Maternal risk factors associated with preterm birth after IVF/ICSI

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Abstract

In vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI).is associated with an increased risk of preterm (33rd - 37th gestational week), and early preterm birth (20th - 32nd gestational week). The underlying general and procedure related risk factors are not well understood so far. 4,328 infertile women undergoing IVF/ICSI were entered into this study. The study population was divided into three groups: a) early preterm birth group (n=66), b) preterm birth group (n=675) and c) full-term birth group (n=3653). Odds for preterm birth were calculated by stepwise multivariate logistic regression analysis. We identified seven independent risk factors for preterm birth and four independent risk factors for early preterm birth. Older (>39) or younger (<25) maternal age (OR:1.504, 95%CI: 1.108-2.042,P=0.009; OR: 2.125, 95%CI: 1.049-4.304,P=0.036, respectively), multiple pregnancy (OR: 9.780, 95%CI: 8.014-11.935,P<0.001; OR: 8.588, 95%CI: 4.866-15.157,P<0.001, respectively), placenta previa (OR: 14.954, 95%CI: 8.053-27.767,P<0.001; OR: 16.479, 95%CI: 4.381-61.976,P<0.001, respectively), and embryo reduction (OR: 3.547, 95%CI: 1.736-7.249,P=0.001; OR: 7.145, 95%CI: 1.990-25.663,P=0.003, respectively) were associated with preterm birth and early preterm birth, whereas gestational hypertension (OR: 2.494, 95%CI: 1.770-3.514,P<0.001), elevated triglycerides (OR: 1.120, 95%CI: 1.011-1.240,P=0.030) and shorter activated partial thromboplastin time (OR: 0.967, 95%CI: 0.949-0.985,P<0.001) were associated only with preterm birth. In conclusion, preterm and early preterm birth risk factors in patients undergoing assisted IVF/ICSI are in general similar to those in natural pregnancy. The lack of some associations in the early preterm group was most likely due to the lower number of early preterm birth cases. Only embryo reduction represents an IVF/ICSI specific risk factor.

Article Summary

- IVF/ICSI is associated with a high risk for both preterm birth (birth <38th week of gestation) and early preterm (birth <33rd week of gestation)
- Maternal age, multiple pregnancy, placenta previa, and embryo reduction surgery were associated with an increased risk for both preterm birthand early pretermbirth after IVF/ICSI.
- Gestational hypertension, higher triglycerides and a shorter activated partial thromboplastin time were only associated with an increased occurrence of preterm birth after IVF/ICSI.
- Strengths and limitations of this study. This is a relatively huge clinical study with detailed patients' characteristics. On the other hand, it is a retrospective single center study.

Summary Box

What is already known on this subject?

IVF/ICSI is associated with a high risk for both preterm birth (birth <38th week of gestation) and early preterm (birth <33rd week of gestation) - a condition with very poor outcome. Underlying risk factors of preterm birth and especially early preterm birth in women undergoing IVF/ICSIwere not compared so far.

What does this study add?

Older (>39) or younger (<25) maternal age, multiple pregnancy, placenta previa, andembryo reduction were associated with an increased risk for both preterm birth and early preterm birth after IVF/ICSI, whereas gestational hypertension, elevated triglycerides and shorter activated partial thromboplastin time were only associated with an increased risk of preterm birth.

Introduction

Preterm delivery accounts for more than 75% of perinatal morbidity and mortality worldwide[1]. Furthermore, those infants who do survive have higher rates of long-term morbidities, including cardiovascular diseases[2] as well as neurologic and developmental disabilities, compared to infants born full term. Known maternal risk factors for preterm birth in the general population include having a previous premature birth, twin pregnancy, an interval of less than six months between pregnancies, history of multiple miscarriages or abortions, smoking cigarettes or using illicit drugs, cardio-metabolic diseases such as hypertension or diabetes, and infections, particularly of the amniotic fluid and lower genital tract[3-7]. Conceiving through in vitro fertilization represents another risk factor in a subgroup of women undergoing assisted reproduction technologies (ART). The risk factors increasing the likelihood for preterm birth in this particular population are, however, as of today, not well understood. Numerous studies[8]analyzed maternal and offspring outcomes after ART, whereas larger sized studies focusing specifically on the risk factors for preterm birthand especially early preterm birth especially associated to poor offspring outcome are lacking. However, this is a clinically important topic, since the understanding of ART related risk factors for preterm birth might identify changeable factors offering potential treatment options to improve offspring short term and long term outcome in women undergoing ART. The aim of the current study was to search for risk factors for preterm and early preterm birth in a large cohort of women who underwent ART.

