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Maternal human papillomavirus infection during pregnancy and preterm delivery: A mother–child cohort study in Norway and Sweden

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Abstract

Introduction: Human papillomavirus (HPV) infection is common in women of reproductive age. Infection and inflammation are leading causes for preterm delivery (PTD), but the role of HPV infection in PTD and prelabor rupture of membranes (PROM) is unclear. We aimed to explore whether HPV infection during pregnancy in general, and high-risk-HPV (HR-HPV) infection specifically, increased the risk of PTD, preterm prelabor rupture of membranes (PPROM), PROM at term, and/or chorioamnionitis.

Abbreviations: CI, confidence interval; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; LR, low-risk; OR, odds ratio; PPROM, preterm prelabor rupture of membranes; PROM, prelabor rupture of membranes; PTD, preterm delivery.

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Material and Methods: In pregnant women, who were participating in a prospective multicenter cohort study from a general population in Norway and Sweden (PreventADALL, ClinicalTrials.gov NCT02449850), HPV DNA was analyzed in available urine samples at mid-gestation (16–22 weeks) and at delivery, and in the placenta after delivery with Seegene Anyplex II HPV28 PCR assay. The risk of PTD, PPROM, PROM, and chorioamnionitis was analyzed using unadjusted and adjusted logistic regression analyses for any 28 HPV genotypes, including 12 HR-HPV genotypes, compared with HPV-negative women. Further, subgroups of HPV (low-risk/possibly HR-HPV, HR-HPV-non-16 and HR-HPV-16), persistence of HR-HPV from mid-gestation to delivery, HR-HPV-viral load, and presence of multiple HPV infections were analyzed for the obstetric outcomes. Samples for HPV analyses were available from 950 women with singleton pregnancies (mean age 32 years) at mid-gestation and in 753 also at delivery.

Results: At mid-gestation, 40% of women were positive for any HPV and 24% for HR-HPV. Of the 950 included women, 23 had PTD (2.4%), nine had PPROM (0.9%), and six had chorioamnionitis (0.6%). Of the term pregnancies, 25% involved PROM. The frequency of PTD was higher in HR-HPV-positive women (8/231, 3.5%) than in HPV-negative women (13/573, 2.3%) at mid-gestation, but the association was not statistically significant (odds ratio 1.55; 95% confidence interval 0.63–3.78). Neither any HPV nor subgroups of HPV at mid-gestation or delivery, nor persistence of HR-HPV was significantly associated with increased risk for PTD, PPROM, PROM, or chorioamnionitis. No HPV DNA was detected in placentas of women with PTD, PPROM or chorioamnionitis.

Conclusions: HPV infection during pregnancy was not significantly associated with increased risk for PTD, PPROM, PROM, or chorioamnionitis among women from a general population with a low incidence of adverse obstetric outcomes.

KEYWORDS
delivery, HPV, infections, preterm birth, rupture of membranes

1 | INTRODUCTION

Preterm delivery (PTD), defined as a birth before 37 weeks of gestation, is the main cause of neonatal mortality as well as lifelong morbidity,[1,2] including increased risks of development of non-communicable diseases.[3] To identify ways to reduce the burden of PTD is therefore of utmost importance.

Although PTD is a multifactorial condition, ascending uterine bacterial infection and inflammatory decidual activation are the most important causes for spontaneous PTD.[4] An intrauterine bacterial infection causing spontaneous PTD is often subclinical; however, PTDs, especially if starting with preterm prelabor rupture of the membranes (PPROM), are at increased risk of infectious complications of the mother and the newborn. This risk is also increased in deliveries starting with prelabor rupture of membranes (PROM) at term.[5] Cervicovaginal dysbiosis confers increased risk for PTD.[4]

Key message

HPV infection during pregnancy was not associated with increased risk for preterm delivery, prelabor rupture of the membranes, or chorioamnionitis among 950 women from a general population with a low incidence of adverse obstetric outcomes.

Why bacteria ascend from the lower genital tract to the uterus and cause PTD and chorioamnionitis in some women remains unexplained, but the mucosal immunity and the microbial ecosystem in the lower genital tract are key factors.[6] It has been suggested that viral infections may reduce the cervical epithelium's capacity to prevent ascending uterine infections[7] and that viral infections of the placenta and decidua, through inflammatory activation, may affect the fetus and cause increased sensitivity to bacterial co-infections, resulting in PTD.[8]

Human papillomavirus (HPV) infection is the most common viral genital tract infection in women of reproductive age,[9] and is often cleared within 2 years.[10] Approximately 40 HPV genotypes have been identified in the genital tract.[9] They are divided into low-risk-HPV (LR-HPV), probable or possibly high-risk HPV, and high-risk-HPV (HR-HPV), according to their association to carcinogenesis.[11] HPV is a sexual transmitted infection. Prevalence depends on age and geographical region studied.[11] HR-HPV was detected in 28% of women aged 23–29 years and 11% of women aged 30–49 years in the Swedish national cervical screening program.[12]

Cervical HR-HPV infection has been associated with an increased risk of PTD,[13,14] PPROM,[14] PROM,[14,15] as well as with placental abnormalities[13] and cervicovaginal dysbiosis.[16,17] Placental HPV infection has also been associated with increased risk of PTD.[18,19] Studies linking HPV infection to obstetric outcomes have shown conflicting results.[20,21] Several previous studies have used HPV exposure before or after pregnancy, as well as abnormal cervical cytology, as proxy for HPV infection during pregnancy. A meta-analysis suggested that HPV infection increased the risk of PTD and PPROM, and more so when restricting the analyses to studies of exposure during pregnancy or to HPV DNA detection.[22] There has been a lack of large prospective studies with HPV DNA testing during pregnancy, but recently a prospective Canadian study (*n* = 899) reported that HPV DNA detection during pregnancy and especially persistent HR-HPV-16/18 infection was associated with PTD.[19] The main aim of the present study was therefore to investigate if genital or placental HPV infection was associated with PTD, and secondarily if HPV infection was associated with PPROM, PROM, and chorioamnionitis.

