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Impact of maternal age on the incidence of obstetrical complications in Japan

Yoshio Matsuda[1], Yayoi Kawamichi[1], Kunihiko Hayashi[2], Arihiro Shiozaki[3], Shoji Satoh[4] and Shigeru Saito[3]

[1]Department of Obstetrics and Gynecology, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, [2]Department of Basic Medical Sciences, Faculty of Medicine, Gunma University, Maebashi, [3]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Toyama, Toyama, and [4]Maternal and Perinatal Care Center, Oita Prefectural Hospital, Oita, Japan

Abstract

Aim: To clarify the effect of maternal age on obstetrical complications through a cohort and case-cohort study.

Methods: We studied 242 715 births at 125 centers of a perinatal network in Japan from 2001 through 2005 as a base cohort. Women with single pregnancies who delivered after 22 weeks of gestation were included in the study. Six classes of maternal age were selected: <20; 20–24; 25–29; 30–34; 35–39; and ≥40 years. The cohort study was used to investigate whether age is related to obstetrical complications. By random selection 3749 births were determined as a subcohort. Risk ratio (RR) was determined using multivariate analysis in the case-cohort study.

Results: The incidence proportion (per 100 births) of pregnancy-induced hypertension, cervical insufficiency, placenta previa, and placental abruption increased with age, whereas the incidence proportion of preterm labor and chorioamnionitis were higher at younger maternal age. The RR of women in the age groups 35–39 years and ≥40 years (with the reference of 1.0 for women in the age group of 20–34 years) were determined: pregnancy-induced hypertension, 1.66, 2.55; placenta previa, 1.76, 2.19; and placental abruption, 1.18, 1.5. The RR of preterm labor for women in the age group of <20 years was 1.78.

Conclusion: The effect of maternal age differs for each obstetrical complication, and thus, it is important to understand these differences for management of individual pregnant patients.

Key words: case-cohort study, cohort study, maternal age, obstetric complication, risk ratio.

Introduction

As the number of advanced maternal age gravida women (i.e. aged 35 years and older at the estimated date of delivery), continues to grow, obstetric care providers require up-to-date data to enhance preconceptual and antenatal counseling.[1-3] Although some studies have found an association between delaying childbirth and adverse maternal and fetal outcomes, other studies have challenged these findings. Moreover, the incidence of obstetric complications is high among women of maternal <20 years.[4]

To effectively manage high-risk pregnancies, it is essential to investigate the risk factors, considering the differences regarding ethnicity, socioeconomical background, or medical systems. However, in Japan, information about these risk factors remains unknown.

Therefore, we performed a cohort and case-cohort study to examine the effect of maternal age on obstetrical complications among Japanese women with single pregnancies between 2001 and 2005.

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Reprint request to: •• Yoshio Matsuda, Department of Obstetrics and Gynecology, Perinatal Medical Center, Tokyo Women's Medical University Hospital, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. Email: ym0709@obgy.twmu.ac.jp

Material and Methods

Data source and study design

The study protocol was reviewed and approved by the Ethics Committee of Tokyo Women's Medical University.

We obtained information from the Japan Perinatal Registry Network database, which was begun in 1974 and is managed by the Japan Society of Obstetrics and Gynecology. In 2001 this database was converted to its present structure, which conforms to uniform coding specifications and has passed rigorous quality checks. The data have been edited and reviewed. We restricted our analysis to women who delivered a single live or stillborn infant at ≥22 weeks, and excluded those for whom data were unavailable. Thus, we studied a base cohort of 242 715 single births occurring at 125 centers of a perinatal network in Japan from 2001 through 2005.

Our study design, including the case-cohort study, was previously reported.[5] A case-cohort study is a variation of the case-control design in which the controls are drawn from the entire base population, regardless of their disease status. The subcohort was randomly selected from the entire base population and included both cases and non-cases.

Case identification and control selection

Gestational age was determined based on the menstrual history, prenatal examination and ultrasound findings during early pregnancy (gestational sac diameter, crown rump length and biparietal diameter).

Cases were identified through the base population using seven obstetric complications. These diagnoses are summarized as follows: patients were diagnosed with pregnancy-induced hypertension if the systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg with or without proteinuria occurring after the 20th week of gestation but resolving by the 12th week of postpartum. Further, patients had preterm premature rupture of the membrane (preterm PROM, <37 weeks) if membranes had been ruptured for over 1 h before 36 weeks of gestation. Preterm labor (<37 weeks) was diagnosed when patients exhibited uterine contractions at regular intervals and exhibited a progressive dilation of the cervix before 36 weeks of gestation. Cervical insufficiency was defined as one of the following clinical signs (a painless cervical dilatation during the second trimester, prolapsed and ballooning of the membranes into the cervical canal or vagina, or funneling or ballooning of the membranes into a dilated internal os; chorioamnionitis was diagnosed when the mother had fever, with body temperature >38.0°C, uterine tenderness, and/or the increase of C-reactive protein irrespective of PROM; placenta previa was defined as a placenta that was located over or very near the internal os, and the diagnosis of placental abruption was based on clinical symptoms, such as abdominal pain and vaginal bleeding, usually confirmed by ultrasonographic findings or fetal heart rate patterns.

The diagnosis of these complications was recorded on a database using a check-box format.

We selected 3749 women for the subcohort. This number represents approximately 1.5% of all registered pregnant women in each hospital. The same subcohort was used for each obstetric complication.

The linked data included information on maternal characteristics: maternal age coded in six classes: <20, 20–24, 25–29, 30–34, 35–39, and ≥40 years and examined as a continuous variable; parity 0, 1 or more; cigarette smoking: smoker or non-smoker; alcohol use during pregnancy; history of treatment for infertility: ovulation induction, artificial insemination from husband (AIH), or *in vitro* fertilization-embryo transfer (IVF-ET); medical complications; pregnancy complications; and fetal and infant outcomes routinely recorded at the time of delivery. The incidence of each obstetric complication was coded as 'yes' or 'no' in the database.

Statistical analysis

Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA). The cohort study was applied to investigate the relationship maternal age and obstetrical complications. The Cochran-Armitage trend test consisted of three components: linearity, lack of fit and total trend.[6–9] Significant 'lack of fit' means that there exists the portion, which is not explained by linear relationship.

As a subcohort, 3749 (1.5%) singleton pregnant women were selected at random. The odds ratio (OR) is not a good estimator of the cumulative incidence ratio (risk ratio, RR) when the incidence of the outcome is not rare in a nested case-control study. Because we applied a case-cohort design to this study, the OR provides an exact estimate of the RR, regardless of cumulative incidence of the outcome.[10] For maternal age, we set the reference of 1.0 for women in the age group of 20–34 years, because teenage pregnancy, meaning a pregnancy occurring when the mother is <20 years old, and elderly gravida, meaning a pregnancy when the mother is ≥35 years old, have been recognized as high risk factors.[11] Our choice of risk factors for inclusion in

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the regression model was based on the results of the univariate analysis. An RR with 95% confidence intervals (CI) was derived from these models to quantify the association between the causative determinant and obstetric complications. Unconditional logistic regression was used for multivariate analysis.

Results

Maternal age and obstetric complications (Table 1)

It became clear that the changes of incidence proportion by maternal age were all significant by the Cochran-Armitage trend test. However, it was only placental abruption, which was explained by only a linear change, and there also existed non-linear trend for other six diseases.

In terms of the relationship between maternal age and obstetric complications, the incidence proportion (per 100 births) of pregnancy-induced hypertension, cervical insufficiency, placenta previa, and placental abruption increased with age. On the other hand, the incidence proportion of preterm labor and chorioamnionitis were higher in women with young maternal age.

RR of maternal age in obstetric complications: Case-cohort study

The RR was determined using multiple logistic regression analysis. We determined RR for elderly and teenage pregnant women, with the reference of 1.0 for women aged 20–34 years. For 35–39 and ≥40 years, these were pregnancy-induced hypertension, RR 1.66 and 2.55; placenta previa, RR 1.76 and 2.19; and placental abruption, RR 1.18 and 1.5. The RR of preterm labor in patients with a maternal age <20 years was determined as 1.78 (Table 2).

Factors other than maternal age are also shown in Table 3 as follows. Statistically significant RR were seen in nullipara (1.78), smoking (1.19), renal disease (2.78), thyroid disease (1.52), chronic hypertension (8.96) and diabetes mellitus (1.97) for pregnancy-induced hypertension; smoking (1.71) for premature rupture of membranes (<37 weeks); multipara (1.23), smoking (1.37), and uterine disease (1.23) for preterm labor (<37 weeks); multipara (1.32), smoking (1.6), IVF-ET (1.53), and uterine disease (1.93) for cervical insufficiency; nullipara (1.91), and smoking (1.73) for chorioamnionitis, multipara (1.25) and IVF-ET (2.59) for placenta previa; and smoking (1.36) and chronic hypertension (2.31) for placental abruption.

Discussion

A case-cohort study contains various advantages with respect to the planning stage of a study. These include a low risk of bias in control selection, the ease of establishing conditions for selecting the controls, and use of the same control group for a large number of diseases.[12,13] Moreover, the case-cohort study is considered to be advantageous with respect to the analytical stage. In a conventional case-control study, it is necessary to assume that the outcome disease under study is rare in order to estimate relevant relative risk. Conversely, in the case-cohort study, the 'rare-disease assumption' is not required because the risks and RR can be estimated directly. Perinatal epidemiological studies are suitable for a case-cohort design.[10,14,15] This is because as outcome measures, the cumulative incidences are generally preferred over incidence rates and it can be assumed that there is no loss to follow-up during pregnancy; moreover, the base cohort population is closed.

