

# Age-Specific Seroprevalence of Human Papillomavirus 16, 18, 31, and 58 in Women of a Rural Town of Colombia

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**Objective:** The study's objective was to estimate human papillomavirus (HPV) genotype-specific seroprevalence to determine population HPV exposure and inform vaccine policy.

**Methods:** This study is a cross-sectional prevalence survey of 878 women of Pueblorrico, a rural town of Colombia. A standardized questionnaire was used to obtain information on demographic characteristics, sexual and reproductive history, and smoking habits. Seropositivity to HPV-16, -18, -31, and -58 was determined by virus-like particles in an enzyme-linked immunosorbent assay.

**Results:** Overall seropositivity to any HPV genotype was 27.9%. The combined seroprevalence of women 15 to 19 and 20 to 24 years old was 35.4% (95% confidence interval [CI], 25.9–46.2) and 36.0% (95% CI, 27.7–45.3), respectively. Seroprevalence for HPV-16 was 17% (95% CI, 14.6–19.6); for HPV-18, 9.8% (95% CI, 8.0–11.9); for HPV-31, 11.4% (95% CI, 9.5–13.7); and for HPV 58, 12.5% (95% CI, 10.5–14.9). Higher HPV seropositivity was associated with the lifetime number of occasional sexual partners (odds ratio, 3.05; 95% CI, 1.26–7.37) and having more than 2 regular sexual partners (odds ratio, 3.00; 95% CI, 1.21–7.45) in women younger than 44 and older than 45 years old, respectively. Use of oral contraceptives and tobacco/cigarettes was significantly associated with reduced HPV seropositivity in women older than 45 but not in women younger than 44 years old.

**Conclusions:** Human papillomavirus seropositivity is associated with measures of sexual behavior, particularly a greater lifetime number of sexual partners. Hormonal and tobacco/cigarette use may be factors influencing the HPV seropositivity in women older than 45 years old.

**Key Words:** Human papillomavirus antibodies, Human papillomavirus vaccine, Colombia, Cervical cancer, Women's health

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In 2008, 529,409 new cases and 274,883 deaths from cervical cancer occurred worldwide. Eighty-five percent of cases and 88% of deaths occur in women living in developing countries.<sup>1</sup> Cervical cancer is caused by persistent infection of oncogenic human papillomavirus (HPV), with HPV-16 and -18 accounting for 60% to 70% of cases worldwide.<sup>2</sup> Human papillomavirus is a sexually transmitted virus that infects the genital epithelium of almost 75% of the sexually active population in the world. Although a high proportion of women develop cervical cytological abnormalities as a consequence of HPV infection, this occurs frequently without clinical evidence. Both HPV infection and its related clinical manifestations clear spontaneously within 1 to 2 years after virus acquisition, but in a small proportion of women infected with high-risk genotypes, persistent infections are established.<sup>3</sup>

Prophylactic vaccines based on “virus-like particles” (VLPs) of HPV-16 and -18 have an efficacy approaching 100% for the prevention of infection and high-grade lesions (cervical intraepithelial neoplasia 2/3) related to these 2 genotypes.<sup>4,5</sup> Because genotype-specific neutralizing antibodies, which prevent the entry of but do not eliminate cells already infected by the virus, mostly mediate this protection, there is no effect of the vaccine in women with a preexistent infection.<sup>6</sup> Therefore, vaccine efficacy is dependent on the level of exposure of the population to infection before vaccination. In addition, vaccine seems to afford partial protection against phylogenetically related genotypes, and this protection seems to be mediated by cross-reactive neutralizing antibodies.<sup>7</sup> Accurate information on the age-specific level of HPV exposure of the population is useful to design optimal strategies for vaccination. With the advent of the HPV vaccine, HPV serology has been useful to predict vaccine benefits on different populations.<sup>8,9</sup> This article reports the age-specific prevalence of antibodies against HPV-16, -18, -31, and -58 genotypes in a population-based sample of women in a rural town of Colombia.

