

Risk of miscarriage with bivalent vaccine against human papillomavirus (HPV) types 16 and 18: pooled analysis of two randomised controlled trials

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ABSTRACT

Objective To assess whether vaccination against human papillomavirus (HPV) increases the risk of miscarriage.

Design Pooled analysis of two multicentre, phase three masked randomised controlled trials

Setting Multicentre trials in several continents and in Costa Rica.

Participants 26 130 women aged 15-25 at enrolment; 3599 pregnancies eligible for analysis.

Interventions Participants were randomly assigned to receive three doses of bivalent HPV 16/18 VLP vaccine with AS04 adjuvant (n=13 075) or hepatitis A vaccine as control (n=13 055) over six months.

Main outcome measures Miscarriage and other pregnancy outcomes.

Results The estimated rate of miscarriage was 11.5% in pregnancies in women in the HPV arm and 10.2% in the control arm. The one sided P value for the primary analysis was 0.16; thus, overall, there was no significant increase in miscarriage among women assigned to the HPV vaccine arm. In secondary descriptive analyses, miscarriage rates were 14.7% in the HPV vaccine arm and 9.1% in the control arm in pregnancies that began within three months after nearest vaccination.

Conclusion There is no evidence overall for an association between HPV vaccination and risk of miscarriage.

Trial registration Clinical Trials NCT00128661 and NCT00122681.

INTRODUCTION

Human papillomaviruses (HPV) cause virtually all of the nearly half million incident cases and quarter million deaths each year from cancer of the cervix.^{1,2} Randomised trials have shown that two vaccines based on an antigen consisting of HPV L1 protein virus-like particles are highly efficacious at preventing end points that are part of the natural course of cervical cancer.³⁻⁵ The Gardasil vaccine targets HPV types 16 and 18, which cause 70% of cervical cancer, and types 6

and 11, which cause genital warts. The Cervarix vaccine targets types 16 and 18. Both vaccines are licensed in Europe, the United States, and many countries around the world.

Because one main target population of the vaccines is women of reproductive age, a meaningful increase in risk of serious adverse effects on reproduction would mitigate some of the public health benefit of reduced morbidity and mortality during adulthood because of lower rates of cervical cancer and its precursors. Miscarriage is the most prevalent adverse outcome of pregnancy. Although vaccines are not known to affect the risk of miscarriage,⁶ Cervarix's new AS04 adjuvant could, in theory, cause alterations in maternal immune function in early pregnancy.⁷ The magnitude of any increased risk of miscarriage and of the set of pregnancies at increased risk would be important factors in personal decisions and public health policy on use of the HPV vaccines. Furthermore, any increase in the baseline rate of miscarriage because of the vaccine would raise concern that it might have other unrecognised biological activity. On the other hand, receipt of a vaccine with no effect on miscarriage might lower a woman's lifetime risk of preterm delivery and infertility by reducing the need for cone excision for treatment of precancerous lesions related to HPV.⁸

We determined the risk of miscarriage in two independent phase three double blind randomised controlled efficacy trials of Cervarix. In a combined analysis, we compared the risk of miscarriage in pregnancies conceived after vaccination with Cervarix or with a hepatitis A vaccine as control. Because the mechanisms by which the vaccine could affect pregnancies are unknown, we also do not know the specific time at risk between conception and vaccination. Therefore, we use a statistical test in our primary analysis that maintains statistical power when the vaccine does have an effect, regardless of the true subset of pregnancies at increased risk, with rigorous control of

the chance of falsely reporting a positive conclusion when the vaccine has no effect.

METHODS

Trials of Cervarix

We included data collected in double blinded randomised clinical trials to evaluate the efficacy of Cervarix for prevention of cervical lesions and persistent infection with HPV types 16 and 18 from two independent large studies. PApilloma TRIAl against Cancer In young Adults (PATRICIA)³ is a multicentre trial sponsored by GlaxoSmithKline Biologicals (GSK). The Costa Rica Vaccine Trial (CVT)⁹ is a study in Costa Rica sponsored by the National Cancer Institute (NCI).