Results

Description of the cohort

From the primary dataset of 4349 treated women, we excluded 13 cases in which the gestational age was unclear and 8 cases that were post-term pregnancies. Thus finally 4,328 cases were included into the study, 3653 of them were full-term deliveries. The prevalence of preterm birth and early preterm birth was 15.5% and 1.8% respectively (Table 1 and Supplementary Figure 1). The median age of the participating women was30 (2733) years old. The median BMI of the women was 21.94(20.08,23.76)kg/m^2.The study cohort consisted mostly of Han Chinese women (87.8%). Caused for infertility in the entire study populations were distributed as follows: primary infertilitywas present in 42.9%and secondary infertility in 57.1%.Theduration of infertility was4(2,6□years. Median birthweight was 3100□2700, 3500□g, Median gestational age was38.4(37.3,39.4□weeks. For more details see Table 1 and 2.

Table 1
Basic parameters before super-ovulation were compared between preterm birth group and full term birth group. [a]:Preterm birth group vs.full-term birth; [b]: Early-preterm birth group vs.full-term birth. BMI,bodymassindex.

Variables	Preterm birth (n=675)	Early-preterm birth (n=66)	Full-term birth (n=3653)	P^a	P^b
Maternal age (year)				0.026	0.021
25~39	599(88.7%)	55(83.3%)	3339(91.4%)		
≥40or20~24	76(11.3%)	11(16.7%)	314(8.6%)		
Maternal BMI (kg/m^2)				0.342	0.506
<18.5	57(8.4%)	7(10.6%)	301(8.2%)		
18.5~23.99	455(67.4%)	41(62.1%)	2523(69.1%)		
24~27.99	139(20.6%)	15(22.7%)	741(20.3%)		
≥28	24(3.6%)	3(4.5%)	87(2.4%)		
Maternal nationality				0.055	0.642
Han	593(90.4%)	59(90.8%)	3115(87.3%)		
Non-Han	63(9.6%)	6(9.2%)	455(12.7%)		
Maternal education level				0.342	0.473
Junior middle school and below	259(39.7%)	34(53.1%)	1439(40.4%)		
Senior high school or Technical secondary school	192(29.4%)	15(23.4%)	999(28.0%)		
Junior college and above	201(30.8%)	15(23.4%)	1125(31.6%)		
Infertility period (year)				0.694	0.156
1~4	426(63.1%)	37(56.1%)	2287(62.6%)		
5~9	201(29.8%)	21(31.8%)	1132(31.0%)		
≥10	48(7.1%)	8(12.1%)	234(6.4%)		
Infertility type				0.269	0.642
Primary infertility	303(44.9%)	30(45.5%)	1556(42.6%)		
Secondary infertility	372(55.1%)	36(54.5%)	2097(57.4%)		

Variables	Preterm birth (n=675)	Early-preterm birth (n=66)	Full-term birth (n=3653)	P^a	P^b
Maternal diastolic blood pressure	70(76-80)	70(77-81)	70(75-80)	0.125	0.086
Maternal systolic blood pressure	109(113-120)	107.5(113-122)	109(114-120)	0.949	0.896

Table 2

Baseline maternal blood test results were compared between preterm birth group and full-term birth group. [a]:Preterm birth group vs. full-term birth; [b]: Early-preterm birth group vs. full-term birth group

