Meta-analysis of pregnancy outcomes in pooled randomized trials on a prophylactic adjuvanted glycoprotein D subunit herpes simplex virus vaccine☆

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ABSTRACT

The primary objective of this investigation was to assess whether the AS04-adjuvanted herpes simplex virus (HSV) glycoprotein D candidate prophylactic vaccine against genital herpes disease increases the risk of spontaneous abortion associated with pregnancy conceived within the vaccination exposure window (vaccine dose received within the period starting 60 days before and ending 20 weeks post-conception day). We performed a meta-analysis of studies designed as part of the clinical development program for this vaccine, to examine the relative risk of abortion (spontaneous or elective) associated with unintended vaccination exposure during pregnancy. Nineteen studies, completed before September 2010, were eligible; 5 matched the inclusion criteria for this analysis (presence of a control arm and at least one adverse pregnancy outcome reported). All vaccinated women ($N = 19,727$) were included, of whom 660 reported a pregnancy during the study period. Overall, 13.3% of pregnancies in the HSV vaccine group and 11.0% in the control group resulted in spontaneous abortion; 24.2% and 20.0% resulted in elective abortion. Among 180 women with a first pregnancy conceived in the vaccination exposure window, 16.7% (HSV vaccine) and 9.5% (control) had a spontaneous abortion and 38.5% and 33.3%, elective abortion. The relative risk for spontaneous abortion associated with vaccine exposure during the risk period for abortion in the course of pregnancy was 1.7 (95% CI: 0.7–4.6). For all women receiving HSV vaccine, this relative risk was 1.3 (95% CI: 0.8–2.1). The corresponding relative risks for elective abortion were 1.2 (95% CI: 0.7–2.0) and 1.3 (95% CI: 0.9–1.8). There was no apparent relationship to dosing and no difference between groups in gestational age at the time of spontaneous or elective abortion. In conclusion there is no statistical evidence that the investigational HSV vaccine increased the risk of spontaneous or elective abortion.

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1. Introduction

In trials evaluating vaccination against sexually transmitted diseases, such as those related to herpes simplex virus (HSV) or human papilloma virus (HPV), the target population often includes women of childbearing age. Even with the stringent precautions that are always put in place to avoid pregnancies in clinical trials, there is nonetheless the potential for participants to become pregnant during the vaccination period, resulting in a potential risk of unintentional exposure to the study vaccine. Only limited data are available to assess this risk. Indeed, vaccination is generally discontinued for women who become pregnant during a study, and

no additional immunizations are administered to these women. Furthermore, unless the vaccine is specifically designed for maternal immunization, clinical studies designed to evaluate pregnancy outcomes would not be included in the vaccine development program. Still, for any licensed vaccine, the magnitude of any increased risk of miscarriage and of the number of pregnancies at risk are important factors in personal and public health decisions regarding vaccination.

In this context, a meta-analysis was performed on studies from the GlaxoSmithKline Vaccines (GSK) HSV vaccine clinical development program, in order to evaluate the potential relative risk (RR) for abortion (spontaneous or elective) associated with HSV vaccination.

2. Material and methods

2.1. Eligibility criteria

All interventional GSK-sponsored studies in the HSV vaccine prophylactic clinical development program that were

completed before September 2010 were eligible for inclusion in the meta-analysis. Data from all females who received either the investigational AS04-adjuvanted herpes simplex virus (HSV) glycoprotein D candidate prophylactic vaccine against genital herpes disease (HSV vaccine) or a control vaccine in eligible studies were included. GSK study numbers for the 19 eligible studies were the following: HPV-001 (208141/001 NCT00698893), HSV-002 (208141/002, NCT00697567), HSV-005 (208141/005), HSV-006 (208141/006), HSV-007 (208141/007), HSV-014 (208141/014), HSV-015 (208141/015, NCT00698490), HSV-016 (208141/016, NCT00698568), HSV-017 (208141/017, NCT00699764), HSV-019 (208141/019), HSV-023 (208141/023), HSV-024 (208141/024), HSV-026 (208141/026), HSV-037 (208141/037), HSV-038 (208141/038), HSV-039 (208141/039, NCT00057330), HSV-040 (208141/040, NCT00224484), HSV-041 (208141/041), HSV-042 (208141/042, NCT00224471). Protocol summaries can be obtained from http://www.gsk-clinicalstudyregister.com/. For the present meta-analysis, the study ID is 'HSV meta analyses'.