Origin of this report

After the planned interim analysis of PATRICIA showed imbalance in the miscarriage rates between the two arms, the data safety monitoring board of CVT asked the trial statistician (SW) to prepare a report on miscarriage based on data in both trials. With the help of two outside consultants (AJW and GM), the trial statistician presented a plan to the board, acquired the required data from the independent statistician for PATRICIA (Lieven Declerck, S-Clinica, Brussels, Belgium), and prepared an analysis. The results were presented separately to the data safety monitoring board for CVT and the independent data monitoring committee for PATRICIA. Members of the two boards asked the statistician to prepare a manuscript for publication. The GSK Biological researchers had opportunities to ask questions and to comment on the research plan, results, and manuscripts, but the

CVT statistician and the outside consultants prepared reports for the monitoring boards and, with the named authors, prepared this manuscript independently.

Participants in the analysis

Eligible women between ages 15 (PATRICIA) or 18 (CVT) and 25 consented to take part and were randomised to receive three doses of vaccine, at baseline and at one and six months. Women received either the HPV 16/18 vaccine formulated with the AS04 adjuvant system or a control hepatitis A vaccine consisting of 720 ELISA units of inactivated viral antigen with Alum, formulated in 0.5 ml doses. Each woman underwent a pregnancy test on a urine sample before each vaccination; vaccination was discontinued when the result was positive. Enrolled women with childbearing potential agreed to use birth control from one month before the first vaccination until two months after the last vaccination, per study protocol. Women are being followed at least annually for a period of four years from first vaccination.

Figure 1 shows follow-up through the data freeze points of 31 January 2008 (PATRICIA) and 20 December 2007 (CVT) among the 26 130 women and 2567 reported pregnancies included in this analysis. Additional details on the two studies are available elsewhere.^{3,9}

Collection of information on pregnancy

Both studies actively monitored participants' safety, including information on pregnancies and their outcomes throughout the entire study period. Specifically, immediately after learning that a woman was pregnant, study staff completed a form documenting the expected delivery date based on report of her last menstrual period. They also contacted the woman after her expected delivery date to learn the pregnancy outcome. Staff coded information on gynaecological history, date of last menstrual period, date of end of pregnancy, gestational age, whether the delivery was vaginal or caesarean, and delivery outcome and weight, length, sex, Apgar score, and outcome of the baby. Women were instructed to report serious adverse events related to pregnancies at any time during follow-up. Any reported abnormality of pregnancy or delivery and any medical condition of the baby at birth were documented with appropriate forms and reported according to the local regulations and the good clinical practice guidelines. All adverse events were coded with MEDRA preferred codes for PATRICIA and with the Spanish edition of ICD-10 (international classification of diseases, 10th revision) for CVT. The date of the last menstrual period was abstracted from charts or inferred from information supplied by the woman. We calculated the estimated date of conception as 14 days after the last period. We defined miscarriage as loss of pregnancy within 20 weeks after the last period. Diagnosis of miscarriage was by self report and clinical judgment of the investigator; a positive pregnancy test result followed by a negative result was usually considered to be loss of

