

process. The release of pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF α) results in production of proteins that promotes myometrial contractility and hormonal receptors (prostaglandin and oxytocin), affects gap junctions and increases the concentration of prostaglandins (138). Prostaglandins can trigger uterine myometrial contraction and also activate cervical ripening and degradation of fetal membranes (138). Expression of matrix metalloproteinase causes breakdown of collagen in the cervix and fetal membranes (138).

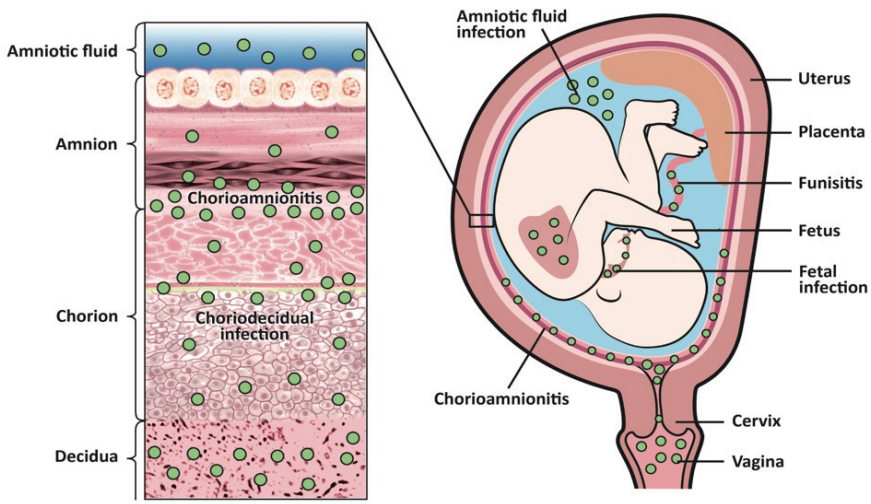
Spontaneous PTD might start due to increased inflammation in fetal or maternal tissues through different mechanisms that are not fully understood. Cervical inflammation is associated with PTD (139) and cervical infection and chemical or mechanical cervical damage has been suggested to be able to promote inflammation and immune cell activation, leading to preterm cervical ripening (140).
Intrauterine inflammation in the fetal membranes and in placenta is associated with spontaneous PTD. It is often caused by microbes but could also occur in the absence of microbes. The “sterile intrauterine inflammation” is thought to be initiated by the release of chemokines, induced by maternal or fetal cellular stress, cellular-injury or cell-death (141).
Inflammation causes activation and weakening of the chorioamniotic (fetal) membranes. Membrane rupture occurs most frequently in the membranes overlying the cervix and this “weak zone” is characterized by increased pro-inflammatory cytokines, matrix degrading enzymes, chemokines, apoptosis and oxidative stress (142). Cell senescence, a mechanism in cell ageing, seems to be an important physiologic process preparing the membranes for rupture (136, 142). Cells with senescence stop to divide without dying, and can continue to produce pro-inflammatory substances and damage nearby cells. Preterm cell senescence in decidua has been reported in preterm labor (138). In the fetal membranes, cell senescence increases with fetal growth/age (142). The inflammatory signals produced due to cell senescence in fetal membranes have been suggested to also reach maternal tissue (decidua, myometrium and cervix) with possible effects on parturition (142). Examination of fetal membranes after pPROM suggests that premature senescence activation, enhanced by oxidative stress, is one of the biological mechanisms contributing to pPROM (142). Infection,

inflammation and environmental and behavioral factors have been suggested to affect senescence in fetal membranes (136, 142).

Infection

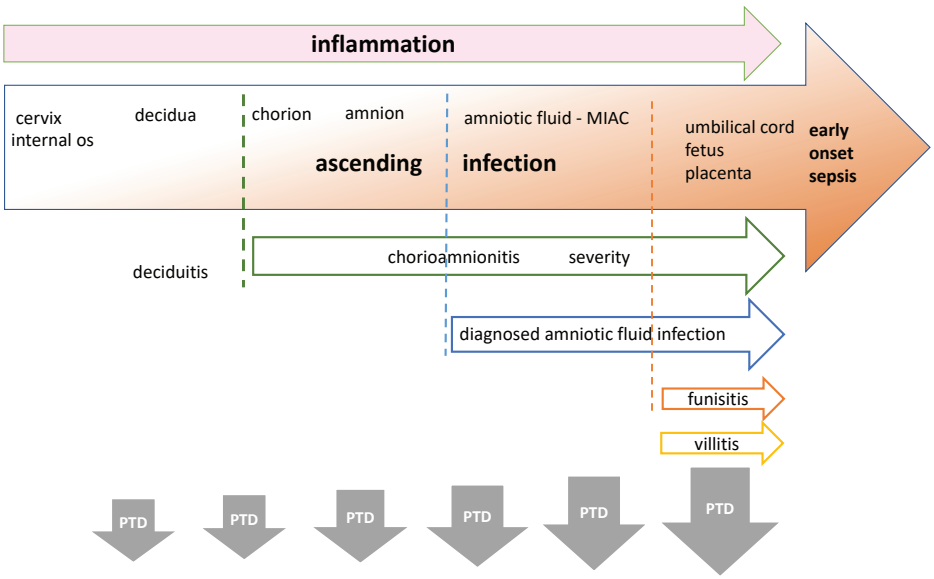
Intra-amniotic bacterial infection has been causally linked to spontaneous PTD and bacterial infection is thought to be the most important reason for spontaneous PTD, especially of early PTD and PTD starting with pPROM (133, 138, 143-146). In about 30 % of all the PTDs is an intra-amniotic infection present, but largely subclinical (138, 146). If the microbes are blood-borne in the mother, then hematogenous spread to the uterus is possible (e.g., shown for the *Cytomegalovirus, Listeria* and *Zika virus*). However, ascending infection from the lower genital tract to the uterus is thought to be the main route of infection since the majority of microorganisms that have been isolated in amniotic fluid are also found in the vagina/cervix (138). The most frequent bacteria found in the amniotic cavity are *Gardnerella vaginalis*, *Ureaplasma species*, genital *Mycoplasma species* and *Fusobacteria species* (147)

Figure 6. Ascending bacterial infection. After cervical passage the infection can ascend to the decidua, the chorioamnioitic membrane, the amniotic cavity, the umbilicus and the fetus. Modified from Goldenberg et al (70), illustration Jan Funke



Ascending microorganisms first infect the decidua of the supracervical region and then, after infection of and passage through the chorioamniotic membranes, the infection is spread to the amniotic cavity and then eventually to the fetus. The infection might also spread to the placenta, causing villitis. Ascending bacterial invasion could thus cause chorioamnionitis, funisitis (inflammation of the umbilical cord) and neonatal infectious complications but also PTD (141).

Figure 7. Ascending infection and inflammation lead to both infectious complications and preterm delivery (PTD). Modified from Keelan (148)



MIAC; microbial invasion of the amniotic cavity, PTD; preterm delivery

Infection causes PTD through inflammation, mainly by activation of the innate immune system. When microbes are sensed by the TLRs in the decidua and fetal membranes it results in production of cytokines and chemokines (e.g., IL-1α, TNF α, IL-8, CCL-2), inflammatory mediators (prostaglandins and reactive oxygen radicals) and proteases leading to activation of the immune system but also to uterine contractions, cervical ripening and membrane rupture (70, 138). The TLRs have also been found in the endocervix, endometrium and in placenta trophoblasts (141). If the infection spreads to the fetus, the fetal immune response may contribute to the inflammatory activation

and start of delivery. Bacteria and viruses have been identified in decidua of the first and second trimesters (149). The timing of migration of bacteria is unknown but since early PTD more often is associated with infection this might happen early in pregnancy or maybe even before conception (70, 144).

The reason why some pregnancies are complicated with ascending bacterial infection and microbial induced PTD is not fully understood. The cervix functions as a barrier against ascending infections during pregnancy through various mechanisms. One mechanism is mucus production, rich of antimicrobial peptides, including the formation of the mucus plug in the cervical canal during pregnancy (150). A barrier against cervical infection is also formed by junction proteins that seal off intercellular spaces between epithelial cells (140). *Lactobacillus* produces hydrogen peroxide, thus affecting pH in the vaginal tract and presence of other microbes. Health in the lower genital tract is associated with dominance by *Lactobacillus spp.* and a low microbial diversity. During pregnancy, the microbiota has been shown to be more stable and less dynamic (151).
Whether the local immune response and the microbiota in the lower genital tract are different between women who deliver at term and those who deliver preterm are important unanswered questions (151, 152). Dominance of *Lactobacillus iners* in first trimester was reported as a risk factor for PTD (153). A highly diverse vaginal microbiota and lack of *Lactobacillus* is considered atypical or a state of dysbiosis (69, 154, 155) and bacterial overgrowth has been linked to PTD (152, 156, 157). Bacterial vaginosis confers risk for intra-amniotic infection and spontaneous PTD (70, 138) but the mechanism by which BV is associated with PTD is unclear (70, 144). It is unknown whether BV through local action in the lower genital tract might cause PTL and PTD, if the organisms do not ascend into the uterus (70). Antibiotic treatment of asymptomatic women with BV has not reduced the incidence of PTD (138). However, in women with a previous PTD and with BV diagnosed in the second trimester, antibiotic treatment (oral metronidazole) resulted in a significant reduction in the incidence of PTD (70, 158-160).
Genital herpes infection can cause ulcerations in the genital tract and has also been associated with PTD (140, 161). Presence of *Lactobacilli*-poor and *Gardnerella*- or *Ureaplasma*- abundant vaginal

microbiota has been observed to have both a dose-response and temporal relationships with PTD (162). Urogenital tract infection with group B *Streptococcus* (GBS) is present in about 20-30% of pregnant women and associated with PTD, pPROM, chorioamnionitis and neonatal sepsis (163).
However, further research on the association between increase or decrease in the relative abundance of specific bacteria in the lower genital tract and PTD is needed (164).

Placenta dysfunction
The uterine spiral arteries in the decidua are remodelled and transformed in early pregnancy to support blood delivery to the intervillous space that surrounds the fetal chorion-villi. The exchange of nutrients and oxygen between maternal and fetal blood occurs here. Preeclampsia is thought to be caused by abnormal placentation and is characterized by abnormal trophoblastic invasion. Preeclampsia is an important cause for indicated PTD.
Abruptio placenta is seen in about 1% of all pregnancies and is more common in spontaneous PTD than in term deliveries (165, 166). It is thought to be initiated by an ischemic lesion in the decidua and has been associated with failure of transformation of the placental spiral arteries (166).
Intrauterine fetal growth restriction (IUGR) is caused by placental dysfunction. More children are born small for gestational age (SGA) in spontaneous PTDs than in term deliveries (167, 168). Failure of transformation of the placental spiral arteries has been reported to be higher in spontaneous PTD with PTL or pPROM than in term deliveries and more pronounced in women with preeclampsia (169).
It has thus been suggested that placental dysfunction might cause both preeclampsia, abruptio placenta, IUGR and also spontaneous PTD (166). Uteroplacental ischemia due to failure of transformation of the placental spiral arteries/placenta vascular lesions could be a key factor but the exact mechanisms remain unclear (166).
Vascular lesions in decidual vessels have been reported to be higher in patients with spontaneous PTD than in term deliveries without complications (170). Placenta ischemic lesions were more common in women with PTL that resulted in PTD than in in PTL followed by a term delivery (171).

Placental dysfunction/chronic under-perfusion leading to IUGR might be accompanied by an accelerated maturation of multiple fetal organ systems and through that mechanism also promote PTD (166). It has previously been suggested that PTD at lower gestational age often occurs due to infection/inflammation while PTD at later gestational ages is caused by vascular lesions /placental dysfunction (166).

Cervical insufficiency, short cervix, prediction of spontaneous PTD
During pregnancy, the inner os of the cervix is normally closed and the opening is sealed with a mucus plug. At the onset of labor, the cervix shortens and begins to dilate preparing for delivery.
Sometimes the cervix starts to open early in the pregnancy, resulting in PTD or late miscarriage. If this occurs in the second or early third trimester it is called cervical insufficiency. For some women, this process recurs in subsequent pregnancies, possibly due to an underlying cervical vulnerability and premature cervical remodeling/cervical ripening.

A short cervix is conventionally defined as a cervical length ≤25 mm in the mid-trimester of pregnancy, measured using transvaginal ultrasound (172). A short cervix has been associated with a previous spontaneous PTD and prior late miscarriage (173, 174) and a short cervical length during pregnancy measured with transvaginal ultrasound (TVU) has been shown to predict PTD (175-177).

Transvaginal ultrasound in women previously treated for CIN
In some studies, previous excisional treatment was not a risk factor for a short cervix measured by TVU (173, 174). Other studies found a shorter cervix in the second trimester in previously treated women (178, 179). Excision of larger cervical volumes has also been associated with shorter cervix, measured with TVU, in subsequent asymptomatic pregnancies (180).

Measurement of cervical length with TVU in asymptomatic women in second trimester has been suggested to predict spontaneous PTD with intermediate accuracy (176, 177), with a similar effect in women with a history of cervical excisional treatment and those without (177). Measurement of cervical length is one of the few possible methods clinicians have to identify threatening PTD. It has been suggested that

by addition of the obstetric history to the information about cervical length (ie history of previous PTD) it is possible to improve the predictive accuracy of PTD (175).

Interventions against PTD

If a short cervical length is found by TVU, interventions can be applied to try to prevent PTD. These interventions include giving the pregnant woman vaginal progesterone, or applying a cervical cerclage (to tighten the opening of the cervix), or introducing a pessary over the cervix. A metanalyses suggested that vaginal administration of progesterone in asymptomatic women with a short cervix (measured with TVU in the second trimester) and a singleton pregnancy may reduce the number of spontaneous PTDs <33 weeks by 30% (172). Targeted cervical cerclage has been suggested to reduce PTD in women previously treated with excision (181). Insertion of cervical pessary has also been suggested to prevent PTD in women with short cervix (182). However, there is still controversy regarding the efficacy and the group of patients that could benefit from these interventions (183-185).

1.2.2 PROM

Prelabor rupture of membranes (PROM) at term (from gestational age 37+0) is commonly defined as membrane rupture before start of uterine contractions.
In a Norwegian register-based study PROM was defined as start of contractions twelve hours or later after membrane rupture, and was reported to be 11.6% (186). In a Swedish retrospective-study PROM was defined as membrane rupture without uterine contractions diagnosed at clinical examination and described in clinical records, and was found in 261/2143 women at term,12.2% (187).
Nulliparity and previous PROM are risk factors for PROM (187). Deliveries starting with PROM – even at term - are more often complicated by fever during delivery (188) and infectious complications of the mother and/or newborn as the amniotic membranes as a barrier against bacterial infection are broken (117). In order to prevent infectious complications for mother and child, these pregnancies are induced medically, and diagnosis and management of PROM is part of the daily work in obstetric care. There is no consensus on how to manage PROM and countries have different national guidelines. If there are no signs of infection or other concerns for the wellbeing of the fetus Norwegian guidelines suggest induction of labor after 24 hours. In Sweden parous women are induced after 24 hours but expectant management with daily clinical check-ups is used in nulli-parous women in up to 72 hours. Identifying measures to prevent PROM, could improve pregnancy outcome for a considerable number of mothers and neonates and free resources in obstetrics care. While multiparous women do not seem to be at higher risk for instrumental delivery or caesarean section in case of PROM, several studies indicate an increase in instrumental deliveries and c-sections for primiparous women with PROM (189).

The mechanisms behind PROM are not fully understood, but senescence and inflammation seem to be important factors for the process leading to membrane rupture as previously described and infection might lead to membrane damage (136, 142, 190).

1.2.3 CHORIOAMNIONITIS AND NEONATAL SEPSIS

Chorioamnionitis
The definition of chorioamnionitis can be either clinical or histopathologic and chorioamnionitis is defined differently across studies and countries, thus limiting comparison between studies.

Clinical chorioamnionitis refers to a clinical syndrome of intrauterine/intramniotic infection. For diagnosis a combination of fever and at least two of the following criteria must be met: maternal leukocytosis, maternal or fetal tachycardia, foul-smelling amniotic fluid and uterine tenderness. None of the included clinical criteria are both sensitive and specific (191) and this syndrome was in a small study of term deliveries associated with microbial invasion of the amniotic fluid in only 61% (192). Of the studied women, 54% had microbial-associated intraamniotic inflammation whereas 24% had intra-amniotic inflammation but no detectable bacteria (192). Diagnosis of clinical chorioamnionitis is an indication for antibiotic treatment and delivery.
Prevalence of clinical chorioamnionitis depends on gestational age and has been reported in 1-4% in US births (193) and in pPROM pregnancies to be about 20% (192).

Histopathologic chorioamnionitis – microscopic evidence of infection or *inflammation* on examination of the chorioamniotic membranes, either occurs due to microbial induced amniotic inflammation (MIAC) or is a sterile inflammation in the absence of detectable microbes induced by chemokines released due to cellular stress, injury or cell death (147). Prevalence of histologic chorioamnionitis depends on gestational age at birth (194). It is present in about 4-5% at term, in 11% at GA 33-36, in 35-40% at GA 25-32, in 94% at GA 21-24 weeks and is more common after membrane rupture (147). It is directly associated with time of membrane rupture before delivery (195).

Histopathologic chorioamnionitis in preterm pregnancies is frequently present without clinical chorioamnionitis and may manifest instead as preterm labor or, more commonly, as pPROM. Most intraamniotic

infections are subclinical and undetected unless the amniotic fluid is examined (147).
In spontaneous PTD is microbial invasion of the amniotic cavity often established before the initiation of preterm labor (147) while in women with spontaneous labor at term microbial invasion also happens, but after initiation of parturition and thus is of shorter duration (147).
Factors associated with chorioamnionitis include PTD, long length of membrane rupture and labor, nulliparity, colonization of group B streptococcus (GBS), BV, multiple vaginal examinations, internal uterine monitoring devices and meconium-stained amniotic fluid (193). Given the longer duration of infection in PTD before clinical signs, the microbial burden is higher and therefore, a more intensive intra-amniotic inflammatory response present in PTD than in term births with microbial invasion (196). Complications with neonatal sepsis are more common in preterm neonates.

Neonatal sepsis
Neonatal sepsis is an important cause of neonatal mortality and morbidity with a fatality rate of about 2 % in term infants and 20% in preterm infants (195, 197). Among survivors, neonatal sepsis is associated with varying developmental delays (197, 198).
Neonatal sepsis is a systemic infection with bacteria in the blood stream or cerebrospinal fluid, (together with for example meningitis, pneumonia, pyelonephritis, gastroenteritis) in infants up to 28 days of life (118). However, the systemic inflammatory response syndrome (SIRS) has also been used when defining neonatal sepsis (195).
Early onset sepsis occurs the first 3 days of life (sometimes instead defined as occurrence the first 7 days of life) and is mainly caused by bacteria transmission from mother to child before and/or during delivery.
In about 30% of pregnancies with intra-amniotic infection are bacteria also identified in the fetal circulation (199). Swallowing/aspiration of infected amniotic fluid by the fetus in utero may lead to pneumonitis and intrapartum sepsis (118, 146).
Incidence of early onset neonatal sepsis (0-6 days) in liveborn children has been reported in Western Sweden to be 1.4/1000 in the period 1997-2007 and 0.9/1000 in the period 2008-2017 (200). Similar incidence has been reported from England and United States (200).