2 | MATERIAL AND METHODS

The present study included women with singleton pregnancies, from the prospective multicenter study PreventADALL (Preventing Atopic Dermatitis and ALLergies in children).[23] Briefly, 2697 women, proficient in Norwegian or Swedish, were recruited in connection with their routine ultrasound examination, gestational age 16–22 weeks, at Oslo University Hospital or Østfold Hospital Trust, Norway or at the Karolinska Institute, Sweden, between December 14, 2014 and October 31, 2016.[23] The women completed comprehensive electronic questionnaires at baseline and at 34 weeks of gestation regarding sociodemographic characteristics, health, lifestyle, and obstetric history. At delivery, obstetric outcomes were registered in study charts and later additional obstetric data were collected from medical charts in Norway and from the Swedish Pregnancy Register.[24]

At mid-gestation, 954 women with singleton pregnancies had urine collected for HPV testing. After one dual pregnancy, invalid HPV results (*n* = 2) and women missing obstetric outcomes (*n* = 2) had been excluded, the final study cohort comprised 950 women (Figure 1). At delivery, valid HPV samples in urine were available from 753 of the 950 included women.

Total nucleic acids for HPV detection were extracted from 1000 μL first-void urine samples and analysis and HPV genotyping were performed on all urine samples with Anyplex II HPV28-PCR assay (Seegene Inc., Seoul, South Korea), as described previously.[25] This method detects 28 genotypes (LR-HPV: types 6/11/40/42/43/44/54/61, Possibly-HR-HPV: types 26/53/66/68/69/70/73/82, and HR-HPV: types 16/18/31/33/35/39/45/51/52/56/58/59) (Table 1), and was also used for HPV analysis of placenta. At delivery, a total of three punch biopsies, diameter 5 mm, were cut all through the placenta (central, middle, and peripheral lobes) for HPV detection. Only placentas from the deliveries with PTD, PPROM, and chorioamnionitis were analyzed for HPV in the present study (see Appendix S1).

The main exposure was presence of any HPV (28 genotypes) and also subgroups of HPV; LR/possibly-HR-HPV-only, HR-HPV-non-16, and HR-HPV-16 (Table 1).

In HR-HPV-positive women (12 genotypes) HR-HPV persistence, HR-HPV viral load, and multiple infection were studied. Women were defined as HR-HPV-persistent if the same genotype was detected at mid-gestation and at delivery. Viral load was classified as high, medium, or low, according to detection thresholds with Anyplex II HPV28-PCR.[25] Multiple HPV infection was defined as being positive for HR-HPV and at least one further HPV genotype.

The primary outcome was PTD, defined as a live birth delivery before 37 weeks (<259 days). Secondary outcomes were spontaneous PTD, defined as PTD starting either with PPROM or contractions, PPROM, defined as preterm spontaneous membrane rupture before contractions, and PROM at term, defined as spontaneous membrane rupture at least 1 hour before the start of the active phase of delivery, at 37 weeks or later (including spontaneous start or induced start after PROM). Tertiary outcomes were treatment with antibiotics due to suspected chorioamnionitis at delivery and diagnosed chorioamnionitis (Table S2). The calculated gestational age used in this study was based on fetal biometric measures at ultrasound, as part of the routine prenatal care at the recruiting centers, or based on embryo transfer if it was an in vitro fertilization pregnancy.

2.1 | Statistical analyses

Women who were negative for HPV infection were compared with (a) women positive for any HPV and (b) HPV-positive women divided into sub-groups of HPV (LR/possibly-HR-HPV-only, HR-HPV-non-16, or HR-HPV-16), concerning obstetric outcomes. Comparisons were performed by univariable and multivariable logistic regression analyses, for HPV status both at mid-gestation and at delivery. Further, sub-analyses including only HPV results from mid-gestation in nulliparous women were performed.