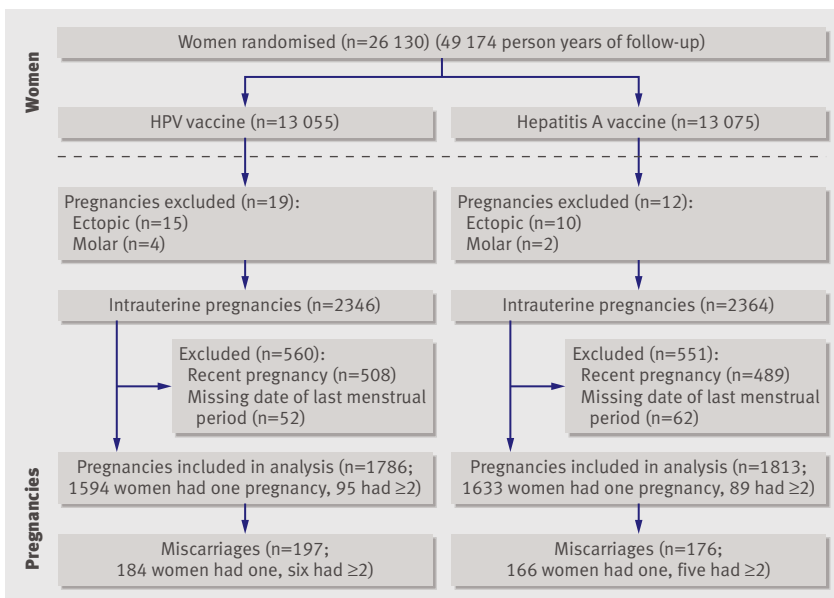


Fig 1 | Follow-up through data freeze points (20 Dec 2007 for CVT and 31 Jan 2008 for PATRICIA) for women and pregnancies leading to 373 miscarriages. In CVT three women received wrong vaccination (one in HPV group and two in hepatitis A group) and were classified according to intention to treat; one woman randomised twice was excluded. Recent pregnancies were defined as those that began within one year of data freeze point

Table 1 | Pregnancy outcomes in pooled data from 26 130 women in two trials of vaccine against human papillomavirus (HPV) types 16 and 18 (with hepatitis A vaccine as control). Figures are numbers (percentages)

	HPV vaccine	Hepatitis A vaccine	Total
Miscarriages	197 (11.0)	176 (9.7)	373 (10.4)
Gestation at miscarriage (weeks):			
0-6	43	38	81
7-12	108	107	215
13-20	44	30	74
Induced abortions*	127 (7.1)	128 (7.1)	255 (7.1)
Stillbirths	15 (0.8)	13 (0.7)	28 (0.8)
Live births	1401 (78.4)	1449 (79.9)	2850 (79.2)
Gestation at live birth (weeks):			
≤33	30	41	71
34-36	75	83	158
≥37	1295	1325	2620
Ongoing pregnancies	46 (2.6)	47 (2.6)	93 (2.6)
Gestation at last contact in ongoing pregnancies (weeks):			
≤20	15	24	39
>20	31	23	54
Total pregnancies	1786	1813	3599

*Information on induced abortion collected only in those countries where it is legal.

pregnancy. We excluded six molar and 25 ectopic pregnancies from all analyses because their causes are likely to be different from most miscarriages. We collected data on induced abortions only in those countries where they are legal. (See appendix 1 on bmj.com for further details on collection of data relating to pregnancy.)

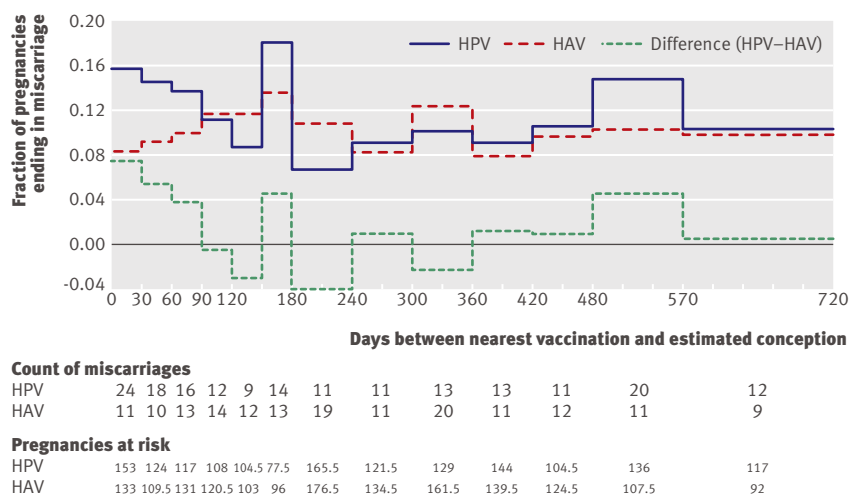


Fig 2 | Miscarriage rates for both arms and differences according to time between nearest vaccination and estimated date of conception. Of 3599 pregnancies included in analysis, 187 (5.2%) had estimated conception date before nearest vaccination. Estimations of conception assume that ovulation occurs on day 14 after last menstrual period, though 10% of cycles have ovulation at day 24 or later.¹³ Among 187 pregnancies with conception ostensibly before vaccination, many had conception dates only a few days before vaccination (median 10 days). Given that all pregnant women had negative hCG test results at time of vaccination (based on highly sensitive β hCG assays), most subsequent pregnancies had probably been conceived after relatively late ovulations and conception had occurred after vaccination. Thus, these data do not provide useful data on the question of vaccine safety before conception (HAV=hepatitis A vaccine)

