R E V I E W

Hormonal contraception and HPV: a tale of differing and overlapping mechanisms

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Abstract: Hormonal contraceptive use is an identified co-factor that modifies cervical cancer risk. The mechanisms by which sex steroid hormones affect the multi-stage natural history of human papillomavirus (HPV) infection and cervical carcinogenesis are still unclear, with no consistent evidence in support of a single biological hypothesis. Understanding the means by which hormonal contraception affects HPV infection and cervical cancer risk may provide critical information to guide future secondary interventions for cancer prevention.

Keywords: hormones, human papillomavirus, cervical cancer

Introduction

Cervical cancer is the second most common cancer among women worldwide, with an estimated 490,000 cases and 250,000 deaths each year.[1] High-risk types of anogenital human papillomavirus (HPV), particularly HPV 16 and 18, have been identified as the cause of cervical carcinogenesis and are associated with other anogenital (eg, penile, vaginal, and anal) and non-anogenital (eg, oropharyngeal and tonsillar) cancers.[2,3] Since their introduction in the late 1960s, the use of hormonal contraceptives that contain either combined estrogen and progesterone (ie, combined oral contraception, COC) or progesterone-only formulations, such as depotmedroxyprogesterone acetate (Depo-Provera; DMPA), has been linked to an increased risk of cervical cancer and is currently considered a co-factor in cervical carcinogenesis.[4]

Long-term use of hormonal contraception is associated with cervical cancer diagnosis in a number of case–control studies which have been analyzed in a series of pooled analyses (Table 1). Large multicenter studies conducted by the International Agency of Research on Cancer (IARC), comprising over 1500 cervical cancer cases and 200 controls, all of whom were HPV positive, identified a nearly 3-fold increase in the risk of cancer diagnosis among women reporting >5 years of COC use.[5] This increased risk was observed for a period of up to 5 years after cessation of use, with a decreasing risk observed with increasing time since cessation of use (ie, a recency effect). This association was replicated in a pooled analysis of additional case–control and cross-sectional studies.[6–8] A similar increased disease risk has also been observed among women reporting long-term use of progesterone-only contraceptives, eg, depotmedroxyprogesterone acetate (DMPA).[6,7] The strength of the association of DMPA use with disease, however, was smaller than the association with COC, suggesting an important role for estrogens in disease risk.

Table 1 Summary of studies examining the relationship between hormonal contraceptive use and cervical cancer risk

Author	Age of study population	Study design	Number of subjects (studies[a])	Number of disease outcomes	Hormonal contraceptive measured	Contraceptive metric	Result
Moreno[5]	>35	CC	1931	1465 (ICC) 211 (ISC)	COC	Ever/never; duration of use; recency	Positive association of long-term COC use with CxCa diagnosis Decreasing risk with increasing time since last use
Appleby[6]	NR	pool	52,082 (24 studies)	16,573 (ICC)	COC	Ever/never; duration of use; recency	Positive association of long-term COC use with CxCa diagnosis among 24 studies Decreasing risk with increasing time since last use
Smith[7]	NR	pool	25 studies	12,531	COC	Ever/never; duration of use; recency	Long-term use of combined oral contraception associated with CxCa diagnosis Decreasing risk with increasing time since last use
ICoESoC[8]	NR	pool	35,916 (12 studies)	8097 (SCC) 1374 (ADC)	COC	Ever/never; duration of use; recency	Per year increase in COC use associated with an increased risk of SCC and ADC Decreasing risk with increasing time since last use
Appleby[6]	NR	pool	3 studies	16,573 (ICC)	DMPA	Ever/never; duration of use; recency	Long-term use of DMPA associated with an increase risk of CxCa Decreasing risk with increasing time since last use
Smith[7]	NR	pool	3 studies	12,531	DMPA	Ever/never; duration of use; recency	Long-term use of DMPA marginally associated with an increase risk of CxCa Decreasing risk with increasing time since last use

Note: [a]For meta-analyses or studies involving pooled data.
Abbreviations: CxCa, cervical cancer; ICC, invasive cervical cancer; ISC, in situ carcinoma; SCC, squamous cell carcinoma; ADC, adenocarcinoma; COC, combined oral contraceptive; DMPA, depotmedroxyprogesterone acetate; NR, not reported; pool, pooled studies.

These case–control studies are limited by their inability to distinguish how and when hormonal contraceptives increase cervical cancer risk. Hormonal contraceptives may affect risk at any point in the 10- to 20-year natural history of cervical cancer, yet case–control designs cannot allow investigation of the temporal effects of exposure on upstream transitions. Prospective and case–control observational studies that test associations at multiple early transitions in the course of cervical cancer development may provide corollary evidence addressing alternative mechanisms by which hormonal contraceptives facilitate carcinogenesis.