Preterm deliveries are at increased risk of chorioamnionitis, which is a risk factor for neonatal sepsis, and preterm infants are also at increased risk for neonatal sepsis due to immaturity of their immune system and a diminished barrier function of the skin and mucus membranes (118). The rate of early-onset neonatal sepsis is greater in preterm than in term neonates (in singleton pregnancies in Sweden 1997-2016 reported as 15.52/1000 liveborn preterm neonates, versus 0.76/1000 liveborn term neonates) (201).
Of the early onset sepsis, 70% has been reported to be due to either *Escherichia coli* or GBS (118). During labor, maternal risk factors include fever, prolonged rupture of membranes, GBS bacteriuria and vaginal colonization with GBS (118). Since the national implementation of risk-based intrapartum antibiotic prophylaxis against GBS in Sweden in 2008 the neonatal early-onset GBS infection has decreased (202).

1.3 WHY IS PREVIOUS TREATMENT OF CIN ASSOCIATED WITH PTD

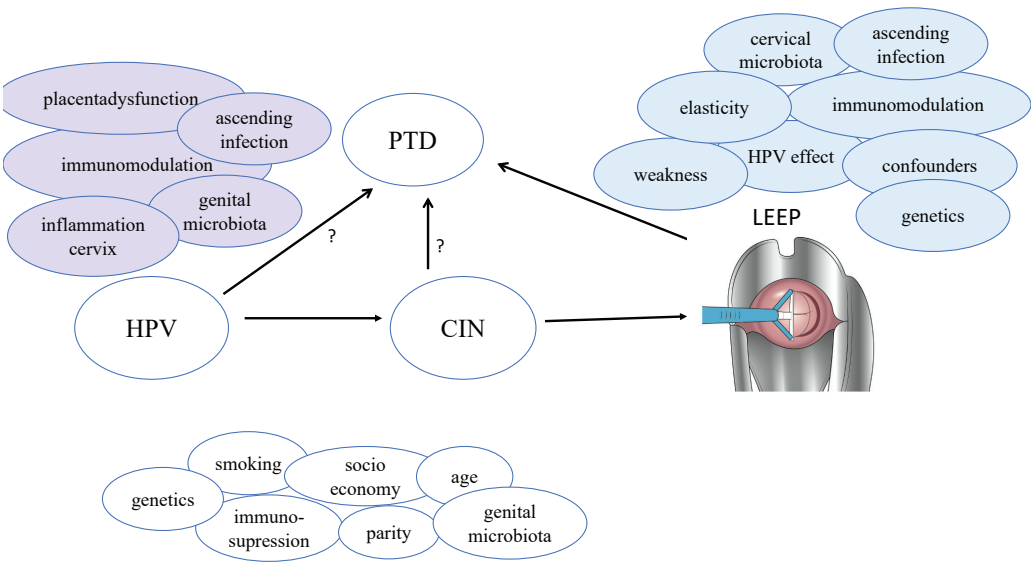
The cervix should withstand the increasing mechanical pressure from the growing uterus during pregnancy, remain closed, but also get prepared for the structural changes needed at time of delivery (soften, shorten and dilate). To do so the cervix undergoes structural and mechanical changes in its muscle and connective tissues. It softens and from being about 5 cm long in first trimester it shortens during pregnancy to about 35-40 mm in the third trimester (203). Throughout pregnancy the cervix acts as a gatekeeper against ascending infections to the uterus from the lower genital tract by the production of mucus, cytokines and anti-microbial peptides (140).

The patho-mechanisms behind the increased risk for PTD seen after surgical treatment for CIN are not fully understood, although several hypotheses have been suggested/discussed;

A direct effect of the excision or the destruction of tissue including glands
-acquired anatomical changes secondary to loss of cervical tissue, cicatrisation of the cervix with changes in the tissue composition and organization of collagen fibers that affects the tensile strength and leads to mechanical weakness (181, 204-208).
-immunomodulation/changed immunological milieu (204, 208, 209).
-alterations of the cervicovaginal microbiota (204, 205, 208, 209).

And/or
An association due other underlying mechanism
-a still present HPV infection affecting parturition pathways and/or the hosts immune response (205).
-An underlying vulnerability for PTD in women that are also vulnerable for HPV persistence and development of CIN, either due to genetics or related co factors (14, 39, 205, 210).

Figure 8. PTD is increased after LEEP but HPV infection and CIN might possibly also increase the risk for PTD.
Hypothetical explanations for the increased risk of PTD after LEEP in blue, possible mechanisms behind an association of HPV/CIN and PTD in purple and background factors associated with both PTD and HPV/CIN in white.



1.3.1 METHOD OF TREATMENT, CONE LENGTH AND VOLUME

The magnitude of risk increase for PTD after treatment of CIN varies across studies (8-14, 116, 211). The surgical method used seems to affect the risk, with highest risk after cold knife excision and higher after excision than after ablation (116) suggesting an acquired mechanical weakness of the cervix secondary to loss of cervical tissue as an explanation for the increased PTD risk. However more radical techniques might also impair the cervical barrier against ascending infections more severely. Laser conization has been reported to remove a larger volume of cervical tissue than LEEP, when stratified for grade of lesion (212).

The risk of PTD seems to increase with the length of the excision (116) indicated by studies of cone length measured on histology specimen by the pathologist after fixation in formalin (10, 213-217). Others found no relationship between volume or depth of excised cervical tissue and gestational length in subsequent pregnancy and suggest that other factors might be of importance (218). However, a recent Cochrane review concluded that the risk for PTD increases with cone length (116). Whether a small cervical excision (≤10 mm) increases the risk of PTD has not been established. An English case control study found no increased risk with cone lengths below 10 mm compared to women taking punch biopsies at colposcopy before delivery (214) while a study from Denmark found an increased risk also for treatments of 10 mm (OR 1.46, 95% CI 1.11-1.91) compared to a general population (217). The Cochrane review concluded that more research on small excisions compared to having untreated CIN is needed (116).

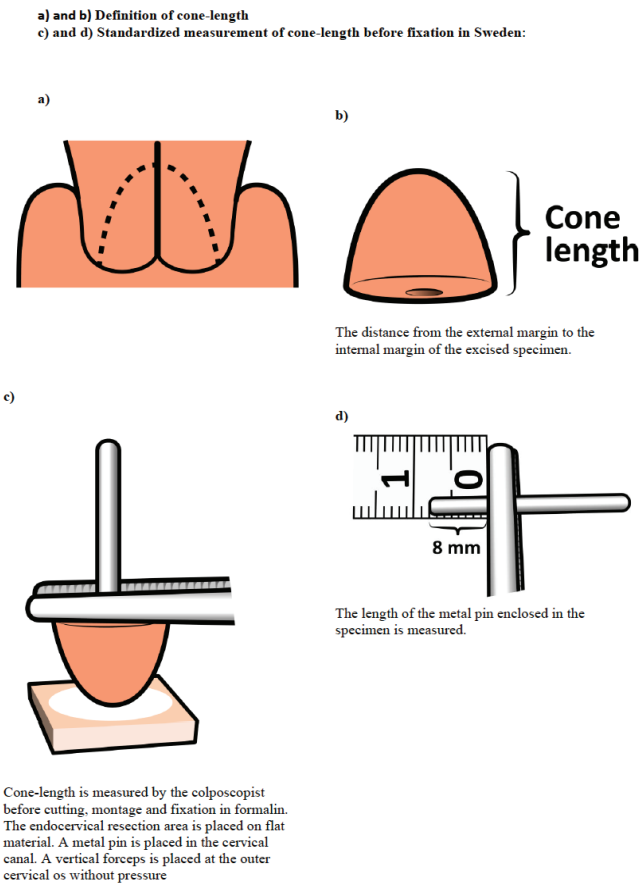
If cone length is associated with other adverse obstetric outcomes is not much studied but a small study found that cone length increased the risk for pPROM (215). If infectious complications after treatment increase with cone-length is so far unknown.

No previous study on cone lengths has compared previous treatment to women with CIN during pregnancy - although using this comparison group would minimize bias from the mere presence of CIN /HPV infection (210).

The association between LEEP and PTD has in clinical practice led to a tendency towards less aggressive treatment in women of childbearing age (11). This in turn has led to concerns of an increased risk of

insufficient treatment, that could result in increased risk of future cancer (212, 219). Gynecologists in Sweden started in 2008 to measure the cone length at treatment in a standardized manner *before* fixation, Figure 9. This gives immediate feedback of the size of treatment to the surgeon. Measurement on fresh instead of formalin fixed samples should also be more accurate since fixation in formalin implies a risk of shrinkage of the specimen. The cone length is, since 2008, recorded in the Process register within the Swedish National Cervical Screening Registry (220). Sweden is therefore in the unique position to study the associations between cone length and adverse obstetric outcomes.

Figure 9. Measurement of cone-length at treatment. (Adapted from Additional file 1: Figure S1 in © 2022 Wiik et al, Feb 22, 2022, BMC Medicine, Springer Nature, (221) https://doi.org/10.1186/s12916-022-02276-6, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)



1.3.2 DOES TREATMENT OF CIN INCREASE THE RISK FOR ASCENDING INFECTION?

The increased risk for PTD seen with more radical techniques might not only be a mechanical factor - a more extensive treatment might also impair the cervical barrier against ascending infections more severely. If excisional treatment is associated with infectious pregnancy complications is not much studied although an increased risk of chorioamnionitis after treatment has been suggested (116). In a small retrospective study of 47 women with previous excisional treatment, 12 women experienced PTL or a late abortion and 8 of these had vaginal culture positive of bacteria and 7 had histopathologic chorioamnionitis (of 9 examined) (222).
It has also been unknown, until recently, if the cervical microbiota is affected by excisional treatment. One previous study found that pregnant women with previous surgical treatment for CIN had less *Lactobacilli* than untreated women (223). The first study to compare microbiota before and after treatment in a small cohort of non-pregnant women found an increase of *Lactobacillus spp* and a decrease of cervical microbial diversity three months after excisional treatment (224). Recently a study of non-pregnant premenopausal women with follow up 6 months posttreatment found no change in vaginal microbiota composition after treatment (87). There were neither any evidence that larger cone lengths/more extensive excisions affected the vaginal microbiota (87).

1.3.3 UNTREATED CIN AND THE RISK OF PTD

The risk-increase with treatment in previous studies depends on whether women with untreated CIN/HPV infection or healthy women served as the comparison group (116).

In one Welsh study, women that had been referred for colposcopy had an increased risk of PTD compared to women with normal cytology, whether they had received treatment or not before given birth (210). It has recently been suggested that untreated CIN also confers a risk of PTD (225, 226), while excisional treatment increases this further (10). El Bastawissi et al reported that women with untreated CIS (n=618) had a slightly increased risk of PTD compared to women without CIS (n=9,201); OR 1.4, (95% CI 1.0, 2.0) (227). A metanalysis of five studies (226-230), including 13,754 women with CIN, suggested an increased risk for PTD in women with CIN compared to no CIN; OR 1.51 (95% CI 1.06-2.16) (231), although the largest study included could not separate previously treated women from untreated women (230). However, Miller found no increased risk for PTD in women with untreated CIN (6.5%) (N=3,023) compared to women with no prior dysplasia (n=14,149) (6.4%); aOR 1.02 (95% CI 0.86-1.22) (232). Lately, a Dutch study reported an increased risk for PTD in women with untreated CIN (6.9% (271/3,940) compared to women with normal cytology (4.8% (1,002/20,969); OR 1.38 (95% CI 1.19-1.60) (233).

To establish if CIN confers a risk for PTD and to understand the causal link and possible patho-mechanism between CIN and PTD further research is needed (116). The possible association between untreated CIN and PTD might be explained by the HPV infection itself or might be due to a vulnerability to both conditions, either by confounding factors or genetic predisposition, in some women (116, 225, 234). Established risk factors for HPV infection/CIN and PTD or pPROM overlap considerably (225, 235). Factors that have been associated with both CIN and PTD includes smoking, parity, immunosuppression and low socioeconomic status (38, 48, 51, 121, 123, 236). *Chlamydia trachomatis* infection has been associated with development of CIN (54) as well as PTD (237) and it has been suggested that the composition of the cervical microbiota confers a risk on the development of CIN (56-61) as well as of PTD (152, 156, 157).

1.3.4 HPV INFECTION AND THE RISK OF PTD

It is still unknown whether an HPV infection itself can cause PTD or other adverse obstetric outcomes.
If HPV can cause adverse obstetric outcomes, the underlying mechanism is unknown but different hypotheses have been suggested.

As previously described have both HPV infection and PTD been associated with genital dysbiosis. Whether HPV causes PTD through an effect on the cervical microbiota is not known.
A cervical HPV infection could induce immunomodulation and hypothetically thereby affect parturition pathways (70, 238), and increase the risk for ascending infections. It has been demonstrated in a mouse model that a cervical viral infection during pregnancy could alter mucosal immunity and also result in reduced capacity to prevent ascending uterine bacterial infection (239).

A retrospective study showed that cervical HPV infection was related to placental abnormalities (226) and HPV has also been found in the placenta (90, 104, 240-243) as early as in the first trimester (244). Several HPV types (16, 11, 18, 31) can complete an entire life cycle in cultured placental trophoblasts (245-247). Trophoblasts are in direct contact with the maternal tissues and critical cells of the placenta. Experimental studies reported higher rates of cell death and failure to adhere to endometrial cell lines in trophoblasts transfected with HPV-16 oncogenes (248). It is proposed that HPV infection might impair trophoblast cell function and cause abnormal implantation and placental dysfunction, leading to obstetric complications like miscarriage, PTD and preeclampsia (226, 242, 249-251). It has also been hypothesized that HPV infection of the trophoblasts might lead to a change in the normal immune modulatory role of the trophoblast, immune sensitization, that might lead to exaggerated inflammatory response to the presence of bacteria or other viral infections, and thereby PTD (251, 252). HPV in the placental decidua has been associated with acute chorioamnionitis (243).

Studies linking positive HPV tests in the genital tract or placenta and/or abnormal cervical cytology to PTD have shown conflicting results.

A recent meta-analysis suggested that HPV infection conferred increased risk for PTD and possibly pPROM (253) but included studies were heterogenous and most of them small. Definition of exposure differed between studies, as well as time of measured exposure.

Previous studies on cervical HPV and PTD have included abnormal tests also before and/or after pregnancy as a proxy for HPV infection during pregnancy (252, 254-256), and several have used abnormal cytology as a surrogate for HPV infection (252, 254), while some have mixed abnormal cytology with positive HPV test (256, 257). Others have included cytology but also studied HPV testing separately (255) and in a minority of studies actual HPV tests during pregnancy have been used as exposure (226, 258, 259). Cytology represent HPV infection to a high degree (260) but in order to study the effect of HPV on pregnancy outcomes, studies with HPV test during pregnancy are preferred.

One retrospective study of abnormal cytology found an association with spontaneous PTD (254) but not with PTD (254) while another found no association with PTD (252). Two retrospective studies combining abnormal cytology with positive hrHPV test found an association with PTD in unadjusted analyses (256, 257), but when results were adjusted this association disappeared (256).
A retrospective register study of cervical hrHPV found an association with PTD that disappeared after adjustment of confounders (255). Another retrospective study showed that positive cervical HPV test was related to placental abnormalities like thrombosis and villitis as well as PTD (226).

Previous studies are mainly retrospective but recently a prospective study (n=899) with vaginal HPV test with genotyping in first and third trimester found that presence and persistence of hrHPV16/18 during pregnancy was associated with increased risk for PTD (258). This study also examined placenta at birth and HPV infection in placenta (any 36 genotypes) was associated with PTD (258).

A few other studies on HPV in placenta and PTD have been published. In a case control study HPV DNA was detected more frequently in

placentas after spontaneous PTD than in placentas from term pregnancies (242) while another found that HPV was present in the same frequency in spontaneous PTDs (9%) as in term pregnancies (9%) (90). A retrospective study found an association between positive HPV test in placenta tissue and PTD, and especially in cases with HPV infection in villous trophoblasts and villitis (243).

A slight decrease in PTD after the implementation of a HPV vaccination program was shown in a recent study from Australia (261).

HPV infection and PROM
A small Indian prospective study of cervical HPV infection during the first trimester, (n=104) found an increased risk for pPROM if positive for vaginal HPV (14.6%) compared to HPV-negative women (3.2%), p=0.026 (259). A retrospective study (n=2,153) of HPV test and/or abnormal cytology up to 3 years before delivery found an increased risk for pPROM (262).
Little information is available regarding the association of HPV infection and PROM at term. One small cross-sectional study (n=311) examined the association of hrHPV in cervical cytology six weeks after birth with PROM, across all gestational ages; showing an increased risk of PROM (aOR 2.32, 95% CI 1.08-4.98) (238).

HPV infection and other obstetric outcomes
HPV infection has also been associated with increased risk for first trimester spontaneous abortions (263) and also with an increased risk for preeclampsia (257) while other outcomes are less studied.

1.4 SUMMARY

In summary, excisional treatment for CIN increases the risk for PTD, but the underlying mechanisms are not yet understood.
A mechanical weakness or an increased risk for ascending infection after treatment have been suggested. Treatment could hypothetically affect the immunological milieu or cervical microbiota and herby the risk for ascending infections. It has also been suggested but not proven that the HPV infection itself could cause PTD. Women with untreated CIN also seem to be at increased risk for PTD although studies of CIN during pregnancy are scarce. Cofactors for PTD and CIN overlap and some women might be vulnerable for both conditions, either due to hereditary or environmental factors. Both PTD and CIN/HPV have been associated with genital dysbiosis. If small excisions increase the risk for PTD, especially if compared to having HPV infection/untreated CIN during pregnancy has not been established yet.

2 AIMS

The overall aim of this thesis was to investigate whether HPV infection, CIN, as well as previous treatment of CIN increase the risk for PTD and for pPROM and PROM.
We further aimed to study possible casual pathways for an association including cone length at treatment, changes in cervical microbiota and infectious complications.

The specific aims were:

Paper I
To investigate whether 1) HPV infection in conjunction with pregnancy 2) previous treatment for CIN and 3) being diagnosed with high grade CIN after delivery - are associated with an increased risk of PTD and other adverse obstetric and neonatal outcomes.

Paper II
To investigate whether CIN during pregnancy and excisional treatment before pregnancy are associated with increased risk for PTD and other adverse obstetric and neonatal outcomes, as well as how risk estimates for excisional treatment vary with cone length at treatment measured on fresh specimens.

Paper III
To investigate whether HPV infection during pregnancy, at mid-pregnancy or at delivery, is associated with PTD, pPROM, PROM and chorioamnionitis.

Paper IV
To compare the cervical microbiota before and after LEEP
and to assess whether cone length at treatment and HPV persistence after treatment affect the bacterial composition post-treatment. Further, this study aimed to compare the microbiota to reference women with normal cervical cytology.

3 STUDY PARTICIPANTS AND METHODS

Paper I and II are register-based studies.
Paper III and IV are prospective clinical cohort studies.