Studies had to meet the following criteria for inclusion in the meta-analysis of spontaneous or elective abortions: the presence of a control group in the study; the presence of at least one pregnancy outcome of interest, i.e. spontaneous or elective abortion with or without apparent congenital anomaly.

The data lock point for the analysis of pregnancy outcome from the HSV program was 27 October 2010. All women of childbearing potential enrolled in the HSV vaccine program were advised to use an effective birth control method during the entire study period and 2 months after the completion of the vaccination series. They were tested for pregnancy with urine kits, immediately prior to each vaccination. The observation period began immediately after vaccination.

2.2. Cohorts analyzed

All women who received at least one vaccine dose in the selected studies (total vaccinated cohort) were included. Men enrolled in 3 of the studies (HSV-007, -016 and -017) were not included. The vaccines received were either the investigational HSV vaccine or a control product (hepatitis A vaccine [HAV] (*Havrix*TM, GSK Vaccines), placebo or AS04 adjuvant system alone used as a control, depending on the selected study).

2.3. Ethics

All studies were conducted in accordance with the 1996 version of the Declaration of Helsinki and with the International Conference on Harmonization (ICH) *Good Clinical Practice* guidelines.

Study protocols were approved by the Institutional Review Board of the institutions taking part and/or local ethics committees. Written informed consent was obtained from all subjects prior to the performance of any study-specific procedures.

2.4. Statistical analyses

The RR estimate and 95% confidence intervals (CIs) were computed using the exact conditional likelihood approach stratified for the study effect (Proc StatXact4 for SAS Users, 1999) [3].

2.5. Endpoints

The primary endpoint was to evaluate the risk of spontaneous abortion associated with pregnancy conceived within the vaccination exposure window.

Secondary endpoints were: to evaluate the risk of elective abortion associated with pregnancy conceived within the vaccination exposure window, or any spontaneous or elective abortion.

Only the first reported pregnancy was considered for subjects who became pregnant more than once during the follow-up period of the study (23 subjects).

According to international consensus, the gestational age is measured from the first day of the last menstrual period (LMP). The pregnancy onset date or estimated date of conception (EDC) is defined as the date of LMP +14 days. In case LMP was unknown, the EDC was calculated as the estimated date of delivery (EDD) by ultrasound minus 266 days. The vaccination exposure window was considered unknown if both the LMP and the EDD were unknown, or it could not be determined based on available parameters obtained from the pregnancy report (e.g. ultrasound examination or clinical assessment of the newborn).

Spontaneous abortion is defined as the termination of a pregnancy without human interference prior to 20 weeks post-conception day (or 22 weeks of gestation) [6]. Elective abortion is defined as the induced termination of a pregnancy due to personal choice or medical reasons prior to 20 weeks post-conception day (or 22 weeks of gestation).

A pregnancy was considered to be in the vaccination exposure window if a vaccine dose was received within a period starting 60 days before and ending 20 weeks post-conception day.

3. Results

3.1. Study selection for the meta-analysis

Nineteen GSK-sponsored interventional studies conducted as part of the HSV vaccine development program and completed before September 2010 were screened for inclusion in the meta-analysis. Five of these met the conditions required for evaluation of the RR of the adverse pregnancy outcomes of interest (Table 1). Study name abbreviations for the selected studies were as follows: HSV-007, HSV-016, HSV-017, HSV-039 and HSV-040. In line with the nature of this analysis, only data from female participants were included. Women who received the candidate HSV vaccine were included in the HSV vaccine group. The control group included women who received AS04 adjuvant alone (study HSV-007) or placebo control (studies HSV-016, HSV-017 and HSV-040), or who received the active comparator HAV (studies HSV-039 and HSV-040).