Statistical analysis

Ongoing pregnancies and induced abortions—To reduce the number of ongoing pregnancies in the analysis, which would require additional statistical assumptions, we excluded all pregnancies with estimated conception dates less than one year before the data lock point; for pregnancies included in the analysis, we included all information available at the time of analysis. Each woman received one to three doses of vaccine. We calculated the days between dates of each vaccination and dates of estimated conception; we used dates of first vaccination dose, most recent vaccination dose before the estimated conception date, and vaccination dose nearest to (whether before or after) the estimated conception dates as three different origins of time scales. For miscarriage analyses we examined the effect of vaccination in subsets of pregnancies defined by time between conception and nearest vaccination. We also analysed the chance of any pregnancy and pregnancy ending in live birth by time after first vaccination.

Rates of miscarriage—We used the ratio of pregnancy losses before 20 weeks' gestational age to total pregnancies, with a correction for pregnancies and induced abortions that might have ended in miscarriage (see appendix 2 on bmj.com), as the definition of the rate of miscarriage. We calculated rates of miscarriage for subsets of pregnancies defined by time between vaccination and conception. We present graphs showing risk of miscarriage by arm and the difference between them for pregnancies conceived at various times since vaccination. The pattern of arm specific rates by time between vaccination and conception might help to distinguish whether a difference is caused by a higher rate in the HPV vaccine arm or a reduced rate in the control arm.

Permutation test to address lack of knowledge of time of conception during which vaccination might confer risk—A standard test of the effect of vaccination on risk of miscarriage requires specification of the timing of pregnancies that might be affected by vaccination—for example, the vaccine might affect only those pregnancies with a conception date within eight weeks of the vaccination date. Specifying the wrong set of pregnancies for analysis can reduce power substantially compared with an analysis based on the correctly specified set of pregnancies. Our test statistic, therefore, is the lowest P value among several tests of the same hypothesis in overlapping subsets of pregnancies defined by time between vaccination and conception. Our null hypothesis was that the vaccine confers no additional risk of miscarriage for pregnancies conceived at any time before or after vaccination. The alternative hypothesis is that the vaccine confers additional risk of miscarriage for a subset of pregnancies defined by time between vaccination and conception. We used a permutation test, sometimes called a randomisation test, to obtain the distribution of the test statistic under the null hypothesis. Tests use one sided P values because a lower miscarriage rate in the Cervarix arm would not be a safety concern. A P value of 0.025 is the standard for significance. This procedure has

Table 2 | Overall and subgroup analyses of pregnancy outcome* by permutation tests of whether rate of miscarriage in HPV vaccine arm is greater than in hepatitis A vaccine arm

Category and subgroup	HPV vaccine		Hepatitis A vaccine		P value (one sided)†
	No of pregnancies	Miscarriage rate (%)	No of pregnancies	Miscarriage rate (%)	
All pregnancies	1708.5	11.5	1727	10.2	0.16
Subset of pregnancies					
Study:					
PATRICIA	972.5	10.6	990	9.0	0.12
CVT	736	12.8	737	11.8	0.69
Maternal age at conception (years):					
≤20	519.5	9.4	531	9.8	0.77
21-23	561.5	11.6	602.5	11.1	0.26
≥24	627.5	13.2	593.5	9.6	0.17
Gestational age at which pregnancy was reported (weeks after estimated conception):					
<8	513.5	16.9	569.5	12.3	0.13
≥8	1192	9.2	1153.5	9.1	0.19
No of vaccinations before estimated time of conception:					
1	154	11.0	142.5	11.2	0.52
2	381.5	13.4	370.5	11.3	0.28
3	1132	11.0	1181.5	9.6	0.44
Interval between estimated date of conception and nearest vaccination (days):					
<90	488	13.7	456.5	9.2	0.033
90‡	394	14.7	373.5	9.1	0.031
≥90	1220.5	10.7	1270.5	10.5	0.68
Subset of miscarriages§					
Gestation at miscarriage (weeks):					
0-6	1708.5	2.5	1727	2.2	0.59
7-12	1708.5	6.3	1727	6.2	0.12
13-20	1708.5	2.6	1727	1.7	0.13

PATRICIA=papilloma trial against cancer in young adults; CVT=Costa Rica vaccine trial.

