

# BMJ Open The mediation effect of multiple gestations on the association between in vitro fertilisation and severe maternal morbidities: a retrospective cohort study

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## ABSTRACT

**Objective** To investigate whether the association between in vitro fertilisation (IVF) and severe maternal morbidity (SMM) was mediated by multiple gestations.

**Design** A retrospective cohort study.

**Setting** The study was conducted at six hospitals in China.

**Participants** Pregnant women at 20 gestational weeks or longer.

**Outcome measure** The outcome was SMM, which was a composite of potential life-threatening conditions, the use of critical medical interventions, or the status of maternal near-miss that occurred during pregnancy, childbirth or within 42 days of pregnancy termination, as defined by WHO.

**Results** In total, 22 368 eligible pregnant women were included, among whom 497 (2.2%) received IVF, and 776 developed SMM (incidence 34.7/1000 live births, 95% CI 32.3/1000 to 37.1/1000). Four multivariable logistic regression models were constructed. Model 1, without including the variable of multiple gestations, showed that IVF was associated with higher risk of SMM (adjusted OR (aOR) 1.54, 95% CI 1.03 to 2.29). Model 2, assessing the association between IVF and multiple gestations, showed that IVF was strongly associated with multiple gestations (aOR 14.75, 95% CI 11.38 to 19.10). Model 3, by adding the variable of multiple gestations to model 1, showed that IVF was not statistically associated with SMM (aOR 0.89, 95% CI 0.58 to 1.36), but multiple gestations were associated with higher risk of SMM (aOR 5.92, 95% CI 4.88 to 7.83). Model 4, investigating the association between IVF and SMM among singleton pregnancies, showed no statistically significant association (aOR 0.70, 95% CI 0.37 to 1.32). An additional analysis by adding the interaction term of IVF by multiple gestations to model 3 showed no statistical significance of the interaction term (aOR 1.15, 95% CI 0.36 to 3.68), confirming the absence of exposure-mediator interaction.

**Conclusions** Using the established rule for judging mediation effect, the results suggested that multiple gestations might mediate the association between the use of IVF and higher risk of SMM. Further prospective studies are warranted to test our finding.

## INTRODUCTION

In vitro fertilisation (IVF), the mainstay of assisted reproductive technology (ART),

## Strengths and limitations of this study

- This was a multicentre cohort study with a large sample size to address the putative association between in vitro fertilisation (IVF) and severe maternal morbidity (SMM) among the Chinese population.
- Rigorous methods were used to collect data and identify SMM defined by WHO.
- The established methods were used to test the mediation effect of multiple gestations on the association between IVF and SMM.
- The admission to intensive care unit and use of blood products may be influenced by physician's experience and medical conditions; such variations may affect the measurement of SMM.
- We were unable to assess the associations between types of IVF (donor vs autologous, fresh vs frozen) and SMM.

has been widely used for treating infertility in the past decades.<sup>1</sup> Each year, more than 200 000 babies are born from this technology worldwide.<sup>2</sup> Several studies, however, suggested that IVF might increase the risk of serious adverse maternal outcomes,<sup>3–7</sup> including severe maternal morbidity (SMM), a composite indicator that measures multiple maternal life-threatening conditions, the use of critical medical interventions or the status of maternal near-miss, as defined by WHO.<sup>8–11</sup> Increasing concerns have arisen regarding the impact of conception with IVF on infertility population.

A few earlier studies reported the association between ART and SMM.<sup>12–15</sup> Among those studies, investigators included pregnant women with singleton and multiple gestations, and assessed both IVF fertility treatment and non-IVF fertility treatment (NIFT). To explore the putative association between ARTs and SMM, investigators typically included multiple gestations as a confounder, after controlling for other confounders

measured at baseline.<sup>14</sup> In the analysis of the singletons subset, one study did not find significant association between ARTs and SMM, partly because of lack of statistical power in some studies;<sup>14</sup> the others reported statistically higher risk of SMM in association with ARTs among singleton subset.<sup>12 13 15</sup>

These analytical approaches may not be optimal. In those analyses, investigators did not explore if there was any difference between IVF and NIFT. Understanding the independent effect of IVF is particularly desirable, since it has become a dominant fertility procedure. Second, in case of multiple gestations, clinicians and patients often choose to transfer multiple embryos to maternal uterus in order to improve the success rate of live births.<sup>16</sup> As a result, multiple gestations are common, and the risk of SMM may be increased. Were this inference real, multiple gestations would act to mediate the undesirable effect of IVF on SMM, and controlling the risk of multiple gestations, while ensuring the success of live birth, would thus be the ideal approach.

Therefore, to examine whether multiple gestations may play a mediate effect on the association between the use of IVF and SMM, we conducted a retrospective cohort study using data from six hospitals in China, and applied established methods for testing the hypothesised mediation effect.