Sweden has unique possibilities for register-based studies.
Every legal citizen in Sweden is given a personal identification number, either at birth or at immigration. Cross-linking different registers based on the personal identification number, enables epidemiological research in a unique manner (264).
The majority of Swedish health care is publicly financed.
Through registration of diseases and medical conditions in mandatory nationwide health-registers and following certain diseases or treatments in national quality registers, health and quality of care in Sweden can be followed up in a population-based way.
It is mandatory for care providers to report data to the health registers run by the National Board of Health and Wealth fare, like the Swedish Medical Birth Registry and the Swedish Cancer Register used in paper I and II. Other national registers from Statistics Sweden were used for collection of background factors.
Of the nearly 100 quality registers in Sweden, we used the Swedish National Cervical Screening Registry (NKCx) in this thesis, which is a quality register with national coverage.
Data from different registers were linked by the National Board of Health and Welfare and pseudonymized by replacing the personal identification number with study identification numbers.

3.1 REGISTERS

The Swedish National Cervical Screening Registry (NKCx)

This quality register, that started in 1978, contains data from 1969 and has been nationwide since 1995. Women are informed in their invitation letter from the screening program about the quality register and record keeping and saved samples in a biobank and can negate to be included. The data in NKCx is reported directly to the register from care-givers/laboratories.
The register is divided into the analysis register (265) and the process register (220). The analyses register includes national data (100% coverage) on invitations, cytological smears, histological samples and HPV tests (265). Cervical HPV tests have been recorded in the database since 2007.
The process register contains real time data on invitations, cytology smears, histology samples and HPV tests. It was first established in western Sweden in 2002 and has after that increased coverage to 85% of Sweden, 19/21 counties (220). The process register also contains data regarding treatment of dysplasia since 2002 and from 2008 records of cone length at treatment measured by the colposcopist before fixation in a standardized manner. The participation in cervical screening is high in Sweden, over 80%, and only a few women have negated to have their data registered in NKCx (108).

The Swedish National Medical Birth Register (MBR)

The Swedish National Medical Birth Register (MBR) was established in 1973 and comprises all births in Sweden from 22 completed weeks of gestation (266). It is held by the Swedish National Board of Health and Welfare. Reporting to the register is mandatory. Initially, a medical report was filled in by the secretaries in each unit for communication between antenatal, delivery and the neonatal units, with a copy to MBR for registration. Since 1982 the data collection to MBR is based on standardized data registration in medical charts. Today the process is computerized (electronic transfer from medical

charts started in 2007), and the information available in the register is extracted from antenatal care units, delivery wards and from pediatric examinations of the newborn by filling in standardized medical records and ICD codes. The current version of included variables was designed in 1998. The MBR includes data on reproductive history, maternal health, pregnancy complications and neonatal outcomes. Data in MBR is considered to be of good quality but varies with time and variables (267).

The Swedish Cancer Register

The Swedish Cancer Register is a mandatory registry established in 1958 and held by the Swedish National Board of Health and Welfare, registering all cancer diagnoses in Sweden, as well as all diagnosis of CIN3 and AIS (268). It is mandatory for both clinicians and pathologists to report to the regional cancer registry at the Regional Cancer Centre, which reports to the register annually. In 1998 the underreporting of gynecological cancers to the register compared to the national patient register was 3.4% (269).

Registers at Statistics Sweden (SCB)

Statistics Sweden is a national administrative authority and responsible for official statistics regarding for example level of education, income, country of birth, immigration, emigration, adoption, child births and deaths.
The Swedish Register of Education, (270) the Total Population Register (271) and the Income and Tax Assessment Register, (272) are examples of registers held by Statistics Sweden.

3.2 STUDY POPULATIONS, EXPOSURE AND OUTCOME DEFINITIONS

Paper I, study population, exposures

The study cohort was identified by linking singleton deliveries 1999-2016 in the MBR to NKCx/Anlysis and the Swedish Cancer Register. Women with chronic inflammatory disease, HIV infection or prior organ transplantation were excluded. Different study groups were defined based on results of cervical HPV tests registered in the NKCx 2007-2016, cervical cytology and histology registered in the NKCx 1978-2016 and/or histological diagnoses registered in the Swedish Cancer Register. Background factors were retrieved from MBR and registers at SCB (Swedish Register of Education, (270) the Total Population Register (271) and the Income and Tax Assessment Register (272).

The study groups were defined as follows:
1) The reference group; a history of exclusively normal cervical cytology results and at least one sample taken in the three years preceding the included delivery, n=338,109.
2) Two partly overlapping HPV infection groups: Women with presumed HPV infection during pregnancy, based on a) abnormal cervical cytology, n=11,727 or b) positive HPV test, recorded within six months prior to conception or during pregnancy, n=2,550.
3) Subsequent CIN2+ group: at presumed risk of persistent HPV infection based on a histological diagnosis of CIN2-3, AIS or cervical cancer any time after delivery, n=33,760.
4) Treated group: histologically diagnosed CIN3 before conception, n=23,189. (CIN3 was used as a proxy for treatment as these women are always treated in Sweden)
The study groups were compared concerning obstetric and neonatal outcomes, see below.

Paper II, study population, exposures

The study cohort was identified by linking singleton deliveries 2008-2016 from western Sweden in the MBR to NKCx/Process and NKCx/Analysis and The Swedish Cancer Register. Different study groups were defined based on results of cervical cytology and histology registered in the NKCx/Process and NKCx/Analysis and treatment data in the NKCx/Process. Women with treatment recorded in NKCx/Process before 2008 or with histological diagnoses of CIN2/CIN2+ before 2008 in the NKCx/Analysis and the Swedish Cancer Register were excluded as well as women with chronic inflammatory disease, HIV infection or prior organ transplantation. Background factors were retrieved from MBR and registers at SCB (Swedish Register of Education, (270) the Total Population Register (271) and the Income and Tax Assessment Register (272).
The study groups were defined as follows:
1) Treated group; cervical excisional treatment performed in western Sweden 2008-2016, n=3,250.
1b) Cone-length group: a subgroup of the treated group, treated with loop electrosurgical excision procedure (LEEP) or with laser excision, and with recorded cone-length > 2mm and ≤50mm, n=2,408.
2) CIN during pregnancy group: CIN or AIS at cervical biopsy during pregnancy in western Sweden, n=1,380.
3) Normal cytology group: history of exclusively normal cervical cytology results and at least one sample taken in western Sweden during the three years preceding the included delivery, n=42,398.
The study groups were compared concerning obstetric and neonatal outcomes, see below.

Obstetric outcomes paper I and II

Obstetric outcomes were defined based on data from the MBR.
For best estimation of gestational age an algorithm was used utilizing ultrasound based gestational age estimation above e.g. estimation based on the last menstrual period. The algorithm is described in detail in the Appendix (Swedish language).
The primary outcome was PTD at 22+0 - 36+6 weeks (154-258 days) of gestation. Also early PTD (22-33 weeks (154-237 days) of

gestation) and very early PTD (22-27 weeks (154-195 days) of gestation) were studied.
Secondary outcomes were pPROM (according to ICD-10 codes in the MBR) and spontaneous PTD (a delivery starting with pPROM or preterm labor, excluding PTD that started with induction or cesarean section). Further outcomes comprised PROM in term pregnancies (≥37 weeks of gestation, intrapartum fever, chorioamnionitis and neonatal sepsis (according to ICD-10 codes in the MBR). The exact ICD-10 codes used are specified in the additional data of paper I and paper II. Also intrauterine fetal death, Apgar score <7 at five minutes, neonatal mortality (1-28 days) and small for gestational age (SGA) (birthweight less than -2 standard deviations (SD) according to Swedish reference curves) were studied (273).

Study population, exposure and outcomes paper III

Study population
All women from the prospective multicenter study PreventADALL (Preventing Atopic Dermatitis and ALLergies in children) clinicaltrials.gov (NCT02449850) (274), who had singleton pregnancies, urine samples collected for HPV detection at mid-pregnancy and reports of obstetric outcomes at delivery were included, n=950. All available data was used and we estimated that we with the sample size of 950 women, with 80% power at 5% significance level, assuming 5% PTD and 12% PROM would be able to detect ORs as low as 2.4 and 1.8 respectively.

Exposure
First-void urine was collected at inclusion in mid-pregnancy and at birth. From the placenta, three punch biopsies through the placenta from the peripheral, middle and central site were collected and stored in RNALater® (Life Technologies, Carlsbad, California, USA).
In urine, HPV DNA was detected and genotyped and semi-quantified for viral load within 36 months, by Seegene Anyplex II HPV 28 PCR assay (Seegene Inc. Seoul, South Korea), as previously reported (1). The same method was used for placenta tissue after homogenizing the tissue and after nucleic acid extraction with Maxwell ® RSC Tissue DNA-kit (PromegaMadison, Winsconsin, USA).

The main exposure was presence of hrHPV or not (12 genotypes), but also presence or absence of HPV (any genotype) and hrHPV16 at mid-pregnancy and at delivery was studied. In hrHPV positive women we also examined if presence of multiple HPV genotypes, persistence of hrHPV or viral load increased the risk for the adverse outcomes.

Outcomes

The primary outcome was PTD and secondary outcomes pPROM, spontaneous PTD, PROM at term, chorioamnionitis and antibiotic treatment at delivery due to suspected chorioamnionitis. Gestational age was based on ultrasound, performed as part of the routine prenatal care, and in IVF pregnancies based on embryo transfer. At birth, GA was systematically recorded in study protocols together with information about start of delivery, indication for induction of labor, time of membrane rupture and active delivery, antibiotic treatment for suspected clinical chorioamnionitis and other obstetric complications. Obstetric background and outcome data was also later quality controlled and complemented through scrutinization of patient journal software systems in Norway and by collection of data from the Swedish Pregnancy Register in Sweden (275). All data was anonymized in the TSD (Service for Sensitive Data) platform (276) and analyses were performed in the TSD.

Study population and outcomes paper IV

Study population

This was a prospective clinical cohort study recruiting women between October 2005 and November 2007. Women that were examined at Østfold Hospital Trust, Health Region East, Norway due to cervical dysplasia and planned for their first LEEP were eligible. Women on long-term antibiotics were excluded.

Eighty-nine women were included in the “LEEP-group” (81 women with CIN3, six with CIN2 and two with persistent CIN1). If the woman required antibiotic treatment after LEEP or became pregnant she was excluded from the follow-up analysis. Two women were lost to follow up at six months and four women at 12 months and thus 77 women were included in the follow up analysis at six months and 72 women at 12 months.

The main analysis was the comparison of cervical microbiota before and after LEEP (6 and 12 months). Additional analyses included comparisons between the LEEP group and a reference group before and after treatment. The reference group consisted of 100 women with normal cervical cytology that were recruited at a private gynecological practice during the same time period.

Background information, collected at inclusion included socio-demographical data, including year of birth, smoking habits, marital status, level of education and medical history including use of recent antibiotics and contraception. The cone length at treatment was documented by the pathologist in millimeters (mm).

Outcome
During sterile speculum examination endocervical swab samples for detection of bacteria were collected prior to treatment and post-treatment (6 and 12 months) in the LEEP group and at base-line for the reference population.
Cultivation and polymerase chain reaction (PCR) was used for detection of bacteria. One swab was transported on Stuart medium for cultivation of aerobic and anaerobic bacteria on blood and lactose agar plates. Growth results were reported as growth or no growth for the following bacteria; *Streptococcus* spp. *Escherichia coli*, *Bacteroides* spp., *Gardnerella vaginalis*, and *Lactobacillus* spp, but also other bacterial species detected were reported. PCR is used to amplify or copy a specific DNA sequence and this technique was used to detect *Chlamydia trachomatis, Mycoplasma hominis*, *Ureaplasma parvum and Ureaplasma urealyticum*. Bacteria were divided into *Lactobacillus* and non-Lactobacillus bacteria species (all other reported species). All women that had at least one type of one non-Lactobacillus bacteria species were included in a constructed group called ”any non-Lactobacillus”.
At follow up cervical LBC was collected for nucleic acid extraction and hrHPV DNA testing (277).

An overview of study populations and outcomes in paper I-IV is presented in Table 2.

Table 2. Study populations and outcomes Paper I-IV

	Paper I	Paper II	Paper III	Paper IV
Study design	Population-based register study	Population-based register study	Prospective cohort study	Prospective cohort study
Sample size (N) Study groups	Total; 400,583 References; 338,109 HPV inf (cyt); 11,727 HPV inf (HPV test); 2,550 Treated; 23,185 Subsequent CIN2+; 33,760	Total; 46,870 Normal cyt; 42,398 Treated; 3,250 Cone-length; 2,408 CIN during pregnancy; 1,380	Total; 950 hrHPV pos; 231 hrHPV neg; 719 HPV-pos; 377 HPV-neg; 573 hrHPV-16-pos; 59 hrHPV-16-neg; 891	Total; 189 LEEP group; 89 References; 100
Study period	1999-2016	2008-2016	2014-2016	2005-2007
Outcomes	Primary; PTD Secondary; early PTD; very early PTD, pPROM, spontaneous PTD PROM at term, Apgar score <7 at five minutes, intrapartum fever, chorioamnionitis, neonatal sepsis, small for gestational age (SGA), intrauterine fetal death, neonatal mortality (1-28 days)		Primary; PTD, Secondary; pPROM, spontaneous PTD, PROM at term, chorioamnionitis, antibiotics due to suspected chorioamnionitis	Presence and number of "non-Lactobacillus bacteria spp" Individual bacteria species *Streptococcus spp, Bacteroides spp, Escherichia coli, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum, Ureaplasma urealyticum, Chlamydia trachomatis.* *Lactoabacillus spp*
Comparisons	*References and – HPV inf (cyt) – HPV inf (HPV test) -Subsequent CIN2+ (incl. stratified for time to CIN) -Treated (incl. stratification for PTD and pPROM studying infectious complications and stratification for infectious complications studying neonatal mortality) *Treated and -HPV inf (cyt) -HPV inf (HPV test) -Subsequent CIN2+ (and paired analyses of women in both groups)	*Normal cyt and -Treated (incl subgroup with benign histology) -CIN during pregnancy -Groups of cone-length (3-5, 6-9, 10, ≤10mm, 11-12 mm, 13-15 mm and >15). *CIN during pregnancy and -cone lengths ≤10mm (with subanalysis of only HSIL) *Cone-length as a continuous variable; in all women, in only cone-lengths ≤10mm and all women with cone lengths truncated at 10 mm.	At mid-pregnancy and delivery: *hrHPV; pos-neg *any HPV; pos-neg *hrHPV16 pos-neg hrHPVpersistence - neg hrHPV At mid-pregnancy: *hrHPV and multiple HPV infection – neg hrHPV *hrHPV viral load	LEEP group before treatment to -6 months after treatment -12 months after treatment LEEP group before treatment -References LEEP group after treatment -References
Comparisons in sensitivity analyses	HPV inf (cyt) was compared to HPV inf (HPV test)	Women with LSILand – HSILwere compared in the CIN during pregnancy group		Change of bacteria with treatment was compared between women; *< 46 yrs and > 45 yrs *with and without hormonal contraception *singles and in a relationship *HPV-pos and HPV- neg at follow-up

BMI; body mass index, CIN; cervical intraepithelial neoplasia, HPV; human papillomavirus, hrhPV; high risk human papillomavirus, HSIL; high grade squamous intraepithelial lesion, inf; infection, LSIL; low grade intraepithelial lesion, mm; millimeter, N; number, neg; negative, pos; positive, PTD; preterm delivery, pPROM; preterm prelabor rupture of membranes, PROM; prelabor rupture of membranes, spp; species, yrs; years

3.3 OVERVIEW OF ANALYSES IN PAPER I-IV

An overview of the statistical analyses applied in paper I-IV is presented in Table 3.

Table 3. Overview of statistical methods.

	Paper I	Paper II	Paper III	Paper IV
Descriptives				
of continuous variables	mean and standard deviation, median and interquartile range			
of categorical variables	Numbers, percentage			
Analysis				
Test of correlation				Pearson correlation test (cone length at treatment - change of n bacteria species)
Comparing continuous variables			Independent t-test (mean) between groups	
Comparing categorical variables			Chi square-test and Fisher´s exact test	
				For paired data (before and after treatment); *Paired t-test for mean number of bacteria spp. *McNemar test for change in detection of individual bacteria species
Of association between binary response and predictors	Univariable logistic regression analysis, OR with 95% CI			
	Multivariable logistic regression analysis, adjusted OR with 95% CI			
	Paired analyses with conditional logistic regression analysis.			
Covariates	Pregnancy characteristics; year of delivery, assisted reproduction. infant's sex. Maternal characteristics; age, BMI, parity, marital status, smoking, country of birth, highest disposable household income three years preceding delivery, education level		Parity and then forward selection of; maternal age, smoking, infant´s sex, history of PTD, BMI, education, ivf, marital status, smoking	Age, marital status, smoking, use of hormonal contraception
	The paired analyses; BMI, marital status, infant's sex, smoking and assisted reproduction	A graphic presentation of cone-lenghts and risk for PTD in a standardized woman; using log reg and covariates; maternal age, parity, smoking, infant´s sex, year of delivery.		
Interaction analyses	In all stratified analyses, differences in ORs between strata were analyzed by an interaction term between the strata and the group in logistic regression.			
Statistical tools	"R" data analysis tool (version 4.0.0, The R Foundation for Statistical Computing, Vienna, Austria) SPSS software (version 26.0, IBM)		SPSS (version 27.0, IBM)	SPSS (version 24.0, IBM)

BMI; body mass index, CI; confidence interval, ivf; in vitro fertilization, n; number, OR; odds ratio, PTD; preterm delivery, spp; species

3.4 ETHICAL PERMISSIONS AND COMMENTS

All studies have been approved by Regional Ethical Committees.

Table 4. Ethical approvals

Paper	Country	Name of Regional Ethics Committee	Name of document	Approval date
I and II	Sweden	The Regional Ethical Committee, Gothenburg	614-15	2015-08-27
			T170-16	2016-03-30
III	Norway	Regional Ethical Committee for Medical and Health Research in South-Eastern Norway	2014/518	2015-05-18
			2017/1053	2017-11-01
	Sweden	Regional Ethical Committee in Stockholm	2014/2242-31/4	2015-03-25
			2018/1437-32	2018-07-13
			2018/2492-32	2018-12-19
IV	Norway	Regional Ethical Committee, Øst-Norway	622-05059	2005-04-13

Since paper I and II are register-based studies no informed consent was collected. The dataset was pseudonymized before being distributed to the research team. Swedish women participating in the national cervical screening program are informed about collection of data to the quality-registers; NKCx Analysis and NKCx Process, and inclusion of data in these registers is voluntary.

All included women consented to participate after oral and written information in paper III and IV.

Paper III is registered at clinicaltrials.gov (NCT02449850). Women and health care givers in paper III were not informed about the results of the HPV tests. The urine was analyzed for HPV within 36 months after collection. During the study period screening of cervical dysplasia through HPV testing had not been established in Norway or Sweden. HPV testing is now part of screening in both countries – however, in cervical smears and not by collection of urine. There were no guidelines for the follow up of a positive HPV test in urine. Since detection of HPV in urine had no clinical implication for the women, neither in form of follow-up or treatment, but, on the contrary, could cause unnecessary concern, results from the HPV testing were not reported back to the women. This was in line with the practice of the National HPV vaccine surveillance program in Norway, where urine samples were monitored for HPV in 17- and 22-years old women, with no feedback to the participants (278). Women enrolled in the

PreventADALL study were included in the Norwegian and Swedish national screening programs for cervix cancer.