Table 1 Incidence of obstetric complications in each age group

	Cases	Maternal age group (years)						Statistical significance		
		<20	20–24	25–29	30–34	35–39	>40			
Obstetric complication								Linearity	Lack of fit	Total trend
Pregnancy-induced hypertension	7371	3.5	3.5	3.4	3.8	5.5	7.6	<0.00001	<0.00001	<0.00001
Premature rupture of membranes (<37 weeks)	6902	4.5	4.5	3.8	3.7	4	4	0.0007	<0.00001	<0.00001
Preterm labor (<37 weeks)	5681	5.9	4.1	3.3	3	2.8	2.4	<0.00001	<0.00001	<0.00001
Cervical insufficiency	2943	1.5	1.4	1.4	1.7	1.9	1.9	<0.00001	0.04	<0.00001
Chorioamnionitis	2508	2.2	1.7	1.5	1.3	1.3	1.3	<0.00001	0.015	<0.00001
Placenta previa	2367	0.4	0.7	1	1.3	2.1	2.4	<0.00001	<0.00001	<0.00001
Placental abruption	1770	0.7	0.9	1	1	1.2	1.2	<0.00001	0.51	0.002

Table 2 Risk ratio [95% CI] of maternal age in each obstetric complication

		Subcohort	Pregnancy-induced hypertension			Premature rupture of membranes (<37 weeks)			Preterm labor (<37 weeks)		
		(*n* = 3749)	(*n* = 7371)			(*n* = 6902)			(*n* = 5681)		
		(%)	(%)	RR	95%CI	(%)	RR	95%CI	(%)	RR	95%CI
Background	Age at delivery (years)										
	<20	1.8	1.3	0.68	0.49–0.95	1.8	0.96	0.71–1.31	2.9	1.78	1.32–2.38
	35–39	17.7	23.9	1.66	1.49–1.85	17.9	1	0.90–1.11	15.6	0.83	0.74–0.93
	≥40	3.0	6.4	2.55	2.04–3.18	3.5	1.14	0.90–1.45	2.5	0.75	0.58–0.98
		Cervical insufficiency (*n* = 2943)			Chorioamnionitis (*n* = 2508)			Placenta previa (*n* = 2367)		Placental abruption (*n* = 1770)	
		(%)	RR	95%CI	(%)	RR	95%CI	(%)	RR	95%CI	(%)	RR	95% CI
Background	Age at delivery (years)										
	<20	1.4	1.32	0.87–1.99	2.3	1.07	0.74–1.54	0.5	0.36	0.19–0.69	1.1	0.67	0.40–1.11
	35–39	21.5	1.04	0.91–1.18	16.5	1	0.87–1.16	28.8	1.76	1.54–2.00	19.9	1.18	1.01–1.37
	≥40	3.8	1.04	0.78–1.38	3.0	1.04	0.76–1.41	6.0	2.19	1.68–2.86	4.1	1.5	1.09–2.07

Although the relationship between maternal age and perinatal outcome has been reported, the relationship between maternal age and obstetric complications has not been thoroughly examined.[16] The primary purpose of this study was to investigate whether the incidence proportion of a few obstetric complications has a significant relationship with maternal age, using Cochran-Armitage trend test. This study clarifies the relationship between maternal age and obstetric complications. As shown, some obstetric complications are related to an advanced maternal age, including pregnancy induced hypertension, cervical insufficiency, placenta previa, and placental abruption. However, other complications are specifically related to a younger maternal age, specifically preterm labor and chorioamnionitis.

Obstetrical complications that arise in patients with older maternal age may also be attributable to higher incidence of underlying medical disease, decreased cardiovascular reserve and diminished ability to adapt to physical stress that may accompany aging.[16–18] Seoud *et al.* reported that preeclampsia shows a bimodel pattern with increases at the lower and upper age extremities depicting a U-shape;[19] however, this finding was not replicated in the current study. The increased incidence proportion of placenta previa may also be related to the relationship between aging and progressive vascular endothelial damage.[20] It has been reported that placental abruption is frequently observed in older women, but it is unclear the mother's advanced age is the primary factor of this condition.[21]

On the other hand, this study showed that the incidence rates of preterm labor and chorioamnionitis decreased with age. Fraser *et al.* reported that the young age of a mother led to an increased risk of adverse pregnancy outcomes that was independent of important, confounding sociodemographic factors,[4] as same as other reports.[22,23] It has also been reported that teenage pregnancy increases the risk of chorioamnionitis.[24,25] Immaturity of the uterine or cervical blood supply may predispose teenage mothers to subclinical infection, and lead to increased prostaglandin production and a consequent increase in the incidence of preterm delivery.

Despite the fact that this analysis was based on a large number of subcohorts of pregnancies, some limitations of this study merit attention. First, our data was limited to information derived from discharge record abstracts, and the procedures could not be simultaneously investigated in the current study. Second, because patients could only enroll in the study if they

Table 3 Risk ratio [95% CI] of maternal background and medical complications in each obstetric complication

		Subcohort (*n* = 3749) (%)	Pregnancy-induced hypertension (*n* = 7371) (%)	RR	95%CI	Premature rupture of membranes (<37 weeks) (*n* = 6902) (%)	RR	95%CI	Preterm labor (<37 weeks) (*n* = 5681) (%)	RR	95%CI
Background	Parity										
	Nullipara	52.2	63	1.78	1.58–2.01	51.3	1.12	0.99–1.26			
	Multipara	47.8							53.5	1.23	1.08–1.39
	Taste										
	Smoking	5.8	6.5	1.19	1.00–1.43	9.3	1.71	1.44–2.02	8	1.37	1.14–1.64
	Alcohol	4.3	4.4	0.96	0.78–1.18	4.7	0.92	0.75–1.13	4.3	0.87	0.70–1.08
	History of treatment for infertility										
	Ovulation induction	2.2	2.6	0.94	0.72–1.24	2.1	0.96	0.73–1.28	2.1	1.03	0.77–1.38
	AIH	1.2	1.5	1.04	0.72–1.50	1.4	1.2	0.83–1.73	1	0.9	0.60–1.34
	IVF-ET	1.8	2.2	0.89	0.66–1.21	2.2	1.29	0.95–1.74	1.8	1.3	0.94–1.79
Medical complication	Renal disease	1.1	3	2.78	1.96–3.95	0.8	0.74	0.48–1.13	1.5	1.26	0.85–1.87
	Thyroid disease	1.5	2.4	1.52	1.11–2.09	1.5	0.99	0.71–1.38	1.3	0.85	0.59–1.22
	Uterine disease	5.3	4.9	0.77	0.64–0.93	6.3	1.19	0.99–1.41	6	1.23	1.02–1.48
	Chronic hypertension	0.6	5.9	8.96	5.86–13.70	0.4	0.67	0.39–1.17	0.4	0.7	0.38–1.27
	Diabetes mellitus	2.1	4.6	1.97	1.52–2.54	2.1	0.96	0.73–1.28	1.8	0.88	0.65–1.19

		Cervical insufficiency (*n* = 2943) (%)	RR	95%CI	Chorioamnionitis (*n* = 2508) (%)	RR	95%CI	Placenta previa (*n* = 2367) (%)	RR	95%CI	Placental abruption (*n* = 1770) (%)	RR	95% CI
Background	Parity												
	Nullipara				63.3	1.91	1.65–2.21				48.4	0.89	0.75–1.07
	Multipara	68.2	1.32	1.14–1.52				57.9	1.25	1.06–1.47			
	Taste												
	Smoking	8.8	1.6	1.31–1.97	9.3	1.73	1.41–2.13	5.6	1.14	0.90–1.45	7.6	1.36	1.07–1.73
	Alcohol	4.5	0.94	0.73–1.22	4.8	0.93	0.72–1.21	2.9	0.66	0.49–0.89	4.8	1.01	0.76–1.34
	History of treatment for infertility												
	Ovulation induction	2.4	1.33	0.94–1.88	1.6	0.63	0.42–0.94	2.7	1.24	0.87–1.76	2.4	1.17	0.79–1.72
	AIH	1.2	1.1	0.68–1.78	1.2	0.94	0.57–1.53	1.8	1.47	0.94–2.30	1.0	0.86	0.48–1.53
	IVF-ET	2.4	1.53	1.06–2.21	2.5	1.34	0.92–1.93	5	2.59	1.88–3.59	1.9	1	0.64–1.56
Medical complication	Renal disease	0.5	0.51	0.28–0.93	0.8	0.72	0.41–1.25	0.5	0.51	0.27–0.97	1.5	1.3	0.77–2.19
	Thyroid disease	1.4	0.93	0.60–1.43	1.6	1	0.66–1.53	1.6	1.19	0.78–1.83	2	1.33	0.86–2.06
	Uterine disease	9.4	1.93	1.58–2.37	5.6	0.98	0.78–1.23	6.8	1.16	0.93–1.45	3.6	0.65	0.48–0.88
	Chronic hypertension	0.5	0.73	0.37–1.44	0.2	0.38	0.15–0.94	0.2	0.31	0.11–0.82	1.5	2.31	1.29–4.11
	Diabetes mellitus	2.7	1.22	0.87–1.71	2.2	0.99	0.69–1.42	1.2	0.55	0.36–0.86	1.4	0.54	0.33–0.87

AIH, artificial insemination from husband; IVF-ET, *in vitro* fertilization-embryo transfer.

started antepartum care in the first trimester at a facility participating this trial, the findings of this study may not be generalized to every obstetric patient of advanced maternal age or younger age.