*Induced abortions counted as half pregnancy in columns of counts and in denominator of rates.

†Permutation test statistic is minimum P value for difference in rates for all pregnancies conceived during selected windows of time between vaccination and estimated date of conception (EDC). By restricting pregnancies considered in permutation test, we reduced number of intervals considered and focused on those most likely to be at risk if vaccine has effect.

‡Estimated date of conception on or after date of vaccination but less than 90 days after nearest vaccination.

§Rates are percentages of all pregnancies with miscarriage in specified interval of gestational age.

reasonable power for a wide range of pregnancy subsets that might be at increased risk of miscarriage, albeit with less power than the standard test with the subset at increased risk specified correctly (see supplemental tables 1a-1e in appendix 6 on bmj.com). (Further details about the permutation test and the power of permutation and standard tests for alternative hypotheses are in appendices 3 and 4 on bmj.com.)

Subgroup and sensitivity analyses—We conducted subgroup analyses thought a priori to have possible importance by applying the permutation method separately for PATRICIA and CVT; maternal age at estimated conception date of ≤20, 21-23, and ≥24 years; pregnancies that came to the attention of investigators within eight weeks and more than eight weeks after the last menstrual period; and the number of vaccinations received by the participant before the estimated date of conception of the pregnancy. We also applied the permutation test on subsets of pregnancy defined by time between vaccination and estimated conception and on subsets of miscarriages defined by gestational age. Because the pregnancy, not the woman, is the unit of observation, we checked whether smoking, parity,

age at enrolment, or age at conception was associated with the assigned group. As no evidence of association was seen (data not shown) we did not perform an analysis adjusting for covariates.

Total pregnancies and live births—We compared the rates of total pregnancy and of live births by time between first vaccination and possible date of conception. Further details about the methods for comparison of rates of total pregnancy and live birth are in appendix 5 on bmj.com.

RESULTS

There were 4710 pregnancies with an estimated date of conception after enrolment among 26 130 randomised women. We excluded 25 ectopic and six molar pregnancies; 19 of these 31 (61%) were in the HPV vaccine arm. We excluded 997 pregnancies with estimated date of conception less than one year from the data freeze date (including 76 (7.6%) miscarriages and 3 (0.3%) stillbirths) and 114 pregnancies with missing date of last menstrual period (among which there were 14 (12%) miscarriages and 2 (2%) stillbirths). Of the remaining 3599 intrauterine pregnancies, 2850

Table 3 | Rates of miscarriage by study arm for pregnancies conceived within specified interval from nearest vaccination. Figures are numbers (percentages) and estimates of effect (difference in percentage, ratio)

Time interval (days) around vaccination	Estimates of effect		
	HPV	Hepatitis A	
-30--1	8 (10.3)	7 (9.9)	0.4, 1.04
-30-29	32 (14.3)	18 (9.3)	5.1, 1.55
-30-59	50 (14.7)	28 (9.3)	5.4, 1.58
-30-89	66 (14.5)	41 (9.7)	4.8, 1.50
-30-849*	196 (12.2)	175 (10.7)	1.5, 1.14
0-29	24 (16.6)	11 (8.9)	7.6, 1.85
0-59	42 (16.0)	21 (9.2)	6.9, 1.75
0-89	58 (15.4)	34 (9.6)	5.8, 1.60
0-849*	188 (12.3)	168 (10.8)	1.5, 1.14
30-59	18 (15.4)	10 (9.4)	6.0, 1.63
30-89	34 (14.7)	23 (10.0)	4.7, 1.47
30-849*	164 (11.9)	157 (10.9)	0.9, 1.09
60-89	16 (13.9)	13 (10.5)	3.4, 1.33
60-849*	146 (11.5)	147 (11.0)	0.5, 1.05
90-849*	130 (11.3)	134 (11.1)	0.2, 1.02
120-849*	118 (11.2)	120 (11.0)	0.3, 1.02
180-849*	95 (10.8)	95 (10.5)	0.3, 1.03
360-849*	60 (12.6)	45 (9.8)	2.7, 1.28

*Maximum number of days between nearest vaccination and conception date is 849.

