

Effect of the Menstrual Cycle and Hormonal Contraceptives on Human Papillomavirus Detection in Young, Unscreened Women

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OBJECTIVE: To estimate the effect of the menstrual cycle and oral contraceptive pill (OCP) use on the prevalence, incidence, and persistence of human papillomavirus (HPV).

METHODS: A longitudinal study was conducted among 2,065 women aged 18–29 years. The women returned a self-collected cervicovaginal sample and filled out a questionnaire. A total of 1,812 women participated at all three time points, month 0, month 6, and month 12.

RESULTS: Low- and high-risk HPV prevalence at study entry was 8.9% and 11.8%, respectively. The annual incidence of low-risk HPV infections was 12.5% and the persistence was 2.0%. For high-risk HPV, the incidence and persistence was 12.1% and 4.5%, respectively. These results did not differ between OCP users and nonusers. A significant relationship between high-risk HPV detection and the timing of sampling was found when OCP users and nonusers were analyzed separately. In the second half of the menstrual cycle, high-risk HPV detection decreased in nonusers ($P=.007$) and increased in OCP users ($P=.021$). When women used OCPs continuously, high-risk HPV detection returned to the level of the first half of the menstrual cycle.

CONCLUSION: High-risk HPV detection was significantly influenced by sample timing in the menstrual cycle

when analyzed separately for OCP users and women with a natural menstrual cycle. This may have implications in the future, when high-risk HPV detection may become a primary screening tool in cervical cancer prevention.

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LEVEL OF EVIDENCE: II

Human papillomavirus (HPV) infection is a necessary event in the carcinogenesis of cervical cancer. However, only a small fraction of women infected with high-risk HPV types will eventually progress to high-grade intraepithelial lesions and cervical cancer. High-parity, long-term oral contraceptive pill (OCP) use, smoking, and coinfection with other sexually transmitted diseases are the most consistently identified cofactors in cervical carcinogenesis.^{1–3}

Oral contraceptive use is associated with invasive cervical cancer. The international collaboration of epidemiological studies of cervical cancer (International Agency for Research on Cancer) showed that for current users of OCP, the risk of invasive cervical cancer increased with increasing duration of use with a relative risk of 1.90 (95% confidence interval [CI] 1.69–2.13) for 5 years or more. This increased relative risk returned to normal after stopping OCP use for 10 years or more.⁴ Although OCPs are defined as a risk factor for cervical cancer, there is no evidence for an association with HPV positivity.⁵

Despite the lack of proof for a relation between OCP use and HPV positivity, there are indications that hormonal factors might influence HPV detection. The detection of HPV related to a woman's last menstrual period (LMP) has been examined in a number of studies. Some studies found a relation between the LMP and HPV detection. However, their results were not concordant because they found dif-

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ferent phases of the menstrual cycle to be optimal for HPV detection.⁶⁻⁹ On the other hand, studies that found no relation between HPV detection and the menstrual cycle suggest that only the sampling technique or the materials used may affect HPV detection.¹⁰⁻¹² These studies on HPV prevalence and LMP are summarized in Table 1.

The mucosal immunity of the female genital tract, which is influenced by immunoglobulin, cytokines, and reproductive hormones, may explain this possible effect of the menstrual cycle on HPV detection. Exogenous and endogenous hormones increase the production of immunoglobulin-producing cells in cervical secretions.¹³ Total immunoglobulin (Ig) A and IgG levels were higher in OCP users than in women with a natural menstrual cycle (nonusers).^{13,14} In OCP

users, the immunoglobulin titer in the cervical mucus is lowest during the week OCP use is stopped and increases with the start of use, whereas in nonusers, the IgA and IgG levels were highest during the follicular phase and lowest around ovulation.¹³⁻¹⁵ These patterns in Ig levels during the menstrual cycle suggest an important role for reproductive hormones in the regulation of mucosal immunity.¹⁴

Because the use of OCP is associated with an increased relative risk of cervical cancer, we expect that the prevalence and persistence of high-risk HPV is higher in women using OCPs compared with nonusers. According to a previous study from our group,⁸ we would expect to find a higher high-risk-HPV detection rate in the second week of the menstrual cycle in nonusers. Whether fluctuating Ig levels truly