Several hypotheses have been proposed to explain the observed relationship between hormonal contraceptive use, HPV, and cervical cancer risk. These hypotheses include: (1) sex steroid hormones mediate cervical ectopy leading to enhanced acquisition of HPV, (2) sex steroid hormones modulate the host immune response to HPV, facilitating viral persistence and development of pre-cancerous lesions, and (3) sex steroid hormones increase risk of development of pre-cancerous lesions and progression to invasive cancer. We review the observational and experimental data addressing these alternative hypotheses to provide a framework by which to evaluate the collective evidence for the role of hormonal contraceptives on HPV and cervical cancer.

Hypothesis 1: sex steroid hormones contribute to cervical ectopy and increase acquisition of HPV

In many populations, women using oral contraceptives may be more sexually active and have more risky sexual behavior (eg, do not use condoms) than women not using hormonal contraception.[9,10] Because of increased sexual risk, it is reasonable to consider that hormonal contraceptive users are more likely to acquire HPV infection and, thus, have an

increased risk of cervical cancer – such that the associations observed between hormonal contraceptive use and cervical cancer are explained by residual confounding variables. However, analyses that were restricted to HPV positive cases and controls only, report a similarly elevated risk of cervical cancer among long duration oral contraceptive users. Thus, residual confounding is unlikely to explain the majority of the increased risk.

Because hormonal contraception has been shown to maintain the presence of columnar epithelial cells in the ectocervical region as well as in the transformation zone in a condition known as cervical ectopy,[11–14] others have hypothesized that hormone contraceptive users are more biologically susceptible to HPV infection given exposure.[4] As reviewed below, while animal models show some support for an increased susceptibility to infection in the ectopic cervix, the observational associations between hormonal contraceptive use, ectopy, and HPV persistence/incidence are less clear.

Murine models of HPV pseudovirus infection: role of progesterone

Mouse models of HPV infection have been developed to identify putative receptors and mechanisms responsible for HPV viral particle entry. In these models, HPV pseudoviral particles are used for experimental infection, and represent fully assembled capsid protein, comprising the two viral structural proteins, L1 and L2, coupled to a fluorescent reporter dye, allowing in vivo tracking of the location of the pseudovirus through infection in the basal epithelium.[15] In these models, experimental animals are treated with DMPA for at least 4 days to thin the squamous epithelium and reduce variability of the epithelium across the menstrual cycle to allow for increased uptake of pseudovirus particles, supporting a hormone-dependent increase in infection susceptibility.[16] Because no change in the maturity state of the cervix or position of the transformation zone has been observed among women using progesterone-only contraceptives, whether the effects of progesterone on virus entry occur in humans remains to be demonstrated.[17,18]

Association of COC use or ectopy and HPV prevalence

Current and long-term use of COCs (>6 years) has been associated with prevalent HPV infection in cross-sectional studies[19] (Table 2). This association was more likely to be observed among women <35 years of age[20–26] than among older women.[5,27,28] Current and previous use of DMPA for at least 1 year was observed in two studies to be associated with

a higher prevalence of HPV among women at or around the age of 30 years.[29,30] Because contraceptive users may engage in riskier sexual behaviors more frequently than non-users, these studies carefully controlled for these behavioral differences, as well as for vaginal cytological abnormalities. Of the three cross-sectional studies that directly assessed ectopy and prevalence of HPV infection, only one showed a significant positive association.[31] Interestingly, hormonal contraceptive use in this study was not associated with increased HPV prevalence, although the authors failed to make a distinction by type of contraceptive method utilized. The remaining two studies either failed to see a significant association[32] or observed an inverse association of ectopy and HPV prevalence.[33]

Association of COC use with new detection of HPV

Combined oral contraception has been the primary contraceptive method explored in relation to new detection of HPV (Table 3). Among college-aged women with 4 years of follow-up, as well as among women 20–29 years of age enrolled in a population-based study with 2 years of follow-up, current and long-term use of COC was positively associated with new detection of HPV, which was significantly attenuated after adjustment for sexual behavior.[34,35] Conversely, among adolescent women recruited from STD clinics with 2 years of follow-up, women who used COCs were at a reduced risk of developing new HPV infections.[36] Another study of women 30–33 years of age reported no significant association of hormonal contraceptive use and HPV infection after adjustment for sexual behavior.[37] Recently, we explored the association between new HPV detection and hormonal contraceptive use in a large prospective study conducted in Zimbabwe, Uganda, and Thailand that was designed to evaluate the association between hormonal contraceptive use and HIV acquisition.[38] We restricted the HPV substudy to the 1135 women enrolled in Thailand which had negligible HIV seroconversion during follow-up, to enhance the generalizability of our results. In this study which purposefully sampled an equal number of non-hormonal contraceptive users, long-term COC users, and long-term DMPA users, the increased risk of new HPV detection among COC users observed in univariate analysis was significantly attenuated after adjustment for sexual risk behavior.[39] In addition, we found no association between cervical ectopy and new detection of HPV in this study.