In summary, the effect of maternal age differs for each obstetrical complication. This study showed the importance of counseling and following patients to check for specific adverse outcomes associated with advancing maternal age as well as those associated with younger aged patients. It is very important to understand these differences for management of every pregnant patient.

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Comparison of risk factors for major obstetric complications between Western countries and Japan: A case–cohort study

Arihiro Shiozaki[1], Yoshio Matsuda[2], Kunihiko Hayashi[3], Shoji Satoh[4] and Shigeru Saito[1]

[1]Department of Obstetrics and Gynecology, University of Toyama, Sugitani, Toyama, and [2]Department of Obstetrics and Gynecology, Tokyo Women's Medical University, Shinjyuku-ku, Tokyo, and [3]School of Health Sciences, Faculty of Medicine, Gunma University, Maebashi-shi, Gunma, and [4]Maternal and Perinatal Care Center, Oita Prefectural Hospital, Bunyo, Oita, Japan

Abstract

Aim: The aim of this study was to demonstrate the differences in risk factors for obstetrical complications between Japan and Western countries.

Material and Methods: Using the Perinatal Database of the Japan Society for Obstetrics and Gynecology, we studied singleton deliveries after 22 weeks of gestation ($n = 242\,715$) at 125 centers of the perinatal network in Japan from 2001 through 2005 as a base cohort. In total, 3749 births (1.5% of the base cohort) were randomly selected as a subcohort. We compared the rate of risk factors in ten cases with obstetrical complications with that in the subcohort (case–cohort study).

Results: Almost all of the evaluated risk factors were common between Western countries and Japan. Older age at pregnancy was a common risk factor for pregnancy-induced hypertension, placental abruption, placenta previa, and placenta accreta/increta/percreta. On the other hand, younger age at pregnancy was a common risk factor for eclampsia and preterm delivery. Smoking during pregnancy was a common risk factor for pregnancy-induced hypertension, preterm premature rupture of the membranes, preterm delivery, cervical insufficiency, chorioamnionitis, and placental abruption. *In vitro* fertilization and embryo transfer was a common risk factor for cervical insufficiency, placenta previa, and placenta accreta/increta/percreta.

Conclusion: This case–cohort study in Japan clarified the common risk factors between Western countries and Japan as well as the risk factors indigenous to Japanese women. To identify the risk factors for a disease in a specific country, we should use data derived from its population.

Key words: case–cohort study, obstetrical complication, pregnancy-induced hypertension, risk factor, smoking.

Introduction

Risk factors for obstetrical complications are reported in many countries, mainly from Western countries.[1–6] Because there is a difference in race, ethnicity, genetic background, lifestyle and socioeconomic status between Western countries and Japan, the risk factors in Western countries are not necessarily consistent with those in Japan.

The Mother and Child Health Handbook is popular among pregnant Japanese women. If an expecting mother could make use of the handbook and know

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Reprint request to: Dr Arihiro Shiozaki, Department of Obstetrics and Gynecology, 2630 Sugitani, Toyama 930-0194, Japan.
Email: s33shio@med.u-toyama.ac.jp

about these risk factors during pregnancy, the rate of these obstetric complications may be lowered. Moreover, it is important for obstetricians to know the risk factors for obstetrical complications to prevent and manage high-risk pregnancies. However, there is no information about risk factors for major obstetrical complications in the Japanese population.

Our objective was to clarify the differences in risk factors between Japan and other countries and to find risk factors specific to Japanese women. Using a case–cohort study, we analyzed data from cases with obstetrical complications versus a cohort of non-complicated births for the same period among singleton births in Japan, and we compared the risk factor profiles between Japan and other countries.

Methods

Case–cohort study

This study design was a case–cohort study.[7–11] The reasons why we did not use a conventional case–control study were as follows. First, a case–cohort study is not subject to selection bias in control sampling. Second, it is easy for condition settings and sampling. Third, a subcohort can be used as a control group for many cases. Fourth, a risk ratio can be directly estimated.

Identification of the study population

The Tokyo Women's Medical University Ethics Committee approved this study. Since 2001 the perinatal database (DB) has been assembled by the perinatal committee in the Japan Society of Obstetrics and Gynecology under a cooperative agreement with secondary or tertiary hospitals in Japan. This DB protects patients' anonymity because of unlinked information. In 2008, 70 082 deliveries (69 470 live births and 612 stillbirths after 22 weeks of gestation) were enrolled from 118 hospitals. Those deliveries accounted for 6.4% of all deliveries (1 094 907 deliveries after 22 weeks of gestation) in Japan.

These data conform to uniform coding specifications and have passed rigorous quality checks. The data have been edited and reviewed, and the current study limited the analysis to women who delivered a singleton live birth or stillbirth at 22 or more weeks, excluding missing data. These exclusions left 242 715 singleton births for analysis. The diagnosis of these complications was recorded on a database using a check-box format.

Cases were selected from among patients admitted to the centers with 10 obstetrical complications as follows: Pregnancy-induced hypertension ($n = 7371$), premature rupture of the membranes (<37 weeks) ($n = 6902$), threatened premature delivery (<37 weeks) ($n = 5681$), cervical insufficiency ($n = 2943$), chorioamnionitis (CAM) ($n = 2508$), placenta previa ($n = 2367$), placental abruption ($n = 1770$), disseminated intravascular coagulation ($n = 343$), placenta accreta/increta/percreta ($n = 197$), and eclampsia ($n = 143$).

A subcohort ($n = 3749$) was randomly selected from the base cohort ($n = 242\,715$) using a computer-generated random number table.

Demographic information

Gestational age was determined based on the menstrual history, prenatal examination and ultrasound findings during early pregnancy (gestational sac diameter, crown–rump length, and biparietal diameter). The DB includes demographic information: (i) age (less than 20, 20–34, 35–39, and 40 years or more); (ii) marital status; (iii) parity (0, and 1 or more); (iv) cigarette smoking during pregnancy; (v) alcohol intake during pregnancy; (vi) work status; (vii) fertility treatment (no or yes: induction of ovulation, artificial insemination with semen [AIH], *in vitro* fertilization and embryo transfer [IVF-ET]); (viii) medical history and medication; (ix) obstetrical history during this pregnancy; (x) major obstetrical complications during this pregnancy (pregnancy-induced hypertension, eclampsia, premature rupture of the membranes, threatened premature delivery, cervical insufficiency, CAM, placental abruption, placenta previa, placenta accreta/increta/percreta, and disseminated intravascular coagulation [DIC]); (xi) mode of delivery; (xii) puerperal complication; and (xiii) neonatal outcomes.

Cases were identified through the base cohort using ten obstetric complications. These diagnoses are summarized as follows:

Patients were diagnosed with pregnancy-induced hypertension if blood pressure was 90 mm Hg diastolic or 140 mm Hg systolic or more on at least two occasions; pre-eclampsia if blood pressure was 90 mm Hg diastolic or 140 mm Hg systolic or more on at least two occasions and if proteinuria was 1+ (30 mg/dL) or more. Eclampsia was defined as the new onset of convulsions during pregnancy or postpartum, unrelated to other cerebral pathological conditions with pre-eclampsia. Further, patients had preterm premature rupture of the membranes (pPROM) (<37 weeks) if membranes had been ruptured for over 1 h before

onset of labor. Preterm delivery (<37 weeks) was diagnosed when patients exhibited uterine contractions at regular intervals and exhibited a progressive dilation of the cervix before 37 weeks of gestation. Cervical insufficiency was defined as a painless cervical dilatation during the second trimester, prolapsed and ballooning of the membranes into cervical canal or vagina, and funneling or ballooning of the membranes into a dilated internal os. Clinical CAM was diagnosed as a maternal temperature more than 38.0°C and at least one of the following four criteria: (i) maternal tachycardia more than 100 beats per minute; (ii) uterine tenderness; (iii) white blood cell count more than 15 000; and (iv) foul smelling of vaginal discharge. If no temperature elevation was present, all four of the other criteria had to be present to diagnose clinical CAM.[12] Placenta previa was defined as sudden, painless, profuse bleeding during the third trimester, at which time the placenta covers the internal os (totally, partially, or marginally) as observed by transvaginal ultrasonography; and placental abruption was defined as unremitting abdominal pain, an irritable, tender and hypertonic uterus, visible or concealed bleeding, bloody amniotic fluid and a non-reassuring fetal status. Placenta accreta/increta/percreta was diagnosed as the placenta being adherent to the uterine wall without easy separation. This definition was based on histological findings, or based on clinical findings if hysterectomy was not performed. DIC was defined as the presence of low platelets (<100 000/μL), low fibrinogen, prolonged prothrombin and partial thromboplastin, and high D-Dimer. The diagnosis of these complications was recorded on a database using a check-box format. The DB records with imperfect data were excluded.