(79.2%) ended in live births (table 1), of which 2620 (91.9%) were term births (37 weeks or more); 373 (10.4%) ended in miscarriage; and 28 (0.8%) in still-birth. No induced abortions were recorded in CVT

(which was conducted in a country where they are not legal); 255 induced abortions were reported in PATRICIA (12.1% of pregnancies in PATRICIA and 7.1% among all pregnancies). When the data file was frozen for this analysis we had no information on the final outcome of 93 (2.6%) pregnancies; 39 (1.1%) had not reached 20 weeks' gestation and remained at risk of miscarriage at the last contact. The estimated rates of miscarriage are 11.5% and 10.1% in the HPV and hepatitis A vaccine arms, which are within the range of international rates.¹⁰

Result of primary test

The one sided P value for the primary permutation test was 0.16, with the nearest vaccination as the reference date (table 2); the more familiar two sided P value was 0.29. Most (58%) of the miscarriages occurred between 7 and 12 weeks' gestation, consistent with published literature.⁴ The permutation test result was also not significant in groups defined by the two trials, by maternal age at estimated conception, by whether the pregnancies were restricted by gestational age at confirmation of pregnancy or by number of vaccinations the participant received before the estimated date of conception; or for subsets of gestational age at miscarriage.

Rates of miscarriage for subsets of pregnancies defined by time between nearest vaccination and conception

Figure 2 shows the counts of miscarriages and pregnancies and the rates by arm according to the time between nearest vaccination and date of estimated conception. Table 3 summarises the underlying rates for selected subsets of pregnancies. The difference was not

Table 4 | Miscarriages by time between nearest vaccination and estimated conception. Figures are numbers (percentages) of miscarriages in each vaccine group by gestation at miscarriage, with estimates of effect (difference in percentage, ratio)*

Time interval (days) around vaccination	0-6 weeks			7-12 weeks			13-20 weeks		
	HPV	Hep A	Estimates of effect	HPV	Hep A	Estimates of effect	HPV	Hep A	Estimates of effect
-30-0	2 (2.6)	2 (2.8)	-0.3, 0.91	2 (2.6)	4 (5.6)	-3.1, 0.46	4 (5.1)	1 (1.4)	3.7, 3.64
-30-29	6 (2.7)	4 (2.1)	0.6, 1.30	16 (7.2)	11 (5.7)	1.5, 1.27	9 (4.0)	3 (1.5)	2.5, 2.61
-30-59	6 (1.8)	6 (2.0)	-0.2, 0.88	31 (9.1)	16 (5.3)	3.8, 1.71	12 (3.5)	5 (1.7)	1.9, 2.12
-30-89	12 (2.6)	9 (2.1)	0.5, 1.24	39 (8.6)	21 (5.0)	3.6, 1.73	14 (3.1)	10 (2.4)	0.7, 1.30
-30-849†	43 (2.7)	38 (2.3)	0.4, 1.15	108 (6.7)	107 (6.6)	0.2, 1.03	44 (2.7)	29 (1.8)	1.0, 1.54
0-29	4 (2.8)	2 (1.6)	1.1, 1.70	14 (9.7)	7 (5.7)	4.0, 1.70	5 (3.4)	2 (1.6)	1.8, 2.12
0-59	4 (1.5)	4 (1.7)	-0.2, 0.87	29 (11.1)	12 (5.2)	5.8, 2.11	8 (3.1)	4 (1.7)	1.3, 1.75
0-89	10 (2.7)	7 (2.0)	0.7, 1.34	37 (9.8)	17 (4.8)	5.0, 2.04	10 (2.7)	9 (2.5)	0.1, 1.04
0-849†	41 (2.7)	36 (2.3)	0.4, 1.16	106 (6.9)	103 (6.6)	0.3, 1.05	40 (2.6)	28 (1.8)	0.8, 1.46
30-59	0 (0.0)	2 (1.9)	-1.9, 0.00	15 (12.8)	5 (4.7)	8.1, 2.72	3 (2.6)	2 (1.9)	0.7, 1.36
30-89	6 (2.6)	5 (2.2)	0.4, 1.19	23 (9.9)	10 (4.3)	5.6, 2.28	5 (2.2)	7 (3.0)	-0.9, 0.71
30-849†	37 (2.7)	34 (2.4)	0.3, 1.13	92 (6.7)	96 (6.7)	0.0, 1.00	35 (2.5)	26 (1.8)	0.7, 1.40
60-89	6 (5.2)	3 (2.4)	2.8, 2.16	8 (7.0)	5 (4.0)	2.9, 1.73	2 (1.7)	5 (4.0)	-2.3, 0.43
60-849†	37 (2.9)	32 (2.4)	0.5, 1.22	77 (6.1)	91 (6.8)	-0.7, 0.89	32 (2.5)	24 (1.8)	0.7, 1.40
90-849†	31 (2.7)	29 (2.4)	0.3, 1.12	69 (6.0)	86 (7.1)	-1.1, 0.84	30 (2.6)	19 (1.6)	1.0, 1.66
120-849†	30 (2.9)	25 (2.3)	0.6, 1.25	60 (5.7)	77 (7.0)	-1.3, 0.81	28 (2.7)	18 (1.6)	1.0, 1.62
180-849†	23 (2.6)	18 (2.0)	0.6, 1.32	53 (6.0)	61 (6.8)	-0.7, 0.89	19 (2.2)	16 (1.8)	0.4, 1.22
360-849†	11 (2.3)	10 (2.2)	0.0, 1.06	39 (8.2)	27 (5.9)	0.0, 1.39	10 (2.1)	8 (1.7)	0.0, 1.2