The relatively large effect of sexual risk on attenuating the strength and significance of the association of COC use with new detection of HPV suggests that differences in the

Table 2 Summary of studies examining the association between hormonal contraceptives and HPV prevalence

Author	Mean age of study population (range)	Study design	Number of subjects	Hormonal contraceptive assessed	Contraceptive metric	Result
Burk[20]	20.4	X	604	COC	Current use	Current COC users had an increased risk of HPV infection
Kjaer[21]	20–29	X	1000	COC	Duration	Users for >5 years increased risk of HPV infection
Ley[22]	22.9	X	467	COC	Ever use; duration	Current, past, short (1 year) and long term use (>6 years) associated with HPV infection
Pham[25]	15–65	X	922	COC	Current; past use	Current use associated with HPV infection
Rousseau[26]	33.3	CC	729	COC	Duration	Short (<6) and long term (>6) duration associated with HR-HPV and HPV 16 infection
Kotloff[24]	22.5	X	414	COC	Current use	Current use marginally associated with HPV
Marks[23]	29.6	X	1070	COC	Current use; duration; ever use	Use for >6 years associated with an increased risk of HPV
Molano[28]	>35	X	1859	COC	Ever use; current use	Positive association among current users (LR HPV types only)
Vaccarella[27]	>35	X	15,145	COC	Current use; duration	No association
Moreno[5]	>35	CC (controls)	1916	COC	Ever use; duration	No association
Harris[29]	18–50	CC	724	DMPA	Recent use; duration of use	>1 year of DMPA use associated with an increased risk of HR-HPV infection
Giuliano[30]	33.1	X	2319	DMPA	Current vs former use	Current use associated with an increased risk of HR-HPV infection
Marks[23]	29.6	X	1070	DMPA	Current use; duration; ever use	No association with current or prior use

Notes: Light gray = null association, white = positive association.
Abbreviations: COC, combined oral contraception; DMPA, depomedroxyprogesterone acetate; HR-HPV, high-risk human papillomavirus; HPV, human papillomavirus; X, cross-sectional; CC, case–control.

sexual practices of hormonal contraceptive users may be driving the univariate associations. Furthermore, the residual positive association observed after adjustment, albeit small, may be a result of unmeasured sexual behaviors and not a result of the biological influence of COC use on facilitating new detection of HPV.

Hypothesis 2: sex steroid hormones modulate the host immune response to HPV, facilitating viral persistence and development of pre-cancerous lesions

If the higher prevalence of HPV among current and long-term hormonal contraceptive users does not appear to be explained by an increased risk of HPV acquisition, it is possible that hormonal contraceptive use leads to an increased risk of persistent infection. Viral persistence is a key risk factor for high grade HPV disease and cervical cancer and appears to be associated with deficiencies in the CD4+ and CD8+ cytotoxic T lymphocyte (CTL) response.[40–42] Women with persistent HPV infection and cervical lesions have reduced in vitro lymphoproliferation responses to immunological stimulants (eg, phytohemagglutinin mitogen or HPV-specific antigens), lower circulating IFN-γ, higher IL-10, and enhanced regulatory T-cell function, as represented by detection of *Foxp3* mRNA transcripts.[43,44] Sex steroid hormones can directly alter immune cell function by binding to intracellular receptors and altering transcription of immune-mediated genes,[45,46] resulting

Table 3 Summary of studies evaluating the association between hormonal contraceptive use and new detection of HPV

Author	Mean age of study population (range)	Number of subjects	Mean length of follow-up (sampling frequency)	Hormonal contraceptive assessed	Contraceptive metric	Result
Moscicki[36]	20	105	2 years (6 months)	COC	Current use at FU	COC users less likely to have an incident HPV infection after adjustment for sexual behavior
Nielsen[34]	20–29	5448	2 years (2 years)	COC	Current use at FU; duration	Positive, marginal increase among current user and user for >6 years associated after adjustment for sexual behavior
Sellors[37]	33	307	1 year (12 months)	COC	Current use at FU	No association
Winer[35]	19	444	4 years (4 months)	COC	Current use at FU	Positive, marginal association after adjustment for sexual behavior
Marks[39]	29.6	1135	18 months (6 months)	COC/DMPA	Current use at FU	Positive, non-significant association after adjustment for sexual behavior

Notes: Light gray = null association, white = positive association, black = negative association.
Abbreviations: COC, combined oral contraceptive; DMPA, depotmedroxyprogesterone acetate; FU, follow-up.

in pronounced effects on the course of infectious diseases.[47–50] Therefore, use of hormonal contraceptives may compromise protective immunity against HPV, leading to a reduced rate of HPV clearance and increased rate persistence.