## MATERIAL AND METHODS

### Design and setting

This was a retrospective cohort study conducted at six hospitals in Sichuan province, China. Among these six hospitals, three were located in Chengdu (capital city of the province, covering about a fifth of the total population), and the other three in the south (Zigong), middle (Suining) and south-west (Panzhihua) of the province. The six hospitals consisted of one academic medical centre (West China Women and Children's Hospital, Sichuan University), two regional hospitals, and three specialty care institutions for women and children. The West China Women and Children's Hospital was the referral care organisation and provided advisory support for the other five hospitals.

At their first antenatal visits, pregnant women were given a unique identifier, which typically took place before the 15th gestational week. They subsequently conducted regular antenatal visits until delivery. All the data at each visit and delivery were recorded into medical charts or electronic medical records (EMRs) and linked by the unique identifier. These data included demographic and gestational characteristics, physical examinations, laboratory tests, gestational comorbidities and clinical outcomes.

### Eligibility criteria

Eligible pregnant women should meet all the following criteria: delivered between 1 January 2009 and 12 December 2010 at one of the six hospitals; were

registered with a unique identifier when conducting the first antenatal visit; and conducted regular antenatal visits until delivery.

Those women were excluded if they met any of the following conditions: had the record of antenatal visits fewer than four times; terminated pregnancy before 20 gestational weeks; or used NIFT during pregnancy (eg, ovarian stimulation with pharmacological agents, with or without intrauterine insemination (IUI)).

### Data collection and quality control

We retrospectively collected the information from medical charts or EMR databases at the six hospitals in 2014. To ensure the quality of data collection, we implemented a rigorous approach. First, we predefined and pilot-tested case report forms. These forms were then manually completed by trained research assistants or clinical staffs, who had adequate understanding about research procedures and the study protocol. They also double-entered data and conducted consistency check. Finally, we checked for the completeness of data of all completed forms; if any missing data occurred, the forms were returned to investigators for data querying.

From each eligible pregnant woman, we collected information regarding demographics, gestational characteristics, physical examinations, laboratory tests, gestational comorbidities, clinical adverse events and mode of conception (IVF versus spontaneous conception).

Demographics and gestational characteristics included maternal age, prepregnancy body mass index (BMI), location of residence, parity, smoking, multiple gestations, gestational age at delivery and delivery mode (vaginal delivery or caesarean section). Physical examinations included height, body weight and blood pressure. Laboratory tests included blood cell count, haemoglobin concentration, serum ferritin level, 75 g or 100 g oral glucose tolerance test, and liver function indicators. Gestational comorbidities included iron deficiency anaemia (IDA), hepatitis B virus (HBV) infection, gestational hypertension disease, cardiac diseases, gestational diabetes mellitus (GDM) and gynaecological diseases during current pregnancy. Clinical adverse events included premature delivery (gestational age <37 weeks), uterine rupture, severe pre-eclampsia, eclampsia, multiple organ dysfunction syndromes, postpartum haemorrhage, blood transfusions, radiology intervention, laparotomy and admission to intensive care unit (ICU).

In defining gestational comorbidities, hypertension included chronic hypertension and gestational hypertension; cardiac diseases included congenital heart disease, rheumatic heart disease, hypertensive heart disease, coronary heart disease, myocarditis, mitral stenosis, inter-ventricular septal defect, nodal tachycardia, atrial fibrillation and sinus irregularities; and gynaecological diseases included fibroid, ovarian cyst and pelvic inflammation. Clinicians recorded gestational comorbidities by medical examinations together with symptoms of patients' self-report at the first antenatal care. Patients were also followed

up for GDM by the American Diabetes Association (ADA) 2003 criteria.<sup>17</sup>

In the regular practice at the six hospitals, obstetricians used a predefined questionnaire to document the mode of conception at the first antenatal visit (before 15 gestational weeks). The response options for the question about conception mode included in vitro fertilisation and embryo transfer (IVF-ET), IUI and others. Pregnant women with IVF during this pregnancy were treated as exposure and the pregnant women with spontaneous conception as control.

### SMM definition and measurement

According to WHO,<sup>8</sup> SMM was defined as a composite of potential life-threatening conditions, the use of critical medical interventions, or the status of maternal near miss that occurred during pregnancy, childbirth or within 42 days of pregnancy termination. The potential life-threatening conditions were those severe maternal complications, which typically required the use of critical interventions (online supplementary table 1). The event of SMM was diagnosed by clinicians at each hospital during pregnancy and delivery at admission. The West China Women and Children's Hospital provided the guidance for the diagnosis of SMM for the other five hospitals. While conducting this study, the other study was ongoing at this region,<sup>18</sup> covering the same six hospitals as our study, in which SMM was also an important outcome measure.