## MATERIALS AND METHODS

### Study Design and Population

The design of this study has been previously described.<sup>10</sup> Briefly, this is a cross-sectional prevalence survey conducted in women of Pueblorrico, a town located in the southwest region of the state of Antioquia. This study used the materials and methods implemented on the surveys conducted by the International Agency for Research on Cancer to examine the risk factors associated with HPV and herpes simplex virus 2 infection worldwide. Based on the data provided by government officials of the town that had carried out a population census in 2002 (6 months before this study), a random sample, with proportional allocation by age (15–19, 20–24, ... 60–64, 65+) and by urban (urbanized center of the town) and rural (countryside rural isolated farms or small villages that by law belong to the town of Pueblorrico) areas of residence, was obtained. One thousand randomly selected women were invited to participate

by individual letters delivered to their addresses. Women who did not reply to the invitation were contacted again and scheduled a hospital visit at their convenience. The sample collection was conducted from September 2002 to April 2003. The Committee on Bioethics from the University of Antioquia approved the study. All women who participated in the project gave informed consent and authorized the use of samples.

### Data Collection

An already standardized questionnaire<sup>11</sup> was used to obtain information on demographic characteristics, smoking habits, and sexual and reproductive history. After the interview, a pelvic examination, including inspection of the external genitalia, was performed, and women provided 10 mL of blood. Blood samples were processed by centrifugation at the site of collection, shipped frozen on dry ice, and stored at  $-70^{\circ}\text{C}$  until testing.

### Human Papillomavirus VLPs Enzyme-Linked Immunosorbent Assay

The production of VLPs of HPV-16, -18, -31, and -58 has been previously described.<sup>12</sup> Briefly, SF21 insect cells were infected with a recombinant baculovirus containing the *L1* gene of each genotype. The VLPs were purified from the nuclear lysate in successive cesium chloride (CsCl) gradients. The quality of VLPs was verified by electrophoresis in sodium dodecyl sulfate polyacrylamide gel electrophoresis and its antigenicity was verified by Western blot with sera from cervical cancer patients. For the enzyme-linked immunosorbent assay (ELISA), VLPs were diluted in phosphate buffer saline and coupled to 96-well Nunc MaxiSorp microplates (Nunc, Life Technologies, Eragny, France) at a concentration of 600 ng per well. Each well was blocked with 200  $\mu\text{L}$  of 2% fetal bovine serum (FBS) (Gibco, BRL, Rockville, MD) for 2 hours at  $37^{\circ}\text{C}$ . The sera were diluted 1:40 in  $5\times$  phosphate buffer saline with 2% Tween-20 and 10% FBS. One hundred microliters of diluted sera was analyzed in duplicate and simultaneously in wells of the same ELISA plate, covered with VLPs or FBS only. To calculate the net absorbance of each serum, the average of optical densities (ODs) of wells with FBS (background) was subtracted from the average of ODs of wells coated with VLPs. Intra-assay and interassay coefficients of variation were calculated, and samples exceeding 20% variation were repeated. The mean ODs  $\pm 3$  SDs of sera from 69 adult women, HPV negative by polymerase chain reaction, tested for each VLP genotype in ELISA under the same mentioned conditions were the cutoff points to consider a serum seropositive.

### Data Analysis

The independent variables were grouped into 3 categories: social and demographic aspects, sexual behavior, and reproductive and smoking history. The overall age-specific HPV seroprevalence for all combined (positive for any HPV type), multiple (positive for at least 2 genotypes), or individual genotypes was estimated. The relationship

between each of the independent variables with the outcome variable (combined HPV seropositivity) was evaluated in a bivariate analysis using logistic regression models adjusted for age. Odds ratios (ORs) with their respective 95% confidence intervals (CIs) as well as the *P* values for trend for independent variables on an ordinal scale were estimated. We also used deviance-based hypothesis tests to evaluate the effects of all our predictors upon the HPV seropositivity (all combined). A step-by-step multivariate logistic regression analysis was performed to select variables for the model, and those variables with a deviance test *P* value  $\leq 0.25$  (Hosmer-Lemeshow criterion)<sup>13</sup> were included in the final model. *P* values  $\leq 0.05$  were considered significant. The R program version 2.12.2 was used for data analyses.<sup>14</sup>