Table 1. Studies on Human Papillomavirus Prevalence and Menstrual Cycle

Author	Sampling Technique	HPV Detection Test Used	HPV Types	Number of Subjects	Result
Fairly et al ¹⁰	Single tampon specimen (in/out specimen)	PCR amplification	High-risk: 16, 18 Low-risk: 6, 11	298	Pellet volume of tampon specimen, but not HPV detection, vary during the menstrual cycle
Harper et al ¹¹	Five HPV screening test: clinician-directed ectocervical swab, clinician-directed endocervical swab, a self-sampled synthetic polyester fiber swab (Dracon), a second self-sampled Dracon swab, and a self-sample tampon, in random order	PCR amplification	High-risk: 16,18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, NM4, NM7, NM9 Low-risk: 6, 11, 40, 42, 53, 54, 57, 66, MM8	103	Percentage of positive samples for HPV did not differ by week of the menstrual cycle
Schneider et al ⁶	Single-point cervical swabs cumulative every 5 weeks in 1 year by clinician	PCR mixture	High-risk: 16	21	HPV 16 detection significantly ($P=.019$) higher in luteal phase
Sherman et al ⁹	Two samples: Papette broom (Wallach Surgical, Orange, CT,) and a Dracon swab by clinician	PCR-based assay	High-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	5060	Small effect of midcycle specimens on highest HPV load
Van Ham et al ⁸	Four samples in one menstrual period by clinician with the Cervix brush (Rovers, Oss, The Netherlands)	SPF ₁₀ -PCR LiPA	25 different HPV types	20	Significantly ($P=.02$) higher rate of HPV positive samples in the follicular phase (7th to 11th day)
Wheeler et al ¹²	12 weekly vulvar and cervical swabs were taken by clinician as well as cervicovaginal saline lavages; 10 were used for evaluation	PCR and the ViraPap (Digene Diagnostics, Silver Spring, MD) DNA dot-blot assay	High-risk; 16, 18, 31, 33, 35, 39, 45, 51, 52, 59 Low-risk: 6, 11, 53, 54	72	No correlation between HPV DNA detection and phase of menstrual cycle was observed

HPV, human papillomavirus; PCR, polymerase chain reaction.



influence HPV detection and the mode of this potential influence needs to be explored. When OCP use and sample timing within the menstrual cycle do affect HPV detection, this may have implications in the future as HPV testing using self samples becomes an important tool in cervical cancer screening programs. We analyzed cervicovaginal self samples and questionnaires of a large population of young, un-screened women to estimate any potential influence from OCP use and sample timing within the menstrual cycle on HPV detection. Additionally, we investigated the HPV prevalence, incidence, and persistence in this group of young, un-screened women. Furthermore, we investigated the potential difference in the HPV prevalence, incidence, and persistence between OCP users and nonusers.

METHODS AND MATERIALS

Study Population and Study Design

This study is part of a 1-year prospective longitudinal study on HPV prevalence, clearance, and persistence performed in The Netherlands in 2007–2008 among 2,065 un-screened women 18–29 years of age.¹⁶ Participants were recruited through advertisements, posters, flyers, the Internet, and active recruitment sites. Exclusion criteria were: not being in the age range of 18–29 years, being pregnant, or not being fluent in Dutch. Of the 2,297 women who responded, 2,065 (90%) were eligible for participation and provided informed consent. A total of 253 (12.3%) women were excluded from further analyses from the 2,065 participants at study entry. These women became pregnant ($n=63$ [3.0%]), were vaccinated against HPV ($n=9$ [0.4%]), or were lost to follow-up ($n=181$ [8.8%]). This resulted in a final number of 1,812 (87.7%) participating women, of whom 1,703 (94.0%) reported to be sexually active at study entry. An additional 26 women became sexually active during the follow-up, ie, 13 every 6 months. Results of the sexually active women were used for further analyses.

Written informed consent was obtained from all participants. This study was approved by the local medical ethics committee.