3.2. Study characteristics

In the 5 selected trials, 19,727 women (10,964 in the pooled HSV vaccine groups and 8763 in the control groups) were vaccinated and thus were included in the meta-analysis. Of the total subjects included in the meta-analysis, the largest single group, 8323 women, came from the HSV-039 efficacy study. The numbers of women vaccinated, reported pregnancies, and pregnancy outcomes for each study included in analysis are shown in Table 1. For the 19,727 women who received at least one vaccine dose during the studies, the mean age was 23.6 ± 10.3 years in the HSV vaccine group and 22.0 ± 9.3 years in the control group (age range: 10–80 years). The majority (86%) of women were of white/caucasian heritage in both groups. Six hundred and sixty women became pregnant during the study period (368 in the HSV vaccine group and 292 in the control group), of whom 370 (56%) came from the HSV-039 efficacy trial. For the 660 women with at least one pregnancy reported in these studies, the mean age was 22.9 ± 5.4 years in the HSV vaccine group and 23.1 ± 5.2 years in the control group (age range: 13–45 years). Demographic characteristics for women who reported at least one pregnancy, for each group, are reported in Supplementary Table 1.

Table 1
Number of women vaccinated and number of women who reported at least one pregnancy and the pregnancy outcomes of interest during the entire study period for selected studies (total vaccinated cohort, females only).

GSK study ref (NCT)	N	≥1 pregnancy (n)	Spontaneous abortion (n)	Elective abortion (n)	Control product[a]	Age range of study population (years)
HSV-007	268	23	4	4	AS04	18–45
HSV-016 (00698568)	4095	90	8	13	Placebo[c]	≥18
HSV-017 (00699764)	1086	90	8	8	Placebo[c]	18–45
HSV-039 (00057330)	8323	370	50	85	HAV	18–30
HSV-040 (00224484)	5955	87	11	37	HAV or Placebo[c]	10–17
Total	19,727[b]	660	81	147	–	10–80

AS04, adjuvant system; HAV, Hepatitis A vaccine; N, number of women who received at least one vaccine dose during the study.
[a] The investigational product in all studies was the gD HSV vaccine.
[b] HSV group: 10,964 women; control group: 8763 women.
[c] Placebo was represented by: aluminum (500 μg aluminum hydroxide), 2-phenoxyethanol as preservative in studies HSV-016 and HSV-017 and saline solution in study HSV-040.

3.3. Pregnancy outcomes

Of the 660 first pregnancies (368 in the HSV vaccine group and 292 in the control group), 49 (13.3%) in the HSV vaccine group and 32 (11%) in the control group resulted in spontaneous abortion; 89 (24.2%) and 58 (20%), respectively, resulted in elective abortion (Table 2).

Of the completed pregnancies reported, 205 in the HSV vaccine group and 169 in the control group resulted in the birth of children with no apparent congenital anomaly (Table 3). Congenital anomaly, ectopic pregnancy or still births were each reported for 0–4 pregnancies in each group. Twenty-three and 28 pregnancies from the HSV vaccine and control groups were lost to follow-up or with unknown outcome (Table 3).

Spontaneous and elective abortions occurred most frequently during the first 8 weeks of gestation for both groups, regardless of the timing of vaccination (Table 2). When considering all pregnancies, the highest proportion of pregnancy outcomes of interest occurred in women who received 3 vaccine doses in both the HSV vaccine and control groups. For pregnancies with onset during the vaccination exposure window the highest pregnancy outcomes occurred after 2 vaccine doses (Table 2).

Table 2
Overall and subgroup analyses of pregnancy outcomes of interest in the HSV group and control group.