## RESULTS

### Characteristics of the Study Population

Eight hundred seventy-one (87%) of the 1000 women voluntarily went to the hospital after the invitation, and 103 of the randomly selected women showed up after the invitation was repeated. Of the 974 women who participated in the study, 945 provided blood samples. Among these, 10 did not have a valid HPV ELISA result, and 57 reported they have never had sexual partners in their entire life. In this analysis, only women who reported sexual experience as well as with valid HPV serology results are included (*n* = 878). The mean (SD) age of the women included was 39.15 (16.2). Fifty

**TABLE 1.** Bivariate analysis of HPV-16, -18, -31, or -58 seroprevalence by selected sociodemographic factors

Factor	Total No. Women (N = 878)		HPV Seropositive			
	n	%	%	OR*	95% CI	<i>P</i>
Age, y (mean [SD], 39.15 [16.2])						
All	878	100.0	27.4	—	24.6–30.5	
≤24	193	22.0	35.8	1.00	—	0.0205
25–34	195	22.2	28.2	0.71	0.46–1.08	
35–44	196	22.3	27.6	0.68	0.45–1.05	
45–54	133	15.1	21.1	0.48	0.29–0.80	
55–64	78	8.9	18.0	0.39	0.21–0.75	
≥65	83	9.5	25.3	0.61	0.34–1.08	
				Trend: <i>P</i> = 0.0028		
Area of residence						
Rural	437	49.8	23.3	1.00	—	0.0446
Urban	331	37.7	29.6	1.40	1.01–1.94	
Missing values	110	12.5	12.5	—	—	
Marital status						
Married/cohabiting	588	67.0	25.9	1.00	—	0.6641
Divorced/separated	59	6.7	30.5	1.40	0.77–2.53	
Widowed	87	9.9	25.3	1.18	0.66–2.09	
Single	144	16.4	34.0	1.15	0.74–1.78	
Socioeconomic status†						
Low	719	81.9	27.7	1.00	—	0.6038
Medium	116	13.2	25.0	0.89	0.56–1.40	
Missing values	43	4.9	4.9	—	—	
Education						
Some elementary school	405	46.1	24.4	1.00	—	0.0479
All elementary school	296	33.7	33.5	1.34	0.95–1.90	
High school	155	17.7	23.9	0.73	0.45–1.16	
Technical	20	2.3	30.0	1.06	0.39–2.90	
Missing values	2	0.2	0.2	Trend: <i>P</i> = 0.8567		

\*Adjusted by age.

†The system for identification of beneficiaries of social programs in Colombia (SISBEN in Spanish) was used for socioeconomic classification. Low, levels 1 and 2. Medium, level 3 or above.

percent lived in rural areas, 67% were married or cohabiting with a partner, 16.4% were single, only few (20%) had attained education beyond high school, and the majority (82%) had a low socioeconomic status (Table 1). The mean (SD) age for sexual debut was 18.9 (4.7) years; on average, the number of lifetime sexual partners was 1.3 (1.1); 57.7% had only 1 sexual partner during their entire life; and only 31% reported history of occasional sexual partners (Table 3).

### Seroprevalence of HPV-16, -18, -31, and -58 Genotypes

Table 1 of the supplementary material shows that overall combined seroprevalence to HPV-16, -18, -31, or -58 was 27.4% (95% CI, 24.6–30.5) (see Table 1, Supplemental Digital Content 1, <http://links.lww.com/IGC/A64>). Seroprevalence for HPV-16 was 17% (95% CI, 14.6–19.6); for HPV-18, 9.8% (95% CI, 8.0–11.9); for HPV-31, 11.4% (95% CI, 9.5–13.7); and for HPV-58, 12.5% (95% CI, 10.5–14.9). There was a statistically significant association between age and HPV seropositivity with a significant trend ( $P_{\text{trend}} = 0.002$ ) of decreased HPV seropositivity on women 55 to 64 years old (OR, 0.39; 95% CI, 0.21–0.75) compared with women 24 years old and younger. Likewise, the same pattern was observed for each HPV genotype (Table 1). The combined seroprevalence of women 15 to 19 and 20 to 24 years old was 35.4% (95% CI, 25.9–46.2) and 36.0% (95% CI, 27.7–45.3), respectively (see Table 1, Supplemental Digital Content 1, <http://links.lww.com/IGC/A64>). We found that seropositivity to HPV-16, -18, -31, or -58 was strongly related with seropositivity to other measured types (Table 2). The highest prevalence ORs were for HPV-18 and -58 (OR, 37.0; 95% CI, 21.4–64.0) and for HPV-18 and -31 (OR, 31.8; 95% CI, 18.7–54.0) followed by prevalence ORs for HPV-58 and -31 (OR, 21.8; 95% CI, 13.3–35.5).