We compared cases with obstetrical complications with the subcohort, with respect to background factors (maternal age, parity, smoking during pregnancy, alcohol intake during pregnancy, ovulation induction, AIH, and IVF-ET) and underlying disorders (essential hypertension, diabetes mellitus, uterine disease [uterine leiomyoma and uterine anomaly], etc.).

Statistical analysis

The data were analyzed statistically with the χ^2-test using a statistical software package (SAS version 9.1). To adjust for the effects of potential confounders, we used logistic regression models to estimate risk ratios (RR) and 95% confidence intervals (CI). The criterion for statistical significance was the 0.05 level.

Results

Overall, pregnancy-induced hypertension (PIH) accounted for the largest proportion ($n = 7371$, 3.04%) of complications and eclampsia accounted for the smallest proportion ($n = 143$, 0.06%) of deliveries. In between were preterm premature rupture of the membranes (<37 weeks) ($n = 6902$, 2.84%), threatened premature delivery (<37 weeks) ($n = 5681$, 2.34%), cervical insufficiency ($n = 2943$, 1.21%), CAM ($n = 2508$, 1.03%), placenta previa ($n = 2367$, 0.98%), placental abruption ($n = 1770$, 0.73%), DIC ($n = 343$, 0.14%), and placenta accreta/increta/percreta ($n = 197$, 0.08%).

Risk factors for obstetrical complications

The RR and 95%CI for these obstetrical complications in Japan are summarized in Table 1.

Risk factors for PIH

PIH was observed at older maternal ages (RR for 35–39 years = 1.66, 95%CI 1.49–1.85; RR for 40 years or older = 2.55, 95%CI 2.04–3.18). Essential hypertension (RR = 8.96, 95%CI 5.86–13.70), renal disease (RR = 2.78, 95%CI 1.96–3.95), diabetes mellitus (RR = 1.97, 95%CI 1.52–2.54), and nulliparity (RR = 1.78, 95%CI 1.58–2.01) were also risk factors. In marked contrast to the previous reports,[13–22] smoking during pregnancy marginally increased the risk of PIH (RR = 1.19, 95%CI 1.00–1.43), as shown in Table 1.

Risk factors for pPROM (<37 weeks)

Smoking during pregnancy (RR = 1.71, 95%CI 1.44–2.02) was overrepresented among deliveries complicated by pPROM.

Risk factors for preterm delivery (<37 weeks)

Preterm delivery was observed at younger maternal ages (RR for 19 years or younger = 1.78, 95%CI 1.32–2.38). Smoking during pregnancy (RR = 1.37, 95%CI 1.14–1.64), multipara (RR = 1.23, 95%CI 1.08–1.39), and uterine disease (RR = 1.23, 95%CI 1.02–1.48) were overrepresented among the pregnancies delivered in the setting of preterm delivery.

We also investigated the risk factors for preterm delivery with CAM and those for preterm delivery with PIH using univariate analysis. Risk factors for preterm delivery with CAM were multiparity and smoking, and risk factors for preterm delivery with PIH were advanced age, essential hypertension, diabetes mellitus, and renal disease.

Table 1 Comparison of significant risk factors for major obstetrical complications

Maternal characteristics			Obstetrical complications									
			Pregnancy-induced hypertension/ Pre-eclampsia		Eclampsia		pPROM (<37 weeks)		Preterm delivery (<37 weeks)		Cervical insufficiency	
			RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI
Maternal background	Maternal age	19 or younger	–	–	6.03	1.72–21.09	–	–	1.78	1.32–2.38	–	–
		35–39	1.66	1.49–1.85	–	–	–	–	–	–	–	–
		40 or older	2.55	2.04–3.18	–	–	–	–	–	–	–	–
	Parity	Nulliparity	1.78	1.58–2.01	2.01	1.19–3.37	–	–	–	–	–	–
		Multiparity	–	–	–	–	–	–	1.23	1.08–1.39	1.32	1.14–1.52
	Smoking during pregnancy		1.19	1.00–1.43	–	–	1.71	1.44–2.02	1.37	1.14–1.64	1.60	1.31–1.97
	In vitro fertilization		–	–	–	–	–	–	–	–	1.53	1.06–2.21
Underlying disease	Essential hypertension		8.96	5.86–13.70	–	–	–	–	–	–	–	–
	Diabetes mellitus		1.97	1.52–2.54	–	–	–	–	–	–	–	–
	Renal disease		2.78	1.96–3.95	–	–	–	–	–	–	–	–
	Uterine disease		–	–	–	–	–	–	1.23	1.02–1.48	1.93	1.58–2.37

Maternal characteristics			Obstetrical complications									
			Chorioamnionitis		Placental abruption		Placenta previa		Placenta accreta/ increta/percreta		Disseminated intravascular coagulation	
			RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI
Maternal background	Maternal age	19 or younger	–	–	–	–	–	–	–	–	–	–
		35–39	–	–	1.18	1.01–1.37	1.76	1.54–2.00	2.50	1.69–3.71	1.87	1.38–2.53
		40 or older	–	–	1.50	1.09–2.07	2.19	1.68–2.86	2.95	1.24–6.99	2.59	1.34–5.01
	Parity	Nulliparity	1.91	1.65–2.21	–	–	–	–	–	–	–	–
		Multiparity	–	–	–	–	1.25	1.06–1.47	2.13	1.35–3.33	–	–
	Smoking during pregnancy		1.73	1.41–2.13	1.36	1.07–1.73	–	–	–	–	–	–
	In vitro fertilization		–	–	–	–	2.59	1.88–3.59	11.65	3.91–34.68	–	–
Underlying disease	Essential hypertension		–	–	2.31	1.29–4.11	–	–	–	–	–	–
	Diabetes mellitus		–	–	–	–	–	–	–	–	–	–
	Renal disease		–	–	–	–	–	–	–	–	–	–
	Uterine disease		–	–	–	–	–	–	2.87	1.51–5.46	–	–

Note: Only RR and 95%CI of the statistically significant risk factors are shown. 95%CI, 95% confidence interval; pPROM, preterm premature rupture of the membranes; RR, relative risk.

Risk factors for cervical insufficiency

Uterine disease (RR = 1.93, 95%CI 1.58–2.37), smoking during pregnancy (RR = 1.60, 95%CI 1.31–1.97), pregnancy by IVF-ET (RR = 1.53, 95%CI 1.06–2.21), and multipara (RR = 1.32, 95%CI 1.14–1.52) were common among women delivering pregnancies complicated by cervical insufficiency.

Risk factors for CAM

CAM was overrepresented among primipara (RR = 1.91, 95%CI 1.65–2.21). The rate of smoking during pregnancy (RR = 1.73, 95%CI 1.41–2.13) was higher among women who presented with cervical insufficiency.

Risk factors for placenta previa

Placenta previa was observed at older maternal ages (RR for 35–39 years = 1.76, 95%CI 1.54–2.00; RR for 40 years or older = 2.19, 95%CI 1.68–2.86). Pregnancy by IVF-ET (RR = 2.59, 95%CI 1.88–3.59), and multipara (RR = 1.25, 95%CI 1.06–1.47) were overrepresented among deliveries complicated by placenta previa and 4.1% of women with placenta previa were complicated by placental accreta/increta/percreta.

Risk factors for placental abruption

Placental abruption was overrepresented at older maternal ages (RR for 35–39 years = 1.18, 95%CI 1.01–1.37; RR for 40 years or older = 1.50, 95%CI 1.09–2.07) and among women with essential hypertension (RR = 2.31, 95%CI 1.29–4.11). The rate of smoking during pregnancy (RR = 1.36, 95%CI 1.07–1.73) was higher among women who presented with placental abruption. In our data, 2.0% of women who experienced placental abruption had suffered from CAM, and 13.9% of women with placental abruption had developed PIH.

Additionally, we examined the risk factors for placental abruption with PIH and those for placental abruption with CAM using univariate analysis. Risk factors for placental abruption with PIH were nulliparity and essential hypertension and the risk factor for placental abruption with CAM was multiparity.

Risk factors for DIC

DIC was observed among women at older maternal ages (RR for 35–39 years = 1.87, 95%CI 1.38–2.53; RR for 40 years or older = 2.59, 95%CI 1.34–5.01).

Risk factors for placenta accreta/increta/percreta

Placenta accreta/increta/percreta was overrepresented among women who sought *in vitro* fertilization (RR = 11.65, 95%CI 3.91–34.68), among multipara (RR = 2.13, 95%CI 1.35–3.33), and at older maternal ages (RR for 35–39 years = 2.50, 95%CI 1.69–3.71; RR for 40 years or older = 2.95, 95%CI 1.24–6.99). The rate of uterine disease (RR = 2.87, 95%CI 1.51–5.46) was higher among placenta accreta/increta/percreta.

Risk factors for eclampsia

Younger maternal age (RR for 19 years or younger = 6.03, 95%CI 1.72–21.09) was most common among women delivering pregnancies complicated by eclampsia. Eclampsia was more common among primipara (RR = 2.01, 95%CI 1.19–3.37).

Comparison of risk factors between Japan and Western countries

To assess the characteristics of obstetrical complications in Japan, we compared the risk factors in Japan with those in Western countries (Table 2).[5,23–48]

Pulmonary edema

In our study, 76 patients suffered from pulmonary edema. The frequency of severe pre-eclampsia, DIC, heart disease, and CAM in patients with pulmonary edema was 61.8% (47/76), 13.2% (10/76), 2.6% (2/76) and 0.0% (0/76), respectively.