### Statistical analysis

Our data set included those women with no missing data across variables of our interest. We described demographics, gestational characteristics and gestational comorbidities by the mode of conception (IVF vs spontaneous conception). We categorised pregnancy BMI into four groups (<18.5 kg/m<sup>2</sup> for underweight, 18.5–23.9 kg/m<sup>2</sup> for normal weight, 24.0–27.9 kg/m<sup>2</sup> for overweight and ≥28.0 kg/m<sup>2</sup> for obese) according to the criteria for Chinese population.<sup>19</sup> Numbers and percentages were used for categorical variables, and mean and SD, or median and IQRs, for continuous variable. The  $\chi^2$  test or the Fisher's exact test was used to compare demographics, gestational characteristics and gestational comorbidities between groups.

We initially explored the association between the use of IVF and SMM using univariable analysis, and conducted a subgroup analysis by the type of gestations (ie, singleton vs multiple gestations). We used Woolf's test to examine homogeneity of ORs between subgroups. We also analysed the association between the use of IVF and multiple gestations.

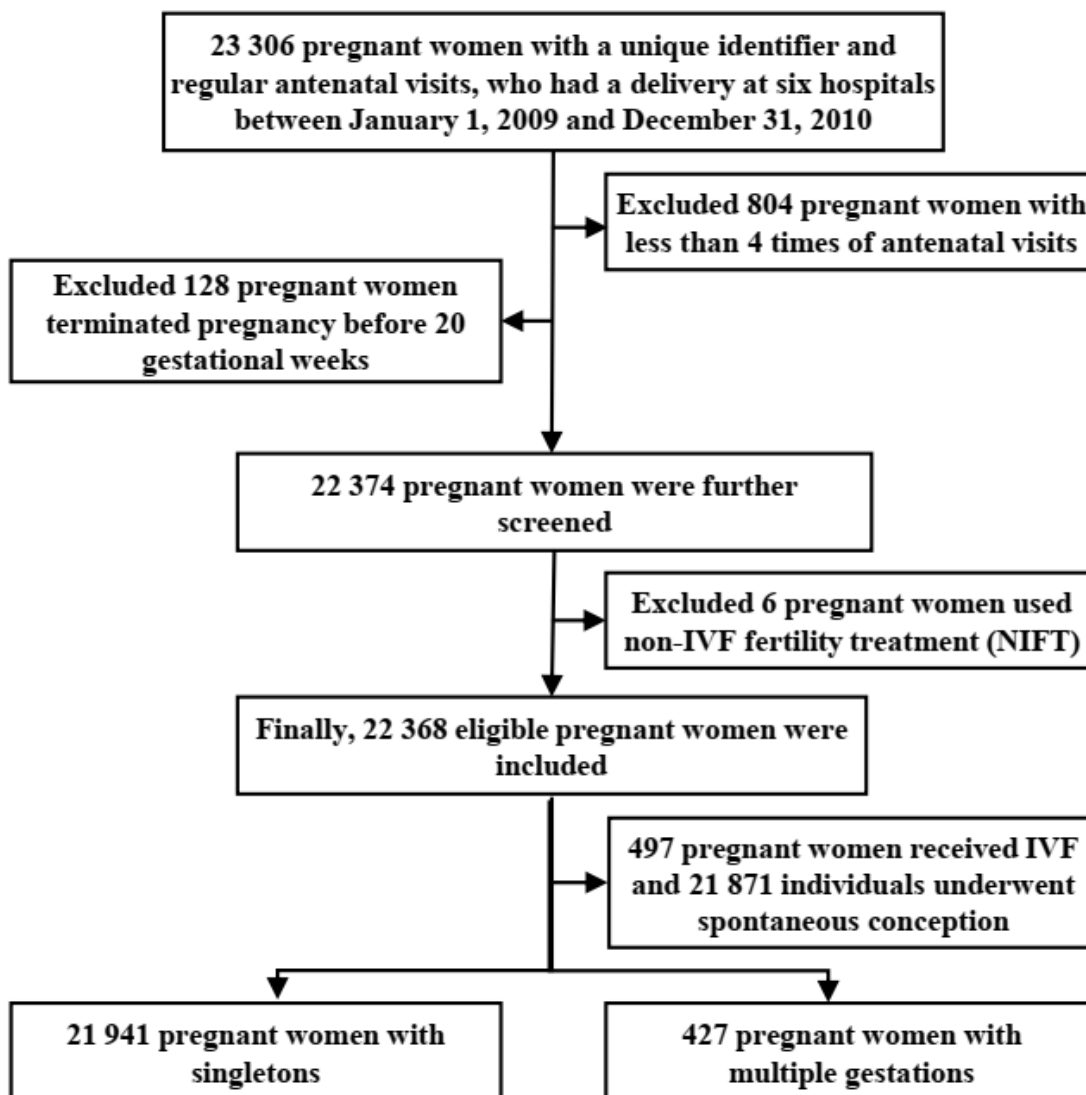
We also conducted multivariable logistic regression analysis to control for confounding effects of potentially important variables, selected based on clinical relevance and prior research evidences. These included maternal age (<35 years vs ≥35 years), prepregnant BMI (underweight vs normal weight vs overweight vs obese),

residence location (rural vs urban area), parity (multiparity vs nulliparity), smoking before pregnancy (yes vs no), and presence of IDA (yes vs no), HBV infection (yes vs no), hypertension (yes vs no), GDM (yes vs no), cardiac diseases (yes vs no) and gynaecological diseases (yes vs no).