All women received a questionnaire and a self sample kit by mail. The self sample kit contained an explanatory letter, an illustrated instruction form on how to perform the cervicovaginal self sample, a small brush in a sterile cover (Rovers Vibabrush, Rovers Medical Devices, Oss, The Netherlands), and a collection tube containing medium (SurePath, Tripath Imaging, Inc., Burlington, NC). To return the self-sample, a leakproof sealed bag, absorption sheet,

and a recloseable plastic return envelope (easyslider, Transposafe Systems Holland BV, Sassenheim, The Netherlands) were added, as described before.¹⁶ The accuracy of cervicovaginal self samples is highly comparable to physician-taken samples and it is inexpensive, feasible, and viewed by women as a convenient and acceptable method.^{17,18}

The questionnaire consisted of two parts. One part concerned demographic information and the other part included questions on sexual behavior as well as questions about current OCP use and the LMP. To ensure privacy, the questionnaires were provided with an anonymous code and all the data were entered in a computer program. The self samples were stored at room temperature and were sent to the Department of Medical Microbiology for processing and HPV detection.

Broad-spectrum HPV DNA amplification was performed using a short polymerase chain reaction fragment assay (SPF₁₀ HPV PCR, Laboratory Bio-Medical Products B.V., Rijswijk, The Netherlands) as described before.^{19,20} HPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, 55, 58, 66, 70, 74, and “X” are defined as low-risk types and HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68, 73, and 82 as high-risk types.

Data at study entry, month 0, were used for the analysis of HPV prevalence. The annual incidence of HPV was a result of the women acquiring a new HPV type at month 6 or month 12. Women positive for any HPV type or group (low-risk or high-risk HPV) at all three measuring points were defined as persistent.

To investigate whether there were any potential confounding factors influencing high-risk HPV, the data on demographics and sexual behavior at study entry were compared between the OCP users and nonusers. At study entry, 72.7% ($n=1,315$) of the 1,812 women were using OCPs.

During the follow-up period of 1 year, 61.1% ($n=1,108$) of the women were using OCPs during the whole year of follow-up and 20.9% ($n=378$) never used OCPs. This results in 18.0% ($n=326$) women changeably using OCPs. These changeable users were excluded from the analysis when comparing the incidence and persistence between OCP users and nonusers. Women who reported using the Nuvaring were registered as OCP users, because this creates local exogenous hormonal fluctuations comparable to OCP use.

The menstrual cycle was divided into the first half (days 1–14) and the second half (days 15–28) of the cycle to examine if the timing of sampling related to the LMP or oral contraceptive withdrawal bleeding influences the HPV detection. The cycle was divided into four weeks



(days 1–7, 8–14, 15–21, and 22–28) for further analysis of these results. The high-risk HPV detection rate in women on continuous OCP use, with a cycle of more than 28 days, was also analyzed.

Of the 5,436 samples (at three time points, 1,812 samples) taken during the follow-up period, 3,904 samples (71.8%) had data on the LMP and oral contraceptive withdrawal bleeding and were taken within 28 days or less of the menstrual cycle. Data on OCP use also needed to be available for the analysis of the combined effect of sample timing related to the LMP and oral contraceptive withdrawal bleeding and OCP use on high-risk HPV detection. This resulted in 3,893 (99.7%) self samples taken within 28 days or less of the menstrual cycle. Specified for OCP users and nonusers, there were 2,893 and 1,000 samples, respectively. From women on continuous OCP use, samples taken more than 28 days after the oral contraceptive withdrawal bleeding, 684 samples were available for analysis.

Analysis on demographic data and HPV prevalence, incidence, and persistence were performed using frequencies, cross tables, and chi square. Data on LMP and OCP use were calculated separately and combined. The binary logistic regression model was used as a univariable test to see if there was a potential confounder between OCP users and nonusers. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Of the 1,812 participants, 1,750 (96.6%) were of Dutch ethnicity with a mean age of 23.2 years (standard deviation 3.3), 335 (18.5%) women smoked, and 1,315 (72.7%) women used OCPs. In total, 1,703 (94.0%) women were sexually active at study entry. From these sexually active women, 356 (20.9%) had one lifetime partner, 809 (47.5%) had two to five, 347 (20.4%) had six to 10, 183 (10.7%) had more than 10 lifetime partners, and for eight (0.5%) women, no data were available on the number of lifetime partners. As shown in Table 2, there are no confounding factors within the demographic characteristics and data on sexual behavior that could have significantly influenced the odds ratio for high-risk HPV prevalence between OCP users and nonusers. Interestingly, this table shows that condom use did not differ significantly between OCP users and nonusers. Thus, condom use was not considered a confounding factor for high-risk HPV detection between these two groups.