Discussion

The goal of this study was to determine risk factors for major obstetrical complications in Japan using the Perinatal Registry Database. We clarified two novel findings. First, we have shown that almost all of the evaluated risk factors were common between Western countries and Japan. Second, we detected a few common risk factors among Asian populations.

In general, there is a racial disparity in the frequency of obstetrical complications. Nevertheless, almost all of the risk factors that we could evaluate in this study were common between Western countries and Japan. These common risk factors may be important in the pathogenesis of obstetrical complications regardless of racial or socioeconomic background. Meanwhile, IVF-ET (a risk factor for placenta previa in Japan) and smoking during pregnancy (a risk factor for CAM in Japan) were not identical to that of Western countries but were identical to that of another Asian country (Taiwan).[44,47] The mechanism for these associations

Table 2 Comparison of risk factors between Western countries and Japan

Obstetrical complications	Risk factors in Western countries	Risk factors in Japan
Pregnancy-induced hypertension/ Pre-eclampsia	History of pre-eclampsia (Duckitt *et al.*, 2005[24])†	Maternal age 35–39
	Prolonged interpregnancy interval (Duckitt and Harrington, 2005;[24] Conde-Agudelo *et al.*, 2007[26])†	Smoking during pregnancy
	Obesity, high body mass index (≧35) (Duckitt and Harrington, 2005;[24] Robinson *et al.*, 2005[27])†	
	Antiphospholipid syndrome (Duckitt and Harrington, 2005;[24] Stella *et al.*, 2006[28])†	
	Family history of pre-eclampsia (Duckitt and Harrington, 2005[24])†	–
Preterm premature rupture of the membranes (<37 weeks)	Previous pPROM (Hadley *et al.*, 1990;[31] Mercer *et al.*, 1999[32])†	Multiparity
	Previous preterm delivery (Harger *et al.*, 1990[29])†	
	Genital tract infection (Parry and Strauss, 1998[33])†	
	Antepartum vaginal bleeding in at least one trimester (Harger *et al.*, 1990[29])†	
Preterm delivery (<37 weeks)	Urinary tract infection (Villar *et al.*, 1998[38])†	Multiparity
	Genital tract infection (Haram *et al.*, 2003[34])†	
	Periodontal disease (Vergnes and Sixou, 2007[39])†	
	Previous preterm delivery (Haram *et al.*, 2003[34])†	
Cervical insufficiency	Previous curettage procedures (Vyas *et al.*, 2006[43])†	Multiparity
	Previous precipitous delivery (Vyas *et al.*, 2006[43])†	Smoking during pregnancy
		In vitro fertilization
Chorioamnionitis	Number of vaginal examinations (Soper *et al.*, 1989[46])†	
	Duration of ruptured membranes (Soper *et al.*, 1989[46])†	
	Use of internal monitors (Soper *et al.*, 1989[46])†	
	Duration of total labor (Soper *et al.*, 1989[46])†	
Placenta previa	Previous cesarean delivery (Faiz and Ananth, 2003[5])†	Maternal age 35–39
Placental abruption	Multiparity (Ananth *et al.*, 2001[48])	Essential hypertension
	Previous placental abruption (Ananth *et al.*, 2001[48])†	Maternal age 35–39

†Risk factors we could not evaluate in present study.

remains unclear, however these risk factors may be unique to Asian populations.

Risk factors for a complication concurrent with another one seem to be different. Multiparous smokers were more likely to develop preterm delivery with CAM, while older-aged women with hypertension, diabetes, or renal disease were predisposed to preterm delivery with PIH. Nulliparous women with hypertension were subject to placental abruption, whereas multiparous women with CAM were more susceptible to placental abruption. Studies using cases with more than two complications might become increasingly important as a means of clinical research.

There are some limitations to this study. First, a history of major obstetrical complications and previous cesarean section or dilatation and curettage was not checked in the study. As a result, we could not compare the rates of a history in Japanese patients with those in Western patients and we could not evaluate the independent effects of this history on the obstetrical complications in the current pregnancy. Many risk factors known in Western countries remain unsolved. Additional studies are needed to clarify the role of a patient's history on the risk of obstetric complications. A revised DB could enable us to see whether a history of an obstetric complication is a real risk factor or not. Second, because the DB was compiled from 125 centers of perinatal research network in which national secondary and tertiary perinatal centers participate, there is a possibility that the database is not representative of the whole birth cohort in Japan. After a new control group was randomly selected from the base cohort and matched with the birth cohort in 2006 ($n = 1\,092\,674$) by gestational weeks at delivery, we compared this new control with the subcohort. As a result, there was no difference in gender of baby, birthweight, and maternal age between them (data not shown). Finally, the DB was assumed to be representative of the whole birth cohort.

Although cigarette smoking during pregnancy increases the risk of adverse outcomes, paradoxically, smoking reduces the risk of pre-eclampsia.[13–22] However, our result that smoking during pregnancy is

a risk factor for PIH was not in line with the previous reports.[13–22] One plausible explanation for this discrepancy is a racial difference of cigarette smoking prevalence during pregnancy. Socioeconomic background and lifestyle of smoking women may be different between Western countries and Japan. In 2002 and 2006, the prevalence of smoking anytime during pregnancy in Japan was 10.0% and 7.5%, respectively.[49] Although the prevalence of smoking during pregnancy has decreased, the prevalence of smoking during pregnancy in Japan is still high. Because the price of cigarettes including tax in Western countries has been higher than in Japan, smoking women in Japan may consume more cigarettes. Use of complete records and use of biochemical markers,[50,51] including exhaled carbon monoxide and urinary cotinine, may help elucidate the effect of smoking during pregnancy on maternal, fetal, and neonatal abnormalities. Although smoking is a risk factor for sexually transmitted infection (STI),[52] data on STI were not included in our DB, therefore we could not examine the incidence of STI in smoking women. To reduce the rate of medical complications, it is important to educate pregnant women about the basic health benefits of quitting smoking before and during pregnancy.

IVF-ET was one of the risk factors for cervical insufficiency. Although the reason why IVF-ET caused cervical insufficiency is unclear, a previous procedure of cervical dilatation as an investigation for infertility or an elective dilatation to facilitate embryo-transfer by a few reproductive medicine specialists may cause cervical insufficiency.

Pulmonary edema as a single obstetrical major complication rarely occurs. It usually occurs secondary to other major complications, such as pre-eclampsia, DIC, heart disease or CAM. In our study, more than 60% of patients with pulmonary edema suffered from severe pre-eclampsia.

Further studies of risk factors in patients with medical complications, including participation of patients from all clinics and hospitals in Japan, are needed to corroborate and extend our findings. Information about risk factors is needed to predict or prevent the onset of major obstetrical complications. For reducing the incidence of these obstetrical complications, the physicians, midwives, and nurses who undertake care of pregnant women should be familiar with those risk factors. Also, we hope the handbook will play a critical role in allowing us to inform pregnant women about complications and in advising them to let their health professionals know about minor symptoms before the onset of complications.

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Impact of fetal sex in pregnancy-induced hypertension and preeclampsia in Japan

Arihiro Shiozaki[a], Yoshio Matsuda[b], Shoji Satoh[c], Shigeru Saito[a,*]

[a] Department of Obstetrics and Gynecology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[b] Department of Obstetrics and Gynecology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjyuku-ku, Tokyo 162-8666, Japan
[c] Maternal and Perinatal Care Center, Oita Prefectural Hospital, Bunyo 476, Oita 870-8511, Japan

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ABSTRACT

The male antigen (HY), the elevated level of fetal antigen in twin pregnancies, and the increased number of MHC mismatches in dizygotic twin pregnancies might affect immunological tolerance during pregnancy. Using the Perinatal Database of the Japanese Society for Obstetrics and Gynecology, we studied the occurrence of pregnancy-induced hypertension (PIH) and preeclampsia in mothers delivering singleton babies and in those delivering monochorionic diamniotic (MD) twin pregnancies and dichorionic diamniotic (DD) twin pregnancies at 125 centers of the perinatal network in Japan from 2001 through 2005. In singleton pregnancies, pregnant women carrying female fetuses had a significantly higher incidence of PIH and preeclampsia compared with those carrying male fetuses. In MD twin pregnancies, compared with mothers carrying male–male fetuses, those carrying female–female fetuses had significantly higher incidences of PIH and preeclampsia and a marked difference was observed in primiparous cases. In DD twin pregnancies, the incidences of PIH and preeclampsia were significantly higher in mothers with female–female fetuses than those with male–male fetuses, while those with male–female fetuses had intermediate values. The incidence of PIH and preeclampsia in MD twin pregnancies was similar to that in DD twin pregnancies with male–male fetuses or female–female fetuses. The male antigen and the increased number of MHC mismatches in DD twin pregnancies were not a risk factor for PIH and preeclampsia. Female fetal sex was a risk factor for PIH and preeclampsia.

1. Introduction

Preeclampsia (preeclampsia) is a pregnancy complication affecting pregnant women and one of the major causes of maternal mortality and morbidity, perinatal death, preterm birth, and fetal growth restriction. Several risk factors for preeclampsia have been identified such as nulliparity, prolonged interpregnancy interval, short cohabitation, condom user, and use of donated embryos (Robillard et al., 2003; Dekker, 2002; Saito et al., 2007).