We hypothesised that the variable of multiple gestations was a mediator of the putative association between the use of IVF and SMM, and used the formal method to test the mediation effect of multiple gestations, as below.<sup>20 21</sup>

- ▶ We first conducted multivariable logistic regression analysis without the variable of multiple gestations (model 1), and the coefficient of IVF in relation to SMM was recorded as  $\beta$ .
- ▶ We then conducted a second multivariable logistic regression analysis to estimate the association between the use of IVF and multiple gestations (model 2), and the coefficient of IVF in relation to multiple gestations was recorded as  $\alpha$ .
- ▶ Subsequently, by adding the variable of multiple gestations to model 1, we conducted multivariable regression analysis (model 3). The coefficient of IVF in relation to SMM was recorded as  $\beta'$  and the coefficient of multiple gestations in relation to SMM was recorded as  $\gamma$ . In order to test for the assumption of absence of exposure-mediator interaction, we additionally conducted an analysis by including an interaction term of IVF by multiple gestations in model 3.
- ▶ To judge whether the mediation effect was present, we used the following rules:
  - If the hypothesis test for  $\beta$  was not statistically significant ( $p > 0.05$ ), the subsequent analyses would not be necessary.
  - Conditional on the statistical significance of  $\beta$  ( $p < 0.05$ ), if  $\alpha$  and  $\gamma$  were both statistically significant ( $p < 0.05$ ), and  $\beta'$  was not statistically significant ( $p > 0.05$ ), the multiple gestations would be judged as a mediator for the association between IVF and SMM.
  - If no statistical significance was identified either for  $\alpha$  or  $\gamma$ , the Sobel method was used to explore the mediation,<sup>22</sup> in which case the statistical significance ( $p < 0.05$ ) suggested the presence of mediation effect. Otherwise, the hypothesis of mediation effect was rejected.

As a supporting exploratory analysis, we further investigated the association between IVF and SMM among singleton pregnancies (model 4), which was a subset analysis of singleton pregnancies based on model 1. Given that one centre only collected data during 1 year (2009), we conducted a sensitivity analysis of removing this centre. In addition, in order to test the robustness of our results, we conducted another sensitivity analysis excluding severe pre-eclampsia and blood transfusion from the SMM event set. We conducted all the statistical analyses by Stata V.12.0 software (StataCorp, College Station, Texas, USA). The test with a two-tailed  $p$  value of less than 0.05 was considered statistically significant.



**Figure 1** Flow chart of the included population. IVF, in vitro fertilisation.

### Patient and public involvement statement

Neither pregnant women nor the public were involved in the design and conduct of the study.

### RESULTS

After screening 23 306 pregnant women, 22 368 met the eligibility criteria and were included in the analyses (figure 1). The number of deliveries at the six hospitals were 358, 1543, 2826, 3591, 4684 and 9366, respectively. Among those, 497 (2.2%) received IVF and 21 871 (97.8%) underwent spontaneous conception; 427 (1.9%) were multiple gestations and 21 941 (98.1%) of singletons occurred during current pregnancy.

There were 776 SMM events (incidence 34.7/1000 live births, 95% CI 32.3/1000 to 37.1/1000), including 525 severe maternal complications (incidence 23.5/1000 live births, 95% CI 21.5/1000 to 25.5/1000), 404 use

of critical interventions (incidence 18.1/1000, 95% CI 16.3/1000 to 19.8/1000), and 6 maternal near-misses (incidence 0.27/1000, 95% CI 0.05/1000 to 0.5/1000). Use of blood products and severe pre-eclampsia were the primary components of SMM, accounting for 370 and 311 events (table 1). The incidences of SMM among six hospitals varied from 14.1/1000 to 51.6/1000. No pregnant woman died during the study period.