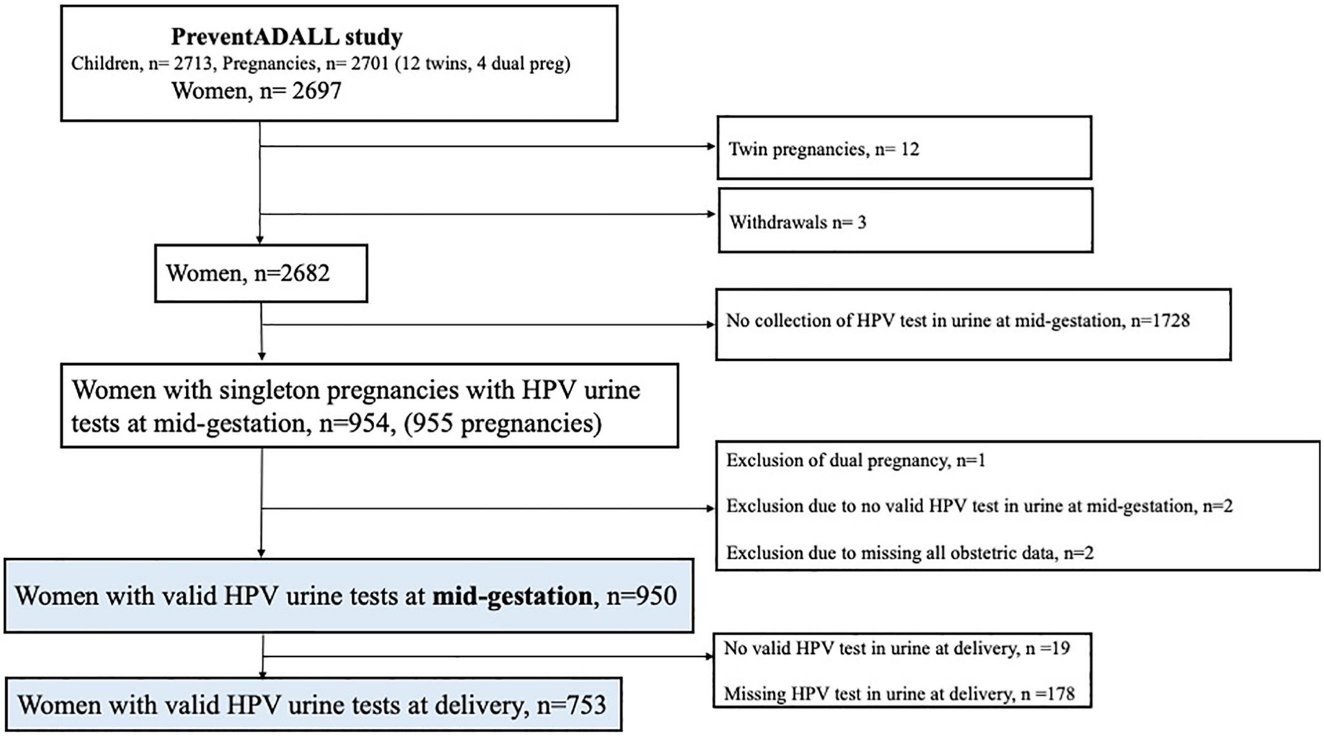

FIGURE 1 Flowchart of the study population. HPV, human papillomavirus; *n*, number; PreventADALL, Preventing Atopic Dermatitis and ALLergies in children.

TABLE 1 Exposure groups and classification of HPV

	Included genotypes and their classification according to IARC[11]		
Exposure groups	**HR-HPV**	**Possibly HR-HPV**	**Low-risk HPV**
Any HPV	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	26, 53, 66, 68, 69, 70, 73, 82	6, 11, 40, 42, 43, 44, 54, 61
LR/Possibly-HR-HPV		26, 53, 66, 68, 69, 70, 73, 82	6, 11, 40, 42, 43, 44, 54, 61
HR-HPV-non-16	18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59		
HR-HPV-16	16		

Abbreviations: HPV, human papillomavirus; HR-HPV, high-risk-human papillomavirus; IARC, International Agency for Research on Cancer; LR, low-risk.

Logistic regression was further used to compare women who were negative for HPV with HR-HPV-positive women with multiple HPV infection at mid-gestation and to analyze obstetric outcomes in relation to HR-HPV viral load at mid-gestation.

Further, women with a positive HR-HPV test result at mid-gestation and a valid HPV test result at delivery were included in an analysis of persistence of HR-HPV. Woman with HR-HPV genotype-specific-persistence between mid-gestation and delivery were compared with women without HR-HPV genotype-specific-persistence for obstetric outcomes with logistic regression analyses.

Candidates for adjustment in multivariable analyses were selected based on previous knowledge of risk factors for PTD and PPROM.[4] Maternal age, parity, education, marital status, and smoking were identified as possible confounders and adjusted for in the multivariable model (Figure S1). A separate category for missing data was constructed for education, marital status, and smoking.

Analyses were performed using IBM SPSS Statistics, version 27.0. A two-sided significance level of 0.05 was applied.

2.2 | Ethics statement

The PreventADALL study overall was approved by the regional ethical committees in Norway (2014/518) on May 18, 2015 and Sweden (2014/2242–31/4) on March 25, 2015 while the present sub-study (InfPreg 2017/1053) was approved on November 1, 2017 (Table S3). The PreventADALL study was registered at clinicaltrials.gov (NCT02449850) on May 18, 2015. All participants signed an informed written consent form at enrollment.

3 | RESULTS

Background information of the 950 women stratified by HPV presence is shown in Table 2. The mean age at inclusion was 32 years, mean body mass index was 25 kg/m^2 and 73% had higher education. Women positive for HPV were more often nulliparous and single/divorced.

At mid-gestation, 377 women (40%) were positive for any HPV and 231 women (24%) were positive for HR-HPV, while at delivery, 208 women (28%) were positive for any HPV and 124 women were positive for HR-HPV (16%). Only 753/950 women had valid HPV results at delivery. Among the 197 women missing a valid HPV test at delivery, 87 (44%) were positive for any HPV and 53 (27%) were positive for HR-HPV at mid-gestation.