Variable	Preterm birth (n=631)	Early-preterm birth (n=66)	Full-term birth (n=3374)	P^a	P^b
Alanine transaminase(U/L)	11.8(15.4-22.2)	12.275(15.45-19.95)	11.4(15.0-20.8)	0.077	0.532
Glutamic oxalacetic transaminase(U/L)	15.9(18.5-22.1)	16.725(19.4-23.125)	15.8(18.2-21.5)	0.089	0.056
Total bile acid(μmol/L)	0.7(1.3-2.7)	0.9(1.3-2.2)	0.7(1.3-2.6)	0.472	0.580
Total protein (g/L)	74.8(77.7-80.3)	74.6(77.25-80.375)	74.6(77.2-79.9)	0.082	0.996
Serum albumin (g/L)	46.3(48.3-50.1)	46.075(48.5-49.95)	46.3(48.0-49.7)	0.059	0.526
Serumglobulin(g/L)	26.9(29.2-31.4)	27.15(29.05-31.225)	27.0(29.1-31.3)	0.366	0.835
Total bilirubin(μmol/L)	7.378(9.5-12.2)	7.775(9.85-12.725)	7.2(9.3-12.4)	0.734	0.316
Direct Bilirubin (μmol/L)	3.0(3.7-4.6)	3.075(3.8-4.925)	3.0(3.8-4.7)	0.493	0.579
Blood urea nitrogen (mmol/L)	3.4(4.0-4.7)	3.30(3.95-4.70)	3.3(4.0-4.7)	0.780	0.772
Creatinine (μmol/L)	54.0(59.0-65.0)	52.0(58.5-66.25)	54(60-66)	0.403	0.644
Uric acid (μmol/L)	228.(266-309.5)	232.0(266.5-297.0)	227(263-302)	0.256	0.732
Cholesterol (mmol/L)	4.00(4.48-4.94)	3.745(4.27-5.01)	3.93(4.39-4.90)	0.022	0.572
Triglyceride(mmol/L)	0.71(0.98-1.39)	0.7325(1.04-1.3975)	0.69(0.91-1.30)	0.009	0.301
High density lipoprotein (mmol/L)	1.25(1.48-1.79)	1.23(1.42-1.63)	1.28(1.50-1.76)	0.628	0.134
Low density lipoprotein (mmol/L)	2.32(2.74-3.29)	2.155(2.65-3.6275)	2.26(2.68-3.17)	0.022	0.957
Apolipoprotein-A1 (g/L)	1.4(1.5-1.7)	1.3(1.5-1.6)	1.4(1.5-1.7)	0.915	0.027
Apolipoprotein-B (g/L)	0.70(0.8-1.0)	0.6(0.8-1.0)	0.7(0.8-0.9)	0.010	0.989

Variable	Preterm birth (n=631)	Early-preterm birth (n=66)	Full-term birth (n=3374)	P^a	P^b
Prothrombin time (s)	9.9(10.40-10.91)	9.9(10.4-10.9)	10(10.4-10.9)	0.945	0.746
International normalized ratio	0.92(0.96-1.00)	0.92(0.955-1.0)	0.92(0.96-1.00)	0.115	0.507
Activated partial thromboplastin time (s)	31.6(34.5-37.58)	31.55(35.0-37.6)	32.4(35.2-38.1)	0.001	0.539
Fibrinogen (g/L)	2.33(2.64-2.975)	2.4(2.69-3.005)	2.33(2.64-2.94)	0.624	0.660
Thrombin time (s)	12.6(13.3-14.1)	12.4(13.1-13.65)	12.6(13.3-14.2)	0.666	0.041

Univariate analysis showed that 14 parameters were significantly different between the full-term birth group and the preterm birth group (P<0.05), including 6 maternal parameters (age, apolipoprotein B, total cholesterol, triglycerides, low density lipoprotein and activated partial thromboplastin time), 5 pregnancy related factors (multiple pregnancy, embryo reduction, placenta previa, gestational diabetes and gestational hypertension), 2 factors related to the IVF/ICSI procedure (blastocyst transfer and number of embryos transferred)and 1 offspring related factor (infant sex) (Table 1- 3).

Table 3

Pregnancy factors after embryo implantation was compared between preterm birth group and full-term birth group. [a]:Preterm birth group vs.full-term birth; [b]: Early-preterm birth group vs. full-term birth.[b]: In the multiple pregnancy, one is female infant, another is male infant.

Variables	Preterm birth (n=675)	Early-pretermbirth (n=66)	Full-term birth (n=3653)	P^a	P^b
Treatment cycle				0.488	0.033
1	500(74.1%)	45(68.2%)	2763(75.6%)		
2	144(21.3%)	21(31.8%)	754(20.6%)		
≥3	31(4.6%)	0(0.0%)	136(3.7%)		
Fertilization method				0.169	0.554
IVF	465(68.9%)	45(68.2%)	2612(71.5%)		
ICSI	210(31.1%)	21(31.8%)	1041(28.5%)		
Blastocyst transfer (%)				0.000	0.027
yes	58(8.6%)	3(4.5%)	512(14%)		
no	617(91.4%)	63(95.5%)	3141(86%)		
Ovulation stimulation protocol				0.463	0.895
Long protocol	373(55.3%)	37(56.1%)	1951(53.4%)		
Extra long protocol	221(32.7%)	20(30.3%)	1203(32.9%)		
others	81(12.0%)	9(13.6%)	499(13.7%)		
Number of transplanted embryos				0.000	0.017
1	31(4.6%)	2(3.0%)	472(12.9%)		
2	644(95.4%)	64(97.0%)	3181(87.1%)		
Multiple pregnancy (%)				0.000	0.000
yes	483(71.6%)	47(71.2%)	792(21.7%)		
no	192(28.4%)	19(28.8%)	2861(78.3%)		
Embryo reduction (%)				0.001	0.004
Yes=0	17(2.5%)	3(4.5%)	35(1.0%)		
no	658(97.5%)	63(95.5%)	3618(99.0%)		
Gestational diabetes				0.034	0.214