The embryo (fetus) and placenta are a semi-allograft to the maternal immune system because half of the embryonal (fetal) genes are paternally derived. In general, the risk of preeclampsia is greatest in primiparous women. Pathogenesis of preeclampsia in primiparous women may differ from that in multiparous women, multifetal gestation, or previous preeclampsia. Subsequent pregnancy with the same partner reduces the risk of preeclampsia (Trupin et al., 1996). Moreover, the risk of preeclampsia seems to be partner-dependent. Subsequent pregnancy with a new partner increases the risk of preeclampsia (Robillard et al., 2003; Dekker, 2002). Conversely, prolonged exposure to

* Corresponding author. Tel.: +81 76 434 7355; fax: +81 76 434 5036.
E-mail addresses: s30saito@med.u-toyama.ac.jp, jri@med.u-toyama.ac.jp (S. Saito).

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a partner's semen may reduce the risk of preeclampsia (Einarsson et al., 2003; Robillard et al., 2003). These phenomena suggest that immunological mechanisms such as induction of tolerance may contribute to the pathogenesis of preeclampsia.

Several studies have identified the association of fetal gender with PIH and preeclampsia, while their results are contradictory. In singleton pregnancies, Toivanen and Hirvonen (1970) reported that the ratio of males to females in babies born to mothers with PIH was 1.24 and the ratio increased up to 1.72 according to the severity of the disease. On the other hand, Hsu et al. (1994) found a predominance of female fetuses in preterm preeclamptic pregnancies compared with preterm normotensive pregnancies ($p = 0.043$), but not those in term preeclamptic and normotensive pregnancies ($p = 0.989$). In multifetal pregnancy, there is no difference in the male/female ratio between normotensive mothers and preeclamptic mothers (Makhseed et al., 1998). However, the sample size in this twin study was very small (<70 cases). Caution is needed when evaluating this result.

There are three hypothesized types of pathogenesis for the risk of preeclampsia in male–male and female–female twins (Tables 1A and 1B). The first hypothesis speculates that immune-incompatibility between mother and fetus ('major histocompatibility complex [MHC] mismatch') contributes to the pathogenesis of preeclampsia (Stevenson et al., 1971). If this were the case, the incidence of preeclampsia should be higher in dichorionic diamniotic (DD) twins compared with monochorionic diamniotic (MD) twins and should be similar in MD twins and in singletons, because all the MD twins are derived from monozygotic twins and 80–90% of the DD twins are derived from dizygotic twins in Japan.

The second hypothesis suggests that increased levels of fetal antigen lead to the pathogenesis of preeclampsia. If this were the case, the incidence of preeclampsia should be twice as high in twins compared with singletons and should be similar in DD twins and MD twins.

The third hypothesis is related to the HY antigen. Recent data suggested that the chance of a subsequent live birth in secondary recurrent miscarriage patients with first-born boys compared with first-born girls was significantly lower in women with HY-restricting HLA class II alleles (Nielsen et al., 2009). Most patients with recurrent placental abruptions had first-born boys and significantly more of these patients carried HLA haplotypes with HY-restricting class II alleles compared with controls (Christiansen et al., 2010). A maternal immune reaction against fetal HY antigens might break the maternal tolerance to semiallograftic fetuses. If this were the case, the male–male twins should have the highest rate of preeclampsia, the female–female twins and female singletons should have the lowest rate, and the male–female twins and male singletons should have an intermediate rate.

The aim of the present study was to evaluate the effects of fetal sex on the pathogenesis of PIH/preeclampsia. To demonstrate that any of the hypotheses above contribute to preeclampsia, we examined the incidence of preeclampsia in twin pregnancies as well as singleton pregnancies and analyzed the relationship among fetal gender, fetal number, and PIH/preeclampsia.

2. Materials and methods

This study was approved by the Tokyo Women's Medical University Ethics Committee.

Detailed descriptions of the database have been published elsewhere (Matsuda et al., in press). Briefly, the attendant physicians at 125 tertiary perinatal centers of Perinatal Research Network in Japan collected yearly data for women in an off-line clinical database with a common format. Data were sent to the Perinatal Committee of the Japanese Society of Obstetrics and Gynecology, and quality control was assessed for the database.

There were 241,672 singleton births and 20,050 twin births (10,025 mothers) that resulted in live birth or fetal death. Fetal death was defined as follows: fetal death before complete expulsion or extraction from the mother of a product of conception with a gestation of at least 22 weeks. All measurements reported in the database were obtained as the usual care provided to high-risk obstetric patients at tertiary perinatal centers. Determination of chorionicity was performed non-invasively during the first trimester of pregnancy by ultrasound examination of the base of the inter-twin membrane for the presence or absence of the lambda sign (Sepulveda et al., 1996).

Gestational age was determined based on the menstrual history, prenatal examination and ultrasound findings during early pregnancy (gestational sac diameter, crown rump length, and biparietal diameter).

Women were classified as having pregnancy-induced hypertension when they had hypertension (systolic blood pressure $\geq$ 140 mmHg or diastolic $\geq$ 90 mmHg) on two occasions. Women were considered to have preeclampsia when they had hypertension (systolic blood pressure $\geq$ 140 mmHg or diastolic $\geq$ 90 mmHg) on two occasions and proteinuria defined as either $\geq$300 mg/24 h urine collection or $\geq$2+ on a dipstick on at least two separate occasions without urinary tract infection. Women were stratified to have severe preeclampsia when they had hypertension (systolic blood pressure $\geq$ 160 mmHg and 100 mmHg) on two occasions and proteinuria defined as either $\geq$2 g/24 h urine collection or $\geq$3+ on a dipstick on at least two separate occasions without urinary tract infection. Women with chronic hypertension were excluded. Variables considered to be of potential importance in the analysis included maternal age, parity, gestational age at delivery, maternal smoking.

We compared numbers and rates of PIH, preeclampsia, preeclampsia with fetal death, severe preeclampsia, and severe preeclampsia with fetal death among mothers (mothers carrying male fetuses; mothers carrying female fetuses; mothers carrying male–male MD fetuses; mothers carrying female–female MD fetuses; mothers carrying male–male DD fetuses; mothers carrying male–female DD fetuses; mothers carrying female–female DD fetuses) and compared background factors (maternal age, gestational age, and parity) among them.

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Table 1A
Hypothesis (1): estimated risks of preeclampsia.

Immunological pathogeneses of preeclampsia		Estimated risks of preeclampsia
I. Increased number of mismatch MHC		Male–male DD twins = male–female DD twins = female–female DD twins
	>	Male–male MD twins = female–female MD twins
s	=	Male singleton = female singleton
II. Elevated level of fetal antigens		Male–male DD twins = male–female DD twins = female–female DD twins
	=	Male–male MD twins = female–female MD twins
	>	Male singleton = female singleton
III. Elevated level of HY antigens		Male–male DD twins = male–male MD twins
	>	Male–female DD twins = male singleton
	>	Female–female DD twins = female–female MD twins = female singleton

MHC, major histocompatibility; HY, male-specific minor histocompatibility; DD, dichorionic diamniotic; MD, monochorionic diamniotic.

2.1. Statistical analysis

The data were analyzed statistically by the Chi-squared test using a statistical software package (SAS version 9.1; SAS Institute, Cary, NC, USA). The criterion for statistical significance was a level of 0.05.

3. Results

3.1. Effect of fetal gender on maternal background

Maternal background, such as maternal age, especially 40 or older, essential hypertension, diabetes mellitus, body mass index (BMI), gestational age, were analyzed for a possible correlation with fetal gender. However, no correlation between any of the clinical parameters and fetal gender was observed (Table 2).

3.2. Effect of fetal gender on PIH/preeclampsia

3.2.1. Singleton: male fetus vs. female fetus

In primipara the frequency of PIH mothers with a female fetus was significantly higher than those with a male fetus (5.2% vs. 4.6%, $p < 0.0001$; Table 3). Of 6199 babies born to PIH mothers, 3009 were male fetuses and 3109 were female fetuses, i.e. 3.3% more female fetuses. And the incidence of primiparous and multiparous preeclampsia mothers with a female fetus was also significantly higher than those with a male fetus (3.8% vs. 3.2%, $p < 0.0001$). Of 4439 babies delivered from preeclampsia mothers, 2333 were female fetuses and 2106 were male fetuses, i.e. 10.8% more female fetuses. When preeclamptic pregnancies were further stratified into mothers with severe preeclampsia, we showed that the rate of female fetuses was significantly higher than that of male fetuses (1.5% vs. 1.3%, $p < 0.0001$). Surprisingly, of 1753 babies from mothers with severe preeclampsia mothers, 929 were female fetuses and 824 were male fetuses, i.e. 12.7% more female fetuses.

3.2.2. MD twins: male–male vs. female–female

From Table 3 it will be seen that the frequency of PIH and preeclampsia in primiparous mothers carrying female–female twins was significantly higher than those carrying male–male twins (9.1% vs. 5.6%, $p = 0.006$; 7.6% vs. 3.6%, $p = 0.0005$), while there was no differences in multiparous mothers.

3.2.3. DD twins: male–male vs. male–female vs. female–female

Also from Table 3 it will be seen that there was no significant difference in the occurrence of PIH/preeclampsia among primiparous mothers with male–male, those with

Table 1B
Hypothesis (2): impact of fetal sex on risk of preeclampsia.