	HSV vaccine				Control group			
	Spontaneous abortion		Elective abortion		Spontaneous abortion		Elective abortion	
	No. of pregnancies (N)	n (%)	No. of pregnancies (N)	n (%)	Total No. of pregnancies (N)	n (%)	Total No. of pregnancies (N)	n (%)
Adverse pregnancy outcomes of interest								
Overall	368	49 (13.3)	368	89 (24.2)	292	32 (11.0)	292	58 (20.0)
Within the vaccination exposure window	96	16 (16.7)	96	37 (38.5)	84	8 (9.5)	84	28 (33.3)
Pregnancy outcomes by gestational age (weeks)								
Overall								
0–8	49	33 (80.5)	89	47 (72.3)	32	19 (73.1)	58	42 (89.4)
9–12	49	7 (17.1)	89	13 (20.0)	32	6 (23.1)	58	4 (8.5)
13–20	49	1 (2.4)	89	3 (4.6)	32	1 (3.8)	58	1 (2.1)
≥20	49	0	89	2 (3.1)	32	0	58	0
Unknown	49	8	89	24	32	6	58	11
Within risk window								
0–8	16	15 (93.8)	37	26 (70.3)	8	6 (75.0)	28	24 (88.9)
9–12	16	1 (6.3)	37	7 (18.9)	8	2 (25.0)	28	3 (11.1)
13–20	16	0	37	3 (8.1)	8	0	28	0
≥20	16	0	37	1 (2.7)	8	0	28	0
Unknown	16	0	37	0	8	0	28	1
Pregnancy outcomes by vaccine doses received before pregnancy onset								
Overall								
1	49	1 (2.5)	89	8 (12.3)	32	4 (15.4)	58	8 (17.4)
2	49	11 (27.5)	89	22 (33.8)	32	5 (19.2)	58	17 (37.0)
3	49	28 (70.0)	89	34 (52.3)	32	16 (61.5)	58	20 (43.5)
4	49	0	89	1 (1.5)	32	1 (3.8)	58	1 (2.2)
Unknown	49	9	89	24	32	6	58	12
Within risk window								
1	16	1 (7.1)	37	7 (20.6)	8	2 (25.0)	28	8 (32.0)
2	16	7 (50.0)	37	20 (58.5)	8	4 (50.0)	28	13 (52.0)
3	16	6 (42.9)	37	7 (20.6)	8	2 (25.0)	28	3 (12.0)
4	16	0	37	0	8	0	28	1 (4.0)
Unknown	16	2	37	3	8	0	28	3

N, total number of pregnant women vaccinated in a given category; n, number of subjects with the pregnancy outcome; %, n/number of pregnancies in women in a given category, with available results × 100; RR, relative risk, 95%CI for RR (exact stratified conditional to total number of cases), p-value = 2-sided exact stratified test conditional to number of cases.

Table 3
Percentage of women with pregnancy and pregnancy outcomes (total vaccinated cohort, women only, pooled data).

	HSV N = 10,964		Control N = 8763	
	n	%	n	%
At least one pregnancy reported	368	3.4	292	3.3
Spontaneous abortion	49	0.4	32	0.4
Elective termination	89	0.8	58	0.7
Other outcome	230	2.1	202	2.3
Live birth with no apparent congenital anomaly	205	1.9	169	1.9
Live birth with congenital anomaly	0	<0.1	4	<0.1
Ectopic pregnancy	1	<0.1	0	<0.1
Still birth	1	<0.1	1	<0.1
Lost to follow-up/unknown	23	0.2	28	0.3

N, total number of women who received at least one vaccine dose during the study; n/%, number/percentage of women in a given category.

3.4. Evaluation of the primary and secondary endpoints: outcomes associated with pregnancies conceived during the vaccination exposure window

In the HSV vaccine group, 96 pregnancies had an onset within the vaccination exposure window, of which 16 (16.7%) resulted in spontaneous abortion and 37 (38.5%) resulted in elective abortion (Supplementary Table 2). Among the 84 pregnancies in the control group with onset within the vaccination window, 8 (9.5%) resulted in spontaneous abortion and 28 (33.3%) in elective abortion (Supplementary Table 2).

For completed pregnancies (i.e. pregnancies with known outcome) with onset within the vaccination exposure window, the calculated RR for spontaneous abortion for HSV vaccine groups relative to the control groups was 1.7 (95% CI: 0.7–4.6) and that for elective abortion was 1.2 (95% CI: 0.7–2.0) (Fig. 1A). Fig. 2A shows the contribution of each study on the assessment of the RR for spontaneous abortion with pregnancy onset within the vaccination exposure window.

For all completed pregnancies, the RR for spontaneous abortion in the HSV vaccine group as compared to the control group was 1.3 (95% CI: 0.8–2.1); for elective abortion the RR was 1.3 (95% CIs: 0.9–1.8) (Fig. 1B). Fig. 2B shows the contribution of each study in the assessment of the overall RR for spontaneous abortion for all completed pregnancies.