Variables	Preterm birth (n=675)	Early-pretermbirth (n=66)	Full-term birth (n=3653)	P^a	P^b
yes	106(15.7%)	5(7.6%)	464(12.7%)		
no	569(84.3%)	61(92.4%)	3189(87.3%)		
Hypertensive disorder complicating pregnancy				0.000	0.705
yes	76(11.3%)	2(3.0%)	144(96.1%)		
no	599(88.7%)	64(97.0%)	3509(3.9%)		
Placenta previa				0.000	0.000
yes	30(4.4%)	3(4.5%)	22(0.6%)		
no	645(95.6%)	63(95.5%)	3631(99.4%)		
Infant sex				0.000	0.000
Male	238(35.3%)	30(45.5%)	1749(47.9%)		
Female	188(27.9%)	16(24.2%)	1532(41.9%)		
Male and female[b]	249(36.9%)	20(30.3%)	372(10.2%)		

Moreover, univariate analysis comparing early preterm birth and full-term birth showed that 10 factors were significantly different between the full-term birth group and the early preterm birth group (P<0.05), including 3 maternal parameters (age, apolipoprotein A1, and thrombin time), 5 pregnancy related factors (multiple pregnancy, embryo reduction, and placenta previa), 3factors related to the IVF/ICSI procedure (blastocyst transfer, treatment cycles and number of embryos transferred), 1 offspring related factor (infant sex) (Table 1- 3).

Multivariate analysis

The above 14 detected factors in the univariate analysis were entered into the Logistic multivariate analysis. No other factors were considered as confounders. After stepwise regression analysis, multivariate analysis showed that 7 factors (older or younger maternal age, multiple pregnancy, embryo reduction,placenta previa, gestational hypertension, higher triglycerides and shorter activated partial thromboplastin time) were left in the multivariate analysis model for preterm birth. With regard to early preterm birth, the above described 10 factors, see above, were entered into the multivariate analysis. After stepwise regression analysis, 4 factors (older or younger maternal age, multiple pregnancy, embryo reduction and placenta previa) remained significant in the multivariate analysis model for early preterm birth (Table 4, supplementary table 1).

Table 4
Stepwise multivariate logistic regression analysis comparing mothers with preterm and full-term births. Fourteen factors showing significant differences in the univariate analysis (see also Tables 1) were entered into the stepwise multivariate logistic regression analysis. The following seven factors showed no significant effect on preterm birth in the the stepwise multivariate logistic regression analysis: Apolipoprotein B, total cholesterol, low density lipoprotein, gestational diabetes,blastocyst transfer, number of embryos transferred, and offspring sex.

Variables	B	OR	95% CI		*P*
			Lower	Upper	
Maternal age*	0.408	1.504	1.108	2.042	0.009
Multiple pregnancy	2.280	9.780	8.014	11.935	<0.001
Embryo reduction	1.266	3.547	1.736	7.249	0.001
Placenta previa	2.705	14.954	8.053	27.767	<0.001
Gestational hypertension	0.914	2.494	1.770	3.514	<0.001
Triglycerides	0.113	1.120	1.011	1.240	0.030
Activated partial thromboplastin time	-0.034	0.967	0.949	0.985	<0.001
*Maternal age: 40 or 20~24 vs. 25-39.					