### Risk Factors for HPV Seropositivity

Factors associated with seropositivity were area of residence, level of education (Table 1), determinants of sexual behaviors, oral contraceptive (OC) use, and use of tobacco/cigarettes (Table 3). However, only factors related to sexual behavior, OCs, and tobacco/cigarette use remained significant in the multiple regression models.

There was a pattern of increased risk for HPV seropositivity as the years of active sexual life increase, although

this trend did not reach statistical significance ( $P = 0.068$ ). In the model that included HPV-16 (Table 4), a statistically significant trend for increased risk for HPV seropositivity ( $P_{\text{trend}} = 0.04$ ) was observed as the years of active sexual life increase after adjustment for age, but it was lost after adjustment for all other variables ( $P = 0.12$ ). Women who had only occasional sexual partners during their lifetime have a higher risk of being seropositive for HPV-16 (OR, 2.25; 95% CI, 1.01–5.02) and HPV-18 (OR, 3.28; 95% CI, 1.33–8.09) as compared with women who had 1 regular sexual partner but had no occasional sex partners.

Table 3 shows that in the bivariate analysis, the use of OCs was inversely associated with HPV seropositivity (OR, 0.68; 95% CI, 0.48–0.97). Even further, there was a significant association of decreasing HPV seropositivity with increasing years of use of OCs ( $P = 0.01$ ). Compared with women who never use OC, the age-adjusted ORs associated with having used OCs for 5 to 9 and more than 10 years were 0.91 (95% CI, 0.52–1.60) and 0.36 (95% CI, 0.17–0.76), respectively, and this trend was statistically significant ( $P_{\text{trend}} = 0.0228$ ). Although a trend of decreased seropositivity in OC users was observed after adjusting for all variables, it did not reach statistical significance (Table 4). An analysis restricted to women younger than 44 years or older than 45 years shows that only in women older than 45 years old, there is a significant decrease of HPV seropositivity associated with the use of OCs (Table 2 Supplemental Digital Content 2, <http://links.lww.com/IGC/A65> and Table 3, Supplemental Digital Content 3, <http://links.lww.com/IGC/A66>).

Finally, women who smoke cigarettes/tobacco were less likely to be HPV seropositive than nonsmokers (OR, 0.70; 95% CI, 0.49–1.00) (Table 3). Although there was a trend for decreased HPV seropositivity with the number of years smoking and number of cigarettes per day, these patterns were not statistically significant (data not shown). In the multiple logistic regression model, cigarette/tobacco users had decreased risk of being seropositive for HPV-31 (OR, 0.67; 95% CI, 0.39–1.14), HPV-58 (OR, 0.68; 95% CI, 0.40–1.13), and HPV-18 (OR, 0.48; 95% CI, 0.26–0.88). Similarly to OC use, a significant decreased risk for seropositivity for any HPV genotype associated to tobacco/cigarette use was observed in women older than 45 years old (OR, 0.40; 95% CI, 0.21–0.76) but not in women younger than 44 years old (Table 2, Supplemental Digital Content 2,

**TABLE 2.** Prevalence, ORs, and 95% CIs of HPV-16, -18, -31, and -58 seropositivity versus seropositivity to other types

HPV Type Seroreactivity	HPV Type Seroreactivity					
	16		18		31	
	OR	95% CI	OR	95% CI	OR	95% CI
18	11.6	7.1–18.9	—	—	—	—
31	8.0	5.1–12.6	31.8	18.7–54.0	—	—
58	7.9	5.2–12.2	37.0	21.4–64.0	21.8	13.3–35.5

**TABLE 3.** Bivariate analysis of reproductive and sexual behavior risk factors for HPV-16, -18, -31, or -58 seropositivity in a sample of women of a rural town of Colombia