*Excludes one woman with irregular menses and two women with missing date of last menstrual period.

†Maximum number of days between nearest vaccination and conception date is 849.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Women of reproductive age are a target for vaccines against human papillomavirus (HPV)
 Generally, non-live vaccines administered around the time of conception or during pregnancy do not increase the risk of miscarriage
 Evidence about the effect of the antigen and adjuvant used in Cervarix on the risk of miscarriage is limited

WHAT THIS STUDY ADDS

There is no overall effect of vaccination with Cervarix on risk of miscarriage
 A small increase seen in risk of miscarriage in the subgroup of pregnancies conceived within three months of vaccination is compatible with chance, but does raise concern for a vaccine that is likely to be administered to millions of women of reproductive age

significant (13.7% in HPV vaccine arm, 9.2% in hepatitis A vaccine arm; $P=0.033$; table 2) by the permutation test for pregnancies with estimated date of conception less than 90 days from nearest vaccination. The difference in estimated miscarriage rates between arms among pregnancies conceived nearer vaccination reflects higher rates in the hepatitis A arm and lower rates in the HPV arm than in the other pregnancies. For an estimated date of conception less than 90 days after vaccination, most of the difference between miscarriage counts in the groups was for miscarriages at 7-12 weeks' gestation (20 of the total difference of 24, table 4). For pregnancies that began 90 days or more after vaccination, the miscarriage rates are similar in the two arms (counts of 130 (HPV) and 134; rates of 10.7% and 10.5%). Results with other time scales also did not show greater risk in the HPV arm (data not shown).

Total pregnancies and live births

The rates of total pregnancies and live births were similar in the two arms, with P values from the permutation test of 0.42 for total pregnancies and 0.23 for live births (see supplementary figs 1a and 1b in appendix 5 on bmj.com).

DISCUSSION

We found no significant increase in miscarriage rate in women who had received the Cervarix vaccine against HPV compared with women assigned to a control arm. We found no evidence of a decrease either in total new pregnancies or in new pregnancies ending in live birth in the HPV arm, and, thus, no evidence that the HPV vaccine affected loss of undetected pregnancies. Among our subgroup analyses, we found a non-significant imbalance in estimated miscarriage rates for pregnancies conceived within three months after the nearest vaccination, the time frame with the highest prior probability of an effect. We found no sign of a detrimental effect of HPV vaccination on miscarriage rates for pregnancies conceived beyond three months after vaccination, even though power to detect an effect with a relative risk of about 2 during this time period was substantial.

Strengths and weaknesses

Despite the reassurance from our overall results and from studies of other vaccines, we looked carefully for signs of increased risk of miscarriage because even a small effect could cause a large number of miscarriages among the millions of sexually active women worldwide whom we expect to receive the HPV vaccine. We used the permutation test to maintain power to find an effect across a range of subsets of pregnancies possibly at increased risk, while strictly controlling the chance of a false positive result that could lead to over-interpretation of an apparent effect found a posteriori in some subsets of pregnancies.