4. Discussion

We describe the results of a meta-analysis performed to evaluate the RR of adverse pregnancy outcomes in women exposed to an AS04-adjuvanted HSV glycoprotein D genital herpes candidate vaccine or control, in studies conducted as part of a clinical vaccine development program.

Among women with a first pregnancy during the vaccination exposure window spontaneous abortion was more frequent in the HSV vaccine group: of 96 reported pregnancies 16 resulted in spontaneous abortions (16.7%). In the control group of 84 reported pregnancies 8 resulted in spontaneous abortions (9.5%). When all women who reported at least one pregnancy were included in the analysis, the proportions were 13.3% in HSV vaccinees (of 368 reported pregnancies, 49 resulted in spontaneous abortions) and 11% in the control groups (of 292 reported pregnancies, 32 resulted in spontaneous abortions). Just over half of the pregnancies analyzed were accounted for by a single study, the HSV-039 study. The RR for the primary and secondary endpoints of the meta-analysis were 1.7 (95% CI: 0.7–4.6) and 1.2 (95% CI: 0.7–1.9) with confidence intervals including 1.

Rates of spontaneous abortion reported in the literature for clinically recognized pregnancies (i.e. having reached at least four to five gestational weeks after LMP) up to 28 weeks of gestation vary

from 11% to 16.0% [9,10,14,16,22,21,24], which are in the same range as the rates described in the present meta-analysis.

We did not identify a dose–response for developing adverse pregnancy outcomes. The proportions of pregnancy outcomes of interest were highest after 3 vaccine doses in both HSV and control groups, when considering all pregnancies, and after 2 vaccine doses when considering pregnancies with onset during the vaccination exposure window. This observation could be explained by the vaccination schedule used in HSV studies: doses 1 and 2 were given one month apart and dose 3 was administered 5 months after dose
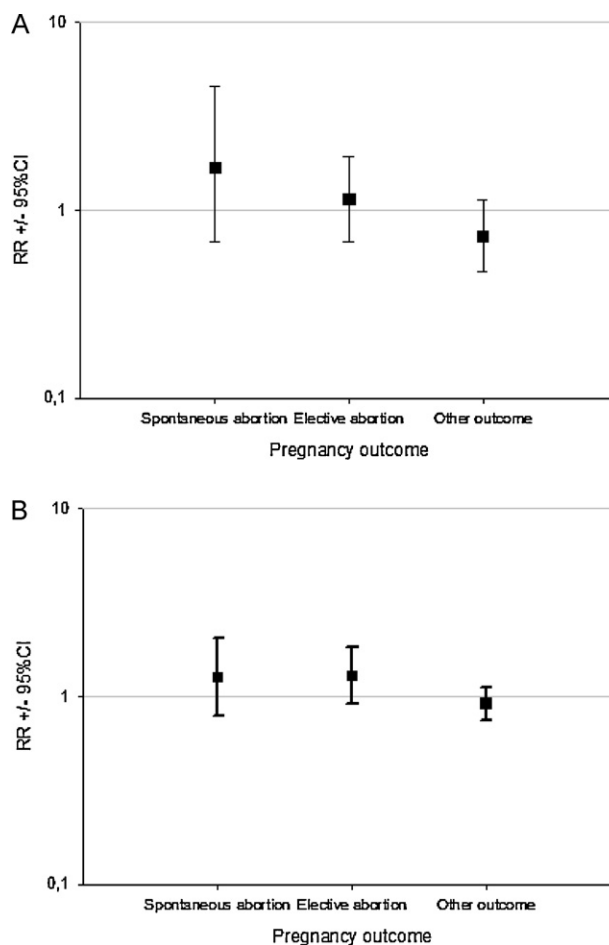