Women in paper IV followed the Norwegian national screening program for cervix cancer. The recommended follow-up cytology within 6 months after treatment, was taken together with the other samples at the 6 months follow-up visit. Women were informed about pathological test results, and if indication for treatment (e.g., positive Chlamydia Trachomatis) the women were offered treatment. For women with free resection margins the follow up could have been done at the general practitioner and the follow up at 12 months could have waited until 18 months according to the guidelines, i.e. for these women the study meant extra follow-up at the hospital.

4 RESULTS AND COMMENTS

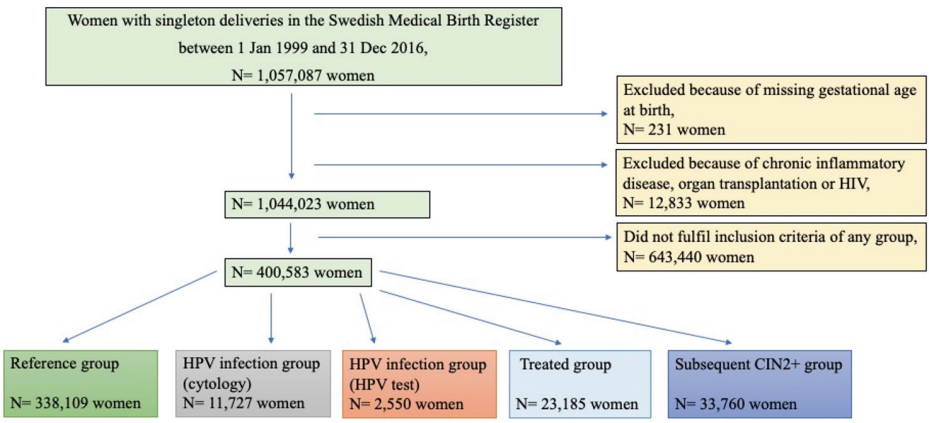
Results from the four studies will be presented followed by comments including comparisons with other studies in the field.

4.1 PAPER I

The aim of this study was to examine whether 1) HPV infection in conjunction with pregnancy 2) previous treatment for CIN and 3) being diagnosed with high grade CIN after delivery - was associated with an increased risk of PTD and other adverse obstetric and neonatal outcomes.

In total 400,583 deliveries, between 1999 and 2016 fulfilled the criteria to be included in one of the study-groups, Figure 10.

Figure 10.
Flowchart of the study population. Study groups were; The reference group (history of only normal cytology), two HPV infection groups; HPV infection (abnormal cytology) and HPV infection (positive HPV test), Treated group (women with CIN3 diagnosed in histology before conception were regarded as previously treated) and Subsequent CIN2+ (women with diagnosed CIN2+ after delivery). CIN, cervical intraepithelial neoplasia; HIV, human immunodeficiency virus; HPV, human papillomavirus.
(Adapted from Fig 1 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2021, (279) https://doi.org/10.1371/journal.pmed.1003641.g001, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)



Background factors differed between study groups regarding for example age, smoking and parity. Women in the treated group were older, nulliparity was most common in the treated group and in the HPV infection groups and smoking during pregnancy was less common in the reference group (279).

Compared to the reference group all groups had an increased risk for PTD. The treated group and the HPV infection groups had increased risk for pPROM and neonatal mortality and the treated group and HPV infection (HPVtest) group for PROM at term, Table 5 (279).

Table 5. Obstetric and neonatal outcomes in exposure groups compared to the reference group – adjusted multivariable logistic regression analyses. (Adapted from Table 2 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2022 (279) https://doi.org/10.1371/journal.pmed.1003641.t002, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)

Outcome	Reference group (*n* = 338,109)	HPV infection groups						Treated group (*n* = 23,185)			Subsequent CIN2+ group (*n* = 33,760)		
		Cytology (*n* = 11,727)			HPV test (*n* = 2,550)								
	n (%)	*n* (%)	aOR (95% CI)	*p*-Value	*n* (%)	aOR[1] (95% CI)	*p*-Value	*n* (%)	aOR (95% CI)	*p*-Value	*n* (%)	aOR (95% CI)	*p*-Value
PTD, <37 weeks	15,661 (4.6)	692 (5.9)	1.21 (1.12–1.31)	**<0.001**	143 (5.6)	1.19 (1.01–1.42)	**0.042**	2,106 (9.1)	1.85 (1.76–1.95)	**<0.001**	1,736 (5.1)	1.12 (1.06–1.18)	**<0.001**
Early PTD, <34 weeks	4,221 (1.2)	221 (1.9)	1.39 (1.21–1.60)	**<0.001**	34 (1.3)	0.99 (0.70–1.40)	0.96	661 (2.9)	2.02 (1.86–2.21)	**<0.001**	488 (1.4)	1.18 (1.06–1.30)	**0.002**
Very early PTD, <28 weeks	820 (0.2)	55 (0.5)	1.73 (1.31–2.28)	**<0.001**	7 (0.3)	0.97 (0.46–2.06)	0.94	139 (0.6)	2.20 (1.82–2.66)	**<0.001**	87 (0.3)	1.10 (0.87–1.39)	0.42
Spontaneous PTD	11,409 (3.4)	493 (4.2)	1.18 (1.07–1.29)	**0.001**	100 (3.9)	1.17 (0.95–1.43)	0.14	1,699 (7.3)	2.06 (1.95–2.17)	**<0.001**	1,291 (3.8)	1.14 (1.07–1.21)	**<0.001**
pPROM	5,110 (1.5)	232 (2.0)	1.22 (1.07–1.40)	**0.004**	64 (2.5)	1.52 (1.18–1.96)	**0.001**	934 (4.0)	2.36 (2.19–2.54)	**<0.001**	521 (1.5)	1.09 (0.99–1.20)	0.09
PROM in deliveries at ≥37 weeks	21,906 (6.8)	828 (7.5)	1.06 (0.98–1.14)	0.13	251 (10.4)	1.24 (1.08–1.42)	**0.002**	1,772 (8.4)	1.11 (1.05–1.17)	**<0.001**	1,719 (5.4)	0.99 (0.94–1.04)	0.64
SGA[2]	6,873 (2.0)	320 (2.7)	1.09 (0.97–1.22)	0.16	65 (2.6)	0.96 (0.75–1.24)	0.76	594 (2.6)	1.02 (0.94–1.12)	0.64	715 (2.1)	0.99 (0.91–1.07)	0.72
Apgar score < 7 at 5 minutes	4,165 (1.2)	191 (1.6)	1.20 (1.04–1.40)	**0.014**	41 (1.6)	1.04 (0.76–1.43)	0.79	366 (1.6)	1.14 (1.02–1.27)	**0.019**	323 (1.0)	0.89 (0.79–1.00)	**0.043**
Neonatal mortality	343 (0.1)	24 (0.2)	1.81 (1.19–2.76)	**0.006**	7 (0.3)	2.69 (1.25–5.78)	**0.011**	47 (0.2)	1.79 (1.30–2.45)	**<0.001**	29 (0.1)	0.71 (0.47–1.05)	0.09
Intrauterine fetal death	711 (0.2)	43 (0.4)	1.55 (1.13–2.12)	**0.006**	6 (0.2)	0.93 (0.41–2.09)	0.86	74 (0.3)	1.35 (1.05–1.72)	**0.019**	50 (0.1)	0.71 (0.53–0.96)	**0.026**
Chorioamnionitis	895 (0.3)	45 (0.4)	1.25 (0.92–1.69)	0.15	10 (0.4)	1.00 (0.53–1.88)	1.00	196 (0.8)	2.75 (2.33–3.23)	**<0.001**	74 (0.2)	1.02 (0.80–1.31)	0.85
Intrapartum fever	2,189 (0.6)	87 (0.7)	1.01 (0.82–1.26)	0.90	37 (1.5)	1.40 (1.00–1.96)	**0.050**	213 (0.9)	1.24 (1.07–1.44)	**0.003**	133 (0.4)	0.89 (0.74–1.06)	0.19
Neonatal sepsis	2,508 (0.7)	97 (0.8)	0.99 (0.80–1.21)	0.89	14 (0.6)	0.67 (0.40–1.14)	0.14	300 (1.3)	1.55 (1.37–1.75)	**<0.001**	216 (0.6)	0.86 (0.74–0.99)	**0.041**

Statistically significant *p*-values in bold. Analyses adjusted for year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level, and assisted reproduction.

[1]Analyses compared to reference group 2007–2016.

[2]Missing data: reference group, *n* = 575; HPV infection (cytology) group, *n* = 24; HPV infection (HPV test) group, *n* = 3; treated group, *n* = 47; subsequent CIN2 + group, *n* = 71.

aOR, adjusted odds ratio; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; pPROM, preterm prelabor rupture of membranes; PROM, prelabor rupture of membranes; PTD, preterm delivery; SGA, small for gestational age.

https://doi.org/10.1371/journal.pmed.1003641.t002

The treated group also had increased risk for chorioamnionitis and neonatal sepsis, Table 5. The risk for PTD, spontaneous PTD and pPROM and infectious complications was higher in the treated group than in the other exposure groups, Table 6.

Table 6. Obstetric and neonatal outcomes in the treated group compared to the HPV infection groups and the subsequent CIN2+group—adjusted multivariable logistic regression analyses (Adapted from Table 2 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2022, (279) https://doi.org/10.1371/journal.pmed.1003641.t004, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/

Outcome	Treated versus HPV infection (cytology) comparison				Treated versus HPV infection (HPV test) comparison				Treated versus subsequent CIN2 + comparison			
	Treated group (*n* = 22,711)	HPV infection (cytology) group (*n* = 11,727)			Treated group (*n* = 14,579)	HPV infection (HPV test) group (*n* = 2,550)			Treated group, (*n* = 18,505)	Subsequent CIN2+ group (*n* = 33,760)		
	n (%)	*n* (%)	aOR (95% CI)	*p*-Value	*n* (%)	*n* (%)	aOR[1] (95% CI)	*p*-Value	*n* (%)	*n* (%)	aOR (95% CI)	*p*-Value
PTD, <37 weeks	2,066 (9.1)	692 (5.9)	1.61 (1.46–1.78)	**<0.001**	1,313 (9.0)	143 (5.6)	1.68 (1.39–2.03)	**<0.001**	1,751 (9.5)	1,736 (5.1)	1.77 (1.63–1.92)	**<0.001**
Early PTD, <34 weeks	645 (2.8)	221 (1.9)	1.53 (1.29–1.81)	**<0.001**	416 (2.9)	34 (1.3)	2.18 (1.50–3.17)	**<0.001**	554 (3.0)	488 (1.4)	1.85 (1.59–2.13)	**<0.001**
Very early PTD, <28 weeks	136 (0.6)	55 (0.5)	1.32 (0.93–1.87)	0.12	98 (0.7)	7 (0.3)	2.79 (1.23–6.35)	**0.014**	118 (0.6)	87 (0.3)	2.01 (1.44–2.80)	**<0.001**
Spontaneous PTD	1,669 (7.3)	493 (4.2)	1.85 (1.65–2.07)	**<0.001**	1,038 (7.1)	100 (3.9)	1,86 (1.49–2.33)	**<0.001**	1,421 (7.7)	1,291 (3.8)	2.00 (1.82–2.19)	**<0.001**
pPROM	918 (4.0)	232 (2.0)	2.09 (1.78–2.45)	**<0.001**	598 (4.1)	64 (2.5)	1.55 (1.17–2.04)	**0.002**	779 (4.2)	521 (1.5)	2.51 (2.20–2.87)	**<0.001**
PROM in deliveries at ≥37 weeks	1,752 (8.5)	828 (7.5)	1.08 (0.98–1.18)	0.12	1,208 (9.1)	251 (10.4)	0.88 (0.75–1.03)	0.11	1,526 (9.1)	1,719 (5.4)	1.21 (1.11–1.31)	**<0.001**
SGA[2]	587 (2.6)	320 (2.7)	0.86 (0.74–1.01)	0.06	383 (2.6)	65 (2.6)	0.93 (0.70–1.25)	0.64	523 (2.8)	715 (2.1)	1.03 (0.90–1.17)	0.72
Apgar score <7 at 5 minutes	355 (1.6)	191 (1.6)	0.94 (0.78–1.14)	0.55	248 (1.7)	41 (1.6)	1.16 (0.81–1.67)	0.43	303 (1.6)	323 (1.0)	1.30 (1.08–1.57)	**0.005**
Neonatal mortality	43 (0.2)	24 (0.2)	1.04 (0.59–1.83)	0.89	21 (0.1)	7 (0.3)	0.56 (0.21–1.45)	0.23	35 (0.2)	29 (0.1)	2.61 (1.45–4.70)	**0.001**
Intrauterine fetal death	71 (0.3)	43 (0.4)	0.72 (0.48–1.09)	0.12	51 (0.3)	6 (0.2)	1.21 (0.49–3.00)	0.69	58 (0.3)	50 (0.1)	1.75 (1.13–2.73)	**0.013**
Chorioamnionitis	192 (0.8)	45 (0.4)	2.21 (1.55–3.15)	**<0.001**	119 (0.8)	10 (0.4)	2.47 (1.24–4.91)	**0.010**	160 (0.9)	74 (0.2)	3.33 (2.41–4.60)	**<0.001**
Intrapartum fever	211 (0.9)	87 (0.7)	1.21 (0.92–1.59)	0.17	154 (1.1)	37 (1.5)	0.79 (0.53–1.17)	0.24	197 (1.1)	133 (0.4)	1.37 (1.06–1.77)	**0.015**
Neonatal sepsis	294 (1.3)	97 (0.8)	1.61 (1.25–2.07)	**<0.001**	171 (1.2)	14 (0.5)	2.34 (1.30–4.24)	**0.005**	257 (1.4)	216 (0.6)	2.02 (1.63–2.51)	**<0.001**

Statistically significant *p*-values in bold. Analyses adjusted for year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level, and assisted reproduction.

[1]Analyses 2007–2016.

[2]Missing data: treated group, *n* = 47; HPV infection (cytology) group, *n* = 24; HPV infection (HPV test) group, *n* = 3; subsequent CIN2+ group, *n* = 71.

aOR, adjusted odds ratio; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; pPROM, preterm prelabor rupture of membranes; PROM, prelabor rupture of membranes; PTD, preterm delivery; SGA, small for gestational age.

https://doi.org/10.1371/journal.pmed.1003641.t004

Women in the subsequent CIN2+ group also had an increased risk of PTD and spontaneous PTD. However, the increase was only significant if the women had been diagnosed with CIN2+ within the first three years after delivery (279).

Also in the paired analyses, comparing obstetric outcomes in women with deliveries both before and after treatment, (n=4,680) there was an increased risk after treatment of PTD: aOR 1.26 (95% CI 1.07-1.49), p=0.007; pPROM: aOR 1.72 (95% CI 1.29-2.29), p<0.001; spontaneous PTD: aOR 1.23 (95% CI 1.02-1.49), p=0.032; chorioamnionitis: aOR 14.22 (95% CI 2.09-96.92), p=0.007 and neonatal sepsis: aOR 1.92 (95% CI 1.02-3.61), p=0.044 (279).

The risk for infectious complications in treated women compared to the references was after stratification for PTD only significantly increased in the subgroup with preterm deliveries; chorioamnionitis: aOR 3.96 (95% CI 3.13–5.02), $p < 0.001$; neonatal sepsis: aOR 1.74 (95% CI 1.43–2.12), $p < 0.001$, - although almost significant for chorioamnionitis also in term deliveries: aOR 1.28 (95% CI 0.98–1.67), $p = 0.07$.
When only including term deliveries there was no increased risk for neonatal sepsis: aOR 1.08 (95% CI 0.91–1.29), $p = 0.36$, in the treated group compared to the references (279).

The risk for chorioamnionitis in treated women compared to the references was after stratification for PTD with pPROM and PTD without pPROM increased in PTD deliveries both with pPROM; aOR 3.68 (95% CI 2.70–5.03), $p < 0.001$ and without pPROM; aOR 2.19 (95% CI 1.32–3.61), $p = 0.002$, but with higher risks in women with pPROM ($p = 0.032$). For neonatal sepsis the risk was only increased in the PTD deliveries with pPROM: aOR 2.70 (95% CI 1.99–3.68), $p < 0.001$ in stratified analyses (279).

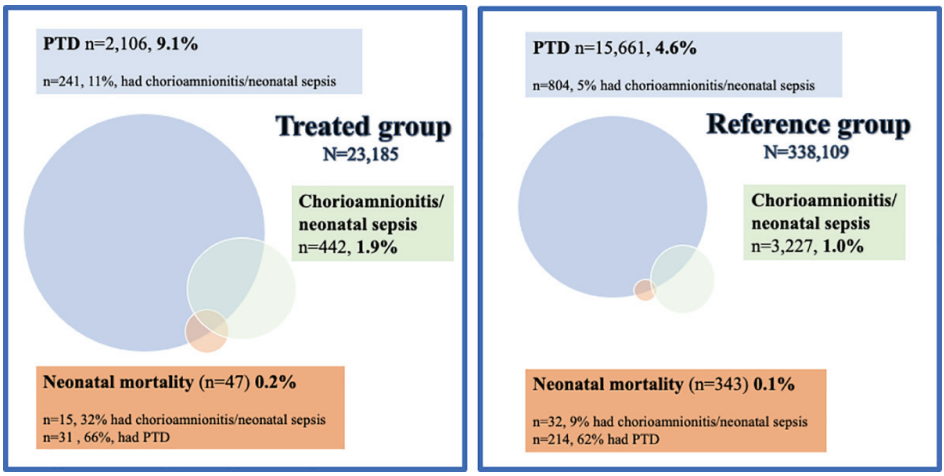
No increased risk of infectious complications was found in the HPV infection groups.

The treated group had increased risk for neonatal mortality compared to the reference group, Table 5. When comparisons were adjusted for

PTD the difference disappeared. Of the neonatal mortality cases in the treated group 14.9% contradicted chorioamnionitis and 25.5% neonatal sepsis while in the group with normal cytology only 4.1% contracted chorioamnionitis and 6.4% had infants with neonatal sepsis.

The risk for neonatal mortality in treated women compared to the references was, after stratification for infectious complications, increased in treated women but only in the subgroup with infectious complications (279).

Figure 11. Venn diagram illustrating the relationship between preterm delivery (PTD), infectious complications (chorioamnionitis/neonatal sepsis), and neonatal mortality in the treated group and the reference group. The treated group more frequently had PTD, neonatal mortality, and infectious complications. The deliveries resulting in neonatal mortality more frequently had infectious complications in the treated group than in the reference group. (Adapted from Fig 3 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2022, (279), https://doi.org/10.1371/journal.pmed.1003641.g005, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)



Women with HPV infection had an increased risk for neonatal mortality (279) compared to references, Table 5, and the risk was still significant in the HPV infection groups after adjustment for PTD; i.e., HPV test: aOR 2.30 (95% CI 1.06–4.99), $p = 0.036$ and cytology: aOR 1.64 (95% CI 1.07–2.50), $p = 0.024$ (279).