A total of 23/950 women (2.4%) had PTD and 20 of them delivered between 34^{+0} and 36^{+6} weeks of GA. Twenty of the PTDs were spontaneous and PPROM was observed in 9/950 (0.9%) women. Of the 23 women with PTD, 10 were positive for any HPV and eight for HR-HPV, six of whom were positive for multiple-HPV, two for HR-HPV-16 and none for HR-HPV-18 (Figure 2).

There was no statistically significant increased risk of PTD in women positive for any HPV at mid-gestation or at delivery compared with HPV-negative women. Neither of the analyses of subgroups of HPV showed significantly increased risk of PTD (Table 3; Tables S4 and S5). Women positive for HR-HPV-non-16 and HR-HPV-16 at mid-gestation had similar frequency of PTD, which was higher than the PTD frequency in HPV-negative women (Table 3; Table S5). However, the comparisons were not statistically significant, nor were they when pooling all HR-HPV-positive women compared with HPV-negative women (odds ratio [OR] 1.55; 95% confidence interval [CI] 0.63–3.78, $p = 0.34$) or when this comparison was done in nulliparous women only (OR 3.18; 95% CI 0.92–11.04, $p = 0.07$).

Neither were there any statistically significant associations between presence of HPV or sub-groups of HPV and spontaneous PTD, PPROM, chorioamnionitis, or antibiotics due to suspected chorioamnionitis at mid-gestation or at delivery (Table 3; Tables S4 and S5).

Of the 927 term births, 59 (6%) missed information about time of membrane rupture or time of active delivery and were excluded from the comparisons of PROM. The prevalence of PROM at term was 25%. HPV status at mid-gestation was not significantly associated with PROM, (Table 3; Table S5). Having any HPV or LR/possibly-HR-HPV at delivery was associated with a lower risk for PROM compared with no HPV, although not significant after adjustments (Table S4).

Among the 753 women with HPV tests both at mid-gestation and delivery, 93/178 (52%) had persistence from mid-gestation to delivery of the same HR-HPV genotype. There was no association between HR-HPV persistence and obstetric outcomes (Table S6). Of 59 HR-HPV-16-positive women at mid-gestation, 45 had available HPV status also at delivery and 29 (64%) had a persistent HR-HPV-16 infection. None of these women experienced PTD, PPROM, or chorioamnionitis.

The frequency of PTD was non-significantly higher in HR-HPV-positive women with multiple-HPV infection (4.3%) than in HPV-negative women (2.3%) (Table 4).

Only 18 women had high viral load and none of them had PTD. We found no association between HR-HPV viral load and PTD or any of the other obstetric outcomes (Tables S7 and S8). In the 18 placentas investigated for HPV detection (12/23 pregnancies with PTD, 8/9 with PPROM, and 5/6 with chorioamnionitis) no HPV was detected in any of the specimens.

4 | DISCUSSION

In this Scandinavian prospective population-based study of 950 singleton pregnancies, HPV infection measured in urine at mid-gestation and at delivery was not significantly associated with increased risk of PTD, PPROM, PROM, or chorioamnionitis.

Recently a Canadian prospective study found increased risk of PTD with vaginal infection with HR-HPV genotypes 16 and 18 in first trimester (prevalence 7.3%) compared with HPV-negative women (12.1% vs 5.6%; OR 2.34; 95% CI 1.02–5.36) and especially if the infection was persistent in third trimester (OR 3.21; 95% CI 1.32–7.82). Women with other HR-HPVs had no increased risk for PTD.[19] In our study, 6.2% were HR-HPV-16-positive at mid-gestation and our comparison gave OR 1.51 (95% CI 0.33–6.87). The frequency of PTD was similar in HR-HPV-16-positive and HR-HPV-non-16-positive women. Women positive only for LR/possibly-high-risk-genotypes had lower frequency of PTD, in line with the Canadian study, and when studying any HPV in the lower genital tract neither our study (including 28 genotypes) nor the Canadian study (including 36 genotypes) found increased risk of PTD compared with HPV-negative women (our study at mid-gestation; OR 1.17; 95% CI 0.51–2.71) and the Canadian study at first trimester (OR 1.25; 95% CI 0.72–2.16).[19] Hence, the HR-HPV genotypes, and especially HR-HPV-16, seem to be the most interesting for further investigations, when assessing if HPV affects the risk for PTD.

A recent register-based Swedish study, in which 2550 women with positive cervical HPV tests (mainly HR-HPV-positive) were compared with a large reference population with normal cytology, suggested an increased risk for PTD in women with HPV (PTD 5.6% vs 4.6%) (OR 1.23; 95% CI 1.04–1.46).[14] Although our comparison of HR-HPV-positive and HPV-negative women at mid-gestation was not statistically significant, our OR of 1.55 (95% CI 0.63–3.78) was larger. However, the definitions of exposure are not fully comparable across these studies.