No effect of the sampling period on high-risk HPV detection was found when analyzing the total group of women and all three sampling times together. However, a significant difference was found in

high-risk HPV detection with regard to the sampling time when analyzing OCP users (3,577 samples) and nonusers (1,000 samples) separately. High-risk HPV detection increased significantly in the second half of the cycle for the OCP users ($P=.021$), whereas a significant ($P=.007$) decrease in the high-risk HPV detection was observed in the second half of the menstrual cycle for the nonusers (data shown in Table 3).

After dividing the menstrual cycle into four sampling weeks, the decrease of high-risk HPV detection in nonusers remained significant ($P=.049$), although the increase of high-risk HPV detection during the contraceptive cycle became a nonsignificant linear trend ($P=.073$), as shown in Figure 1.

At study entry, HPV DNA was detected in 18.9% (95% CI 17.15–20.81) of all 1,812 women. For low-risk HPV and high-risk HPV, the prevalence was 8.9% (95% CI 7.61–10.29) and 11.8% (95% CI 10.31–13.33), respectively. No significant difference in HPV prevalence between OCP users and nonusers was found. Furthermore, the prevalence of low-risk HPV and high-risk HPV was also equally divided within the OCP users and nonusers, 9.1% (95% CI 7.62–10.81) compared with 8.1% (95% CI 5.84–10.84) and 11.7% (95% CI 10.02–13.57) compared with 11.9% (95% CI 9.20–15.10), respectively.

Of all women, 67.2% (95% CI 65.00–69.38) never had any type of HPV at time months 0, 6, and 12. This was 80.0% (95% CI 78.11–81.84) for low-risk HPV and 77.3% (95% CI 75.32–79.23) for high-risk HPV. Newly detected HPV at months 6 and 12 was 16.1% (95% CI 14.45–17.89) for any HPV type and 12.5% (95% CI 11.04–14.14) for low-risk HPV and 12.1% for (95% CI 10.67–13.73) for high-risk HPV. At months 0, 6, and 12, HPV DNA was persistently detected 8.7% (95% CI 7.41–10.06) of all women. In the year of follow-up. For low-risk HPV and high-risk HPV, this was 2.0% (95% CI 1.40–2.74) and 4.5% (95% CI 3.62–5.59), respectively. The results on HPV incidence and persistence did not significantly differ between OCP users and nonusers.

Concluding, OCP use did not influence HPV prevalence, incidence, or persistence. Additionally, sample timing related to the menstrual cycle had no significant effect on HPV detection in the total group. However, a significant relation between high-risk HPV detection and sample timing related to the menstrual cycle was found when separate analysis for OCP users and nonusers was performed. In the second half of the menstrual cycle, high-risk HPV detection decreased in nonusers ($P=.007$) and increased in OCP users ($P=.021$).



Table 2. Demographic Characteristics and Data on Sexual Behavior for Oral Contraceptive Pill Users and Nonusers