Comments Paper I

HPV infection

In paper I, we observed that HPV infection in conjunction with pregnancy was associated with an increased risk for PTD, spontaneous PTD, pPROM, PROM at term and neonatal mortality.

A recent meta-analysis found a risk estimate of aOR 1.50 for PTD while we in paper I found lower aORs; 1.21 (cytology) and aOR 1.19 (HPV test), although our results are within the CI of the meta-analysis study (1.19 to 1.88) (253).

Previous studies have shown conflicting results.

In a recent population-based study no significant increased risk for HPV infection was found when 2,276 hrHPV-negative women (PTD 6.7%) were compared to 419 hrHPV-positive women without CIN2+ (PTD 7.6%); aOR 1.11 (95% CI 0.75-1.66), and neither when the hrHPV-negative women were compared to 834 women with low grade cervical abnormalities (PTD 7.3%); aOR 1.06 (95% CI 0.78-1.45) (255). However, the sample size of that study was smaller than in paper I.

A previous study combining HPV test and cytology performed within three years before delivery supports our results that HPV infection increases the risk for pPROM (262). That study found an increased risk for pPROM in 829 women with HPV infection compared to 1,324 HPV negative women, also after for adjustment of previous treatment; aOR 2.07 (95% CI 1.03-4.14) (262).

In paper I was there a significant increased risk for PROM at term in the HPV infection group with HPV test but not in the cytology group (279). Previous studies on the association between HPV infection and PROM at term are scarce, but a small study found increased risk for PROM across all gestational ages (238).

The observation of increased neonatal mortality in women with HPV-infection has to our knowledge not been showed before.

Previous treatment

In paper I, women with previous treatment for CIN had greater risks of PTD, spontaneous PTD and pPROM when compared to the other study groups.

The risk increase for PTD after treatment was comparable to previous population-based register-studies from Denmark (1997-2005) (12) and

Finland (1998-2009) (13). Both compared deliveries in women with previous excisional treatment to women without treatment from a general population; aOR, 2.00 (95% CI 1.83-2.20) (12) and OR 1.61, (95% CI 1.47–1.75) (13).

The results from paper I confirms that the association between treatment and PTD cannot solely be explained by having HPV infection or CIN or background factors associated with CIN.

Paper I is as far as we know the first study to demonstrate that the risk for PROM at term is increased after treatment for CIN.

Furthermore, paper I showed that previous treatment confers increased risk for infectious complications like chorioamnionitis and neonatal sepsis. An increased risk for chorioamnionitis was suggested by a recent meta-analysis (116), but the four included studies comprised only 314 previously treated women (risk ratio (95% CI); 3.43 (1.36-8.64).
There was an increased risk for chorioamnionitis after treatment in PTDs, especially in deliveries that started with pPROM but also in those that did not. There was also a trend of increased risk for chorioamnionitis in term deliveries after treatment. The risk for neonatal sepsis was increased after treatment in PTDs.

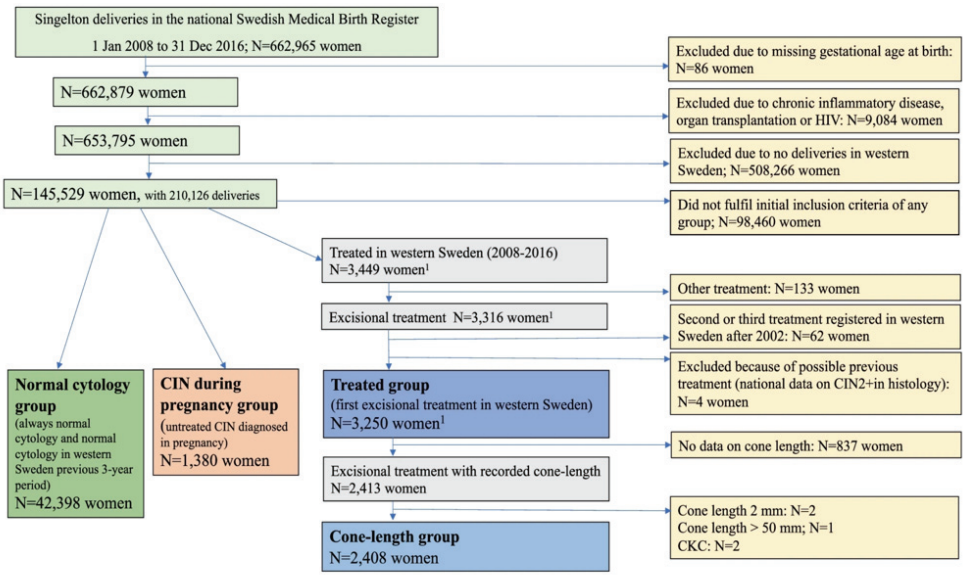
The results suggest that there might be an increased risk for ascending bacterial infection after treatment, leading to pPROM/PTD and infectious complications.

4.2 PAPER II

The aim was to investigate whether CIN during pregnancy and excisional treatment before pregnancy are associated with increased risk for PTD and other adverse obstetric and neonatal outcomes, as well as how risk estimates for excisional treatment vary with cone length at treatment measured on fresh specimens.

A total 46,870 women were included in any of the study groups, Figure 12. Of the treated women 2,408 with LEEP/Laser excision had recorded cone length.

Figure 12. Flowchart of the study population
(Adapted from Fig 1 in © 2022, Johanna Wiik et al, BMC Medicine, Springer Nature, Feb 22, 2022 (221) *https://doi.org/10.1186/s12916-022-02276-6, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)*



Flowchart of the study population. The different study groups were the normal cytology group, the CIN during pregnancy group, and the treated group with the subgroup cone-length group. Inclusion into the groups was based on the history of cervical cytology, cervical histology, previous treatment for CIN, and recorded cone-length at treatment. [1] *n*=158 women had a previous delivery in the CIN during the pregnancy group and were excluded from the treated group when compared to the CIN during the pregnancy group. CIN, cervical intraepithelial neoplasia; CKC; cold-knife conization; HIV, human immunodeficiency virus; *N*, number

Study groups differed regarding some background factors. Women in the CIN during pregnancy group were younger and less often pregnant

after assisted reproduction, women in the normal cytology less often nulli-parous and more often non-smokers and the treated group gave birth more frequently in 2014-2016 than the other study groups (221). The group with untreated CIN had a slightly increased risk for PTD compared to the normal cytology group, OR; 1.28 (95% CI 1.01-1.63), although non-significant after adjustments (221). When women with low-grade lesions and women with high-grade lesions in the CIN during pregnancy group were compared, they had a similar risk for PTD; aOR 0.90 (95% CI 0.55-1.47), p=0.67 (221).
Previous treatment was associated with increased risk for PTD, spontaneous PTD, pPROM and PROM at term compared to women with normal cytology, and also when compared to women with CIN (Table 7). The treated group also had increased risk of chorioamnionitis and neonatal sepsis, when compared to the normal cytology group, although results were non-significant after adjustments (221).

Table 7. Adverse obstetric and neonatal outcomes in the treated group, compared to the CIN during pregnancy group, unadjusted and adjusted multivariable logistic regression analyses.

Outcome	CIN during pregnancy group (*N*=1380)	Treated group (*N*=3092)[a]	Unadjusted analyses		Adjusted analyses[b]	
	n (%)	*n* (%)	OR (95% CI)	*p* value	aOR (95% CI)	*p* value
PTD <37 weeks	74 (5.4)	239 (7.7)	1.48 (1.13–1.93)	**0.004**	1.60 (1.21–2.12)	**0.001**
Early PTD <34 weeks	15 (1.1)	74 (2.4)	2.23 (1.28–3.90)	**0.005**	2.36 (1.32–4.23)	**0.004**
Very early PTD <28 weeks	6 (0.4)	15 (0.5)	1.12 (0.43–2.88)	0.82	0.90 (0.33–2.49)	0.84
Spontaneous PTD	49 (3.6)	189 (6.1)	1.77 (1.28–2.44)	**<0.001**	1.95 (1.40–2.72)	**<0.001**
pPROM	20 (1.4)	103 (3.3)	2.34 (1.45–3.80)	**0.001**	2.74 (1.66–4.51)	**<0.001**
PROM, delivery at ≥ 37 weeks	69 (5.3)	205 (7.2)	1.39 (1.05–1.84)	**0.022**	1.38 (1.03–1.85)	**0.030**
SGA[c]	35 (2.5)	63 (2.0)	0.80 (0.53–1.22)	0.30	0.88 (0.56–1.37)	0.57
Apgar score <7 at 5 min	16 (1.2)	37 (1.2)	1.03 (0.57–1.86)	0.92	1.00 (0.54–1.85)	1.00
Neonatal mortality	1 (0.1)	2 (0.1)	0.89 (0.08–9.85)	0.93	0.74 (0.06–9.07)	0.81
Intrauterine fetal death	2 (0.1)	7 (0.2)	1.56 (0.32–7.54)	0.58	1.62 (0.31–8.42)	0.57
Chorioamnionitis	5 (0.4)	14 (0.5)	1.25 (0.45–3.48)	0.67	1.35 (0.46–4.00)	0.58
Intrapartum fever	9 (0.7)	19 (0.6)	0.94 (0.43–2.09)	0.88	0.68 (0.29–1.57)	0.37
Neonatal sepsis	22 (1.6)	62 (2.0)	1.26 (0.77–2.06)	0.35	1.35 (0.81–2.25)	0.25

Statistically significant *p* values in bold type

[a]158 women also had a previous delivery in the CIN during pregnancy group and were excluded from the treated group in the analyses

[b]Analyses adjusted for: year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level and assisted reproduction

[c]Missing data: CIN during pregnancy group, *n*=0, treated group, *n*=2

aOR, adjusted odds ratio; *CI*, confidence interval; *CIN*, cervical intraepithelial neoplasia; *N*, number; *OR*, odds ratio; *pPROM*, preterm prelabor rupture of membranes; *PROM*, prelabor rupture of membranes; *PTD*, preterm delivery; *SGA*, small for gestational age

The risk was similar in cone lengths up to 10 mm and then risk increased with cone length for PTD, spontaneous PTD, pPROM, PROM at term and neonatal sepsis (221). When all cone lengths up to 10 mm were grouped into 10 mm (truncated to 10 mm), the risk for PTD increased with 15% with every millimeter above 10 mm (221).

Table 8. Associations between cone-length and adverse obstetric and neonatal outcomes, truncated analyses of ≤ 10mm, unadjusted and adjusted multivariable logistic regression analyses. *(Adapted from Table 6 in © 2022, Johanna Wiik et al, BMC Medicine, Springer Nature, Feb 22, 2022, (221) https://doi.org/10.1186/s12916-022-02276-6, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)*

	n	OR (95% CI)	*p*	aOR (95% CI) [a]	*p*
PTD, <37 weeks	187	1.16 (1.09–1.22)	**<0.001**	1.15 (1.09–1.23)	**<0.001**
Spontaneous PTD	149	1.16 (1.09–1.24)	**<0.001**	1.18 (1.10–1.25)	**<0.001**
pPROM	83	1.17 (1.08–1.26)	**<0.001**	1.18 (1.09–1.28)	**<0.000**
PROM, delivery at ≥ 37 weeks	162	1.11 (1.04–1.19)	**0.003**	1.14 (1.05–1.22)	**0.001**
Chorioamnionitis	9	1.06 (0.79–1.42)	0.69	0.99 (0.74–1.34)	0.97
Neonatal sepsis	45	1.16 (1.05–1.28)	**0.003**	1.19 (1.07–1.33)	**0.002**

Statistically significant *p* values in bold type

[a]Analyses adjusted for: year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level and assisted reproduction

aOR, adjusted odds ratio; *CI*, confidence interval; *N*, number; *OR*, odds ratio; *pPROM*, preterm prelabor rupture of membranes; *PROM*, prelabor rupture of membranes; *PTD*, preterm delivery

Small excisions, ≤ 10mm, were associated with increased risk for PTD and pPROM when compared to the normal cytology group, Table 9, and also when compared to women with CIN; PTD aOR 1.41, 95% CI 1.02–1.94, pPROM aOR 2.44, 95% CI 1.40–4.28, spontaneous PTD aOR 1.73, 95% CI 1.18–2.54 (221). Sub-group analyses, including only high-grade lesions in the treated and CIN during pregnancy group, showed similar results.

Table 9. Adverse obstetric and neonatal outcomes in cone-length groups up to 10 mm, compared to the normal cytology group, adjusted multivariable logistic regression analyses. *(Adapted from Table 5 in © 2022, Johanna Wiik et al, BMC Medicine, Springer Nature, Feb 22, 2022, (221) https://doi.org/10.1186/s12916-022-02276-6, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)*

	Normal cytology group *N*=42,398	Treated, cone-length <6 mm *N*=212			Treated, cone-length 6–9 mm *N*=1219			Treated, cone-length 10 mm *N*=374		
	N (%)	*N* (%)	aOR[a]	*p*	*N* (%)	aOR[a]	*p*	*N* (%)	aOR[a]	*p*
PTD, <37 weeks	1794 (4.2)	16 (7.5)	1.72 (1.03–2.89)	**0.038**	77 (6.3)	1.47 (1.16–1.87)	**0.002**	26 (7.0)	1.57 (1.05–2.36)	**0.028**
Spontaneous PTD	1264 (3.0)	12 (5.7)	1.83 (1.02–3.31)	**0.044**	61 (5.0)	1.68 (1.29–2.20)	**<0.001**	20 (5.3)	1.75 (1.11–2.76)	**0.017**
pPROM	479 (1.1)	7 (3.3)	2.55 (1.19–5.49)	**0.016**	36 (3.0)	2.36 (1.66–3.34)	**<0.001**	10 (2.7)	2.07 (1.09–3.92)	**0.026**
PROM, delivery at ≥ 37 weeks	2084 (5.1)	11 (5.6)	0.93 (0.50–1.71)	0.81	79 (6.9)	1.23 (0.97–1.55)	0.09	23 (6.6)	1.14 (0.74–1.75)	0.55
Chorioamnionitis	97 (0.2)	0	–	–	4 (0.3)	1.11 (0.40–3.05)	0.84	3 (0.8)	2.73 (0.85–8.76)	0.09
Neonatal sepsis	591 (1.4)	3 (1.4)	0.79 (0.25–2.48)	0.69	22 (1.8)	1.09 (0.71–1.68)	0.70	6 (1.6)	0.95 (0.42–2.14)	0.90

Statistically significant *p* values in bold type

[a]Analyses adjusted for: year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level, and assisted reproduction

aOR, adjusted odds ratio; *CI*, confidence interval; *mm*, millimeter; *N*, number; *OR*, odds ratio; *pPROM*, preterm prelabor rupture of membranes; *PROM*, prelabor rupture of membranes; *PTD*, preterm delivery.

Comments Paper II

Treatment in paper I was defined as CIN3 in histology. In paper II we had verified treatment-data but in a smaller cohort. Treatments after 2008 were included. Colposcopists today aim to achieve removal of the CIN at treatment in order to prevent the development of cancer but try to, especially in women of reproductive age, preserve as much healthy tissue as possible. For the most common clinical situation, i.e. TZ 1, a cone-length of 6–9 mm is recommended, in order to include the cervical crypts (107). The mean cone-length in this study, 9 mm, well- reflects these Swedish guidelines.

The findings based on verified treatment data in paper II support the results from paper I. Excisional treatment was associated with increased risk for PTD, pPROM, spontaneous PTD and PROM at term compared to women with normal cytology. Risk estimates were similar as in paper I. The risk for chorioamnionitis and neonatal sepsis was also increased in treated women in paper II, although not significant after the thorough adjustments in this smaller cohort, and we suspect this might be due to a lack of power in this study. The neonatal mortality was too seldom to yield conclusive results in this study. Risk estimates for spontaneous PTD after treatment compared to women with normal cytology were comparable to a recent Dutch study (2005–2015); aOR 2.07 (95% CI 1.85–2.33) (233).

In paper II the risk for PTD and pPROM was also increased after treatment compared to women with CIN, verified with a biopsy during pregnancy. The Dutch study also found increased risk for spontaneous PTD in treated women compared to women with CIN; aOR 1.51 (95% CI 1.29-1.76). The aOR 1.51 in that study was lower than in paper II (1.95), although within the CI of our study (1.40-2.72).
Untreated CIN in that study (n=5,940) included also CIN diagnosed outside pregnancy and was in comparison to women with normal cytology associated with increased risk of spontaneous PTD; aOR 1.38 (95% CI 1.19-1.60) (233). We found increased risk for PTD in the CIN during pregnancy group compared to women with normal cytology, although not significant in the adjusted analyses. The group included only 1,380 women and thus the analyses had limited power.

Cone length

Novel findings are that the risk was increased but similar in treatments 3-10 mm, and then increasing with cone length for PTD, pPROM, PROM at term and neonatal sepsis. There were only 9 women with chorioamnionitis and recorded cone lengths, limiting our possibility to assess the effect of cone length on the risk of chorioamnionitis.

Minor treatments (≤10mm) increased the risk for PTD and pPROM compared to women with CIN during pregnancy and also if restricted to HSIL lesions in the compared groups.

Our results are supported by a previous study from Denmark that found an increased risk for PTD after small treatments (cone length 10 mm), aOR 1.46 (95% CI 1.11–1.92) compared to untreated women (217). Opposed to our results, no increased risk for PTD was found in women with a minor excision (cone-length <10 mm) compared to women with a punch biopsy taken before delivery, in an English case-control study (214).

The Danish study suggested a 6% increase in risk for PTD per each additional millimeter of tissue excised (217). In paper II there was a 10% increase in risk for PTD per each extra millimeter cone length, or 15% per each extra millimeter cone length above 10 mm.

4.3 PAPER III

The aim was to investigate whether HPV infection during pregnancy, at mid-pregnancy or at delivery, is associated with PTD, pPROM, PROM and chorioamnionitis.

Of 954 women with singleton pregnancies that provided urine for HPV testing at mid-pregnancy 950 had valid HPV test and known obstetric outcomes and were included in the study. Of these 753 yielded valid HPV results at delivery.

At mid-pregnancy 40% were positive for any HPV and 24% for hrHPV. Women positive for hrHPV were more frequently singles and nulli-parous than those negative for hrHPV.

The overall numbers and frequency of adverse outcomes was low; PTD (n=23, 2.4%), pPROM (n=9, 0.9%), chorioamnionitis (n=6, 0.6%), antibiotics due to suspected clinical chorioamnionitis (n=19, 2%), except for PROM at term; (n=219, 25%).

Women positive for hrHPV (n=231) at mid-pregnancy had higher frequency of PTD than women negative for hrHPV (n=719) (3.5%. vs 2.1%), although this was not a significant difference, OR and aOR are presented in paper III.
Sub-analyses in 518 nulli-parous women found a borderline-significant increased risk in women positive for hrHPV.
Women positive for hrHPV-16 at mid-pregnancy also had a higher frequency of PTD than women negative for hrHPV-16 (3.4% vs 2.4%) and the frequency of PTD was higher in women with multiple HPV infection compared to no hrHPV (4.3% vs 2.1%), although comparisons were not significant.

No association between persistence of hrHPV and PTD was found.