The average maternal age was 27.6 years and the average gestational age was 38.4 weeks; 99.4% were Han nationality; and the average prepregnant BMI was 26.6 kg/m<sup>2</sup>. Women who were aged 35 years or older ( $p<0.001$ ), resided in urban area ( $p<0.001$ ), had nulliparity ( $p=0.003$ ) and smoked before pregnancy ( $p<0.001$ ) were more likely to receive IVF than spontaneous pregnancy. Among those who received IVF, 18.71% had multiple gestations, as opposed to 1.53% of

**Table 1** The category and number of SMMs in the study population

Category	Subtype	Number (%)
Severe maternal complications	Severe postpartum haemorrhage	216 (0.97)
	Severe pre-eclampsia	311 (1.39)
	Eclampsia	9 (0.04)
	Sepsis or severe systemic infection	0 (0)
	Ruptured uterus	4 (0.02)
	Severe complications of abortion	0 (0)
Critical interventions or intensive care unit use	Admission to intensive care unit	27 (0.12)
	Interventional radiology	4 (0.02)
	Laparotomy	45 (0.20)
	Use of blood products	370 (1.65)
Life-threatening conditions (near-miss criteria)	Cardiovascular dysfunction	5 (0.02)
	Respiratory dysfunction	2 (0.01)
	Renal dysfunction	2 (0.01)
	Coagulation/haematological dysfunction	6 (0.03)
	Hepatic dysfunction	2 (0.01)
	Neurological dysfunction	2 (0.01)
	Uterine dysfunction	6 (0.03)
<b>Total</b>		<b>776 (3.47)</b>

SMM, severe maternal morbidity.

the population who experienced spontaneous conception ( $p<0.001$ ) (table 2).

Multiple gestational comorbidities during current pregnancy, including IDA ( $p=0.001$ ), HBV ( $p=0.044$ ), GDM ( $p<0.001$ ) and gynaecological diseases ( $p<0.001$ ), were statistically associated with IVF. The use of IVF was also associated with gestational age ( $p<0.001$ ) and caesarean section delivery ( $p=0.020$ ) (tables 2 and 3).

Univariable analysis showed statistically significant association between the use of IVF and higher risk of SMM (OR 1.69, 95% CI 1.14 to 2.49). Among the singleton pregnancy subgroup, the use of IVF was not associated with risk of SMM (OR 0.77, 95% CI 0.41 to 1.45); among the multiple gestation subgroup, IVF was not associated with SMM either (OR 1.10, 95% CI 0.61 to 1.97); homogeneity Woolf's test:  $p=0.42$  (table 4). The use of IVF was associated with more multiple gestations (OR 14.84, 95% CI 11.56 to 19.06).

We conducted four multivariable logistic regression models. Model 1, without including the variable of multiple gestations, showed that IVF was associated with higher risk of SMM (adjusted OR (aOR) 1.54, 95% CI 1.03 to 2.29). Model 2, assessing the association between the use of IVF and multiple gestations, showed that IVF was strongly associated with multiple gestations (aOR

**Table 2** Demographic and gestational characteristics in the included population (n, %)

Characteristics	IVF (n=497)	Spontaneous conception (n=21 871)	P values
<b>Maternal age, years</b>			
<35	383 (77.06)	19 302 (88.25)	<0.001
≥35	114 (22.94)	2 569 (11.75)	
<b>Prepregnancy BMI (kg/m<sup>2</sup>)</b>			
<18.5	1 (0.20)	39 (0.18)	0.118
18.5–23.9	91 (18.31)	4 176 (19.09)	
24.0–27.9	236 (47.48)	11 298 (51.66)	
≥28.0	169 (34.00)	6 358 (29.07)	
<b>Residence location</b>			
Urban area	339 (68.21)	13 039 (59.62)	<0.001
Rural area	158 (31.79)	8 832 (40.38)	
<b>Parity</b>			
Nulliparity	410 (82.49)	16 787 (76.75)	0.003
Multiparity	87 (17.51)	5 084 (23.25)	
<b>Smoking before pregnancy</b>			
Yes	49 (9.86)	1 190 (5.44)	<0.001
No	448 (90.14)	20 681 (20681)	
<b>Multiple gestations</b>			
Yes	93 (18.71)	334 (1.53)	<0.001
No	404 (81.29)	21 537 (98.47)	
<b>Gestational age, weeks</b>			
≥37	405 (81.49)	20 018 (91.53)	<0.001
<37	92 (18.51)	1 853 (8.47)	
<b>Delivery mode</b>			
Vaginal delivery	132 (26.56)	6 878 (31.45)	0.020
Caesarean section	365 (73.44)	14 993 (68.55)	

BMI, body mass index; IVF, in vitro fertilisation.

14.75, 95% CI 11.38 to 19.10). Model 3, by adding the variable of multiple gestations to model 1, showed that IVF was not statistically associated with SMM (aOR 0.89, 95% CI 0.58 to 1.36), but multiple gestations were associated with higher risk of SMM (aOR 5.92, 95% CI 4.88 to 7.83). An additional analysis by adding the interaction term of IVF by multiple gestations to model 3 showed that the interaction term was not statistically significant (aOR 1.15, 95% CI 0.36 to 3.68).

Using established rules for judging the mediation effect, these models suggested that multiple gestations were a mediator for the association between IVF and SMM. As a supporting and exploratory analysis, model 4, which included singleton pregnancies only, further suggested that IVF was not associated with SMM (aOR 0.70, 95% CI 0.37 to 1.32). In model 1, model 3 and model 4, we controlled for an identical set of confounders (table 5).