Factor	Total No. Women (N = 878)		HPV Seropositive			
	n	%	%	OR*	95% CI	P
Age at first intercourse, y (mean [SD], 18.9 [4.7])						
≥20	277	31.5	24.2	1.00	—	0.8344
16–19	431	49.1	27.8	1.06	0.74–1.52	
≤15	170	19.4	31.8	1.15	0.73–1.82	
				Trend: P = 0.5504		
Years of active sexual life (mean [SD], 20.2 [15.4])						
≤5	168	19.1	32.7	1.00	—	0.7229
6–10	119	13.6	31.1	1.29	0.71–2.34	
11–15	117	13.3	28.2	1.53	0.71–3.32	
16–20	96	10.9	26.0	1.64	0.67–4.00	
21–25	101	11.5	26.7	1.97	0.74–5.25	
26–30	75	8.5	24.0	2.60	0.84–8.01	
≥31	202	23.0	22.8	3.12	0.91–10.65	
				Trend: P = 0.0682		
No. sexual partners before 20 y (mean [SD], 1.3 [5.2])						
0	275	31.3	23.3	1.00	—	0.3720
1	467	53.2	29.8	1.22	0.85–1.74	
2+	136	15.5	27.9	0.95	0.57–1.58	
				Trend: P = 0.9392		
No. lifetime occasional sexual partners (mean [SD], 1.1 [6.1])						
0	605	68.9	26.5	1.00	—	0.9052
1	130	14.8	29.2	1.07	0.70–1.64	
2+	142	16.2	30.3	1.08	0.72–1.63	
Missing values	1	0.1	—	—	—	
				Trend: P = 0.6698		
No. lifetime regular sexual partners (mean [SD], 1.3 [1.1])						
0	45	5.1	42.2	1.00	—	0.1155
1	657	74.8	25.3	0.59	0.31–1.14	
2+	171	19.5	31.6	0.80	0.40–1.63	
Missing values	5	0.6	0.6	—	—	
				Trend: P = 0.5956		
No. lifetime regular/lifetime occasional sexual partners						
1/0	507	57.7	24.7	1.00	—	0.1321
2+/0	97	11.0	35.1	1.61	1.01–2.58	
1+/1+	224	25.5	27.2	1.10	0.76–1.59	
0/1+	45	5.1	42.2	1.73	0.89–3.37	
Missing values	5	0.6	0.6	—	—	
				Trend: P = 0.1791		
OC use						
Never	264	30.1	32.6	1.00	—	0.0336
Sometimes	544	62.0	25.2	0.68	0.48–0.97	
Missing values	70	8.0	—	—	—	

(Continued on next page)

TABLE 3. (Continued)

Factor	Total No. Women (N = 878)		HPV Seropositive			
	n	%	%	OR*	95% CI	P
Time of OC use, y						
0	264	30.1	32.6	1.00	—	0.0153
≤4	246	28.0	25.2	0.65	0.52–1.60	
5–9	88	10.0	30.7	0.91	0.17–0.76	
10+	70	8.0	14.3	0.36	0.43–0.98	
Missing values	210	23.9	—	—	—	
Use of tobacco/cigarette						
Never	585	66.6	30.4	1.00	—	0.0490
Ever	292	33.3	21.6	0.70	0.49–1.00	
Missing values	1	0.1	—	—	—	

\*Adjusted for age.

<http://links.lww.com/IGC/A65> and Table 3, Supplemental Digital Content 3, <http://links.lww.com/IGC/A66>).

## DISCUSSION

This article presents data of a population-based cross-sectional survey of HPV seroprevalence on a sample of women of a rural town of Colombia. Compared with other studies conducted in Colombia, this study included younger women who live in a rural setting. This population reports a highly monogamous sexual life with only few women showing occasional sexual partners.