Eight of the hrHPV negative women (1.1%) and one of the hrHPV positive women (0.4%) at mid-pregnancy had pPROM.

There were no significant associations between presence of hrHPV, any HPV or HPV-16 at mid-pregnancy and any of the examined outcomes.
At delivery, any HPV was associated with PROM at term, but with a lower risk of PROM, OR is presented in paper III.

Of the 23 women with PTD nine had no urine collected at delivery and 11 no placenta sampled at delivery. There was no detection of HPV-DNA in any of the placentas collected from women with PTD, pPROM or chorioamnionitis.

Comments Paper III

Paper III found no statistic significant association between hrHPV infection, measured in urine at mid-pregnancy and at delivery, and PTD, pPROM, PROM or chorioamnionitis.

However, the frequency of PTD was higher in hrHPV-positive than in hrHPV-negative women as well as in women positive for hrHPV16 compared to those negative for hrHPV16 at mid-pregnancy. There was a tendency of increased risk for PTD in hrHPV-positive women in the nulliparous group. Although hrHPV positive women at mid-pregnancy had no significant risk for PTD the adjusted odds ratio 1.74 was larger than the aOR found in the comparisons of women with HPV infection and normal cytology in paper I (aOR 1.21).

To our knowledge, only one prospective study of similar size has examined if genital HPV infection and placenta HPV infection is associated with PTD (258). That study found increased risk in women with vaginal hrHPV 16/18 infection in first trimester; aOR 2.55 (95% CI 1.07-6.04), and in women with persistence of hrHPV16/18; aOR 3.72 (95% CI 1.47-9.39), when compared to HPV negative women. Any 36 genotypes of HPV in placenta were also associated with PTD (258). Genital detection of other types of hRHPV, low-risk-HPV or any-HPV of 36 genotypes, were not significantly associated with increased risk for PTD (258).

When we compared women with any HPV (28 genotypes) to HPV negative women in paper III the frequency of PTD was similar in women negative and positive for any HPV, in line with results from the study by Niyibizi et al (258).

In paper III, none of the 17 women that had hrHPV-18 at mid-pregnancy had PTD. If we use our data in paper III at mid-pregnancy to study hrHPV16/18 compared to HPV negative women, like Niyibizi et al did, we get an OR of 1.21 (95% CI 0.27-5.49).
We combined the data of women positive for hrHPV16/18 and women negative for HPV in paper III with data from the study by Niyibizi et al (258) to see if the evidence for hrHPV16/18 as a risk factor for PTD increased. The evidence rather decreased and there was no longer a significant association; OR (Mantel Haenszel test) 1.97 (95% CI 0.96-4.04), p=0.07.

In paper III, the frequency of pPROM was higher in women negative for hrHPV, although not significant, and only 9 women had pPROM limiting the conclusions that can be drawn. One previous smaller prospective study of vaginal HPV detection in first trimester in 104 women in India found a significant increased risk for pPROM in HPV-positive women (pPROM 14.6%) compared to HPV- negative women (3.2%), p=0.026 (259).

For PROM at term the risk-estimates were not increased but rather the opposite and women with any 28 HPV at delivery had significantly lower risk for PROM. This result was not expected and we have no biological explanation for this.

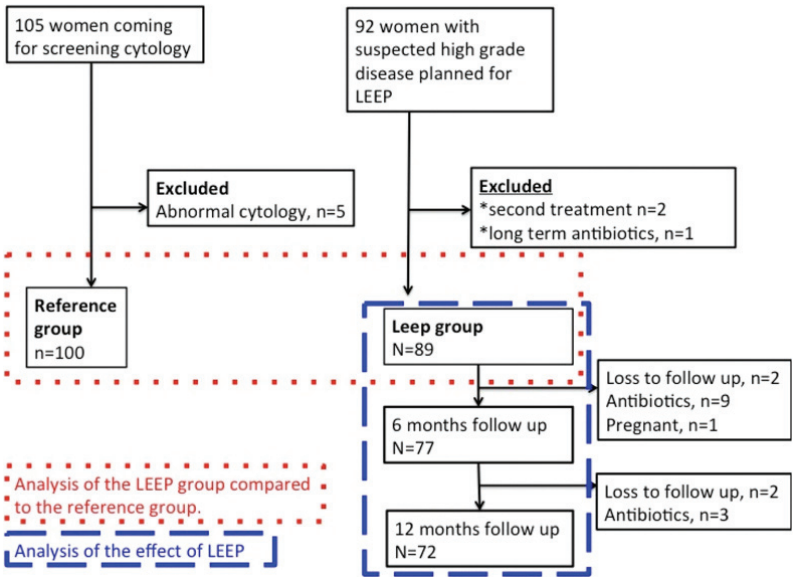
The definition of PROM in paper III was broad (membrane rupture at least one hour before active delivery) since we did not have information regarding time of start of contractions. This definition limits our possibility to compare the results to other studies. A Korean study that defined PROM as membrane rupture prior to labor in 311 women reported increased risk in hrHPV-positive women for PROM aOR 2.32 (95% CI 1.08-4.98) (238).

4.4 PAPER IV

The aim was to compare the cervical microbiota before and after LEEP and to assess whether cone length at treatment and HPV persistence after treatment affect the bacterial composition post-treatment. Further, this study aimed to compare the microbiota to references with normal cervical cytology

The LEEP group was constructed of 89 women and the reference group of 100 women, Figure 13.

Figure 13. Flowchart of formation of the study groups and follow up (Adapted from Fig 1 © 2019, Johanna Wiik et al, BMC Women's Health, Springer Nature, Feb 6, 2019, (277) https://doi.org/10.1186/s12905-019-0727-0 CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)



Women in the LEEP group were younger, more likely to use contraception, smoke and being single (277).

Compared to before treatment, there was a significant reduction in the mean number of non-*Lactobacillus* bacterial species six and 12 months after LEEP (277). There was also a tendency towards an increase in detection of *Lactobacillus* at 12 months (277).

More women had cleared than aquired *Ureaplasma parvum* and *Streptococcus spp* at the control at six months after LEEP, however there were no significant changes in individual bacteria species after 12 months (277), Table 10.

Table 10. The cervical microbiota six and 12 months after LEEP compared to before LEEP. (Adapted from Table 3, © 2019, Johanna Wiik et al, BMC Women´s Health, Springer Nature, Feb 6, 2019, (277) https://doi.org/10.1186/s12905-019-0727-0, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)

	6 months after LEEP (*N*=77)			12 months after LEEP (*N*=72)		
Bacteria	Pos->Neg n	Neg.->Pos n	p[1]	Pos->Neg n	Neg->Pos n	p[1]
Bacteroides spp	5	1	0.22	5	3	0.73
Escherichia.coli	2	1	1,00	3	1	0.63
Gardnerella vaginalis	8	8	1.00	7	7	1.00
Mycoplasma hominis	5	1	0.22	5	2	0.45
Streptococcus spp	8	1	0.04	8	3	0.23
Ureaplasma parvum	15	5	0.04	10	5	0.30
Ureaplasma urealyticum	5	1	0.22	5	3	0.73
Lactobacillus spp	12	17	0.46	8	18	0.08

[1]Mc Nemar test

Abbreviations; *LEEP* Loop Electrosurgical Excisional Procedure, *N* numbers, *neg* negative, *pos* positive

There was no association between persistence of HPV at follow up or cone length at treatment and change in cervical microbiota (277). More types of bacterial species were detected in women planned for LEEP than in the reference group and *Bacteroides* spp., *Ureaplasma parvum, Gardnerella vaginalis and Mycoplasma hominis* were significantly more often detected in women in the LEEP group (277).

Table 11. The cervical microbiota in the LEEP group and the Reference group (Adapted from Table 5, © 2019, Johanna Wiik et al, BMC Women´s Health, Springer Nature, Feb 6, 2019, (277) https://doi.org/10.1186/s12905-019-0727-0, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)

	LEEP group *N*=89		Reference group *N*=100		Unadjusted[1]			Adjusted[2]		
Bacteria	n	%	n	%	OR	CI	p	OR	CI	p
Bacteroides spp	7	8	0	0	∞		0.005	∞		0.008
Chlamydia trachomatis	3	3	1	1	3.5	0.4–33.8	0.34	2.3	0.2–26.0	0.52
Escherichia.coli	4	4	9	9	0.5	0.1–1.6	0.26	0.3	0.1–1.4	0.12
Gardnerella vaginalis	28	32	9	9	4.6	2.1–10.5	<.001	3.5	1.4–9.0	0.009
Mycoplasma hominis	17	19	3	3	7.6	2.2–27.0	0.001	2.4	0.6–9.5	0.21
Streptococcus spp	12	14	10	10	1.4	0.6–3.4	0.50	1.9	0.7–5.5	0.24
Ureaplasma parvum	41	46	16	16	4.5	2.3–8.8	<.001	3.9	1.6–9.3	0.002
Ureaplasma urealyticum	9	10	3	3	3.6	1.0–13.9	0.07	2.2	0.5–10.5	0.32
Lactobacillus spp	32	36	39	39	0.9	0.5–1.6	0.76	0.8	0.4–1.7	0.58
any non-*Lactobacillus*	59	66	41	41	2.8	1.6–5.1	0.001	2.2	1.1–4.5	0.03

[1]Fisher's exact test

[2]Logistic regression adjusted for Marital status, Hormonal Contraceptive Use, Smoking and Age. 188 cases analysed, 1 missing case in the reference group because of missing data on smoking

Abbreviations; *CI* Confidence Interval, *LEEP* Loop Electrosurgical Excisional Procedure, *N* numbers, *OR* Odds Ratio

Comments Paper IV

We found in paper IV a more diverse cervical microbiota before compared to after LEEP-treatment, measured with culture and PCR. A previous study, including only 26 non-pregnant women, found decreased cervical microbial diversity as well as an increase in *Lactobacillus spp* after excisional treatment (224). That study was published after recruitment and analyses of data in paper IV. The follow up in that study was after 3 months and the microbiota was measured by16sRNA NGS techniques (224). Recently a study of non-pregnant premenopausal women (n=103) found no change in vaginal microbiota composition 6 months after treatment (87). Neither were there any evidence that larger cone lengths/more extensive excisions affected the vaginal microbiota and this is comparable to our results (87).
These three studies suggest that increased cervical dysbiosis after treatment is not the underlying mechanism for the increased risk for PTD after treatment. However, all these studies were performed in non-pregnant cohorts and it is unknown if results are different during pregnancy and parturition.

Women planned for LEEP due to CIN had a more diverse cervical microbiota than women with normal cytology smear in paper IV both before and after treatment. These comparisons were however limited by the fact that the groups differed in many background factors. The results are however supported by the findings by Mitra et al who found high-diversity vaginal microbiota composition, an increased prevalence of *Lactobacillus* sp. depletion and higher levels of proinflammatory cytokines in women with CIN before and after treatment compared to normal controls (87). Other studies of the vaginal microbiota using NGS techniques have also suggested that the microbiota is important for acquisition and persistence of HPV as well as for development of CIN and cervical cancer. An increased bacterial diversity and reduced levels of *Lactobacillus spp* seems to confer increased risks (56-58, 61).
The association between CIN/HPV infection and PTD, pPROM might hypothetically be related to a concomitant dysbiotic cervical microbiota. However, the microbiota might be different during pregnancy and parturition and the suggested association between cervical microbiota and PTD needs to be further explained.

5 DISCUSSION

First, a general discussion of the findings in this thesis will be presented. Thereafter, methodological considerations, strengths and limitations of the included studies will be discussed.

5.1.1 EXCISIONAL TREATMENT, RISK OF PTD, PPROM, AND POSSIBLE MECHANISMS

The increased risk of PTD after treatment is an effect of the excision
We demonstrated in paper I that the risk for PTD and pPROM increased after treatment, defined as previous CIN3, not only in comparison to women with normal cytology but also compared to women with HPV infection in conjunction with pregnancy and in women that later developed high grade CIN.

Figure 14. ***Risk of preterm delivery and spontaneous preterm delivery—adjusted logistic regression.*** *Preterm delivery (2a); spontaneous preterm delivery (2b). Women with HPV infection had an increased risk of preterm delivery and spontaneous preterm delivery; treatment increased the risk further. aORs are given, with 95% confidence intervals in parentheses. Analyses adjusted for year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level, and assisted reproduction. aOR, adjusted odds ratio; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.*
(Adapted from Fig 2 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2022, (279) https://doi.org/10.1371/journal.pmed.1003641.g002, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)

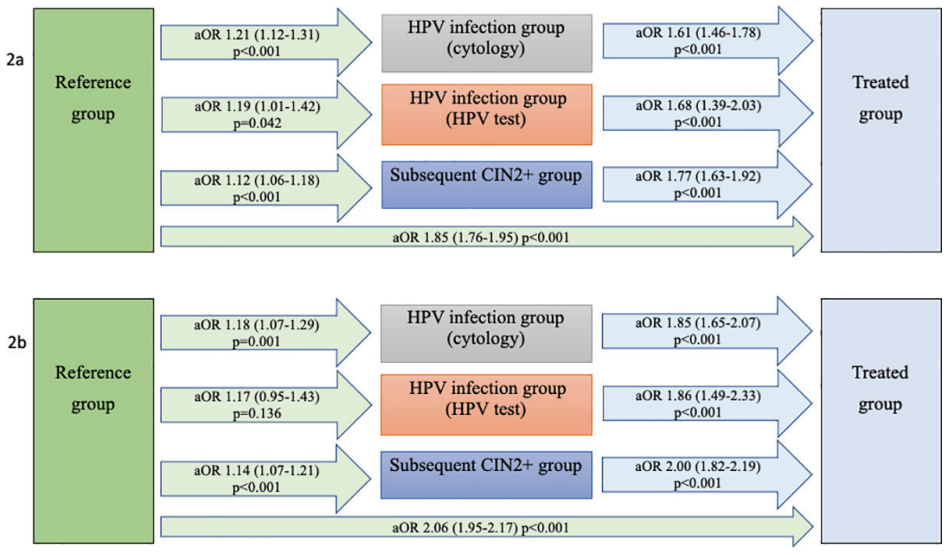


Figure 15. ***Risk of pPROM—adjusted logistic regression.*** *Women with HPV infection had an increased risk of pPROM; treatment increased the risk further. aORs are given, with 95% confidence intervals in parentheses.*
Analyses adjusted for year of delivery, maternal age, parity, BMI, marital status, country of birth, infant's sex, smoking, income, education level, and assisted reproduction. aOR, adjusted odds ratio; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; pPROM, preterm prelabor rupture of membranes.(Adapted from Fig 3 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2022 (279),
https://doi.org/10.1371/journal.pmed.1003641.g003
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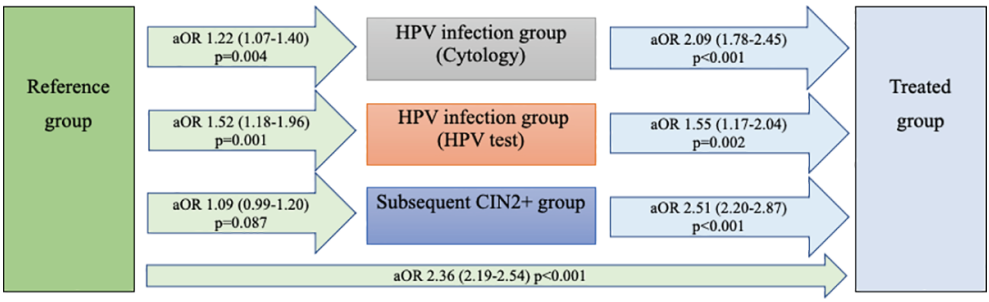


In paper II women with registered excisional treatments were compared to women with CIN during pregnancy and to women with a history of normal cytology and also in these comparisons had previously treated women an increased risk for PTD and pPROM. The risk for PTD and pPROM in treated women increased with cone-length at treatment.

Also, the paired analyses in paper I showed an increased risk for PTD and pPROM after treatment.

Several previous studies have reported increased risk for PTD and pPROM after treatment but it has been questioned if this risk could be due to underlying risk factors in women with CIN, environmental factors, genetic factors or the HPV infection itself.
The comparisons in paper I and II confirm that the increased risk for PTD and pPROM after treatment cannot be explained solely by confounding factors in women with CIN or by a present HPV infection.

Larger excisions increase the risk

In paper II, we found that the risk for PTD and pPROM increased with cone length. The risk for PTD was increased for all treatments, in small excisions up to 10 mm with about 50% and thereafter with 15% with every extra mm.

It has earlier been questioned if small excisions confer increased risk for PTD compared to having untreated CIN. In our cohort were we able to compare a large number of treatments up to 10 mm to women with CIN during pregnancy. We conclude, based on our results in paper II, that all excisional treatments confer increased risk for PTD i.e. there is no safe cone length below which there is no increased risk (221).

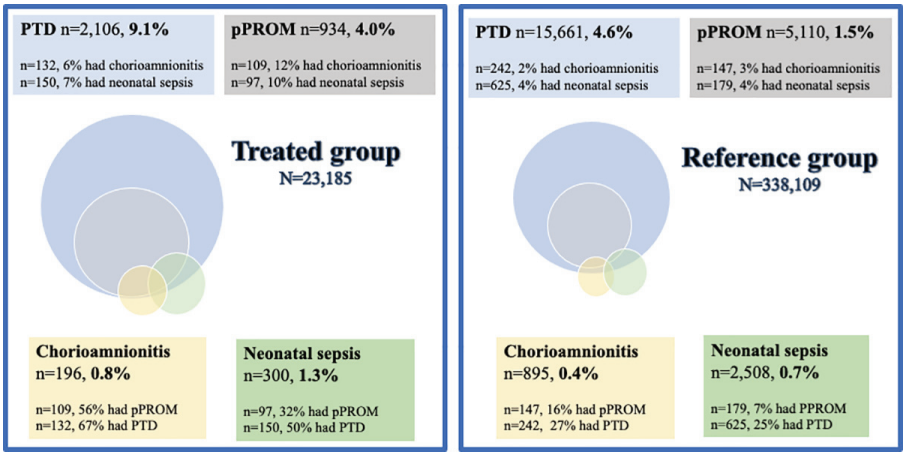
As previously suggested the increased risk seen with larger excisions might hypothetically be due to a mechanical weakness; a shorter cervix and/or cicatrization. It might also be due to an increased risk for ascending infection.

Infectious complications

We found that women with previous treatment for CIN had an increased risk of chorioamnionitis and neonatal sepsis in paper I. This might indicate that there is an increased risk for ascending bacterial infection after treatment causing both PTD, pPROM, chorioamnionitis and neonatal sepsis.

Infection is an important cause of PTD and pPROM while membrane rupture - pPROM and PROM - also increases the risk for infectious complications. However, the increased risk for chorioamnionitis in the treated group could not only be explained by pPROM since there was also an increased risk for chorioamnionitis in other PTDs.