**Table 3** Gestational comorbidities between IVF and spontaneous population (n, %)

Gestational comorbidities	IVF (n=497)	Spontaneous conception (n=21 871)	P values
<b>IDA</b>			
No	459 (92.35)	20 904 (95.58)	0.001
Yes	38 (7.65)	967 (4.42)	
<b>HBV infection</b>			
No	467 (93.96)	20 953 (95.80)	0.044
Yes	30 (6.04)	918 (4.20)	
<b>Hypertension</b>			
No	488 (98.19)	21 583 (98.68)	0.341
Yes	9 (1.81)	288 (1.32)	
<b>Cardiac diseases</b>			
No	491 (98.79)	21 577 (98.66)	0.793
Yes	6 (1.21)	294 (1.34)	
<b>GDM</b>			
No	408 (82.09)	20 090 (91.86)	<0.001
Yes	89 (17.91)	1 781 (8.14)	
<b>Gynaecological diseases</b>			
No	441 (88.73)	20 326 (92.94)	<0.001
Yes	56 (11.27)	1 545 (7.06)	

GDM, gestational diabetes mellitus; HBV, hepatitis B virus; IDA, iron deficiency anaemia; IVF, in vitro fertilisation.

The sensitivity analyses—by removing one centre with a small number of pregnant women, or excluding severe pre-eclampsia and blood transfusion from the SMM event set—showed similar results (online supplementary tables 2 and 3).

## DISCUSSION

### Findings and interpretations

Our study, through established statistical tests, suggests that the association between the use of IVF and higher

risk of SMM may be mediated by multiple gestations. That is, in the population that used the IVF technique, the resulting multiple gestations would lead to a statistically significant higher risk of SMM; this did not occur among singleton pregnancies, although the relatively wide CIs cannot preclude the possibility of risk increase (in this case aOR 0.37 to 1.32), suggesting a moderate level of uncertainty. This finding was consistent with previous findings that multiple gestations were an established risk factor for severe adverse outcomes, both for pregnant women and fetuses.<sup>23 24</sup> In an ideal situation, singleton pregnancy may be the goal of IVF.<sup>25–27</sup>

Our findings refute the hypothesis that IVF itself is a risk factor for increased risk of SMM. It also intrigues an important question as to what would be the best approach for embryo transfer. Clinicians should carefully assess the possibility of multiple gestations for those women planning to receive the IVF technique. In particular, the likelihood of multiple gestations is not low in the population using IVF. In our analysis, 18.71% (93/497) pregnant women of those receiving IVF were multiple gestations; in USA and Europe, about 40% and 21% of treatment cycles are transferred at least three embryos, respectively, resulting 20%–30% of conceptions by IVF are multiple gestations.<sup>28–30</sup>

Although several authorities have recommended that a limited number of embryos should be transferred,<sup>27 31</sup> no definitive conclusion has been achieved as to the number of embryo transfers. Some have proposed elective single-embryo transfer,<sup>32 33</sup> and recommended a number of factors for clinical assessment when choosing the number of embryos, including maternal age, embryo stage, coexisting medical condition and sub-type of IVF (eg, donor-oocyte cycles and frozen-embryo transfer cycles).<sup>27 34</sup> In the Chinese setting, no clear recommendations are available. ART is not reimbursed by National Basic Medical Insurance in China. Patients who choose ART are typically wealthy, and prefer to transfer multiple embryos to improve the one-time success rate. Thus, despite the informed

**Table 4** Univariable analysis of SMM associated with IVF among the whole population and subgroups (n, %)

Mode of conception	SMM (n=776)	Non-SMM (n=21 592)	OR (95% CI)
<b>Overall (n=22 368)</b>			
Spontaneous	748 (96.39)	21 123 (97.83)	
IVF	28 (3.61)	469 (2.17)	1.69 (1.14 to 2.49)
<b>Singleton subgroup*(n=21 941)</b>			
Spontaneous	688 (98.57)	20 849 (98.15)	
IVF	10 (1.43)	394 (1.85)	0.77 (0.41 to 1.45)
<b>Multiple gestation subgroup*(n=427)</b>			
Spontaneous	60 (76.92)	274 (78.51)	
IVF	18 (23.08)	75 (21.49)	1.10 (0.61 to 1.97)

\*Woolf's test of homogeneity:  $\chi^2=0.652$ ,  $p=0.42$ .

IVF, in vitro fertilisation; SMM, severe maternal morbidity.