Compared with HPV-negative women, a higher frequency of PTD was particularly seen in HR-HPV-positive women in the

TABLE 2 Maternal background characteristics in women with negative or positive test result in urine for any of 28 genotypes of HPV at mid-gestation

	All *N* = 950	HPV-negative [a] *N* = 573		HPV-positive [b] *N* = 377	
Age (years)					
Mean ± SD	32.0 ± 4.5	32.2 ± 4.3		31.6 ± 4.8	
Median (IQR)	32.0 (29–35)	32.0 (29–35)		32.0 (29–35)	
	N (%)	*n*	%	*n*	%
Marital status					
Married/cohabitants	825 (86.8)	503	87.8	322	85.4
Single/separated or divorced/other	17 (1.8)	4	0.7	13	3.4
Missing	108 (11.4)	66	11.5	42	11.1
Education					
Preliminary school or high school	140 (14.7)	68	11.9	72	19.1
Higher education, <4 years	295 (31.1)	183	31.9	112	29.7
Higher education, ≥4 years /PhD	400 (42.1)	252	44.0	148	39.3
Unspecified or missing	115 (12.1)	70	12.2	45	11.9
Smoking					
Never	660 (69.5)	399	69.6	261	69.2
Before pregnancy	139 (14.6)	84	14.7	55	14.6
During pregnancy[c]	41 (4.3)	23	4.0	18	4.8
Missing	110 (11.6)	67	11.7	43	11.4
BMI kg/m^2					
Underweight (<18.5)	7 (0.7)	6	1.0	1	0.3
Normal weight (18.5–24.9)	514 (54.1)	312	54.5	202	53.6
Overweight (25–29.9)	291 (30.6)	180	31.4	111	29.4
Obese (≥30)	116 (12.2)	64	11.2	52	13.8
Missing	22 (2.3)	11	1.9	11	2.9
Parity					
0	518 (54.5)	284	49.5	234	62.1
≥1	430 (45.3)	288	50.3	142	37.7
Missing	2 (0.2)	1	0.2	1	0.2
IVF pregnancy					
Yes	69 (7.3)	46	8.0	23	6.1
No	873 (91.9)	524	91.4	349	92.6
Missing	8 (0.8)	3	0.5	5	1.3
History of PTD excluding nulliparous women (%)	*N* = 432	*N* = 289		*N* = 143	
No previous PTD	291 (67.4)	197	68.2	94	65.7
Previous PTD	16 (3.7)	10	3.5	6	4.2
Missing history PTD	125 (28.7)	82	28.4	43	30.1
Infant sex					
Girl	454 (47.8)	310	54.1	185	49.1
Boy	495 (52.1)	263	45.9	191	50.7
Missing	1 (0.1)	0	0	1	0.2
Country of origin					
Norway	504 (53.0)	300	52.4	204	54.1
Sweden	246 (25.9)	150	26.2	96	25.5
Other	90 (9.5)	56	9.8	34	9.0
Missing	110 (11.6)	67	11.7	43	11.4

(Continues)

TABLE 2 (Continued)

	All N = 950	HPV-negative[a] N = 573		HPV-positive[b] N = 377	
Site of inclusion					
Oslo University Hospital, Norway	282 (29.7)	170	29.7	112	29.7
Østfold Hospital Trust, Norway	391 (41.2)	236	41.2	155	41.1
Karolinska University Hospital, Sweden	277 (29.2)	167	29.1	110	29.2

Abbreviations: BMI, body mass index; HPV, human papillomavirus; IQR, interquartile range; IVF, in vitro fertilization; *N*, number; *SD*, standard deviation.

[a]Negative for 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, and 61, in urine.

[b]Positive for 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, and/or 61, in urine.

[c]Data collected at gestational age 34 weeks. Only seven of the 41 women that smoked during pregnancy continued to smoke after recognizing pregnancy.