Figure 16. Venn diagram illustrating the relationship between preterm delivery (PTD), preterm prelabor rupture of membranes (pPROM), and infectious complications in the treated group and the reference group. The treated group had PTD, pPROM, and infectious complications more frequently. PTD and deliveries after pPROM were more often complicated by infections in the treated group than in the reference group. The deliveries with infectious complications were more frequently preterm and complicated by pPROM in the treated group than in the reference group.
(Adapted from Fig 4 in © 2021, Johanna Wiik et al, PLOS Medicine, May 10, 2022, (279). https://doi.org/10.1371/journal.pmed.1003641.g004, CC BY 4.0/http://creativecommons.org/licenses/by/4.0/)



We found in paper II that the risk for neonatal sepsis increased with cone length. While this association was not seen for chorioamnionitis, there were only nine women with chorioamnionitis with reported cone length in the study so we could not draw any firm conclusions from this result.

The risk for ascending infection after treatment, and thus risk for PTD, pPROM and infectious complications, might increase with size of the excisions possibly due to a shorter way for bacteria to travel between the cervix outer and inner os or due to an impaired immunological response after destruction of cervical tissue. It might also be due to a combination of these suggested factors or unknown factors.

The cervix function as a barrier against ascending infections through different mechanisms. The cervical columnar epithelium produces mucus with important antibacterial properties and the mucus also protects the cervical epithelium against mechanical and chemical injury (140). It is unknown how the mucus is affected by excisional treatment but deep excisions (longer cone lengths) will remove more columnar epithelium and possibly result in an impaired mucus production.

The cervical epithelium is important for the cervical barrier defense.

In an animal study of pregnant mice was damage of the cervical epithelium followed by the ascendence of *Ureaplasma spp* bacteria – which in turn led to an intrauterine inflammatory response (280). The tight junctions between cervical epithelial cells are part of the cervical barrier defense against infections. The cervical epithelium regulates expression of tight junction proteins during pregnancy in a temporal manner. It has been suggested that impairment of the epithelial barrier, possibly due to alterations caused by inflammation, could lead to increased permeability/access to underlying cervical tissue, for pathogens, toxins, inflammatory agents and/or water (281). Loss of the tight junctions might therefore lead to cervical remodeling and PTD. Recruitment and activation of inflammatory cells by secretion of cytokines and chemokines by cervical cells are also important defense mechanisms. Previous excisional treatment could hypothetically affect all these mechanisms.

An increased risk for ascending infection after treatment might be multifactorial and genetic or other environmental factors might increase the risk in a synergistic way.

Excision, modulating the immunological milieu, might also lead to a change of the composition of cervical microbiota, another possible explanation for ascending infection.
We did not find an increased diversity of bacteria or less *Lactobacilli* after treatment compared to before treatment in non-pregnant women, but rather a decrease of non-*Lactobacilli* species in paper IV. This contradicts the hypothesis that treatment leads to dysbiosis. Neither Zhang (224) or Mitra (87) found more dysbiosis after treatment compared to before. Neither was there any association between cone length at treatment and change of microbiota (87, 277).

5.1.2 CIN AND HPV INFECTION, RISK OF PTD, PPROM, AND POSSIBLE MECHANISMS.

It has previously been suggested that women with untreated CIN have an increased risk for PTD, but studies have been scarce. If this association is due to underlying risk factors in women with CIN or due to a persistent HPV infection is unknown.

We found an increased risk for PTD in women with CIN during pregnancy in paper II, that was no longer significant in the adjusted analyses, but the group was small (1,380 women). A recent Dutch study found an increased risk for PTD in women with untreated CIN (n=5,940), however that study included also CIN before and after pregnancy (233).
We defined a group of women that were diagnosed with CIN after delivery in paper I to study the risk in a group of women that have vulnerability for persistent hrHPV infection and high-grade CIN. We found an increased risk for PTD in women that were diagnosed with CIN after treatment but when dividing this group at the median in time to diagnosis (3 years) only the group of women with a diagnosis of CIN the first 3 years after delivery had a higher risk for PTD compared to women with normal cytology. The increased risk in this early diagnosis group might be explained by an already existing HPV infection during the studied pregnancy.

We found in paper I that HPV infection in conjunction with pregnancy was associated with an increased risk of PTD and spontaneous PTD and pPROM. HPV infection was defined by either abnormal cytology or a positive HPV test (mainly hrHPV) 6 months before conception until delivery and results were similar for these two exposure groups.

We found no significant association between HPV infection during pregnancy and PTD or pPROM in paper III but this study was limited by the number of participants, and especially by the low number of women with PTD in the cohort. However, the frequency of PTD was higher in women positive for hrHPV infection at mid-pregnancy than in women negative for hrHPV and sub-analyses in only nulli-parous women were borderline significant, p=0.050. For any HPV (28

genotypes) the frequency of PTD was similar in HPV-positive and HPV-negative women. This suggest that only hrHPVs infections might confer increased risk for PTD.
Another recent prospective study from Canada (n=899) found an increased risk for PTD in women with hrHPV 16 and 18 – the most oncogenic types, but not for other HPV genotypes (258). In paper III women with hrHPV 16 compared to no hrHPV 16 infection had a higher frequency of PTD but there were only a few cases and no firm conclusions can be drawn. None of the women with PTD were positive for hrHPV 18 in paper III.
The hrHPV 16 genotype persists longer compared to many other genotypes of HPV, is associated with the majority of cervical cancer cases (about 50%) and leads to a faster progression to cancer (15, 37) while HPV 18 has been associated with about 20% of cervical cancer lesions (24).
The HPV interferes with immune signaling pathways and HPV infection has a direct effect on the expression of pro-inflammatory genes (32). Previous studies suggest that this complex interaction between HPVs and different inflammatory cytokines differs for HPV genotypes and also depends on expression of oncogenes (32).
The hrHPV associated interference with the expression of TLRs differ between genotypes HPV 16 and 18 (37). Hypothetically could the hrHPV16 specific immunomodulation affect the risk for PTD, as opposed to some of the other genotypes.
Different HPV genotypes have also been associated with different bacterial strains and bacterial combinations (282), and HPV associated changes in the microbiota might hypothetically cause an immune-inflammatory process resulting in PTD (258).
We suggest future studies that especially focus on the hrHPV16 genotype to further test these hypotheses.

There are theories that HPV infection might cause PTD by dysfunctional placentation (242). The Canadian study by Niyibizi et al reported increased risk of PTD if HPV was detected in placenta swabs or biopsies (258). We did not find HPV in any of the examined placentas in paper III. However, we only had results for 12 of the 23 placentas in PTD cases, limiting our ability to draw conclusions from this finding.

Apart from PTD, placental dysfunction could lead to IUGR of the fetus or preeclampsia. We had no information about IUGR but we found no association between HPV infection and children born as SGA in paper I, arguing against this hypothesis of placental dysfunction.

Another hypothesis is that HPV infection might increase the risk for ascending bacterial infection to the uterus. We found no increased risk for chorioamnionitis in women with HPV infection during pregnancy in paper I or in paper III or in women with CIN during pregnancy in paper II. However, chorioamnionitis is a rare event and there were only 5 women in the CIN during pregnancy group in paper II and only 2 women with hrHPV infection in paper III that had chorioamnionitis, limiting the possibility to draw conclusions from these two studies. Paper I was larger and the frequency of chorioamnionitis was similar in the reference group (0.3%) as in the HPV infection (cytology) group (0.4%) and the HPV infection (HPVtest) group (0.4%)(279).
We did not find an increased risk for neonatal sepsis in women with HPV infection in paper I or in women with CIN during pregnancy in paper II. These results do not support the hypothesis that HPV infection itself increases the risk for ascending bacterial infections, however one should bear in mind that intrauterine infections are often subclinical and often manifest as PTL or pPROM instead of chorioamnionitis and neonatal sepsis.
A paper including 100 pregnancies complicated by pPROM studied the association between cervical HPV infection (21 genotypes) at admission and microbial invasion of the amniotic cavity (MIAC) and intra-amniotic inflammation (IAI) in women with pPROM. Women with HPV infection (24%) had similar rates as women without HPV regarding MIAC (21% vs 22%) or IAI (21% vs 18%) or levels of the inflammatory marker IL-6 in amniotic fluid (283), suggesting that cervical HPV infection does not increase the risk for ascending infection. They did however find a higher rate of MIAC and IAI as well as higher amnionic fluid IL-6 levels in 6 women with more than one HPV type (283), suggesting that women with multiple HPV infection might be at higher risk for MIAC and thereby PTD.
We found in paper III that women with multiple HPV infection had a non-significant tendency of increased risk for spontaneous PTD. Having more than one HPV genotype has also been associated with persistence of HPV/dysplasia (284). Multiple HPV infection has

previously also been associated with increased vaginal microbial diversity (285).
We found in paper IV that women with CIN had a more diverse microbiota compared to women with normal cytology, both before and after treatment, although the study included also postmenopausal women. However similar results were published by Mitra et al for premenopausal women (87). Several other studies found that persistent hrHPV infection and CIN are associated with dysbiosis, BV and CST IV (58, 76, 286). CST IV in pregnant women has been associated with both a short cervix, measured by TVU, and with PTD (287). Dysbiosis has been associated with inflammation (81, 82) and also with increased risk for PTD (70, 138).
Ureaplasma spp and *Gardnerella vaginalis* have both been associated with PTD (280) and were more often detected in the LEEP group than the reference group in paper IV both before and after treatment.
The pathomechanism linking hrHPV infection and CIN to PTD might involve changes in the cervicovaginal microbiota, although this was not studied in this thesis.

When summarizing our results, the register-based study (paper I) suggested that HPV infection confers an increased risk for PTD and pPROM. This could however not be confirmed in our prospective clinical study (paper III), but this study was underpowered. However, the comparison of women with hrHPV to those without hrHPV at mid-pregnancy in paper III gave an aOR 1.74, which was higher than in paper I (aOR 1.21) - and thus compatible with the results in paper I.

The other register-based study (paper II) found an increased risk for PTD in women with CIN, although not significant in the adjusted analyses.

Further prospective clinical studies are needed to assess if HPV infection and CIN increases the risk for PTD and pPROM.

5.1.3 EXCISIONAL TREATMENT, HPV INFECTION AND THE RISK OF NEONATAL MORTALITY

Neonatal mortality refers to deaths of liveborn neonates 1-28 days after birth.
Previous studies have suggested an increased risk for neonatal mortality after treatment of CIN, which was confirmed in paper I.

Since PTD is the main reason for neonatal mortality and treatment is associated with PTD this was expected. When comparisons were adjusted for PTD the difference disappeared, indicating that the risk increase was mediated by gestational age.

The main cause of spontaneous PTD is infection, and infection is also an important cause of neonatal mortality.
Of the neonatal mortality cases in the treated group, 14.9% contracted chorioamnionitis and 25.5% neonatal sepsis in paper I. Only 4.1% of the infants with neonatal mortality in the reference group contracted chorioamnionitis and 6.4% neonatal sepsis. The risk for neonatal mortality in treated women compared to the references was, after stratification for infectious complications, increased in treated women but only in the subgroup with infectious complications.

This indicates that infectious complications and PTD might link previous treatment for CIN to neonatal mortality.

Neonatal mortality was also increased in women with HPV infection in paper I, and also after adjustment of PTD. Due to the observational character of paper I, no conclusions regarding possible underlying pathomechanisms were possible.

5.1.4 EXCISIONAL TREATMENT, HPV INFECTION AND THE RISK OF PROM

In paper I we found that previous treatment was associated with an increased risk for PROM at term and in paper II PROM at term increased with excised cone length.
Further, HPV infection (HPV test) was associated with PROM at term in paper I, but the comparison with the reference group was not significant for the HPV infection (cytology) group.
HPV status was not associated with an increased risk for PROM at term in paper III, instead the results pointed towards lower risk of PROM in women with HPV infection.
The most common definition of PROM is membrane rupture at least one hour prior to contractions.
In paper I and paper II PROM at term was based on GA and ICD-10-codes (O756A, O756B, O756X). These codes are used for membrane rupture without contractions that later is followed by spontaneous contractions or induction of labor, or sometimes acute caesarean. No exact time between membrane rupture and contractions is specified but these ICD codes are mainly used when membrane rupture leads to clinical check-up. In paper I, the prevalence of PROM at term in HPV positive (HPV test) women was 10% and in the reference group 7%. The frequency of PROM at term has previously been reported to be 12 % in Sweden (187). In paper III a broader definition was used leading to a prevalence of 25% in the clinical cohort study. PROM was in paper III defined as start of active delivery at least one hour after membrane rupture. A delivery was considered active if at least 2 of 3 criteria were fulfilled; spontaneous membrane rupture, regular contractions (>2/10 minutes) and cervix effaced and dilated 4cm.

Infection and/or cell senescence are possible mechanisms for membrane damage resulting in PROM, but the exact pathomechanisms behind PROM at term have not yet been established.
Our results suggest that the risk for PROM at term increases after excisional treatment. Hypothetically could this be due to ascending infection or due to a more rapid process of cervical ripening, effacement and dilation after treatment.
More research is needed to establish whether HPV infection during pregnancy increases the risk of PROM.

5.2 STRENGTHS AND LIMITATIONS AND METHODOLOGICAL CONSIDERATIONS

This section will address methodological considerations for register based cohort studies (paper I and II) and clinical cohort studies (paper III and IV).

Register-based studies, methodological considerations.
Population-based register-studies have the possibility to include many study participants and minimize loss of follow-up. Large sample size increases precision and enables studies of rare exposures and outcomes (288). The population-based design prevents self-selection bias. The MBR used in paper I and II is mandatory and includes almost all Swedish women giving birth. Participation in the quality register NKCx is also high and the Swedish Cancer Register is mandatory. The data used from the NKCx; cytology, histology, HPV tests and cone lengths were reported directly to the register from care-givers/laboratories and were not influenced by earlier or later screening-data. The antenatal care data in MBR is collected before knowledge of the pregnancy outcome. Therefore, even if we collected the data from the registers retrospectively one could argue that the studies are prospective since data was reported into the registers prospectively.
Population based register studies are often considered of good external validity with generalizable results. However, to rely on data in registers, entered by others, limits the researchers control of the variables, for example outcomes. Only data available from the register are available for studying a certain research question. If ICD codes are studied - then data collection is only as good as ICD coding is in clinical routine. Misclassification causes information bias. If misclassification is unrelated to the studied exposure, it probably occurs for participants in all comparison groups, and this will then tend to attenuate a true association. Another limitation in register-based studies is missing data. Last but not least, there is always a risk for residual confounding in register based cohort studies (288).

Clinical cohort studies, methodological considerations.
In clinical cohort studies, it is possible to longitudinally follow study participants and to study causal effects.
The researcher has the possibility to collect and define the precise variables of interest and to control the collection of data in a better way than in register-based studies.

However clinical cohort studies are more expensive, thus often smaller and could be limited by self-selection of participants. Sub optimal participation rates limits the possibility to reject the null-hypothesis and thereby increases the risk for type II errors. Another limitation is loss to follow-up.
Self-reported information on confounders might sometimes be less reliable than data in registers (for example regarding income and education).

5.2.1 PAPER I

The main strength of paper I was the size and the population-based data collection. More than 400 000 women were included in the study groups.
When paper I was published, it was to our knowledge, - the largest study to date examining whether HPV infection in conjunction with pregnancy and previous treatment for CIN was associated with obstetric and neonatal outcomes. It included also the largest number of deliveries before and after treatment for CIN in the same woman at the time point of publication. The smallest group was the HPV infection group (n=2,550), defined by a positive HPV test, but the results for that group were similar as those in the larger HPV infection – cytology group (n=11,727).
Paper I included adjustments for many potential confounders.
The exposure and outcome definitions are well-established world-wide.
Because of its size and design, we think the study has good external validity and the results are generalizable to the general Swedish population as well as to other countries/populations.

The main limitation of paper I was the observational register-based design. In observational studies, there is always a risk for residual confounding despite thorough adjustments for confounders. The study groups differed regarding some background factors for example, age, smoking, parity, education and year of delivery. We adjusted for these and six other possible confounders, based on prior knowledge. However, residual confounding can exist despite thorough adjustments for possible confounders. There might be unknown confounders not adjusted for and some women also missed information regarding some background-data.
The missing frequency was low for background factors reported from Statistics Sweden (education, country of birth, income) but higher for some background-factors from the MBR. Missing data varied for background factors and between study groups; BMI (5-10%), country of birth (0.1%), smoking (2-4%), employment (8-12%), marital status (4-5%), education (0.2-1.2%). For the adjusted analyses, a missing category was constructed for each variable with missing data. Smoking in late pregnancy is especially underreported in the MBR, during the

study period missing in MBR for about 12-30%, while smoking before pregnancy and/or in early pregnancy is missing for around 5-6% (266, 267). We constructed a missing category if all data regarding smoking was missing and assumed that if the woman did not smoke before or in early pregnancy, she did not become a smoker in late pregnancy. Only 0.1% of women in the MBR missed estimated GA and 0.1-0-4% maternal age during the study period (267).

Although residual confounding might exist, our findings of increased risk of PTD, pPROM, spontaneous PTD, and chorioamnionitis when the treated group was compared to the other groups is strengthened by similar findings in the paired analyses in which deliveries before and after treatment in the same woman were compared. However, the paired analyses were limited by the fact that we could not adjust for time-dependent confounders like maternal age and parity. Nulli-parity is a risk factor for PTD and to not adjust for parity might thus attenuate a true association between treatment and PTD.

There are also some limitations regarding the exposure definitions that could have caused bias/systematic errors in this study, thus affecting internal validity.
The definition of HPV infection in this study relies on positive screening tests. HPV infections can occur and clear between testing and this may have led to an underestimation of the true incidence and prevalence of HPV infection. HPV results are registered as positive or negative. The tests used at Swedish laboratories have predominantly detected hrHPV but a minority of HPV tests in this study might have been positive due to detection of low-risk HPV genotypes only (279). Since HPV test results only became available in 2007, we also used abnormal cytology as a surrogate for HPV infection, as has been done in previous studies. An earlier study from Sweden reported that 72% of women, under 40 years of age, with low-grade abnormal cytology were hrHPV positive (260). High-grade abnormality in cytology represents to a higher degree presence of hrHPV and was also included in this study and therefore we assume that abnormal cytology in our study to a high degree represent the presence of hrHPV. We included positive HPV tests and cytology up to six months before conception, and therefore some women might have cleared their HPV infection

before pregnancy. Such misclassification could have attenuated a true association between HPV infection and PTD (279).
Some women in the reference group might have had an undetected HPV infection, since we did not require a negative HPV test for inclusion into that group. Such misclassification could have attenuated the increased risk of adverse outcomes found in the exposure groups when compared to the reference group (279).
Since no national treatment data was available, we chose to use CIN3 as a surrogate for treatment, since these women – if not pregnant - are always treated in Sweden. Due to this were all women treated for high-grade lesions in Sweden during the study period not included in the treated group (e.g., treatment of CIN2 was not included in the study).

Information bias could be present due to incorrect classifications of outcomes in MBR. ICD codes for the adverse outcomes might be missing and practice in ICD-coding might vary between caregivers and departments and over time. Further PTD might also be wrongly classified into spontaneous or indicated in the MBR. The large size of the study groups minimizes the eventual effect of such misclassification. We adjusted for time periods in our analyses to correct for possible changes in coding over time, and the HPV infection (HPV test) group was only compared to deliveries in other groups between 2007-2016.
Our definition of neonatal sepsis includes all ICD codes for neonatal sepsis reported to the MBR. Since we missed information regarding debut of neonatal sepsis relative to time of delivery our outcome was not restricted to early onset sepsis that is of most interest, since this represents infections acquired before delivery.