**Table 5** Multivariable logistic regression analysis of IVF associated with SMM

Variables in the models	Model 1 (all pregnancies) aOR (95% CI)	Model 2 (all pregnancies) aOR (95% CI)	Model 3 (all pregnancies) aOR (95% CI)	Model 4 (singletons) aOR (95% CI)
Dependent variable	SMM	Multiple gestations	SMM	SMM
Mode of conception				
IVF versus spontaneous	1.54 (1.03 to 2.29)	14.75 (11.38 to 19.10)	0.89 (0.58 to 1.36)	0.70 (0.37 to 1.32)
Multiple gestations	–	–	5.92 (4.48 to 7.83)	–
Maternal age, years				
≥35 vs <35	1.54 (1.26 to 1.88)	1.41 (1.07 to 1.86)	1.52 (1.24 to 1.86)	1.62 (1.31 to 2.00)
Prepregnancy BMI				
Underweight versus normal weight	0.78 (0.10 to 5.77)	–	0.81 (0.11 to 6.04)	0.78 (0.10 to 5.78)
Overweight versus normal weight	1.03 (0.83 to 1.27)	1.48 (1.06 to 2.07)	1.00 (0.81 to 1.24)	0.96 (0.77 to 1.19)
Obese versus normal weight	1.48 (1.19 to 1.84)	2.84 (2.04 to 3.96)	1.38 (1.10 to 1.72)	1.27 (1.02 to 1.60)
Residence location				
Rural area versus urban area	1.19 (1.02 to 1.38)	1.23 (1.00 to 1.51)	1.17 (1.00 to 1.36)	1.20 (1.03 to 1.40)
Parity				
Multiparity versus nulliparity	1.41 (1.19 to 1.67)	0.83 (0.64 to 1.07)	1.45 (1.22 to 1.71)	1.46 (1.23 to 1.75)
Smoking before pregnancy				
Yes versus no	0.56 (0.38 to 0.83)	0.30 (0.15 to 0.60)	0.60 (0.40 to 0.88)	0.63 (0.43 to 0.94)
IDA				
Yes versus no	3.22 (2.54 to 4.09)	–	3.10 (2.44 to 3.95)	3.00 (2.32 to 3.87)
HBV infection				
Yes versus no	1.59 (1.19 to 2.13)	–	1.59 (1.18 to 2.13)	1.61 (1.18 to 2.20)
Hypertension				
Yes versus no	4.83 (3.49 to 6.69)	–	4.76 (3.43 to 6.62)	4.91 (3.48 to 6.90)
Cardiac diseases				
Yes versus no	4.00 (2.81 to 5.70)	–	3.87 (2.70 to 5.55)	4.07 (2.81 to 5.90)
GDM				
Yes versus no	1.17 (0.92 to 1.49)	–	1.13 (0.88 to 1.44)	1.21 (0.94 to 1.56)
Gynaecological diseases				
Yes versus no	0.90 (0.68 to 1.20)	–	0.88 (0.66 to 1.18)	0.90 (0.66 to 1.22)
Individuals	22368	22368	22368	21941
Number of variables	14	7	15	14
Likelihood ratio $\chi^2$	314.8	381.4	433.5	273.1

Model 1 estimated the association between IVF and SMM by multivariable logistic regression analysis without the variable of multiple gestations in all pregnant women.

Model 2 estimated the association between IVF and multiple gestations by multivariable logistic regression analysis in all pregnant women.

Model 3 estimated the association between IVF and SMM by multivariable logistic regression analysis including the variable of multiple gestations in all pregnant women.

Model 4 estimated the association between IVF and SMM by multivariable logistic regression analysis without the variable of multiple gestations in a subset of singletons.

BMI, body mass index; GDM, gestational diabetes mellitus; HBV, hepatitis B virus; IDA, iron deficiency anaemia; IVF, in vitro fertilisation; SMM, severe maternal morbidity.

risk associated with the technology, transfer of multiple embryos is commonly practised.

### Comparison with previous studies

A few studies previously explored the association between the use of IVF and risk of SMM, all of which were conducted in North America. One study,<sup>14</sup> enrolling 6543

women who delivered live births >20 gestational weeks at one centre, suggested that the use of ART, compared with spontaneous conception, increased the overall risk of SMM; however, ART did not increase the risk among singleton pregnancies. The second study,<sup>13</sup> collecting 1016 618 deliveries from Truven Health MarketScan

Commercial Claims and Encounters Databases between 2008 and 2012, concluded that ART was associated with increased risk of SMM among singletons, but not among multiple gestations. By linking ART treatment records with birth certificates and maternal and infant hospitalisation records in Massachusetts between 2004 and 2010, the third study included 458918 pregnant women aged 18 years or older who had live-born singletons or twins, and suggested that ART was associated with increased risk of SMM among singletons, and the risk was even higher among twins.<sup>12</sup> In addition, a study testing the association among singletons in 114409 pregnancies from the Ontario birth registry (BORN Information System) and Canadian Assisted Reproductive Technologies Register in Canada showed that IVF was associated with an increased risk of SMM.<sup>15</sup>

In assessing the association between the use of IVF and SMM, previous studies either explicitly or implicitly considered multiple gestations as a confounder. However, either multiple gestations or singleton pregnancy was a consequence of IVF, that is, a characteristic not measured at baseline. Thus, an appropriate interpretation is that the resulting multiple gestations, due to the use of IVF, may determine the undesirable effect on SMM. As a result, our analyses have supported this hypothesis. Our study proposed and empirically examined the mediation effect of multiple gestations between IVF and SMM. In retrospect, the mediation effect of multiple gestations was neither adequately recognised nor explored.