The results of the multivariate analyses showed that compared to the maternal age group of 25~39 years, younger mothers (20~24 years) and also older mothers (>40 years old) displayed a significantly increased preterm birth ratio by 0.50 times (OR=1.504, 95%CI:1.108-2.042,*P*=0.009) and early preterm birth ratio by 1.13 times (OR=2.125, 95%=CI: 1.049-4.304, *P*=0.036). Compared to singleton pregnancies, mother with multiple pregnancies had a significantly increased preterm birth ratio by 8.78 times (OR=9.780, 95%CI:8.014-11.935, *P*<0.001) and early preterm birth ratio by 7.58 times (OR=8.588, 95%CI: 4.866-15.157, *P*<0.001). Embryo reduction significantly increased preterm birth ratio by 2.54 times (OR=3.547, 95%CI: 1.736-7.249, *P*=0.001) and early preterm birth ratio by 6.14 times (OR=7.145, 95%CI: 1.990-25.663, *P*=0.003). Placenta previa increased also significantly both preterm birth ratio by 13.95 times (OR=14.954, 95%CI:8.053-27.767, *P*<.001) and early preterm birth ratio by 15.48 times (OR=16.479, 95%CI:4.381-61.976, *P*<.001). The presence of gestational hypertension, higher triglycerides andashorteractivated partial thromboplastin was significantly associated with preterm birth (OR=2.494, 95%CI:1.770-3.514, *P*<0.001; OR=1.120, 95%CI:1.011-1.240, *P*=0.030; OR=0.967, 95%CI:0.949-0.985, *P*<0.001, respectively) but not early preterm birth (*P*>0.05) (Table 4, Supplementary Table 1).

Discussion

Our study showed that older (>39) or younger (<25) maternal age, multiple pregnancy, placenta previa, and embryo reduction surgery were associated with an increased risk for both preterm birth and early preterm birth after IVF/ICSI. Gestational hypertension, higher triglycerides and a shorter activated partial

thromboplastin time were only associated with an increased occurrence of preterm birth but not early preterm birth. However, the lack of some associations in the early preterm group was most likely due to the lower sample size.

In several studies it was already shown that ART is associated with adverse pregnancy outcomes, such as preterm birth[9]. The underlying risk factors for preterm birth in this population, however, are not fully established so far. The current study, investigating a cohort of women who underwent ART, identified several factors which were associated with preterm birth. The majority of the identified factors, such as maternal age[10], multiple pregnancies, placenta previa, gestational hypertension, high triglycerides and hypercoagulability[11, 12], have previously been shown to be associated with an increased risk for preterm birth in the general population as well.

Our study showed an increased risk for preterm delivery in association with maternal age. Bothyounger and older women after ART treatment had an increase risk for preterm birth in our study as it was likewise seen in studies addressing this topic in the general population. Somewhat smaller study done in an ART populations mainly reported similar associations [13]. However, there is also a study with a similar study design as our study showing that women aged 25-29 were at an increased risk for preterm birth in comparison to women aged 30-34. Women aged ≥ 35 years did not display an increased risk of any type of preterm birth[14]. These previous findings may suggest that while there is a positive association between maternal age and the risk for preterm birth, younger women who conceived via ART may display a higher risk for preterm birth compared to older women who conceived undergoing ART. As the proportion of women who conceive with ART also shows an age related increase, these results may also reflect the increased clinical risk of adverse birth outcomes among young women who needed ART to conceive[13].

The current study also identified gestational hypertension as risk factor for preterm birth in women who needed ART to conceive. This finding is in line with the current literature, gestational hypertension was shown to be associated with an increased risk for preterm birth in both the general population and women who underwent ART. Moreover, it was shown that ART is associated with a higher frequency of gestational hypertension and preeclampsia as compared to natural pregnancy [15, 16].The underlying reasons for a higher frequency of gestational hypertension in ART pregnancies remain incompletely understood. Wang et al. showed in a large cohort comparing ART pregnancies to natural conception that ART is associated with a higher prevalence for gestational hypertension, yet this association disappeared once data was stratified by multiple birth cases[17]. The authors concluded that multiple pregnancy which is associated with ART is the single most likely explanation for the increased rate of gestational hypertension among ART mothers.

Another risk factor for preterm birth found by the current study is placenta previa. Placenta previa is associated with preterm birth in the general population as well [18]. A study that investigated mothers who had conceived both naturally and via ART, showed that the risk of placenta previa was three-fold higher in

the ART pregnancy[19]. The mechanisms underlying this phenomenon still have to be elucidated. It is hypothesized that ART related procedures, such as an induction of uterine contractions due to transcervical catheter insertion or the unique endocrinological environment with high estradiol concentrations following ART cycles might be responsible[20].