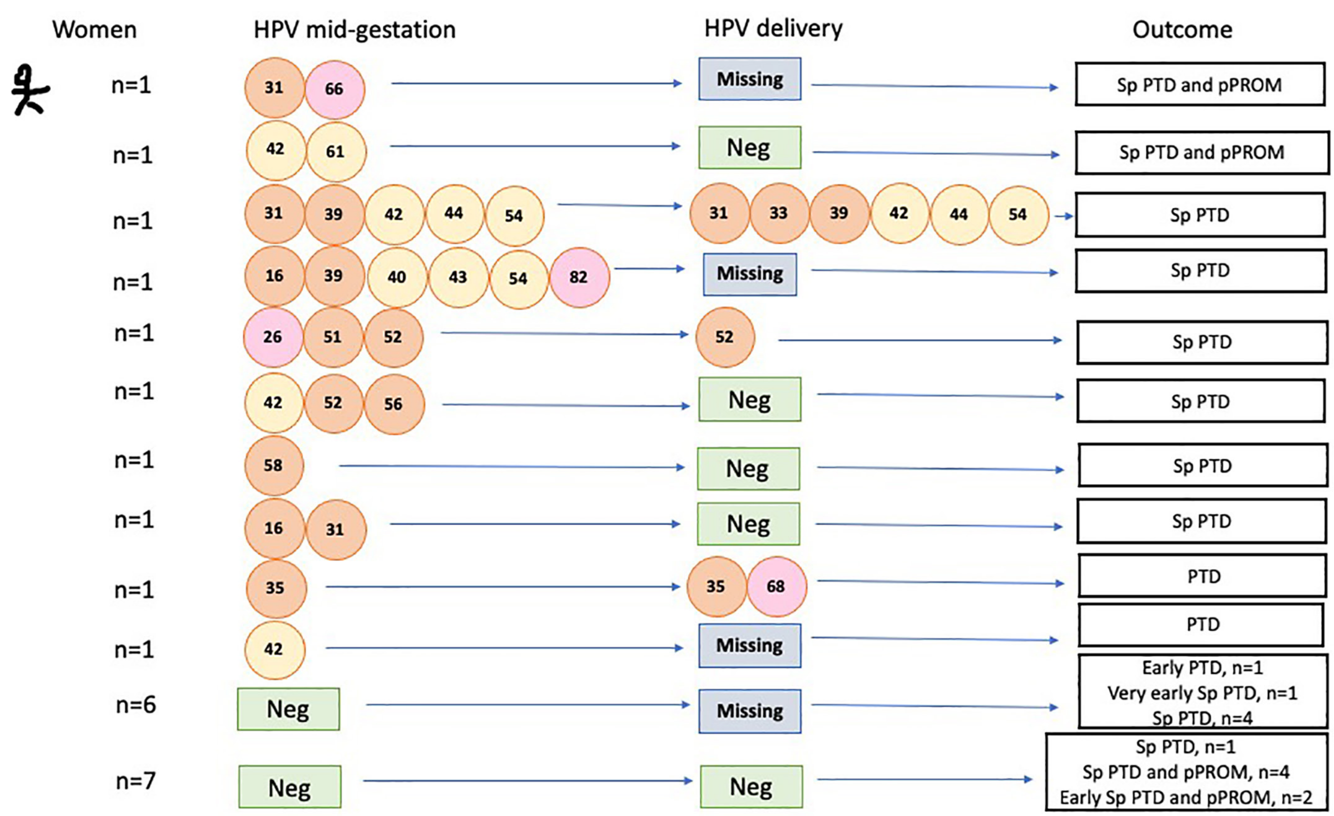

FIGURE 2 Women with valid HPV test in urine at mid-gestation and preterm delivery. A total of 23/950 women had PTD, 10 of whom were positive for HPV in urine at mid-gestation. Illustration of the different genotypes of HPV that were detected in first-void urine at mid-gestation and at delivery in the preterm deliveries. Orange = HR-HPV, yellow = low-risk HPV, pink = possibly HR-HPV. Early PTD was defined as gestational age less than 238 days and very early PTD as gestational age less than 196 days. HPV, human papillomavirus; HR-HPV, high risk human papillomavirus; N, number; Neg, negative; PPROM, preterm prelabor rupture of membranes; PTD, preterm delivery; Sp PTD, spontaneous preterm delivery.

sub-analyses of nulliparous women and in HR-positive women with multiple HPV infections. However, the comparisons included limited numbers of PTDs and the differences were statistically non-significant. Multiple compared with single HPV infection has been associated with HPV-persistence and cervical dysplasia.[26] The role of multiple HPV infection concerning PTD needs further study.

The Canadian study detected any HPV in 91/819 (11.1%) placentas (pooled swabs and biopsies) and placental HPV infection was associated with PTD (OR 2.17; 95% CI 1.01–4.68).[19] Our study included only placenta biopsies to minimize risk of contamination from the vaginal tract. We did not detect HPV in any of the examined placentas in the women with PTD. However, placenta biopsies were missing for 11 of 23 women, limiting our ability to exclude the possibility that the presence of HPV in placenta increases the risk for PTD.

The results of the present study do not support that HPV infection increases the risk for PPROM or PROM, although the small

TABLE 3 Evaluation of the association between HPV prevalence at mid-gestation and PTD, spontaneous PTD, PPROM, PROM at gestational age 37 weeks or later, and maternal infectious complications, by using unadjusted and adjusted logistic regression analyses