Fig. 1. Relative risk for completed pregnancies with onset within the vaccination exposure window (Panel A) and for all completed pregnancies (Panel B), classified by pregnancy outcome of interest. *Notes*: 'Other outcomes' refers to ectopic pregnancy, live infant with congenital anomaly, live infant with no apparent congenital anomaly, lost to follow up/unknown or stillbirth. RR, relative risk; CI, confidence interval.
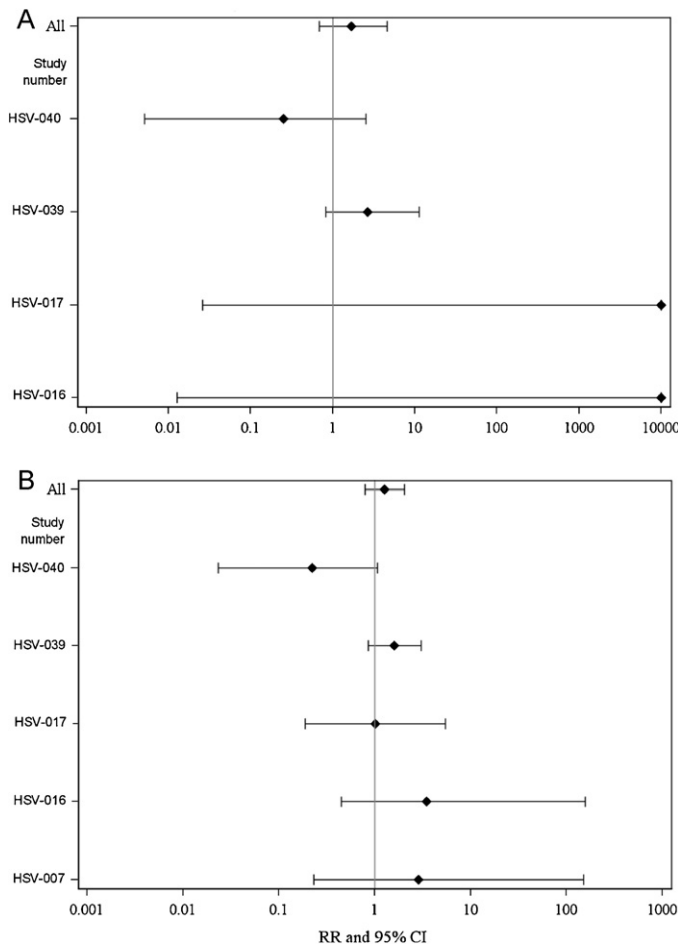
Fig. 2. Forest plot of relative risk of spontaneous abortions for completed pregnancies with onset within the vaccination exposure window (Panel A) and for all completed pregnancies (Panel B). *Note*: In the HSV-007 study, 4 spontaneous abortions were reported, none of which were associated with the vaccination exposure window. This study was not included in the Panel A of the figure.

2, which provides a long reporting period after dose 2. Additionally, the proportion of spontaneous abortions in this meta-analysis was highest early in pregnancy (during the first 8 weeks of gestation), with a substantial decrease after 8–12 weeks. This is consistent with the expected distribution of spontaneous abortions in the general population [22,23].

There are some limitations to this meta-analysis. Pregnancy is classically an exclusion criterion for participation in vaccine clinical trials. The HSV trials included in this meta-analysis enrolled either women of childbearing potential, who had a negative pregnancy test at enrollment and prior to each vaccination and who used an accepted method of birth control, or women of non-childbearing potential. Among a large number of women in the trials selected here (19,727 women vaccinated), 660 reported at least one pregnancy during the conduct of the study, and of these, 180 became pregnant during the vaccination exposure window (i.e. a susceptible period for spontaneous abortion). Although almost nothing is known about the time-window during which the likelihood of pregnancy loss would be highest if there would be a potential vaccine-related effect, one may assume that this period would be close to vaccination, the period when one may expect unintended pregnancies to occur in these clinical trials. An unintended vaccination earlier during pregnancy is an important issue, as the probability of loss is much higher earlier in gestation, thus it may falsely increase the risk of adverse outcomes in the vaccinated cohort. Several authors have reported some bias in analyses of

spontaneous abortion rates, for example the underreporting of elective abortions in countries in which abortion is prohibited [7,11,17]. Misreporting elective abortions as spontaneous abortions (by women in either group) could also falsely increase the rate of spontaneous abortions in the subset of women who conceived close to vaccination. It is reassuring that despite these potential sources of bias against the vaccinated cohort, no safety signal was detected. Other limitations of this analysis include the variability between the studies in terms of demographic characteristics of the study population (e.g. age, socioeconomic status and obstetric history), and duration of follow-up. Additionally, it was not possible to adjust the analysis for confounders or risk factors (e.g. older maternal age, assisted conception, low pre-pregnancy body mass index, regular or high alcohol consumption, older paternal age, previous reproductive history, thyroid abnormalities, diabetes, congenital uterine abnormalities and smoking). Finally, a clear limitation of the study is that over half of the cases came from one study.