Regarding maternal laboratory parameters, the current study demonstrated a positive association between maternal triglycerides, shorter activated partial thromboplastin time and preterm birth. Both high triglycerides and hypercoagulability were already shown to be associated with an increased risk for preterm birth in the general population[21, 22]. Hypercoagulabilty and preterm birth has not yet been investigated extensively. Results from one small prospective study analyzing the relationship between maternal hypercoagulability and preterm labor in 76 women demonstrated a statistically significant procoagulant activity, expressed by a shorter prothrombin time and activated partial thromboplastin time, in pregnant women with premature uterine contractions who gave birth prematurely[22]. The presence of hypercoagulation before starting an IVF treatment was shown to be associated with negative IVF outcomes such as pregnancy loss. Our study clearly established hypercoagulability as an risk factor for preterm birth in ART and hence may help to clarify ongoing debates on this subject[23].

With regard to triglycerides it was demonstrated that high maternal triglycerides levels during pregnancy are related to an increased risk for pretermbirth in both obese women and in women with normal BMI in the general population conceiving naturally [21]. There are studies that investigated lipid levels and ART outcomes showing that maternal triglyceride are inversely associated with live birth rate, however data regarding the relationship between maternal triglycerides and premature birth in the setting of ART are scarce[24].

Our study is in good agreement with previous studies indicating that multiple pregnancy is a strong risk factor for preterm birth, both in the general population as well as in women who conceived trough ART[25]. Multiple pregnancy is considered one of the largest hazard of ART. Until now ART is associated with a high number of multiple pregnancies due to the current policy transferring multiple embryossimultaneously to achieve a high pregnancy rate[26]. To reduce the risks associated with multiple pregnancy, embryo reduction is performed frequently. Previous meta-analyses have shown that embryo reduction improves outcomes in triplet pregnancies, but never to that degree of singleton pregnancies. Aneffective method to reduce the risk of multiple births in ART is an elective single embryo transfer, a policy that is adopted by an increasing number of guidelines. However, studies also demonstrated that elective single embryo transfer is not associated with a reduction in the risk for preterm delivery[27].

In conclusion, our studydemonstratedthat maternal age, multiple pregnancy, embryo reduction and placenta previa could increase the risk of preterm birth in womenundergoing IVF/ICSI. During ART treatment, the numbers of embryo transfers per cycle should be reduced to two or even to one thus reducing the need for embryo reduction procedures or multiple pregnancy. Strengthening antenatal care is necessary during pregnancy, especially for the patients with placenta previa. The finding that coagulation

abnormalities are linked to preterm birth needs independent confirmation and if confirmed may stimulate clinical trials testing drugs interfering with the coagulation system as it is for example done in pregnant women suffering from activated protein C resistance[28].

Methods

Ethics, inclusion and exclusion criteria, data collection

The retrospective study was approved by the ethics committee of Reproductive & Genetic Hospital of Citic-Xiangya, Changsha, China (approval document number LL-SC-2019-003). The need of informed consent was waived by the ethics committee of Reproductive & Genetic Hospital of Citic-Xiangya, Changsha, China.

A total of 4349 infertile women who had undergone IVF/ICSI treatment and obtained live birth in the Reproductive & Genetic Hospital of Citic-Xiangya from January 1^{st,}2016 to December30th,2017 were collected.

Inclusion criteria were as follows:

a) Women treated with super-ovulation protocols exactly as described previously[29]

b) Fresh embryo transfer recipients, who received IVF/ICSI treatment

c) Giving live birth after ART

Exclusion criteria were as follows:

a) Using donor sperms or donor eggs for ART

b) Complete clinical data were not available

c) Post-term birth (>42nd week of gestation) cases were excluded

d) Infertile couples with known female or male genetic causes of infertility

Gestational age was calculated by adding 2 weeks (14 days) to the number of days since fertilization[30]. Note: Gestational age was determined as the gestational 17thday when 6-8 cell embryo was transferred into the uterus, and gestational 19thwhen blastocyst was transferred into the uterusafter treatment in the fresh embryo transfer recipients.

Full-term birth was defined as a live birth with a gestational age between 37 but not over 42 weeks (37 weeks ≤gestational age<42 weeks). Preterm birth was defined asalivebirthwith a gestational age of at least 20but not over 37 weeks (20 weeks ≤gestational age<37 weeks)[31]. Early-preterm birth was defined

as a live birth with a gestational age between 20 but not over 32 weeks (20 weeks ≤gestational age<32 weeks)[32].