Exposure	Outcome *n/N*	%	OR (95% CI)	*p*	aOR (95% CI)[a]	*p*[a]
	PTD					
HPV-negative	13/573	2.3	1 (Reference)		1 (Reference)	
Any HPV +[b]	10 /377	2.7	1.17 (0.51–2.71)	0.71	1.16 (0.49–2.71)	0.74
HPV-negative	13/573	2.3	1 (Reference)		1 (Reference)	
LR/Possibly-HR-HPV +[c]	2/146	1.4	0.60 (0.13–2.68)	0.50	0.58 (0.13–2.63)	0.48
HR-HPV-non-16 +[d]	6/172	3.5	1.56 (0.58–4.16)	0.38	1.54 (0.56–4.26)	0.40
HR-HPV-16 +	2 /59	3.4	1.51 (0.33–6.87)	0.59	1.62 (0.35–7.58)	0.54
	Spontaneous PTD					
HPV-negative	12/573	2.1	1 (Reference)		1 (Reference)	
Any HPV +[b]	8/377	2.1	1.01 (0.41–2.50)	0.98	0.96 (0.38–2.43)	0.93
HPV-negative	12/573	2.1	1 (Reference)		1 (Reference)	
LR/Possibly-HR-HPV +[c]	1/146	0.7	0.32 (0.04–2.50)	0.28	0.31 (0.04–2.41)	0.26
HR-HPV-non-16 +[d]	5/172	2.9	1.40 (0.49–4.03)	0.53	1.32 (0.44–3.94)	0.62
HR-HPV-16 +	2/59	3.4	1.64 (0.36–7.51)	0.52	1.71 (0.36–8.12)	0.50
	PPROM[e]					
HPV-negative	6/573	1.0	1 (Reference)		1 (Reference)	
Any HPV +[b]	3/377	0.8	0.76 (0.19–3.05)	0.70	0.76 (0.19–3.14)	0.71
HPV-negative	6/573	1.0	1 (Reference)		1 (Reference)	
LR/Possibly-HR-HPV +[c]	2/146	1.4	1.31 (0.26–6.57)	0.74	1.37 (0.27–6.98)	0.70
HR-HPV-non-16 +[d]	1/172	0.6	0.55 (0.07–4.62)	0.58	0.53 (0.06–4.54)	0.56
HR-HPV-16 +	0/59	0	NA	NA	NA	
	Chorioamnionitis					
HPV-negative	4/573	0.7	1 (Reference)		1 (Reference)	
Any HPV +[b]	2/377	0.5	0.76 (0.14–4.16)	0.75	0.61 (0.10–3.65)	0.59
HPV-negative	4/573	0.7	1 (Reference)		1 (Reference)	
LR/Possibly-HR-HPV +[c]	0/146	0	NA		NA	
HR-HPV-non-16 +[d]	2/172	1.2	1.67 (0.30–9.22)	0.55	1.26 (0.20–7.86)	0.80
HR-HPV-16 +	0/59	0	NA		NA	
	Antibiotiotics due to suspected choriamnionitis					
HPV-negative	10/573	1.7	1 (Reference)		1 (Reference)	
Any HPV +[b]	9/377	2.4	1.38 (0.55–3.42)	0.49	1.27 (0.50–3.25)	0.61
HPV-negative	10/573	1.7	1 (Reference)		1 (Reference)	
LR/Possibly-HR-HPV +[c]	1/146	0.7	0.39 (0.05–3.06)	0.37	0.39 (0.05–3.10)	0.37
HR-HPV-non-16 +[d]	7/172	4.1	2.39 (0.90–6.37)	0.08	2.13 (0.77–5.91)	0.15
HR-HPV-16 +	1/59	1.7	0.97 (0.12–7.72)	0.98	0.89 (0.11–7.35)	0.91
	PROM ≥37 weeks[f]					
HPV-negative	141/528	26.7	1 (Reference)		1 (Reference)	
Any HPV +[b]	78 /340	22.9	0.82 (0.60–1.12)	0.21	0.79 (0.57–1.09)	0.15
HPV-negative	141/528	26.7	1 (Reference)		1 (Reference)	
LR/Possibly-HR-HPV +[c]	33/134	24.6	0.90 (0.58–1.39)	0.63	0.94 (0.60–1.46)	0.77

TABLE 3 (Continued)

Exposure	Outcome n/N	%	OR (95% CI)	p	aOR (95% CI)[a]	p[a]
HR-HPV-non-16 +[d]	31/152	20.4	0.70 (0.45–1.09)	0.12	0.62 (0.39–0.98)	0.04
HR-HPV-16 +	14/54	25.9	0.96 (0.51–1.82)	0.90	0.94 (0.49–1.82)	0.85

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; LR, low-risk; *n*, number; NA, non applicable; OR, odds ratio; PPROM, preterm prelabor rupture of membranes; PROM, prelabor rupture of membranes; PTD, preterm delivery.

[a]Adjusted logistic regression, adjusted for maternal age, smoking (never/before pregnancy/during pregnancy/missing), marital status (married or cohabitants/single or separated or divorced / missing), education (preliminary school or high school/higher education <4 years / higher education ≥4 years or PhD/missing) and parity (0/≥1).

[b]Positive for 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, and/or 61, in urine.

[c]Positive for 26, 53, 66, 68, 69, 70, 73, 82, 6, 11, 40, 42, 43, 44, 54, and/or 61, in urine.

[d]Positive for 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and/or 59, in urine.

[e]One of those had PPROM and delivered at term.

[f]Of 927 term pregnancies 59 had missing information about PROM and were not included in the analyses (of the HPV-negative 32/560 [5.7%] were missing and of the HPV-positive 27/367 [7.4%] were missing).

TABLE 4 Evaluation of the association between multiple HPV prevalence at mid-gestation and adverse obstetric outcomes by using unadjusted and adjusted logistic regression analyses

	Multiple HPV[a] N = 139		HPV-negative N = 573		Unadjusted		Adjusted[b]	
	n	%	n	%	OR (95% CI)	p	aOR (95% CI)[b]	p[b]
PTD	6	4.3	13	2.3	1.94 (0.73–5.21)	0.19	2.11 (0.74–5.96)	0.16
Spontaneous PTD	6	4.3	12	2.1	2.11 (0.78–5.72)	0.14	2.26 (0.79–6.50)	0.13
PPROM	1	0.7	6	1.0	0.69 (0.08–5.74)	0.73	0.62 (0.07–5.59)	0.67
Chorioamnionitis	1	0.7	4	0.7	1.03 (0.11–9.30)	0.98	0.81 (0.08–8.62)	0.86
Antibiotics due to suspected chorioamnionitis	4	2.9	10	1.7	1.67 (0.52–5.40)	0.39	1.48 (0.43–5.07)	0.53
PROM ≥37 weeks[c]	27	21.6	141	26.7	0.76 (0.47–1.21)	0.24	0.67 (0.41–1.10)	0.12

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; PPROM, preterm prelabor rupture of membranes; PROM, prelabor rupture of membranes; PTD, preterm delivery.

[a]Positive for more than one HPV whereof at least one HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and/or 59) in urine.