Concerns about the risk of vaccination on pregnancy outcome are considered to be primarily theoretical [13]. Indeed, certain vaccinations during pregnancy are either recommended (e.g. flu) or common practice. Any theoretical concern on a possible role of an adjuvant or adjuvant system, including AS04, should consider the mechanism of action of the adjuvant and its impact on the innate immune response. AS04 transiently induces local NF-κB activity and cytokine production. This leads to an increased number of activated antigen-loaded dendritic cells and monocytes in the lymph node draining the injection site, which further increase the activation of antigen-specific T cells [5]. Therefore, since the effect of AS04 after the intramuscular injection is local, not systemic, and time-limited [5], any effect on the course of a pregnancy is unlikely. Safety data from studies evaluating other vaccine formulations containing AS04 confirm this. In the development program of a HPV-16/-18 AS04-adjuvanted vaccine; *Cervarix*™, GlaxoSmithKline Vaccines), a numerical imbalance in spontaneous abortions was noted in a large phase III trial which included women of childbearing potential [15]. This initial observation triggered the pooled analysis of 2 randomized controlled trials (Papilloma TRIal Against Cancer In young Adults (PATRICIA) [15] and The Costa Rica Vaccine Trial [12]) aiming to evaluate the risk of spontaneous abortion based on data from both trials [20]. Eligible women aged between 15 and 25 years received either the HPV-16/-18 adjuvanted vaccine or the control hepatitis A vaccine. Spontaneous abortion was diagnosed by self-report and clinical judgment of the investigator; or a positive pregnancy test followed by a negative result. This analysis concluded that there was no overall effect of the HPV vaccine on risk of spontaneous abortion (11.5% in HPV vaccine group and 10.2% in HAV vaccine group). A small numerical difference in spontaneous abortion rates between arms in the subset of pregnant women who conceived closer to vaccination was reported with the HPV-16/-18 vaccine (13.7% in HPV vaccine group compared with 9.2% in HAV vaccine group) [20]. However, a statistical analysis by permutation test (aiming to account for potential effect of timing of vaccination relative to time of conception) found that the rate of spontaneous abortion in women who conceived within 3 months after receiving the HPV study vaccine was not significantly higher than in women assigned to a control arm [20]. To date, studies assessing safety of vaccines that contain AS04 have consistently demonstrated clinically acceptable safety profiles [1,2,4,8,19]. Previously reported pharmacovigilance data for other adjuvanted vaccines that are administered during pregnancy also show no sign of unusual or severe adverse events for either the mother or the fetus [18,25].

This meta-analysis showed that the RR of spontaneous abortions and elective abortions for patients inadvertently exposed to HSV vaccination were not significantly increased. Women of childbearing age are a major target group for vaccine trials, not only for HSV vaccines but for any trial assessing prophylactic vaccines against

potential sexually transmitted diseases. It is therefore essential to continue to monitor the safety of vaccines intended for women of childbearing age in terms of pregnancy outcomes.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.01.002

References

[1] Beran J. Safety and immunogenicity of a new hepatitis B vaccine for the protection of patients with renal insufficiency including pre-haemodialysis and haemodialysis patients. Expert Opin Biol Ther 2008;8:235–47.

[2] Bernstein DI, Aoki FY, Tyring SK, Stanberry LR, St-Pierre C, Shafran SD, et al. Safety and immunogenicity of glycoprotein D-adjuvant genital herpes vaccine. Clin Infect Dis 2005;40:1271–81.

[3] Cytel Software Corporation. Proc-StatXact 4 for SAS Users, User Manual. Cambridge: Cytel Software Corporation; 1999.