	n	OCP Users	n	Nonusers	OR (95% CI) of OCP Users for High-Risk HPV
Age (y)	1,315	22.8±3.1	495	24.5±3.2	1.13 (0.82–1.58)
Ethnicity					
Dutch	1,315	1,277 (97.1)	495	471 (95.2)	0.98 (0.71–1.35)
Other		38 (2.9)		24 (4.8)	
Current smoking					
Yes	1,308	231 (17.7)	494	104 (21.1)	1.01 (0.73–1.40)
No		1,077 (82.3)		390 (78.9)	
Living with parents					
Yes	1,309	264 (20.2)	492	52 (10.6)	1.02 (0.74–1.41)
No		1,045 (79.8)		440 (89.4)	
Relationship					
Married	1,311	43 (3.3)	494	35 (7.1)	0.87 (0.62–1.22)
Living together		294 (22.4)		123 (24.9)	
Couple, living apart		632 (48.2)		122 (24.7)	
Single		342 (26.1)		214 (43.3)	
Sexual activity ever					
Yes	1,314	1,274 (97.0)	494	428 (86.6)	0.89 (0.64–1.23)
No		40 (3.0)		66 (13.4)	
Age at first intercourse					
13 or younger	1,274	22 (1.7)	426	14 (3.3)	0.91 (0.66–1.27)
14–16		626 (49.2)		200 (46.9)	
17–19		524 (41.1)		173 (40.6)	
20 or older		102 (8.0)		39 (9.2)	
Lifetime sex partner(s)					
1	1,269	293 (23.1)	425	63 (14.8)	1.24 (0.88–1.75)
2–5		628 (49.5)		181 (42.6)	
6–10		240 (18.9)		106 (24.9)	
More than 10		108 (8.5)		75 (17.7)	
Gender of sex partner(s)					
Male	1,271	1,261 (95.7)	523	381 (90.1)	0.95 (0.68–1.33)
Female		2 (0.1)		3 (0.7)	
Both		53 (4.2)		39 (9.2)	
Sex partner(s) in past 6 mo					
0	1,268	98 (7.7)	428	56 (13.1)	0.94 (0.67–1.31)
1		990 (78.1)		301 (70.3)	
2		123 (9.7)		46 (10.7)	
More than 2		57 (4.5)		25 (5.9)	
Sexual contact in past 6 mo (frequency)					
0	1,234	83 (6.7)	420	50 (11.9)	0.98 (0.71–1.40)
1–6		127 (10.3)		75 (17.9)	
7–24		140 (11.3)		59 (14.0)	
25–54		502 (40.7)		138 (32.9)	
More than 54		382 (31.0)		98 (23.3)	
Ever been diagnosed with a STI?					
No	1,273	1,168 (91.8)	425	365 (85.9)	1.00 (0.71–1.38)
Yes		105 (8.2)		60 (14.1)	
Condom use					
Never (0%)	1,270	639 (50.3)	423	159 (37.6)	0.88 (0.63–1.24)
Sometimes (0–50%)		351 (27.6)		93 (22.0)	
Most of the time (50–100%)		198 (15.6)		88 (20.8)	
Always (100%)		82 (6.5)		83 (19.6)	
Sexual age (y)	1,273	6.1±3.3	526	8.1±3.4	1.06 (0.75–1.49)

OCP, oral contraceptive pill; OR, odds ratio; CI, confidence interval; HPV, human papillomavirus; STI, sexually transmitted infection. Data are n, mean±standard deviation, or n (%) unless otherwise specified.



Table 3. High-Risk Human Papillomavirus Prevalence in Sample Moments of the Total Group and for Oral Contraceptive Pill Users and Nonusers Divided by Last Menstrual Period Sample Moments

Day of Last Menstrual Period or Day After Start of Last OCP Withdraw Bleeding	OCP Users (n=3,577)	Nonusers (n=1,000)
1–14	159/1,378 (11.5%)	65/500 (13.0%)
15–28	219/1,515 (14.5%)	39/500 (7.8%)
More than 28	75/684 (11.0%)	NA
<i>P</i>	.021	.007

OCP, oral contraceptive pill; NA, not applicable.

Menstrual cycle is divided into the first and second half for nonusers. The OCP cycle is divided into the first and second half of the cycle with an extra category added (more than 28 days) for the continuous OCP users.