[b]Adjusted logistic regression, adjusted for maternal age, smoking (never/before pregnancy/during pregnancy/missing), marital status (married or cohabitants / single or separated or divorced / missing), education (preliminary school or high school / higher education <4 years / higher education ≥4 years or PhD / missing) and parity (0 / ≥1).

[c]Analyzed in 653 term pregnancies (125 with multiple HPV and 528 negative for HPV), Information about PROM was missing in 8/133 term pregnancies with multiple HPV infection (6.0%) and in 19/560 term pregnancies negative for HPV (3.4%).

number of women with PPROM ($n = 9$) limits our possibility to draw firm conclusions. To our knowledge the association between HPV and PPROM/PROM has previously only been prospectively studied in a small Indian study ($n = 104$), which reported that women with vaginal HPV infection during pregnancy had increased risk for PPROM compared with HPV-negative women (14.6% vs 3.2%, $p = 0.03$).[27]

In our study, the estimates for PROM were lower in HPV-positive women, with a significantly lower risk for PROM at term in women with any HPV or LR/possibly-HR-HPV in unadjusted analyses at delivery. This finding was unexpected and we do not have any biological explanation for this. In contrast to this, a Korean study ($n = 311$) found an increased risk for PROM (defined as rupture of membranes before labor) in women positive for HR-HPV in the cervix 6 weeks after birth (adjusted OR 2.32, 95% CI 1.08–4.98).[15] An increased risk for PPROM and PROM in HPV-positive women compared with women with normal cytology was also suggested by the Swedish population-based study.[14] In that study the PROM diagnosis at term was based on International Classification of Diseases codes, whereas in the present study a broader definition was used for PROM, resulting in a higher prevalence of PROM and limiting the possibility to compare the results.

We did not find any association between HPV infection and chorioamnionitis. Although our findings are in accordance with the larger Swedish population-based study,[14] the small number of women with chorioamnionitis in the present study is a limitation.

Although persistence of HR-HPV in our study was as high as 52%, there were only a few cases of adverse outcomes in each comparison group and no definite conclusions can be drawn.

The main strength of this study is the prospective design with test of 28 HPV genotypes, including all high-risk-genotypes, both at mid-gestation and at delivery as well as examination of placentas. HPV testing was performed on first-void urine, which in several studies has been shown to represent genital infections.[28] The percentage of pregnant women positive for HPV (40%) and HR-HPV (24%) at mid-gestation in our study was comparable to vaginal detection of HPV (42%) and HR-HPV (28%) in first trimester in the Canadian study.[19] Maternal age was comparable in these cohorts (mean 32 vs 31 years).

The most important limitation of this study is the low incidence of PTD and other adverse outcomes in the cohort. The percentage of PTD is generally low in Scandinavia, around 5% in singleton pregnancies.[14] In the present cohort, only 2.4% delivered preterm. Of the 753 women with an HPV test at delivery, the frequency of PTD was even lower (1.9%) and we assume that the results at delivery, and hence also the persistence analyses, are biased by missing urine samples at delivery from pregnancies with adverse outcomes. We therefore focused on the test results at mid-gestation.

Even if this study is larger than the Canadian study,[19] the frequency of PTD was more than double in that study (6.1%) and our study is limited by power owing to the low incidence of PTD. Selection bias, by self-selection of participants, might explain the low incidence of PTD. Although this study aimed to include a general population, more than 70% in our cohort had higher education and only a few smoked during pregnancy. This could affect the generalizability of our results.

Another limitation of this study is that we lacked information regarding previous treatment for cervical dysplasia, which is associated with an increased risk of PTD.[29] We had no information about presence of other genital infections or composition of genital microbiota, and some women lacked information for covariates used in our adjusted model. Due to these limitations, residual confounding is possible.

A possible association between HPV infection and PTD cannot be ruled out by this study because of its limited sample size with rare events. As there is a great need to prevent PTD and as vaccination can prevent infection with several HPV genotypes[30] further studies are warranted. To further study the effect of HPV on obstetric outcomes, we suggest measurement of genotype-specific HPV infection during pregnancy in larger prospective studies, including analyses of persistence and presence of multiple HPV infections as well as analyses of genital microbiota and inflammatory markers.

5 | CONCLUSION

HPV infection during pregnancy was not associated with increased risk of PTD, PPROM, PROM, or maternal infectious complications. This study was limited by the low number of adverse obstetric outcomes. Whether HPV infection affects other aspects of pregnancy and infant outcomes remains to be explored.

AUTHOR CONTRIBUTIONS

JW contributed to conceptualization, data curation, methodology, formal analysis, writing the original draft, and writing—review and editing. MV contributed to conceptualization, data curation, and writing—review and editing. CMJ contributed to conceptualization, data curation, methodology, writing—review and editing, and funding acquisition. AS— conceptualization, methodology, and writing—review and editing. KLC was project leader and contributed to data curation, methodology, writing—review and editing, and funding acquisition. BJ contributed to conceptualization, methodology, and writing—review and editing. SN contributed to methodology, formal analysis, and writing—review and editing. BG, GHa, GHe, BN, ER, HS, BS, CS, KH, and RV contributed to data curation and to writing—review and editing. AR contributed to data curation, methodology, and writing—review and editing. VS contributed to conceptualization, methodology, and writing—review and editing. KS contributed to conceptualization, data curation, methodology, writing—review and editing, supervision, project administration, and funding acquisition. All authors read and approved the final. manuscript.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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