Between 4 and 6% of the groups had their gestational age estimated by last menstrual period instead of ultrasound based, but fewer women in the HPV infection (HPVtest) group (1.8%), probably because it included only data from 2007.

5.2.2 PAPER II

The main strength in Paper II was the population-based design with comparison of women with cone-length before fixation to both women with normal cytology and to women with CIN during pregnancy. It included many treatments ≤10mm (n=1,805). Cone length was measured in a standardized manner on fresh tissue before fixation which limits the risk of underestimation of cone length due to specimen shrinkage.

The risk for PTD after treatment was highlighted in a meta-analysis in 2006 (8). Treatments from 2008-2016 were included in paper II. Of those with cone length 85% were performed with LEEP and the rest with laser thus representing the standard methods applied today. Mean cone-length was 9.1 mm and we assume that our data represent modern treatment undertaken by colposcopists aware of the need not to remove unnecessary healthy tissue in women of reproductive age. The methods of treatment and definitions of exposure and outcomes used in this study are used world-wide. The majority had their GA estimated by ultrasound and only 1% by last menstrual period. We believe that this study should have good external validity.

There are however important limitations which must be considered when interpreting the results. As discussed for paper I there is always a risk for residual confounding despite thorough adjustments for confounders in observational studies. The limitations regarding residual confounding, discussed in paper I can be applied also for paper II.
The study groups differed also in paper II regarding some background factors, for example smoking, parity and assisted reproduction. Some women missed information regarding background factors but missing data for background factors was similar between the study groups; BMI (10-12%), Smoking (6%), employment (10%), marital status (7%), education (0.3-1.0%).
We constructed missing categories used in the adjusted analyses and adjusted for the same possible confounders as in paper I.

Information bias might be present due to incorrect classification of outcomes in MBR, as discussed for paper I. Such possible

misclassification could have had a higher impact in paper II, since study groups were smaller. However, paper II studied a shorter time period and only one health care region – with probably more homogenous use of ICD codes.

The CIN during pregnancy group included only 1,380 women. The adjusted analyses regarding PTD risk compared to women with normal cytology were no longer significant and we suspect that these analyses might be limited by power. Only 9 women with chorioamnionitis had recorded cone length and the analysis whether cone length affect the risk for chorioamnionitis is therefore of limited value.
Treatment-data in the NKCx/Process register in western Sweden is available from 2008, and this defined the study period. We used all available data from the register in western Sweden during the study period and therefore no sample size calculation was performed for paper II.

5.2.3 PAPER III

The strength of this study is the prospective design with HPV detection of 28 genotypes in urine both at mid-pregnancy and at delivery as well as in placenta at delivery. Previous studies have mainly been retrospective and have included HPV detection both before and after pregnancy. To our knowledge only one previous prospective study of similar size has assessed whether HPV infection, defined by HPV test during pregnancy and at birth is associated with PTD (258).
The prospective design lowers the risk for information bias, for example incorrect classification of the studied exposures and outcomes.
The use of first void urine for detection of genital HPV detection has previously been used in several studies (289). Detection of HPV in urine can originate from the cervix but also from vagina and vulva. The frequency of HPV infection in our study was comparable to other studies (258).

The main limitation of this study, based on a general Scandinavian population, is the low incidence of adverse outcomes in this cohort, which was lower than in the general population.
Only 2.4% of the women with HPV test in mid-pregnancy had PTD. The frequency of PTD in singleton pregnancies in Norway/Sweden is around 5%, suggesting self-selection bias for participation in this study. We therefore did not have power to study PTD risk.

Women were invited to participate either at an university hospital (Oslo, Norway), a region hospital (Østfold, Norway) or at maternal care units in Stockholm, Sweden. There were no selection criteria other than that the women should understand Swedish or Norwegian and not plan to move during the study period. The presence of self-selection is supported by high educational levels in the cohort (more than 70% had higher education and only 0.9% preliminary school only) and low frequency of smoking. Self-selection might affect the external validity.

We have focused on the HPV detection at mid-pregnancy since we suspect that the results at delivery are biased, due to a failure to collect

samples from women with adverse outcomes. As many as 9 of the 23 women (39%) with PTD that had test at mid-pregnancy did not provide urine samples for HPV test at delivery, while 20% (188/927) of the women with term deliveries that had test at mid-pregnancy did not provide urine samples at delivery. Placenta was not collected from 11 of the 23 women with PTD.
The presence of HPV DNA was not evaluated in the entire placenta but only from punch biopsies from three sites. We did not detect HPV in any of the studied placentas and this might be an underestimation of the true incidence.

Due to the size of the dataset and number of adverse events it was not possible to adjust for many confounders. We adjusted for parity and used forward logistic regression in order to adjust for the most important background factors but at the same time limit the use of unnecessary adjustments. Some women missed background-factors and missing categories were constructed for some background-factors. Residual confounding is still possible. Another important limitation is the lack of information regarding earlier CIN or previous treatment.

The most common definition used for PROM is contractions at least one hour after membrane rupture. In this study, no information about time of start of contractions was collected, only start of active delivery. PROM was defined as start of active delivery at least one hour after membrane rupture, resulting in a high frequency of PROM in the cohort (25%). Of the 927 term births 6% missed information about time of membrane rupture or time of active delivery, (8% in hrHPV positive and 6% hrHPV negative). We assumed missing at random and omitted these missing cases from the analyses.

5.2.4 PAPER IV

The main strength in paper IV was the prospective design with assessment of microbiota before and at two serial time points after LEEP. The paper also included a reference population with normal cytology. The study included also cone-length at treatment and HPV detection at follow up. It was the second study published, and the study with the largest sample size, that examined if LEEP changes the cervical microbiota, as far as we are aware.

There are however several important limitations in the study design. Data was collected between 2005-2007 and this study relied on culture and PCR instead of NGS technique, which use has increased during the last decade. Detection of bacteria using NGS techniques, mainly based on bacterial 16S rRNA, permits a possibility to detect and differentiate bacteria to a level of detail that is not possible with culture techniques. For example, classification into different species of *Lactobacillis* was not possible in paper IV. It has been suggested that *Lactobacillus iners* is associated with a less stable microbiota than *Lactobacillus crispatus* (290) and dominance of *Lactobacillus iners* in first trimester was reported being a risk factor for PTD (153).

The stability and composition of cervicovaginal microbiota is influenced by numerous factors including ethnicity (290), hygiene practice (291), smoking (292), recent sexual intercourse (293), and hormones. Presence of *Lactobacillus spp*. is influenced by estrogen-driven maturation of the vaginal epithelium (294) and consistent with this the highest instability and diversity of the microbiota is reported at the time of menstruation when estrogens are at their lowest (295, 296). Reduced estrogen and resulting vaginal atrophy following menopause are thought to lead to *Lactobacillus spp*. depletion, increased pH and increased diversity (297). The vaginal microbiome becomes more stable and less diverse in pregnancy (151) while the decrease of estrogen postpartum is associated with an increase in vaginal microbial diversity (155). A meta-analysis has also shown that hormonal contraceptive use is associated with reduced incidence of BV and reduced risk for recurrent BV (298).
Some factors that may have an impact on the cervical microbiota were unfortunately unknown in paper IV. There was no detailed collection

of information about use of hormone replacement therapy, menopausal status or the exact time of menstruation. However, none of the samples were collected during menstruation. Background factors regarding sexual behavior, history of recent sexual activity, number of sexual partners or use of vaginal douching or menstrual hygiene practices were not collected.

Since information about menopausal status was missing, we assumed women up to 45 years of age to be premenopausal and included them in subgroup analyses with similar results. However, the sample size was small in the subgroup analyses, facing the risk of type II-errors, especially for the analysis of specific bacteria species.

There were important differences in background characteristics between the reference group and the LEEP group. The reference group was for example older, more often married and nonsmoking than the LEEP-group, factors that might affect the microbiota and our results. We adjusted for age, marital status, smoking and use of contraception in the comparisons of the two groups but residual confounding is still possible.
Furthermore, follow-up of possible changes in microbiota composition over time in the reference group would also have been valuable, but collection of cervical microbiotas was only made once in this group.

6 CONCLUSION

To reduce spontaneous PTD and its associated long-term morbidity and mortality is one of the major challenges in modern obstetrics. It is also important to reduce the burden of PROM and maternal and neonatal infectious complications.
Below is a summary of results in boxes, followed by conclusions:

Previous excisional treatment was associated with an increased risk of PTD and pPROM compared to women with normal cytology (paper I and II), women with HPV infection in conjunction with pregnancy (paper I), women that developed HSIL after delivery (paper I) and women with CIN during pregnancy (paper II).

Previous excisional treatment was also associated with an increased risk of neonatal mortality, PROM at term, chorioamnionitis and neonatal sepsis (paper I).

Small excisions, up to 10 mm, were associated with an increased risk of PTD, of about 50%, and the risk increased further with cone-length with about 15% for every extra millimeter cone length (paper II).

Increasing cone length at excisional treatment was associated with increased risk of pPROM, PROM and neonatal sepsis (paper II).

Excisional treatment as such increases the risks of PTD and pPROM and larger excisions confer higher risks.

Excisional treatment is also associated with increased risks of maternal and neonatal infectious complications and neonatal mortality.

If the increased risks of PTD, pPROM, PROM, chorioamnionitis, neonatal sepsis and neonatal mortality after treatment could be explained by an increased risk for ascending bacterial infections to the uterus after treatment, should be explored in future research.

Clinicians should aim to remove all CIN at treatment, in order to prevent development of cancer, but minimize destruction of healthy cervical tissue, especially in women of reproductive age.

In women that have not finished their family planning, active surveillance instead of direct removal of moderate CIN, might be preferable, since CIN in many women regresses spontaneously. In Sweden women under 25 years of age with CIN2 are monitored instead of treated, while women aged 25 or older are treated directly. Other countries have used initial surveillance of CIN2 in women under 30 years of age. The policy in Sweden is under revision, and one reason for that is to reduce the number of treatments of fertile women, considering the high age for maternity in modern society.

Our results highlight that diagnosis, surveillance, and treatment of CIN, should be managed by educated and well-trained colposcopists.

Cone-length should be measured on fresh tissue in a standardized manner for immediate clinical feedback to the colposcopist, and also be recorded for future use in pregnancy counselling /antenatal care planning and in further studies.

Pregnancies after treatment of CIN, especially after large excisions, should be regarded as high-risk pregnancies for PTD and infectious complications. History of previous excisional treatment should be added to triage systems used for prioritization of women seeking for preterm labor. The recorded cone length at treatment should be available for risk estimation at antenatal surveillance, to tailor risk-reducing interventions for those with the highest risk of PTD.

Our findings support the benefit of HPV vaccination programs to reduce future need of excisional treatments.

HPV infection in conjunction with pregnancy was associated with an increased risk of PTD in a large register-based study (paper I).

To have CIN during pregnancy was associated with increased risk of PTD in a smaller register-based study, but only in unadjusted analyses (paper II).

That HPV infection confers increased risk for PTD could not be confirmed in a prospective cohort study of pregnant women (paper III) but the study had limited power. Women positive for hrHPV at mid-pregnancy in that study had a higher frequency of PTD than hrHPV negative women, although the comparison was not significant.

Women that have CIN during pregnancy seem to have an increased risk of PTD. Our results suggest that this might be due to the co-existing HPV infection, since we found that HPV infection in conjunction with pregnancy was associated with increased risk of PTD.
Only high-risk genotypes of HPV seem to confer increased risk for PTD.
However further prospective clinical studies are needed to confirm that hrHPV infection increases the risk of PTD.
Future studies should further explore the impact of different HPV genotypes, especially hrHPV16, and the impact of infection with multiple HPV genotypes on the PTD-risk.

If an association between HPV infection and an increased risk of PTD is due to the virus itself, due to an inflammatory response, due to the cervical microbiota or other factors still needs to be established.

Our findings support the benefit of HPV vaccination programs. These are not only beneficial for cancer prevention but also for prevention of PTD - both through the reduction of HPV infections / CIN in pregnant women and through reduction of excisional treatments of CIN.

HPV infection was associated with an increased risk of pPROM and PROM at term in a large register-based study (paper I) but results were not supported by a smaller prospective cohort study (paper III).

HPV infection seems to be associated with increased risk of pPROM and possibly also of PROM at term but more studies are needed.

In a small prospective study, a tendency of decreased bacterial diversity and increase of Lactobacillus spp. was observed six and 12 months after LEEP compared to before treatment (paper IV).

There was no association between persistence of HPV at follow up or cone length at treatment and change in cervical microbiota (paper IV).

A higher number of bacterial species was found in the cervix in women with CIN prior to LEEP and also after LEEP compared to women with normal cytology (paper IV)

Our data, derived from a nonpregnant cohort, suggest that excisional treatment does not lead to a more dysbiotic cervical microbiota. This suggest that the increased risk of PTD and infectious complications after treatment is not explained by increased dysbiosis caused by treatment. However, women with CIN harbor a more diverse cervical microbiota than women with normal cytology and our data suggest that this might also prevail after treatment. If this increases the risk for PTD is unknown.

7 FUTURE PERSPECTIVES

The magnitude of risk increase for PTD after excisional treatment may not only depend on the cone-length at excision, but also on the volume of excised tissue and/or the preoperative cervical volume/length i.e. proportion of excised tissue (208, 209).
Future prospective studies should include measurement of the cervix before treatment, standardized measurement of cone-length and excised volume before fixation at treatment and follow up of pregnant women post treatment, including surveillance with standardized TVU of cervical length (208, 209).

Our results support general HPV vaccination programs. These might not only reduce PTD by lowering the number of excisional treatments but also by a reduced burden of HPV infections / CIN during pregnancy. In the future, studies in cohorts of vaccinated women will ascertain whether PTD and neonatal mortality decrease when the burden of HPV decreases.

We also suggest that women with verified CIN during pregnancy are compared to previously treated women and women with normal cytology in more comprehensive studies. Larger population-based register-studies on the effect of HPV infection during pregnancy, on pregnancy outcomes, will also be possible in the near future since HPV-testing in cervical cancer screening has increased rapidly during the last decade.

We also suggest larger prospective studies of HPV detection in the cervix and placenta. These should include HPV genotyping to further study if there are certain genotypes of HPV that are associated with PTD or if multiple HPV infections confer higher risk for PTD. If this is the case, implementation of new genotypes in the HPV vaccination program might be of benefit.

Further experimental studies to understand the causal pathways between HPV infection, CIN, treatment of CIN and the risk for PTD and other adverse obstetric outcomes are needed. This may also, in a

broader perspective, be a key in understanding biological mechanisms leading to PTD. Key elements here seem to be the cervico-vaginal microbiota, HPV genotypes, the inflammatory response and ascending infections.
We suggest future longitudinal studies in pregnant cohorts for comparison of obstetric outcomes, measurement of inflammatory markers and examination of cervico-vaginal as well as intrauterine microbiota with 16S rRNA NGS technique. Comparisons should include women with previous treatment, women with genotyped HPV infection, women with verified CIN during pregnancy and women with normal cytology/no HPV infection during pregnancy.

There might be an underlying genetic vulnerability, possibly in the inflammatory response, that cause some women to be more vulnerable to both persistent HPV infection and CIN and also to PTD and pPROM and future genetic studies are therefore also warranted.

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APPENDIX

Estimation of gestational age paper I and paper II.

Our variable *Gestational age estimation method* in table 1 in paper I and table 1 in paper II was constructed using the description of estimation of gestational age in the Swedish Medical Birth Register.

Gestational age estimation method
Ultrasound; GRMETOD = 1 or, 5 or 6 or 7
Last menstrual period; GRMETOD = 2 or 3 or 8 or 9 or 10
Other; GRMETOD = 4 or 11 or 12

Description from MBR;

GRMETOD

Värde	Innehåll
0	Ingen uppgift fanns
1	Förlossningsdatum enligt ultraljudsundersökningen (BPULDAT) stöds av graviditetslängden skattad i FV2 (GRVFV och GRDFV). Förlossningsdatum enligt ultraljudsundersökning används.
2	Mödravårdcentralens beräknade förlossningsdatum på senaste menstruationen (BPSMDAT) stöds av graviditetslängden skattad av FV2 (GRVFV och GRDFV). Beräknad förlossning enligt senaste menstruationen används.
3	Graviditetslängden beräknad från sista menstruations datum (SMDAT) i MHV1 stöds av graviditetslängden skattad i FV2 (GRVFV och GRDFV). Förlossningsdatum beräknad på angivet SM-datum används.
4	Endast förlossningsvårdens skattning av graviditetslängden (GRVFV och GRDFV) finns att tillgå. Denna skattning används.
5	Förlossningsdatum enligt ultraljudsundersökning (BPULDAT) överensstämmer med mödravårdens beräkning på senaste menstruation (BPSMDAT). Skattning enligt ultraljudsundersökning används.
6	Förlossningsdatum enligt ultraljudsundersökning (BPULDAT) överensstämmer med beräkning gjort från sista menstruations datum (SMDAT) i MHV1. Skattning enligt ultraljudsunder-sökning används.
7	Endast förlossningsdatum grundat på ultraljudsundersökning (BPULDAT) finns att tillgå. Detta datum används för graviditetslängsbestämning.
8	Endast förlossningsdatum grundat på mödravårdens beräkning på senaste menstruation (BPSMDAT) finns att tillgå. Detta datum används för graviditetslängsbestämning.
9	Endast förlossningsdatum grundat på variabeln SM-datum (SMDAT) finns att tillgå. Detta datum används för graviditetslängsbestämning.
10	Endast förlossningsdatum grundat på mödravårdens beräkning på senaste

menstruation (BPSMDAT) finns att tillgå samt förlossningsdatum grundat på variabeln SM-datum (SMDAT) finns att tillgå. Om detta överensstämmer används mödravårdens beräkning på senaste menstruation.

11 Om uppgift om kön eller födelsevikt saknas eller om markering av flerbörd eller rensad missbildning finns görs skattning av gravlängd enligt följande hierarki:

a. Skattning enligt ultraljudsundersökning (BPULDAT).
b. Mödravårdcentralens beräknade förlossningsdatum på senaste menstruationen (BPSMDAT).
c. Förlossningsdatum beräknat på variabeln SM-datum (SMDAT).
d. Förlossningsvårdens skattning av graviditetslängden (GRVFV och GRDFV).

12 Minst antal standardavvikelser mellan födelsevikten och för-väntad födelsevikt enligt respektive metod för skattning av graviditetsbestämning. För beräkning av avvikelsen, se särskild bilaga.

Beräkningarna görs utifrån en algoritm där följande antaganden görs;
1. endast graviditetsskattningar större än 139 samt mindre än 316 dagar ses som rimliga.
2. för skattningar som grundas på minsta avvikelse mot förväntad vikt, typ 12, krävs graviditetslängdavvikelse större än 181 och mindre än 301 dagar (from 26 tom 42 fullbordade veckor).
3. för att graviditetslängd beräknad i förlossningsjournal 2 - FV2 (GRVFV och GRDFV) ska ge stöd åt övriga beräkningar krävs att skillnaden i dagar måste vara högst 14 dagar. För att övriga uppgifter ska kunna stödja varandra får skillnaden vara högst 7 dagar.