DISCUSSION

The findings of this longitudinal study among un-screened, young women support the idea that reproductive hormones may influence high-risk HPV detection. In women with a natural menstrual cycle, high-risk HPV detection decreased with the duration of the cycle, whereas in OCP users, the high-risk HPV detection increased during the second half of the OCP cycle, as depicted in Figure 1. This opposite trend in high-risk HPV detection during the menstrual cycle between OCP users and nonusers explains why high-risk HPV detection in the total study population was not significantly influenced by the sample timing within the menstrual cycle. Another large study investigating high-risk HPV detection within the menstrual cycle showed a small midcycle increase of HPV detection.⁹ However, data on OCP use are not reported and therefore it is difficult to

compare this study with our results. A smaller study by our group, in nonusers, found a higher rate of HPV-positive samples in the follicular phase. Because the follicular phase is in the first half of the menstrual cycle, these data are similar to our findings in the nonusers.⁸ This may have implications in the future, when high-risk HPV detection may become a primary screening tool in cervical cancer prevention.

An explanation for the fluctuation in high-risk HPV detection in the natural menstrual cycle may be found in the changes in the mucosal immunity. The mucosal immunity is influenced by reproductive hormones and reaches the lowest level around ovulation in the natural menstrual cycle.^{13–15} A lower mucosal immunity may lead to an increase of HPV replication and therefore HPV detection. However, there is no direct evidence for this assumption in the literature. We hypothesize that there may be some delay between the actual fluctuations in the mucosal immunity and the level of high-risk HPV detection. The higher mucosal immunity in the follicular phase may lead to the decrease of high-risk HPV detection in the second half of the natural menstrual cycle, whereas the lower mucosal immunity during ovulation may induce increased high-risk HPV detection in the first half of the next menstrual cycle. According to this hypothesis, the increase of high-risk HPV detection shown during the second half of the OCP cycle may be a delayed effect of the lower mucosal immunity in the week OCP use is stopped. To illustrate this hypothesis, we used the results on the mucosal immunity (IgG and IgA levels) as previously shown by Franklin et al¹³ and displayed them in Figure 2. As shown in this figure, the mucosal immunity shows opposite patterns for OCP users and nonusers.

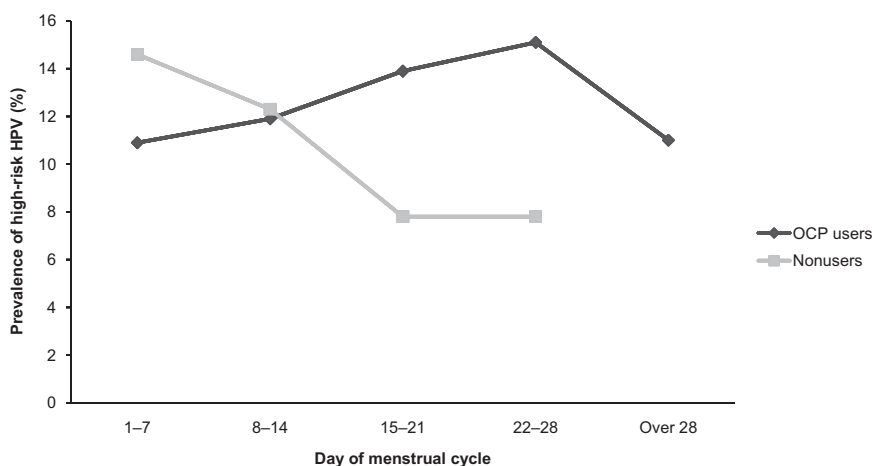


Fig. 1. Human papillomavirus detection for oral contraceptive pill users and nonusers with respect to the last menstrual period and withdrawal bleeding. Menstrual cycle is divided in 4 weeks for nonusers; the decrease of high-risk human papillomavirus (HPV) detection in the second half of the menstrual cycle is significant ($P=.049$). The increase of high-risk HPV detection in the second half of contraceptive cycle lost significance ($P=.073$). OCP, oral contraceptive pill.