Sex steroid hormones and host immunity

The effects of sex steroid hormones such as estradiol and progesterone on host immunity have been evaluated using both in vitro model systems and direct measures in women using exogenous hormones. Higher estradiol and progesterone serum concentrations in pregnant women correlate with higher concentrations of IgG, IgA, and IL-1β in the cervical mucus collected during each trimester, suggesting that changes in sex steroid hormones can alter the cervical immunological microenvironment.[51] Functional differences in T-cell response have been reported in pregnant versus non-pregnant women, and in exogenous hormone users relative to non-users. Peripheral T-cells isolated from pregnant women produce more anti-inflammatory cytokines, including IL-4 and IL-10, than do cells from non-pregnant women.[52] Women taking hormone replacement therapy (estradiol alone or combined with progesterone) had lower concentrations of IL-1β,[53] TNF-α,[53,54] IFN-γ,[55,56] IL-6,[54,57] and IL-2[55] reduced natural killer cell activity,[58,59] and higher concentrations of IL-1ra[60] and total immunoglobulin[61] in cultured peripheral blood mononuclear cells. The differences in the concentrations of circulating sex steroid hormones in pregnancy

and among exogenous hormone users limit the ability to draw any conclusions about the mechanistic influence of estradiol and progesterone on the immune system. In fact, estradiol has been reported to have qualitatively different effects on peripheral blood mononuclear cells (PBMCs) according to concentration used. At concentrations simulating pregnancy levels (10^{-7} M), estradiol reduces TNF-α[53,54,62–64] and IL-1β,[53,65–67] induces the production of polyclonal antibodies,[68,69] and upregulates somatic hypermutation and class-switch recombination in B-cells.[70] Estradiol at pregnancy-level concentrations also induces IL-10 production[64,68,69,71] and increases in CD4+CD25+ regulatory T cell function.[72,73] At lower concentrations relevant to the menstrual cycle among non-pregnant women (10^{-8} M–10^{-10} M), estradiol increases production of IL-1β, TNF-α, IFN-γ, and IL-12.[62,66,67,71,74] Lastly, we found that estradiol and progesterone alone, in combination at concentrations relevant to COC use (E2: 10^{-8} M; P4: 10^{-6} M, or 10^{-10} M), increased the concentration of IL-10, TGF-β, and FoxP3 and decreased the concentration of IFN-γ, TNF-α, and IL-12p70 in PBMCs isolated from healthy donor women stimulated with HPV 16 VLP in vitro.[75]

Although experimental data on the effects of progesterone are scarce, it is regarded primarily as immunosuppressive. Progesterone at pregnancy concentrations suppresses regulatory T cell function and increases production of IL-4 and IL-5 in vitro.[76,77] Lastly, in mouse models of herpes simplex type-2

(HSV-2) infection, progesterone has been shown to alter mucosal immune responses including suppression of local immunoglobulin production in response to vaginal HSV-2 challenge, leading to enhanced susceptibility of infection as well as enhanced inflammation and pathology and mortality.[78]

Sex steroid hormones and HPV persistence

Persistence of HPV is a pivotal step in the natural history of cervical cancer (Table 4).[79] The association between current use of COCs or DMPA and HPV persistence is highly variable across study populations. Three studies, two conducted among college age women with 2[80] and 10 years[81] of follow-up and another among early adolescent females[82] attending inner-city family planning clinics with 2 years of follow-up showed no association between current COC use and HPV persistence. A population-based study conducted among 20- to 29-year-old women in Denmark with up to

2 years of follow-up observed an association of current COC use with an increased risk of HPV persistence.[83] We observed similar associations in our prospective study of women aged 20–30 years recruited from family-planning clinics in Thailand who reported longer term use of hormonal contraception.[39] Among women >30 years of age, a large prospective study of 1700 individuals from Colombia with more than 6 years of follow-up reported no association of COC use with HPV persistence.[84,85] Conversely, DMPA use was associated with an increased risk of HPV clearance in a single study conducted in a triage population of young women with mild cervical abnormalities.[86]

The elevated risk of HPV persistence among COC users in population-based studies of women 20–30 years of age or women accessing family planning services suggests that, similar to measurement of the effects of hormonal contraception on invasive cancer risk, duration of exposure to exogenous

Table 4 Summary of studies examining the relationship between hormonal contraceptives and HPV persistence

Author	Age of study population (range)	Number of subjects	Infection types included	Mean length of FU (sampling frequency)	Hormonal contraceptive assessed	Contraceptive metric	Result
Maucort-Boulch[86]	25	2408	Both	2 years (6 months)	COC	Current use at enrollment	No association with COC use
Molano[85]	29	227	Prevalent	6 years (6 months)	COC	Ever/never	Ever use was positively, marginally associated with HPV viral clearance
Munoz[84]	>35	2139	Both	9 years (6 months)	COC	Ever/never	No association
Nielsen[83]	20–29	1166	Prevalent	2 years (2 years)	COC	Current/former	Current use of COCs associated with HPV persistence
Richardson[80]	17–27	327	Both	2 years (6 months)	COC	Current use; duration of use	No association
Shew[82]	15	49	Both	2 years (3 months)	COC	Current use	No association
Sycuro[81]	19	147	Incident	10 years (10 years[a])	COC	Current use at long-term FU visit	No association
Marks[39]	29.6	1135	Both	18 months (6 months)	COC	Current use at FU	Current use of COCs associated with HPV persistence
Maucort-Boulch[86]	25	2408	Both	2 years (6 months)	DMPA	Current use at enrollment	DMPA associated with HPV clearance
Marks[39]	29.6	1135	Both	18 months (6 months)	DMPA	Current use at FU	No association

Notes: [a]Since end of initial study period which had 4 years of FU with 6-month sampling; light gray = null association, white = positive association.
Abbreviations: COC, combined oral contraception; DMPA, depotmedroxyprogesterone acetate (Depo-Provera); FU, follow-up; HPV, human papillomavirus.

hormones is an important metric. If this is the case, the lack of association between COC use and HPV persistence in studies conducted among adolescent and college-aged women could be explained by a selection bias, where study participants may not have accrued adequate exposure time since sexual debut to observe a longer term effect on persistent infection. The possible impact of duration of hormone use reinforces the need to measure prior and current use of hormonal contraception in order to stratify study subjects by their estimated cumulative exposure.