The absence of a vaccine effect is consistent with other information about risk of miscarriage from vaccination with Cervarix and Gardasil or other vaccines before or during pregnancy. The May 2008 update of guidelines from the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention states that risk from vaccinating pregnant women is "primarily theoretical," and that no evidence has shown a risk with vaccines that are not live.¹¹ ACIP guidelines for Gardasil, which uses the same type of antigen as Cervarix but with a different adjuvant, do not recommend use during pregnancy, though they note that the limited data on vaccination during pregnancy do not show a causal association with adverse outcomes or adverse events in the developing fetus.¹²

The power of our study to detect a moderate effect of the vaccine in subsets of pregnancies, the absence of established effects of other vaccines on miscarriage, and the low prior probability that Cervarix has an effect on miscarriage combined with the limited statistical evidence of effect lead us to conclude that the HPV vaccine probably does not cause miscarriage. The observed variation in the differences between rates of miscarriage between arms in the subset of pregnancies conceived closer to vaccination is consistent with a small effect of vaccination or statistical noise. The similarity of miscarriage rates in the two arms for conceptions that began beyond three months after vaccination provides some reassurance that there is no long term effect of the vaccine.

Nonetheless, we cannot completely rule out the possibility of an increased risk among pregnancies conceived within three months of vaccination. It is unlikely that postmarketing surveillance will find small but important effects of vaccination on miscarriage because of the difficulties of ascertaining miscarriages in comparable unvaccinated women and determining timing of last menstrual period in vaccinated women, which is needed to investigate whether any effect is restricted to pregnancies conceived near vaccination. We recommend further analyses of data on miscarriage from randomised controlled trials to help to clarify further whether vaccines have any effect on miscarriage: pooling reported and as yet unreported data on both vaccines with the VLP antigen—that is, Cervarix and Gardasil—and pooling all data from Cervarix and other GSK vaccines containing the same novel adjuvant as Cervarix.

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Contributors: SW, AW, and GM designed the pooled analysis. PG, ACR, RH, AH, and BB were responsible for data collection. SW, BEC, AW, GM, and BB analysed the data. SW wrote the paper. SW, BEC, AW, GM, PG, BB, AH, ACR, DS, RH, and MS interpreted the data. SW, BEC, AW, GM, PG, AH, ACR, DS, RH, and MS critically reviewed all material. SW is guarantor.

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Competing interests: Vaccine was provided for CVT by GSK Biologicals, under a clinical trials agreement with NCI. GSK also provided support for aspects of the trial associated with regulatory submission needs of the company under FDA BB-IND 7920. Douglas Lowy and John Schiller from NCI are named inventors on US government owned HPV vaccine patents that are licensed to GSK and Merck, and so are entitled to limited royalties as specified by federal law. None of the other NCI and Costa Rica co-authors have any potential conflicts of interest to report. The researchers are completely independent from the non-government funders and sponsors. After a planned interim analysis, including safety review, of PATRICIA in December 2006,³ the data and safety monitoring board (DSMB) of CVT requested an assessment of possible effects of the vaccine on miscarriages by performing a pooled post hoc analysis of data from the two parallel trials. GSK provided data on pregnancy for this

miscarriage analysis from PATRICIA³ to the statistician for CVT (SW). Two outside consultants (GM and AW), who are experts in reproductive epidemiology, helped in the preparation of the analysis and report. Both the data and safety monitoring board and the independent data monitoring committee (IDMC), which oversees CVT and PATRICIA trials, respectively, recommended that NCI statistician prepare a manuscript describing the results for publication in the scientific literature. GSK scientists provided background information and data from PATRICIA, and provided suggestions on the methods, analysis and interpretation. CVT investigators from NCI and Costa Rica prepared this manuscript with input from the expert and consultants. GSK scientists commented on draft manuscripts, but the named authors made the final decisions about its content.

Ethical approval: The NIH Office of Human Subjects Research granted an exemption from institutional review board review to NCI investigators to use data from PATRICIA for this pooled analysis. The study protocol, amendments, informed consent, and other information regarding PATRICIA that required pre-approval were reviewed by a national, regional or investigational centre independent ethics committee or institutional review board.

Data sharing: No additional data available.

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