	Male–male MD twins	Female–female MD twins
I. MD twins (monozygote) vs. singleton		
Number of fetal MHC mismatches	Twice	Twice
Amount of fetal MHC antigen	Same	Same
Amount of fetal HY antigen	Twice	Not evaluated

	Male–male DD twins	Male–female DD twins	Female–female DD twins
II. DD twin (dizygote [90%] or monozygote [10%]) vs. singleton			
Number of fetal MHC mismatches	Twice	Twice	Twice
Amount of fetal MHC antigen	Twice (90%)Same (10%)	Twice	Twice (90%) Same (10%)
Amount of fetal HY antigen	Twice	Same	Not evaluated

	Male–male	Female–female
III. DD twins vs. MD twins		
Number of fetal MHC mismatches	DD > MD	DD > MD
Amount of fetal MHC antigen	DD = MD	DD = MD
Amount of fetal HY antigen	DD = MD	Not evaluated

MD, monochorionic diamniotic; DD, dichorionic diamniotic; MHC, major histocompatibility; HY, male-specific minor histocompatibility; 'twice' means twice as much as singletons, 'same' means same as singletons.

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Table 2
Fetal gender and maternal background.

	Singleton			MD Twins			DD Twins			
Fetal gender	Male	Female	*p*	Male–male	Female–female	*p*	Male–male	Male–female	Female–female	*p*
I. Primipara										
Maternal age (mean ± SD) (years)	29.4 ± 5.1	29.4 ± 5.1	0.3133	28.7 ± 4.6	29.1 ± 4.7	0.0672	31.2 ± 4.7	32.0 ± 4.7	31.4 ± 4.7	<0.005*1, <0.001*2, 0.408*3
Maternal age: 40 or older	2.6% (1675/65,351)	2.7% (1633/61,136)	0.229	1.4% (12/828)	1.8% (16/891)	0.7067	3.1% (43/1376)	4.6% (87/1883)	4.0% (50/1263)	0.292*1, 0.039*2, 0.423*3
Gestational age (mean ± SD; weeks)	38.1 ± 2.9	38.4 ± 2.8	<0.001	34.1 ± 3.7	34.3 ± 3.7	0.2074	35.2 ± 3.0	35.2 ± 3.2	35.3 ± 3.1	0.6541*1, 0.9875*2, 0.7060*3
BMI (mean ± SD) (kg/m²)	24.9 ± 3.5	24.8 ± 3.5	0.5917	24.7 ± 2.5	25.1 ± 3.6	0.9657	25.2 ± 3.2	25.3 ± 3.7	25.3 ± 3.4	0.9997*1, 0.9999*2, 0.9999*3
Diabetes mellitus	1.8% (1170/65,268)	1.7% (1066/61,045)	0.1315	0.8% (7/833)	1.1% (10/899)	0.7415	0.9% (13/1383)	1.4% (26/1909)	0.9% (16/1296)	0.5440*1, 0.3465*2, 0.9315*3
Renal disease	1.1% (734/65,280)	1.2% (707/61,071)	0.5777	0.1% (1/834)	0.9% (8/899)	0.0583	0.8% (11/1388)	0.5% (10/1912)	0.7% (9/1276)	0.9715*1, 0.4597*2, 0.6742*3
Smoking	5.3% (2574/48,407)	5.3% (2432/45,501)	0.8626	4.0% (24/607)	3.7% (25/678)	0.917806	3.1% (31/996)	2.4% (34/1.393)	2.4% (22/924)	0.4020*1, 0.3857*2, 0.9631*3
II. Multipara										
Maternal age (mean ± SD; years)	31.8 ± 4.6	31.8 ± 4.6	0.6064	31.2 ± 4.4	30.8 ± 4.6	0.1068	32.2 ± 4.3	32.5 ± 4.1	31.9 ± 4.3	0.2869*1, 0.2999*2, 0.0073*3
Maternal age: 40 or older	4.2% (2942/58,847)	4.3% (2413/56,200)	0.6212	2.6% (18/696)	2.6% (18/694)	0.993	3.7% (26/707)	3.6% (30/840)	2.9% (19/647)	0.5305*1, 0.9680*2, 0.5708*3
Gestational age (mean ± SD; weeks)	37.7 ± 2.9	37.9 ± 2.9	< 0.001	34.4 ± 3.7	34.6 ± 3.4	0.1558	35.0 ± 3.4	35.6 ± 2.8	35.3 ± 3.1	0.3410*1, 0.001 *2, 0.1229*3
BMI (mean ± SD) (kg/m²)	25.1 ± 3.5	25.1 ± 3.7	0.9561	25.3 ± 3.5	25.7 ± 3.1	0.9679	25.4 ± 3.3	25.5 ± 3.9	25.4 ± 3.1	1*1, 0.9997*2, 0.9998*3
Diabetes Mellitus	1.9% (1134/58,769)	1.9% (1043/56,158)	0.3686	0.8% (6/710)	1.8% (13/703)	0.1592	2.4% (17/704)	2.0% (17/840)	1.1% (7/646)	0.1004*1, 0.7284*2, 0.2233*3
Renal Disease	0.9% (504/58,782)	0.9% (491/56,128)	0.7506	0.0% (0/709)	1.0% (7/704)	0.0224	0.8% (6/707)	0.8% (7/839)	0.6% (4/651)	0.8519*1, 0.8035*2, 0.8519*3
Smoking	6.4% (2829/43,965)	6.4% 2674/41,999)	0.6949	4.0% (21/520)	5.3% (27/507)	0.4071	6.0% (31/517)	6.4% (40/624)	5.5% (26/474)	0.8348*1, 0.8688*2, 0.3989*3
III. Primipara + multipara										
Maternal age (mean ± SD; years)	30.5 ± 5.0	30.5 ± 5.0	0.6338	29.8 ± 4.7	29.8 ± 4.7	0.9368	31.5 ± 4.6	32.1 ± 4.5	31.6 ± 4.6	0.9636*1, <0.001*2, <0.001*3
Maternal age: 40 or older	3.4% (4167/124,198)	3.4% (4046/117,336)	0.2071	2.0% (30/1524)	2.1% (34/1585)	0.8256	3.3% (69/2079)	4.3% (117/2715)	3.6% (69/1910)	0.6743*1, 0.0921*2, 0.2663*3
Gestational age (mean ± SD; weeks)	38.0 ± 2.9	38.2 ± 2.9	<0.001	34.2 ± 3.7	34.5 ± 3.6	0.0669	35.1 ± 3.1	35.3 ± 3.1	35.3 ± 3.1	0.2801*1, 0.1046*2, 0.9291*3
BMI (mean ± SD; kg/m²)	25.0 ± 3.5	25.0 ± 3.6	0.9981	25.0 ± 3.0	25.4 ± 3.4	0.8317	25.2 ± 3.2	25.3 ± 3.7	25.3 ± 3.3	0.9999*1, 0.9998*2, 1*3
Diabetes Mellitus	1.9% (2304/124,037)	1.8% (2109/117,203)	0.2875	0.8% (13/1543)	1.4% (23/1602)	0.1628	1.4% (30/2087)	1.6% (43/2749)	1.2% (23/1915)	0.6064*1, 0.8111*2, 0.3644*3
Renal Disease	1.0% (1238/124,062)	1.0% (1198/117199)	0.5506	0.1% (1/1543)	0.9% (15/1603)	0.0015	0.8% (17/2095)	0.6% (17/2751)	0.7% (13/1927)	0.7487*1, 0.5315*2, 0.9578*3
Smoking	5.8% (5403/92,372)	5.8% (5106/87,500)	0.9091	4.0% (45/1127)	4.4% (52/1185)	0.7113	4.1% (62/1513)	3.7% (74/2017)	3.4% (48/1398)	0.3999*1, 0.5707*2, 0.7867*3

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Table 3
Fetal gender and PIH/preeclampsia.

	Singleton			MD Twins			DD Twin			
Fetal gender	Male	Female	p	Male–male	Female–female	p	Male–male	Male–female	Female–female	p
I. Primipara										
PIH	4.6% (3009/65,380)	5.2% (3190/61,158)	<0.0001	5.6% (47/834)	9.1% (82/899)	0.0058	7.1% (98/1389)	7.7% (148/1914)	8.8% (112/1276)	0.0993[a]
Preeclampsia	3.2% (2106/65,380)	3.8% (2333/61,158)	<0.0001	3.6% (30/834)	7.6% (68/899)	0.0005	4.7% (65/1389)	5.4% (104/1914)	6.0% (77/1276)	0.1198[a]
Severe preeclampsia	1.3% (824/65,371)	1.5% (929/61,134)	<0.0001	1.2% (10/834)	2.3% (21/899)	0.11	1.2% (16/1388)	1.6% (31/1913)	1.8% (23/1276)	0.2174[a]
II. Multipara										
PIH	3.0% (1771/58,892)	3.3% (1875/56,242)	0.0017	4.8% (34/710)	4.3% (30/704)	0.6334	4.0% (28/707)	3.8% (32/840)	5.2% (34/651)	0.2656[a]
Preeclampsia	1.9% (1127/58,892)	2.2% (1224/56,242)	0.0018	2.7% (19/710)	2.1% (15/704)	0.6201	2.3% (16/707)	2.7% (23/840)	3.5% (23/651)	0.1198[a]
Severe preeclampsia	0.7% (434/58,877)	0.9% (480/56,229)	0.0283	0.4% (3/710)	0.6% (4/704)	0.9912	0.8% (6/706)	1.1% (9/840)	0.9% (6/651)	0.2174[a]
III. Primipara + multipara										
PIH	3.8% (4780/124,272)	4.3% (5065/117,400)	<0.0001	5.2% (51/1544)	7.0% (112/1603)	0.0419	6.0% (126/2096)	6.5% (180/2754)	7.6% (146/1927)	0.0483[a]
Preeclampsia	2.7% (3233/124,272)	3.0% (3557/117,400)	<0.0001	3.2% (49/1544)	5.2% (83/1603)	0.0066	3.9% (81/2096)	4.6% (127/2754)	5.2% (100/1927)	0.0428[a]
Severe preeclampsia	1.0% (1258/124,248)	1.2% (1409/117,363)	<0.0001	0.8% (13/1544)	1.6% (25/1603)	0.0931	1.1% (22/2094)	1.5% (40/2753)	1.5% (29/1927)	0.2522[a]