Hypothesis 3: sex steroid hormones increase risk of development of pre-cancerous lesion and progression to invasive cancer

In vitro exposure to sex steroids alters E6/E7 HPV gene expression

Interpretation of the observational studies assessing the relationship of sex steroid hormones on HPV-related disease endpoints have been guided primarily by mechanistic hypotheses that sex steroids increase carcinogenic potential through interaction with either the virus or host. Cell culture models utilizing immortalized epithelial-derived cells have been the most efficient system to assess the effects of estradiol and progesterone on viral factors (Table 5). The effect of estradiol and progesterone on HPV transcription has been evaluated in epithelial cell lines immortalized through transfection with whole genomes of HPV 16 or 18, or with high-risk E6/E7 oncogenes alone. Estradiol, at concentrations similar to late-term pregnancy (10^{-6} M or 10^{-7} M), increases the expression of HPV 16 E6/E7 transcripts in HPV containing SiHa and CaSki cell lines.[87–90] Estradiol also increases cellular proliferation and reduces apoptotic potential in both SiHa and CaSki cell lines.[90,91] Exposure of SiHa and CaSki cells to progesterone at concentrations consistent with those found during late-term pregnancy (10^{-6} M or 10^{-7} M) also increases HPV gene expression and cell proliferation.[87,89] Treatment of these cell lines with progesterone and estrogen

Table 5 Summary of studies evaluating the effects of sex steroid hormones on HPV in vitro

Author	Experimental model	Hormone treatment [C]	Result
Mitrani-Rosenbaum[88]	SiHa cells (HPV 16)	17β-estradiol [10^{-6} M]	Estrogen stimulates HPV 16 transcript expression. Estrogen response elements identified on HPV 16 genome
Hwang[92]	SFR (CaSki derivative) (HPV 16)	Tamoxifen [10^{-9} thru 10^{-11} M]	Tamoxifen induces HPV 16 mRNA expression and E7 expression
Chen[87]	HeLa cells w/plasmids containing LCR for HPV 16 and 18 (HPV 18)	17β-estradiol [10^{-7} M] progesterone [10^{-7} M]	Both sex steroid hormones as well as derivatives increased HPV 16 LCR expression in the CAT reporter assay system
Kim[91]	HeLa (HPV 18), CaSki (HPV 16), C33a (control)	17β-estradiol [10^{-6} M]	HeLa and CaSki were stimulated by estradiol at [10^{-6} M] and [10^{-7} M] Estradiol suppressed HeLa proliferation
Ruutu[90]	CaSki and SiHa (HPV 16)	17β-estradiol; progesterone; tamoxifen; RU486	No effect of hormone treatment on HPV 16 E6/E7 expression Progesterone increased cell proliferation in both cell lines while 17β-estradiol in SiHa only 17β-estradiol protected CaSki cells from apoptosis while progesterone enhanced apoptosis in CaSki cells RU486 abrogated progesterone effects while tamoxifen had no effect on estrogen
Mittal[89]	SiHa cells (HPV 16)	Progesterone [10^{-6} M]	Progesterone increased HPV 16 E6/E7 mRNA expression
Marks[75]	Human PBMCs	E2 [10^{-8} M] P4 [10^{-6} M/10^{-10} M]	Upon stimulation with HPV 16 VLP single and combined treatments of E2 and P4 resulted in: 1) Decrease [c] of IL-12p70, TNF-α, and IFN-γ 2) Increased [c] of IL-10, IL-6, IL-1ra, TGF-β, and Foxp3

Abbreviation: PBMCs, peripheral blood mononuclear cells.

antagonists, including RU486 and tamoxifen, respectively, has shown differential effects. Although RU486 was effective at reversing progesterone-induced HPV gene expression and cellular proliferation, tamoxifen was significantly less effective.[89,90] At low concentrations, tamoxifen stimulated growth of CaSki cells, and induced HPV 16 E7 gene expression.[91,92] These data illustrate the complexities of the relationship between sex steroid hormone signaling.