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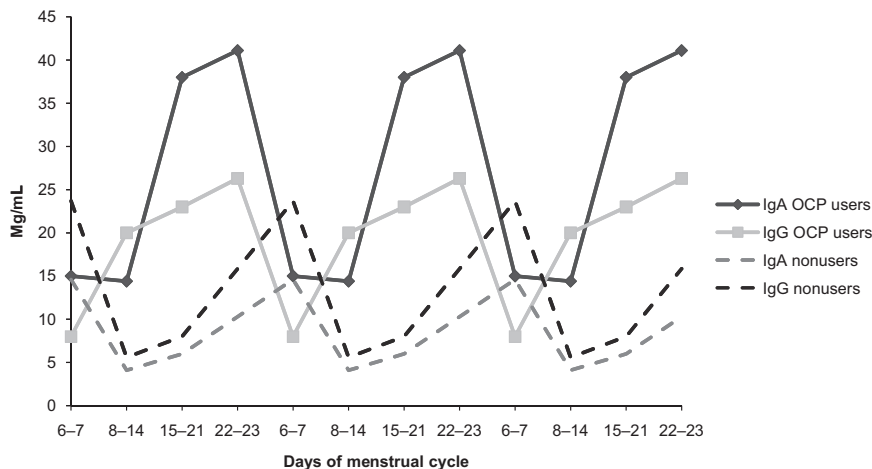


Fig. 2. Fluctuation of the immunoglobulin (Ig) A and IgG levels during the menstrual cycle. This figure displays the weeks of three menstrual cycles to visualize the fluctuations. OCP, oral contraceptive pill. Based on data from Franklin RD, Kutteh WH. Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. *J Reprod Immunol* 1999;42: 93–106.

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Another possible explanation for the increase of high-risk HPV detection in the second half of the OCP cycle may be a result of the positive influence of estrogens on the viral transcription and the expression of the viral oncogenes (E6 and E7).²¹ However, these two explanations do not explain the decrease of high-risk HPV detection in women on continuous OCP use. Perhaps the continuous positive stimulation of OCP on the mucosal immunity and the influence of the estrogens on the viral oncogenes balance each other after several weeks.

The lack of evidence for an association between OCP use and high-risk HPV prevalence is confirmed in several other studies. In some of these studies, only the median number of sex partners seemed to influence the high-risk HPV prevalence significantly.^{7,16,22–25} Additionally, studies that did show a significant association between high-risk HPV prevalence and OCP use suggested a greater exposure to HPV resulting from a presumed association between OCP use and sexual behavior.^{26–28} Because we did not detect a difference in sexual behavior between the groups of OCP users and nonusers, sexual behavior was not a confounding factor in this study.

Mouse models have shown that exogenous estrogens play an essential role in the carcinogenesis of cervical cancer. The estrogens stimulate the viral oncoproteins E6 and E7 and the coexpression of these oncoproteins contributes to the development of cervical cancer.²⁹ Additionally, they have shown that estrogens, besides their role in the development of cervical lesions, also contribute in the persistence and continued development of cervical cancer.³⁰ Because epidemiological studies support the carcinogenic effect of OCP use, it does not prove that OCPs influence high-risk HPV prevalence per se.^{5,7,16,31} The role

of OCPs in carcinogenesis might be explained by a possible role in facilitating HPV reactivation or persistence.^{1,24,30,32} Exogenous hormones enhance HPV transcription and decrease HPV clearance in women, which may lead to more persistent high-risk HPV infections resulting in cervical neoplasia and cervical cancer.^{4,33}

Despite that HPV persistence is expected to be higher among OCP users, we did not find any significant difference in HPV incidence and persistence between OCP users and nonusers. This finding is supported by others and may be explained by the short duration of follow-up (1 year) or a lack of data on OCP use in our study.^{24,34–36} Because the questionnaire only asked about current OCP use, data on previous use for nonusers or the duration of use for OCP users were not available. Because a longer duration of OCP use is associated with an increased risk of cervical cancer, the lack of data on the duration of OCP use might be a caveat in this study.^{37,38} This relatively young group of women may have a shorter duration of OCP use compared with women with cervical abnormalities. However, the increased risk with the duration of OCP use is relative, because the risk is reversible 10 years after use has stopped.^{4,38}

In conclusion, high-risk HPV detection measured with the highly sensitive SPF₁₀ Lipa is influenced by the sampling period related to the LMP or OCP withdraw bleeding. Further studies need to investigate whether this effect remains with a less sensitive test to elucidate the clinical implications of HPV detection in primary cervical cancer screening. Because OCP use does not significantly influence HPV prevalence, incidence, or persistence, its increased risk for cervical cancer may be explained by a direct hormonal effect on the carcinogenesis.



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