All patient's data (clinical data as well as laboratory data) used in our study were extracted from the routine electronic patient records used in our hospital.

Clinic data collection

A structured medical history was taken. The following risk factors for preterm birth were examined in this study:

Maternal risk factors: (1) Basic parameters before super-ovulation: nationality, education, age, body height, body weight, infertility duration, types of infertility, causes of infertility (maternal causes, paternal causes, maternal and paternal causes, unknown causes), blood pressure readings. (2) Pregnancy history: parity, artificial abortion, drug abortion, spontaneous abortion, ectopic pregnancy, number of deliveries, vaginal delivery, cesarean section. (3) Blood test result before super-ovulation: liver and kidney function, lipid items, blood coagulation function. (4) Pregnancy related factors: multiple pregnancy, embryo reduction, gestational diabetes, gestational hypertension, placenta previa.

Relevant risk factors during IVF/ICSI procedure: cycle count, fertilization way, blastocyst transfer, ovulation induction scheme, source of sperm, transferredembryocount, dosage of gonadotropin, ovulation inducing days.

Offspring data: gestational age at delivery, gender, birth weight.

Basic parameters about the mother, pregnancy history, gynecological complications, and relevant risk factors during IVF/ICSI procedure came from the case report in the hospital. Furthermore, blood test results from maternal blood taken before the beginning of superovulation was extracted from the case report. Pregnancy related factors and offspring data were followed up strictly by special nurse.

Patient and Public Involvement

This study is a retrospective study. Data were obtained through the electronical medical record system of the hospital. Patients were not directly involved in this study. The patients were unaware of the results of the study.

Statistical analysis

Continuous variables are represented as mean±standard deviation for normally distributed variables and student's unpaired t-test was used for comparison of variables between two groups. Continuous variables are represented as median and quartiles M(Q_1-Q_3) for non-normally distributed variables and Mann-Whitney nonparametric test was used for comparison of variables between groups. Categorical variables

are described as frequency and percentages. Pearson's chi-square test was used for testing qualitative data and Fisher's exact test was used when the expected frequencies were <5%.

Multivariate Logistic regression analyses with step forward selection using the likelihood method were applied to examine the association between the patient's characteristics and the risk of preterm birth. Analyzed variables with $P<0.05$ in the univariate analysis were entered into the multivariate analysis. No other factors were considered as confounders. Results are represented as ORs with corresponding 95% CIs and *P* values.

Statistical package for social sciences (SPSS version 22.0, Chicago, IL, USA) was used to perform all data analyses and a two-sided *P* value <0.05 was considered to be statistically significant.

All methods were carried out **in accordance with relevant guidelines and regulations of the People Republic of China.**

Declarations

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Author contributions

Jian Li, Fei Gong, Ge Lin and Berthold Hocher designed the study. Jinhua Shen Suimin Zeng Jing Li collected the data. Jinhua Shen, Xiaoli Zhang, Yangqin Peng, Berthold Hocher, and Qin Zhang checked quality of the data. JinhuaShen and YangqinPeng performed the statistical analysis. JinhuaShen drafted the manuscript. Liang Hu Christoph Reichetzeder and Mei Tian contributed to the data interpretation and revised drafts of the manuscript.

Competing interests

The authors declare no conflicts of interest.

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Ethics approval

This study was approved by the ethics committee of the Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China (approval number: ll-sc-2019-003). The data of this study is only used for this study, and the data of patients are strictly confidential. This study will not cause any harm to the patients' body and mind.