Transgenic murine model of cervical carcinogenesis reveals direct effects of sex steroids on carcinogenesis

Transgenic mouse models that contain HPV 16 E6/E7 genes under the control of a keratin promoter (K14-HPV16E6/E7) have been useful in identifying a potential role of estradiol on host-mediated cervical carcinogenesis (Table 6). The development of cervical tumors in these mice is dependent on treatment with estradiol for at least 6 months and the presence of HPV 16 E6.[93–95] The dose of estradiol is positively correlated with tumor size and cessation of estradiol treatment after 6 months leads to tumor regression.[93] Interestingly, estradiol exposure does not appear to be associated with increasing HPV 16 mRNA oncogene expression as measured using in situ hybridization.[95] Treatment of these mice with ICI 182,780, an estrogen receptor antagonist, or raloxifene,

a selective estrogen receptor modulator, is highly effective at clearing tumors and precursor lesions.[96] However, it is important to consider that these experimental models focus primarily on the impact of estradiol alone (without progesterone) on HPV oncogene-associated carcinogenesis and do not explore the effects of sex steroids on the host immune responses to tumor surveillance and/or infection.

Effect of sex steroid hormones on increased risk of cervical intraepithelial neoplasia grade 2 or 3

The association of hormonal contraceptive use on the development of cancerous precursor lesions such as high grade squamous intraepithelial lesions or cervical intraepithelial neoplasia grade 2 or 3 (CIN 2/3) among women with pre-existing HR-HPV infections has been inconsistent across studies (Table 7). Prospective studies that followed HR-HPV positive women between the ages of 20–30 without evidence of prevalent cervical disease for development of CIN 2/3 have been unable to identify a consistent positive association with COC use, but did observe a positive association with CIN 2/3 among users of DMPA.[97,98] Among adolescent women diagnosed with CIN 2/3, current use of COC was observed to reduce the likelihood of lesion regression.[99] Case–control studies that included HR-HPV positive women with histologically confirmed CIN 2/3 compared with age- and

Table 6 Summary of the effects of estradiol on HPV-mediated cervical carcinogensis in mice

Author	Experimental model	Hormone treatment[a]	Result
Shai[94]	K14E6/E7 transgenic mice	17β-estradiol for 9 months (0.05 mg/60 days)	E6 alone (without E7) cooperates with 17β-estradiol to induce lesions after 9 months
Arbeit[95]	K14-E6/E7 transgenic mice	17β-estradiol for 6 months (0.25–0.72 mg/60 days)	Estradiol increases development of dysplasia and hyperplasia only among mice with HPV 16 E6/E7 transgenes HPV 16 E6/E7 mRNA expression was not affected by estradiol trt
Chung[96]	K14-E6/E7 transgenic mice	17β-estradiol for 6 months (0.05 mg/60 days)	Treatment of mice with ICI, an estrogen receptor antagonist and raloxifene, a selective ER modulator, cleared cancer and precursors of the cervix and vagina ICI was capable of preventing progression of lesions
Brake[93]	K14E6/E7 transgenic mice	17β-estradiol for 6 months (0.05 mg/60 days)	Estrogen treatment induced tumor formation in transgenic mice Tumor number and size was positively correlated with the amount of exposure to estrogen Removal of estrogen exposure after 6 months led to tumor regression

Note: [a]17β-estradiol administered subcutaneously as a pellet every 60 days.
Abbreviations: ICI, ICI 182,780; ER, estrogen receptor; HPV, human papillomavirus.

HPV-matched controls also showed inconsistent associations between CIN 2/3 and hormonal contraceptive use.[29,100–103] A single case–control study conducted in Costa Rica that included women with both high grade cervical lesions and invasive disease identified an association between use of COCs for >5 years and CIN 2/3 diagnosis; this association, however, was observed only among women with 3 or more pregnancies.[102]

Although the observational and experimental data suggest a distinct role of sex steroids on HPV-associated carcinogenic processes, limitations in the experimental design make it difficult to rule out a role of hormonal contraception on other endpoints in the natural history of HPV, particularly those related to HPV infection and tumor surveillance prior to the development of cancer. The cell culture and animal models showing sex steroid hormone modulation of HPV gene expression and tumorigenicity focus primarily on single-dose hormone treatments administered in pregnancy-range concentrations, making it difficult to translate these results to in vivo exposures in non-pregnant women. Additionally, a majority of epidemiologic studies measured hormonal contraceptive use at a single time point at study enrollment with no consideration of duration of use (ie, "ever/never" classification used), thereby combining long- and short-term

Table 7 Summary of studies examining the relationship between hormonal contraceptive use and CIN 2/3

Author	Age of study population (range)	Study design	Number of subjects	Number of disease outcomes (type)	HPV status assessed at single or multiple timepoints	HC method Assessed	Contraceptive metric	Result
Castle PE[97]	<30	Pr(P)	1,812	58(CIN3)	single	COC	Current use at enrollment	Current OC use not associated with CIN3
Castle PE[98]	25	Pr(A)	5,060	499(CIN3) 361(CIN2)	multiple	COC	Current use; former use	No association with OC use
Deacon J[100]	25–35	CC	898	232(CIN3)	single	COC	Current use; past use	No association
Hildesheim A[102]	34	CC	989	116(HSIL)	single	COC	Current use; duration of use	Positive association of COC use >5 years among those with >3 pregnancies
Kjellberg L[103]	37	CC	390	137(CIN2/3)	single	COC	Duration of use	No association after adjustment for HPV
Munoz N[101]	35	CC	1,037	525(CIN3)	single	COC	Ever/Never	No association
Moscicki A[99]	20.4	Pr(A)	95	65(CIN 2/3)	multiple	COC	Current use	COC use associated with a reduced risk of regression
Castle PE[98]	25	Pr(A)	5,060	499(CIN3) 361(CIN2)	multiple	DMPA	Current use; former use	Current DMPA use associated with CIN3
Harris T[29]	18–50	CC	724	173(CIN2/3)	single	DMPA	Current use; duration	Use of DMPA for >2 years was non-significantly protective against CIN2+