[4] Descamps D, Hardt K, Spiessens B, Izurieta P, Verstraeten T, Breuer T, et al. Safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for cervical cancer prevention: a pooled analysis of 11 clinical trials. Hum Vaccin 2009;5:332–40.

[5] Didierlaurent AM, Morel S, Lockman L, Giannini SL, Bisteau M, Carlsen H, et al. AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. J Immunol 2009;183:6186–97.

[6] European Medicines Agency (Committee for Medicinal Products for Human Use). Guideline on the Exposure to Medicinal Products during Pregnancy: Need for Post-authorisation Data. London, UK: EMEA; 2005.

[7] Figa'-Talamanca I, Repetto F. Correcting spontaneous abortion rates for the presence of induced abortion. Am J Public Health 1988;78:40–2.

[8] Garcon N, Segal L, Tavares F, Van Mechelen M. The safety evaluation of adjuvants during vaccine development: the AS04 experience. Vaccine 2011;29:4453–9.

[9] Goldhaber MK, Fireman BH. The fetal life table revisited: spontaneous abortion rates in three Kaiser Permanente cohorts. Epidemiology 1991;2:33–9.

[10] Goldhaber MK, Fireman BH. Re: Estimates of the annual number of clinically recognized pregnancies in the United States, 1981–1991. Am J Epidemiol 2000;152:287–9.

[11] Hammerslough CR. Estimating the probability of spontaneous abortion in the presence of induced abortion and vice versa. Public Health Rep 1992;107:269–77.

[12] Herrero R, Hildesheim A, Rodriguez AC, Wacholder S, Bratti C, Solomon D, et al. Rationale and design of a community-based double-blind randomized clinical trial of an HPV 16 and 18 vaccine in Guanacaste, Costa Rica. Vaccine 2008;26:4795–808.

[13] National Center for Immunization and Respiratory Diseases. General recommendations on immunization: recommendations of the advisory committee on immunization practices (ACIP). MMWR Recomm Rep 2011;60:1–64.

[14] Nybo Andersen AM, Wohlfahrt J, Christens P, lsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320:1708–12.

[15] Paavonen J, Jenkins D, Bosch FX, Naud P, Salmerón J, Wheeler CM, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet 2007;369:2161–70.

[16] Saraiya M, Berg CJ, Shulman H, Green CA, Atrash HK. Estimates of the annual number of clinically recognized pregnancies in the United States, 1981–1991. Am J Epidemiol 1999;149:1025–9.

[17] Susser E. Spontaneous abortion and induced abortion: an adjustment for the presence of induced abortion when estimating the rate of spontaneous abortion from cross-sectional studies. Am J Epidemiol 1983;117:305–8.

[18] Tavares F, Nazareth I, Monegal JS, Kolte I, Verstraeten T, Bauchau V. Pregnancy and safety outcomes in women vaccinated with an AS03-adjuvanted split virion H1N1 (2009) pandemic influenza vaccine during pregnancy: a prospective cohort study. Vaccine 2011;29:6358–65.

[19] Verstraeten T, Descamps D, David MP, Zahaf T, Hardt K, Izurieta P, et al. Analysis of adverse events of potential autoimmune aetiology in a large integrated safety database of AS04 adjuvanted vaccines. Vaccine 2008;26:6630–8.

[20] Wacholder S, Chen BE, Wilcox A, Macones G, Gonzalez P, Befano B, et al. Risk of miscarriage with bivalent vaccine against human papillomavirus (HPV) types 16 and 18: pooled analysis of two randomised controlled trials. BMJ 2010;340:c712.

[21] Wilcox AJ. Surveillance of pregnancy loss in human populations. Am J Ind Med 1983;4:285–91.

[22] Wilcox AJ, Treloar AE, Sandler DP. Spontaneous abortion over time: comparing occurrence in two cohorts of women a generation apart. Am J Epidemiol 1981;114:548–53.

[23] Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319:189–94.

[24] Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. Fertil Steril 1996;65:503–9.

[25] Zuccotti G, Pogliani L, Pariani E, Amendola A, Zanetti A. Transplacental antibody transfer following maternal immunization with a pandemic 2009 influenza A (H1N1) MF59-adjuvanted vaccine. JAMA 2010;304:2360–1.