PIH, pregnancy-induced hypertension; preeclampsia, preeclampsia; MD, monochorionic diamniotic; DD, dichorionic diamniotic.
[a] Male–male vs. female–female.

male–female, and those with female–female twins. There was also no significant difference among multiparous mothers. In total (primiparous and multiparous) mothers, however, there was a significant difference in the occurrence of PIH and preeclampsia between mothers with female–female and those with male–male twins (7.6% vs. 6.0%, $p = 0.0483$ and 5.2% vs. 3.9%, $p = 0.043$, respectively), while mothers with male–female twins have intermediate values (6.5% vs. 4.6%).

3.2.4. MD twins vs. DD twins

There was no significant difference in the occurrence of PIH/preeclampsia between primiparous mothers with MD male–male twins and those with DD male–male twins (5.6% vs. 7.1%, $p = 0.189$ and 3.6% vs. 4.7%, $p = 0.266$ respectively) (Table 3). There was also no significant difference in the occurrence of PIH/preeclampsia between primiparous mothers with MD female–female twins and those with DD female–female twins (9.1% vs. 8.8%, $p = 0.782$ and 7.6% vs. 6.0%, $p = 0.159$ respectively; Table 3). No differences were observed in the occurrence of PIH/preeclampsia between multiparous mothers carrying MD male–male twins and those carrying DD male–male twins (4.8% vs. 4.0%, $p = 0.446$ and 2.7% vs. 2.3%, $p = 0.742$ respectively; Table 3), or between total (primiparous + multiparous) mothers with MD male–male twins and those with DD male–male twins (5.2% vs. 6.0%, $p = 0.324$ and 3.2% vs. 3.9%, $p = 0.267$ respectively; Table 3).

3.3. Effect of fetal gender on preeclampsia/severe preeclampsia with fetal death

Irrespective of parity and fetal number, no significant differences in the frequencies of male and female fetuses were found among mothers with preeclampsia and fetal death, and among those with severe preeclampsia and fetal death (Table 3).

4. Discussion

The goal of this study was to ascertain whether fetal sex affects the frequency of PIH/preeclampsia or whether DD twin and MD twin pregnancies affect the incidence of PIH/preeclampsia. Of 9845 singleton babies born to PIH mothers, 4780 were boys and 5065 were girls (6.0% more girls) and of 6790 singleton babies born to preeclampsia mothers, 3233 were boys and 3557 were girls (10.0% more girls). This female preponderance in primiparous mothers with PIH/preeclampsia was also seen in multiparous mothers. In MD twin pregnancy, compared with mothers carrying male–male fetuses, those carrying female–female fetuses had significantly higher incidences of PIH and preeclampsia. In DD twin pregnancies, the incidences of PIH and preeclampsia were significantly higher in mothers with female–female fetuses than in those with male–male fetuses, while those with male–female fetuses had intermediate values. This was precisely the opposite of the report, which showed that male gender is associated with an increased risk of preeclampsia in singleton pregnancies (Toivanen and Hirvonen, 1970). But caution is necessary because the sample size of this study is very small. More-

over, two Danish Birth Registries (Basso and Olsen, 2001; Nielsen et al., 2010) also showed that male gender is a risk factor for preeclampsia. In a Danish study carried out between 1980 and 1994, the male-to-female sex ratio in birth among mothers with preeclampsia was 1.10 (95% CI = 1.07–1.12; Basso and Olsen, 2001), while the risk for of was equal in male–male twins and in female–female twins, although they did not classify the twin pregnancies between MD twins and DD twins. In another Danish Birth Registry (1982–2005), the male-to-female sex ratio was 1.05 and the overall frequencies of preeclampsia in births among nulliparous mothers with male fetuses and female fetuses were 4.3% and 4.1% respectively ($p = 0.0001$) and those among multiparous mothers were 1.9% and 1.8% respectively ($p = 0.44$; Nielsen et al., 2010). These previous findings were the opposite result of our study.

The reason why the results in Denmark are different from those in Japan is not clear. In our study, the male/female ratio in singletons was 1.06, which was similar to that in Australia, 1.06 (1991–1998), and that in all cases in Japan, 1.05 (2008). This male-to-female ratio was rather low compared with that in Denmark (1.10). Furthermore, as shown in Table 3, there were no significant differences in the frequencies of male and female fetuses among mothers with preeclampsia and fetal death and among those with severe preeclampsia and fetal death, irrespective of parity and fetal number. Therefore, the possibility that more male babies had already been rejected in miscarriage during early pregnancy and in stillbirth during late pregnancy is not taken into account in our study. Also, the higher proportion of male fetuses does not explain the preponderance of female fetuses in mothers with preeclampsia. It is still unclear why mothers with preeclampsia have slightly more female babies in Japan and why mothers with preeclampsia have slightly more male babies in Denmark. This result could be due to differences in racial and ethnic backgrounds or racial differences in immunological response to HY antigens.

To test the hypotheses discussed above as a possible cause of preeclampsia, we categorized PIH/preeclampsia mothers into seven groups (male singletons, female singletons, male–male MD twins, female–female MD twins, male–male DD twins, male–female DD twins, and female–female DD twins) according to fetal gender and zygosity, and compared the rate of PIH/preeclampsia among those groups.

As a first hypothesis, Stevenson et al. (1971) speculated that immune-incompatibility between mother and fetus (mismatching of MHC) contributes to the pathogenesis of preeclampsia. In HLA-C mismatch cases, decidual $CD8^+$ T cells are activated, functional decidual regulatory T cells regulate these $CD8^+$ T cell, resulting in normal pregnancy (Tilburgs et al., 2009). The combination of maternal KIR AA genotype and fetal HLA-C2 genotype may be a risk factor for preeclampsia (Hiby et al., 2004). The frequency of fetal HLA-C2 genotype is twice as high in DD twin pregnancies as that in MD twin pregnancies. If these were the case, the incidence of preeclampsia should be higher in DD twins than in MD twins and should be similar in MD twins and in singletons. The present study has demonstrated that the incidence of preeclampsia is similar in both types of twins (DD twins, 4.5% vs. MD twins, 4.2%) and consequently does not support 'the MHC mismatching theory' in the pathogenesis of PIH/preeclampsia.

The second hypothesis suggests that the difference in the amount of fetal antigens leads to the pathogenesis of preeclampsia. If this were the case, the incidence of preeclampsia should be twice as high in twins as in singletons and should be similar in DD twins and MD twins. In this study we found that the occurrence of preeclampsia is similar in both types of twins (DD twins, 4.5% vs. MD twins, 4.2%) and these twins are at 1.5–1.6 times increased risk of preeclampsia. Our result supports 'the fetal antigen theory' in the pathogenesis of PIH/preeclampsia.

The third hypothesis is related to the HY antigen. Recent reports suggest that HY antigens are recognized by maternal lymphocytes, and may induce abortion or placental abruption (Nielsen et al., 2009; Christiansen et al., 2010). If this were the case, the male–male twins should have highest preeclampsia rate, the female–female twins and female singletons should have the lowest rate, and the male–female twins and male singletons should have the intermediate rate. We observed that the female–female DD twins had the highest preeclampsia rate (5.2%), male–female (opposite-sex) DD twins had the intermediate rate (4.6%), and the male–male DD twins had the lowest rate (3.9%). Additionally, we identified that the frequency of preeclampsia was higher in female–female MD twins than in male–male MD twins. Finally, this finding does not support the 'HY antigen theory' in the pathogenesis of PIH/preeclampsia. Hiby et al. (2004) reported the prevalence of preeclampsia decrease accompanying the increase in the activation of receptors on KIR. Therefore, maternal immune cell activation against fetal HY antigens might reduce the risk of PIH or preeclampsia. Further studies are needed to clarify these points.

There is a limitation of this study. The fetal gender of previous pregnancies in multiparous women was not checked in the study. As a result, we could not compare the rates of preeclampsia in first pregnancies with those in second pregnancies and we could not evaluate the independent effect of fetal gender on the preponderance of preeclampsia. Additional studies are needed to clarify the role of fetal gender in the first pregnancy on the risk of preeclampsia in subsequent pregnancies.

This is a first attempt to study the paradox of maternal tolerance based on fetal gender and zygosity in large samples. The present study suggests that fetal HY antigen and numbers of mismatched MHC antigens are not risk factors in PIH and preeclampsia. Further studies are needed to clarify the pathogenesis of PIH and preeclampsia with regard to the immunological aspect.

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