Notes: *X = cross-sectional; CC = case-control; Pr = prospective (P = passive follow-up ; A = active follow-up); Light gray = null association, white = positive association, black = negative association.

users into a single category, potentially diluting any effect among long-term users.

Conclusions

The relationship between hormonal contraception and HPV infection and cervical cancer risk is far from clear. At least three separate, non-mutually exclusive hypotheses have been proposed for hormone action on the various endpoints related to the natural history of HPV (Figure 1). While case–control studies make a compelling argument for the direct role of sex steroid hormones on carcinogenesis, other case–control and prospective studies, conducted to assess the association of hormonal contraceptive use with HPV-infection-related and pre-cancerous endpoints, are inconsistent. Experimental models measuring the direct effect of sex steroid hormones on HPV-related disease processes have focused primarily on the effects of these hormones in single, super-physiological concentrations on tumor formation and viral gene expression and not on infection or host response to infection. In light of the growing interest in translational medicine, collaboration between clinical investigators, epidemiologists, and laboratory scientists is needed to move this field forward.

The identification of HPV as the necessary cause of cervical cancer has facilitated the development of a highly effective prophylactic vaccine and implementation of secondary preventative strategies that utilize molecular based assays for the detection of HPV infection. Given that vaccination is prophylactic and not effective in treating pre-existing HPV infection, and that HPV DNA detection in conjunction with cytologic testing has a relatively poor positive predictive value, identification of therapeutic targets for non-surgical interventions to treat carcinoma in situ and invasive cancer still remains an important goal. This is particularly important in countries of the developing world where the burden of illness is high and access to appropriate facilities for surgical interventions is low.

While prophylactic vaccination has shown high efficacy in preventing the developing of pre-cancerous lesions, efforts to induce sterilizing immunity against a pre-existing HPV infection through the development of a therapeutic vaccine have not been successful.[104] The select estrogen receptor modulator raloxifen, used for treatment of breast cancer, has recently been suggested as a therapeutic treatment for cervical carcinoma, based on the experimental and observational data
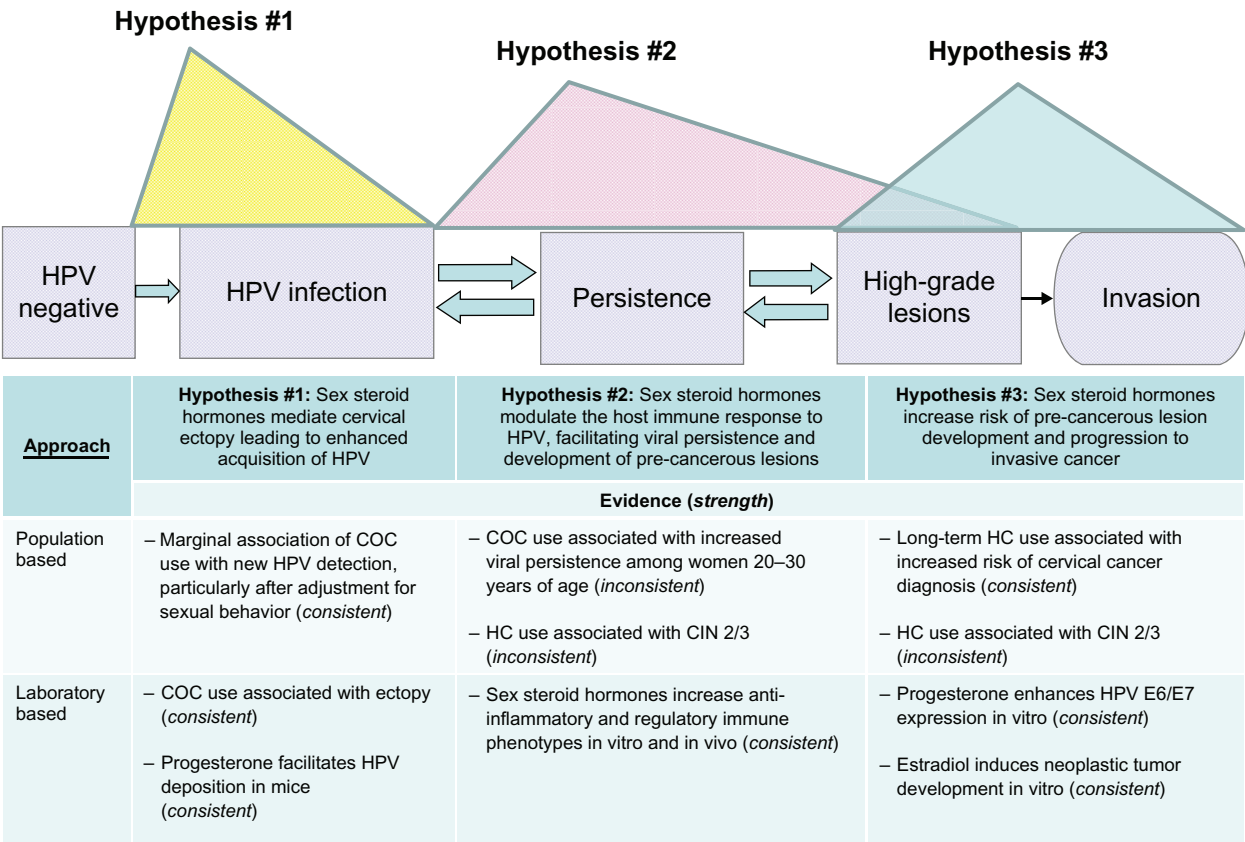