The overall seroprevalence to HPV-16, -18, -31, or -58 was 27.4% (95% CI, 24.6–30.5). Seventeen percent, 9.8%, 11.4%, and 12.5% of women were HPV-16, HPV-18, HPV-31, and HPV-58 seropositive, respectively. The combined seroprevalence of women 15 to 19 and 20 to 24 years old was 35.4% (95% CI, 25.9–46.2) and 36.0% (95% CI, 27.7–45.3), respectively; seroprevalence decreased slightly with age up to 55 years and then showed an increase in women older than 65 years. These observations are within the broad range found in previous reports but slightly higher than those reported for women younger than 24 years in other Latin American countries<sup>15</sup> and in Colombia.<sup>16</sup> The high rate of HPV exposure in the absence of an increased number of sexual partners may reflect continuing sexual exposure through the male partners. It has been shown that vaccination does not reduce progression to HPV-16/18–related high-grade cervical lesions in women who are seropositive and DNA positive to HPV-16 or HPV-18 prevaccination.<sup>17,18</sup> Our findings may be helpful to formulate appropriate strategies for HPV vaccination in this population.

In the multivariate model, the number of sexual partners remained significantly associated with HPV-16 and -18 seropositivity, and although there was no statistically significant association, a dose-response relationship was observed for HPV-16 antibodies with years of active sexual life. This is in agreement with previous reports that the amount of time

and opportunity for exposure to HPV are determinants of seropositivity.<sup>19</sup> Several studies have tried to correlate seropositivity to a genotype with that of another genotype to understand if there is antibody cross-reactivity in the natural immune response and if this response is protective.<sup>20,21</sup> However, it has been difficult to answer this question in epidemiological studies because seroprevalence estimates seem to be influenced by several factors such as limitations in the assays used, presence of multiple infections, and lack of correlation between detection of HPV DNA and seropositivity. Nevertheless, we found that seroreactivity to HPV-16, -18, -31, or -58 was strongly associated with seroreactivity to the other measured types. The risk was higher for the nongenetically related (HPV-18 and -31 and HPV-18 and -58) and low for the genetically related (HPV-16 and -31 and HPV-16 and -58) genotypes. It is possible that there was overestimation due to a certain degree of assay unspecificity especially for HPV-31 and -58. It has been recently observed that HPV vaccine induces cross-neutralizing antibodies for HPV types for which evidence of vaccine efficacy has been demonstrated.<sup>7</sup> Identification of natural- and vaccine-induced cross-reactivity suggests the possibility in the formulation of vaccines able to neutralize a wide range of oncogenic genotypes.

The most striking finding of our study is the significant inverse association between use of OCs and cigarette/tobacco smoking and HPV seropositivity. In the bivariate analysis, reduced HPV seropositivity was observed in OC users compared with never users. There was also a pattern of decreasing seropositivity as years of use of OCs increased, and this was statistically significant. In the multivariate analysis, a 20% to 30% reduction in the probability of seropositivity was observed in ever users of OC compared with never users but did not reach statistical significance. After adjustment for years of active sexual life, the number of sexual partners, and tobacco/cigarette use, a decreased probability of seropositivity to HPV associated to the use of OCs was observed in women older than 45 years old but not in women younger than 44 years old. Our results are similar to those observed

**TABLE 4.** Logistic regression models showing relations for seropositivity to multiple or single HPV-16, -18, -31, or -58 HPV genotypes and years of active sexual life, number of sexual partners, and smoking with adjustments for other risk factors among women of Pueblorrico, Antioquia, 2003