In our study, we found no statistically significant association between the use of IVF and singletons, but a few earlier studies otherwise reported statistically significant association. We have thoroughly compared these studies with our study, as shown in online supplementary table 4. The apparent differences may be explained as below. First, those published studies conducted in North America defined SMM by 5–25 indicators using International Classification of Diseases (ICD)-9 or ICD-10 codes.<sup>12–15</sup> In contrast, our study used SMM defined by WHO,<sup>8</sup> which included 15 indicators. Our definition of SMM had a broader spectrum of serious adverse events, such as severe postpartum haemorrhage, severe pre-eclampsia, laparotomy and admission to ICU, four of which accounted for a large proportion of total events (77.2%) in our data set. Thus, the incidence of SMM—the outcome of interest—in our study was much higher than that in other studies (3.5% vs 1.1%–1.5%).<sup>12–15</sup>

Second, the definition of exposure seemed different between earlier studies versus ours. In the two studies by Martin *et al* and Belanoff *et al*, they included a diversity of ARTs, such as ovarian stimulation by drug treatments, IUI and IVF. The increased risk of SMM among singletons may be a result of combined effects of several interventions.<sup>12–14</sup> In comparison, our analysis exclusively included IVF.

Third, the difference in results is possibly due to the varying adjustments for confounding effects across those

studies. For instance, some important confounders, such as maternal IDA and HBV infection identified at the first antenatal visit, were included in our analysis, mainly because of high prevalence of IDA and HBV infection in the Chinese population.<sup>35 36</sup>

Finally, one potentially important difference is the study population. In our study, we exclusively included pregnant Chinese women; in other studies, the population was North American. Another potentially important difference, which is closely related to the population, is that there is a difference between the healthcare systems in China and USA. Such differences may have contributed to the apparent differences in the findings.

Therefore, with all these variations, one may interpret the findings cautiously. Our findings do not offer a definitive answer as to the mediation effect of multiple gestation on SMM; instead it generates an important hypothesis, which warrants further testing by well-designed, rigorously conducted prospective studies.

### Strength and limitations

Our study has several strengths. First, this is the first multi-centre cohort study addressing the putative association between IVF and SMM among the Chinese population. We included 22368 consecutive deliveries in a naturalistic medical environment; the finding is thus generalisable. Second, we used established methods to examine the mediation effect of multiple gestations between IVF and SMM, by which we were able to clearly clarify the role of multiple gestations. The results suggested that transferring a limited number of embryos may reduce SMM. Third, we used rigorous methods to collect data and strictly followed the WHO approach to measure and identify SMM. In particular, we checked the definitions of required indicators, and confirmed the consistency between our available data and the standard. Last, the population receiving IVF is more likely to have specific demographic and social characteristics,<sup>37</sup> as well as health and fertility problems due to gestational comorbidities (eg, IDA and other haematological diseases, HBV infection and other hepatic diseases, cardiopathy, and hypertension).<sup>18 36 38</sup> We have used a more rigorous approach by controlling for these confounders in our analyses, compared with previous studies.

Our study has a few limitations also. Given the limited sample size of multiple gestations in our population, we did not conduct multivariable regression among multiple gestations to explore the association between IVF and SMM. Besides, similar to previous studies, the data were retrospectively collected from medical records and EMRs at six hospitals. Although the conception mode of this pregnancy was strictly recorded, there was a small likelihood of misclassification about IVF, since some pregnant women may not fully report the use of IVF. The number of pregnant women varied among the six hospitals, and one centre had a small number of pregnant women. Nevertheless, sensitivity analysis by excluding this centre showed similar findings. In addition, the admission to



ICU and use of blood products may be influenced by physician's experience and medical conditions; such variations may affect the measurement of SMM. Finally, some important confounding factors, such as uterine status and male factor, were not available in our data set and thus not included in the adjustment analyses. We were also unable to assess the associations between types of IVF (donor versus autologous, fresh versus frozen) and SMM, as such information was unavailable.

## CONCLUSION

In summary, our study suggested that the apparent effect of IVF on SMM may be mediated by multiple gestations. The finding also suggested the need for careful consideration of the number of embryo transfers during the practice of IVF, since transferring more embryos may increase the risk of multiple gestations. Given the limitations of our study, however, the findings are not definitive. Further studies, particularly using prospective designs, should be conducted to confirm the likely mediation effect of multiple gestations.

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