Figure 1 Summary of the potential biological hypotheses of the effects of sex steroid hormones on the natural history of HPV infection and cervical cancer.
Abbreviations: COC, combined oral contraception; HC, hormonal contraception; HPV, human papillomavirus; CIN 2/3, cervical intraepithelial neoplasia 2/3.

on the role of sex steroid hormones on neoplasia and cervical cancer risk.[96,105] A recent sub-analysis of a large National Cancer Institute-funded trial of breast cancer and treatment with either raloxifen or another estrogen receptor antagonist, tamoxifen, was performed to evaluate the potential for these drugs in reducing cervical cancer and carcinoma in situ.[106] Although the number of cervical cancer outcomes was small, these drugs showed no overall effect. While larger trials designed specifically to explore the therapeutic effects of these drugs on cervical cancer are needed to confirm these findings, first it is important to consider whether the total body of existing data on the effects of sex steroid hormones on HPV and cervical cancer provide a strong enough case to warrant such investigations.

Hormonal contraceptive use and the natural history of HPV and cervical cancer appears to be a highly dynamic interaction that is dependent on not only the duration of sex steroid hormone exposure but the stage or stages of the natural history during which this exposure occurs. In observational studies, the primary effects of hormonal contraceptive use on endpoints such as persistence as well as cancer diagnosis are observed among women who report longer-term use. In experimental models utilizing HPV16 transgenic mice, only long-term exposure to estradiol results in both the development and progression of neoplastic lesions. Conversely, short-term use or current use among short-term users of hormonal contraceptives appears to be more closely related to virologic endpoints such as increased prevalence and new detection of HPV in observational studies. Removal of sex steroid hormone exposure, either through cessation of COC use or elimination of estradiol treatment in mouse models in case–control and experimental studies, respectively, appears to reduce the risk of cervical cancer diagnosis in case–control studies and results in spontaneous regression of lesions in mice. These data suggest that timing of hormonal exposures could be crucial.

To help clarify the temporal relationships between hormonal exposure and cervical cancer risk, observational studies exploring the role of hormonal contraception on HPV and cervical cancer should consider alternative biological mechanisms in both exposure assessment and overall study design. For example, if duration of use is a critical component of risk, studies should include women who have accumulated the immortal person-time required to estimate risks associated with long duration hormonal contraceptive use. Similarly, prospective studies designed to estimate risk of progression to CIN2+ should allow for the possibility that the association between hormonal contraceptive use and cervical cancer is mediated through an increased risk of HPV acquisition and/or persistence. Under these biological

models, study designs which follow prevalently-detected HR-HPV positive women for development of CIN2+ are susceptible to significant selection bias. Cross-sectionally identified HR-HPV positive women will overselect "prevalent persistors" and will inadvertently partially match on the direct impact of hormonal contraceptive use.[107]

Sex steroid hormones act in complex regulatory systems that may not be observable using frequentist approaches in observational designs or reductionist experimental designs. Moving forward, elucidating the role of hormonal contraceptive use on cervical cancer development would benefit from interdisciplinary approaches. Experimental data should inform the development of biological models that can be tested in observational studies, and the observational data should be used to refine the experimental models to best reflect the in vivo state. However, the subtle, yet critical, limitations to both experimental and observational studies are often difficult to recognize across disciplines. A simple modification to the status quo that might help reconcile some of the inconsistencies reviewed here is preparation of a section of the discussion in manuscripts directed toward a broader interdisciplinary audience. A clear description of the main conclusions and an honest review of the limitations of the study, free of jargon, would be a welcome first step. Identifying mechanisms associated with increased cervical cancer risk remains a high priority, specifically because they can suggest therapeutic targets – in this case, estrogen modulators. Future research must acknowledge and accommodate the complexity of natural biological systems if we are to be effective in translating results to interventions.

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