Factor	HPV-16		HPV-18		HPV-31		HPV-58		Multiple HPV		Any HPV	
	OR*	95% CI	OR*	95% CI	OR*	95% CI	OR*	95% CI	OR*	95% CI	OR*	95% CI
Years of active sexual life												
≤5	1.00		1.00		1.00		1.00		1.00		1.00	
6–10	1.92	0.87–4.22	1.47	0.61–3.55	0.87	0.36–2.10	0.98	0.43–2.23	1.37	0.57–3.30	1.40	0.73–2.71
11–15	1.96	0.71–5.42	0.72	0.21–2.47	0.64	0.21–1.99	0.72	0.25–2.08	0.94	0.31–2.87	1.46	0.62–3.43
16–20	1.95	0.59–6.39	0.54	0.13–2.21	0.54	0.15–2.00	0.43	0.11–1.59	0.50	0.13–1.98	1.40	0.52–3.76
21–25	3.29	0.90–11.96	0.22	0.04–1.11	0.35	0.08–1.50	0.24	0.05–1.14	0.32	0.07–1.46	1.66	0.57–4.84
26–30	4.01	0.91–17.60	0.19	0.03–1.30	0.36	0.07–1.92	0.95	0.20–4.48	0.52	0.10–2.67	2.58	0.76–8.69
≥31	3.18	0.60–16.80	0.34	0.05–2.28	0.94	0.17–5.25	1.06	0.19–6.02	0.73	0.13–4.21	3.36	0.88–12.79
$P_{\text{trend}}^{\dagger}$	0.04		0.08		0.47		0.49		0.23		0.068	
$P_{\text{trend}}^{\ddagger}$	0.12		0.07		0.55		0.68		0.34		0.103	
No. regular/occasional partners												
1/0	1.00		1.00		1.00		1.00		1.00		1.00	
2+/0	1.02	0.54–1.92	1.89	0.89–4.01	1.30	0.61–2.76	1.42	0.72–2.82	0.93	0.41–2.10	1.57	0.95–2.60
1+/1+	1.09	0.68–1.74	1.45	0.80–2.64	1.58	0.94–2.67	1.19	0.70–2.02	1.45	0.85–2.47	1.03	0.69–1.52
0/1+	2.25	1.01–5.02	3.28	1.33–8.09	2.30	0.93–5.72	1.72	0.72–4.10	3.21	1.36–7.58	1.65	0.80–3.37
OC use												
Never	1.00		1.00		1.00		1.00		1.00		1.00	
Ever	0.79	0.50–1.24	0.64	0.37–1.11	1.04	0.61–1.78	0.61	0.37–1.00	0.74	0.44–1.23	0.69	0.47–1.01
Use of tobacco/cigarette												
Never	1.00		1.00		1.00		1.00		1.00		1.00	
Ever	1.05	0.68–1.62	0.48	0.26–0.88	0.67	0.39–1.14	0.68	0.40–1.13	0.83	0.50–1.40	0.65	0.44–0.94

\*Adjusted for the following variables: age, years of active sexual life, number of regular/occasional sexual partners, OC use, and tobacco/cigarette use. ‡Adjusted for the following variables: years of active sexual life, number of regular/occasional sexual partners, OC use, and tobacco/cigarette use.

†Adjusted for age only.

by Marais et al<sup>22</sup> in Africa. The role of hormones in suppression of HPV immune response has been demonstrated in vitro. Exposure of peripheral blood mononuclear cells from healthy women to progesterone and 17-estradiol either alone or in combination decreases the levels of lymphoproliferation and production of proinflammatory cytokines (interferon  $\gamma$ , interleukin 12p70, tumor necrosis factor  $\alpha$ ) and increases the levels of interleukin 10 and transforming growth factor  $\beta$  and expression of *Foxp3* in response to HPV-16 VLPs.<sup>23</sup>

In addition, women who smoke cigarettes/tobacco were less likely to be HPV seropositive than nonsmokers. In the logistic regression model, tobacco/cigarette use remained statistically significantly associated with decreased risk of being seropositive for HPV-18 only. In a study conducted in Finland, young women who smoked were less likely to either seroconvert or maintain detectable HPV-16/18 antibodies over time than nonsmokers.<sup>24</sup> Oral contraceptive use and tobacco

smoke are well-established HPV cofactors for the development of cervical precancer and cancer,<sup>25,26</sup> but the molecular mechanisms by which these factors increase the risk of cervical precancer and cancer remain unknown. Similarly to the decreased seropositivity observed in OC users, a significant decreased probability of being seropositive for HPV associated to the use of tobacco/cigarette was observed only in women older than 45 years. The age of sexual debut of women aged younger than 45 and older than 46 years was 18.2 (3.8) and 20.7 (6.1), respectively, and this was statistically different (Mann-Whitney  $P$  value < 0.001). Similar to our observations in the same population regarding herpes simplex virus 2 seropositivity,<sup>10</sup> an age cohort effect seems to explain the differences on risks factors of HPV seropositivity in this population. Our results suggest that at least in women older than 45 years, OCs and cigarette smoking seem to interfere with the development of an adequate immune

response against HPV. The possible association of OCs and cigarette smoking with establishment of HPV persistence and development of high-grade cervical intraepithelial lesions by impairment of immune response deserves further evaluation.

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