References

1. Slattery, M. M. & Morrison, J. J. Preterm delivery. *Lancet (London, England)*. **360**,1489–1497(2002).
2. Tian, M., Reichetzeder, C., Li, J. & Hocher, B. Low birth weight, a risk factor for diseases in later life, is a surrogate of insulin resistance at birth. *Journal of hypertension*, **37**, 2123–2134 (2019).
3. Smid, M. C. *et al.* Maternal race and intergenerational preterm birth recurrence. American journal of obstetrics and gynecology. 217, 480.e481-480.e489(2017).
4. Orbach, H. *et al.* Hypertension and antihypertensive drugs in pregnancy and perinatal outcomes. *American journal of obstetrics and gynecology*, **208**, 301301–301306 (2013).
5. Mutsaerts, M. A. *et al.* Effects of paternal and maternal lifestyle factors on pregnancy complications and perinatal outcome. A population-based birth-cohort study: the GECKO Drenthe cohort. *Human reproduction (Oxford, England)*. **29**,824–834(2014).
6. Margerison-Zilko, C. E., Talge, N. M. & Holzman, C. Preterm delivery trends by maternal race/ethnicity in the United States, 2006-2012. *Annals of epidemiology*. **27**,689-694.e684(2017).
7. Goisis, A., Remes, H., Barclay, K., Martikainen, P. & Myrskylä, M. Advanced Maternal Age and the Risk of Low Birth Weight and Preterm Delivery: a Within-Family Analysis Using Finnish Population Registers. *American journal of epidemiology*, **186**, 1219–1226 (2017).
8. Sha, T., Yin, X., Cheng, W. & Massey, I. Y. Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. Fertility and sterility. 109, 330-342.e339(2018).
9. Pinborg, A. *et al.* Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update*, **19**, 87–104 (2013).
10. Fuchs, F., Monet, B., Ducruet, T., Chaillet, N. & Audibert, F. Effect of maternal age on the risk of preterm birth: A large cohort study. *PloS one*, **13**, e0191002 (2018).
11. Zlatnik, M. G., Cheng, Y. W., Norton, M. E., Thiet, M. P. & Caughey, A. B. Placenta previa and the risk of preterm delivery. *The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet*, **20**, 719–723 (2007).
12. Khazaeipour, Z., Shirazi, M., Niromanesh, S., Dastgerdy, E. & Sharbaf, F. R. Association of hypertriglyceridaemia with gestational diabetes and adverse pregnancy outcomes. *Endocrine Practice*, **23**, 7 (2017).
13. Ogawa, K. *et al.* Association between very advanced maternal age and adverse pregnancy outcomes: a cross sectional Japanese study. *BMC Pregnancy Childbirth*, **17**, 349 (2017).

14. Xiong, X., Dickey, R. P., Pridjian, G. & Buekens, P. Maternal age and preterm births in singleton and twin pregnancies conceived by in vitro fertilisation in the United States. *Paediatr Perinat Epidemiol*, **29**, 22–30 (2015).
15. Almasi-Hashiani, A. *et al.* Assisted reproductive technology and the risk of preeclampsia: an updated systematic review and meta-analysis. *BMC Pregnancy Childbirth*, **19**, 149 (2019).
16. Xu, X. K., Wang, Y. A., Li, Z., Lui, K. & Sullivan, E. A. Risk factors associated with preterm birth among singletons following assisted reproductive technology in Australia 2007-2009–a population-based retrospective study. *BMC Pregnancy Childbirth*, **14**, 406 (2014).
17. Wang, Y. A. *et al.* Increased incidence of gestational hypertension and preeclampsia after assisted reproductive technology treatment. *Fertility and sterility*, **105**, 920–926922 (2016).
18. Vahanian, S. A., Lavery, J. A., Ananth, C. V. & Vintzileos, A. Placental implantation abnormalities and risk of preterm delivery: a systematic review and metaanalysis. *American journal of obstetrics and gynecology*, **213**, S78–90 (2015).
19. Jackson, R. A., Gibson, K. A., Wu, Y. W. & Croughan, M. S. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstetrics and gynecology*, **103**, 551–563 (2004).
20. Farhi, J. *et al.* High serum oestradiol concentrations in IVF cycles increase the risk of pregnancy complications related to abnormal placentation. *Reproductive biomedicine online*, **21**, 331–337 (2010).
21. Lin, X. H. *et al.* Maternal High Triglyceride Levels During Early Pregnancy and Risk of Preterm Delivery: A Retrospective Cohort Study. *The Journal of clinical endocrinology and metabolism*, **104**, 1249–1258 (2019).
22. Keren-Politansky, A., Breizman, T., Brenner, B., Sarig, G. & Drugan, A. The coagulation profile of preterm delivery. *Thrombosis research*, **133**, 585–589 (2014).
23. Ata, B. & Urman, B. Thrombophilia and assisted reproduction technology-any detrimental impact or unnecessary overuse? *Journal of assisted reproduction and genetics*, **33**, 1305–1310 (2016).
24. Jamro, E. L. *et al.* Preconception serum lipids and lipophilic micronutrient levels are associated with live birth rates after IVF. *Reproductive biomedicine online*, **39**, 665–673 (2019).
25. Stock, S. & Norman, J. Preterm and term labour in multiple pregnancies. *Seminars in fetal & neonatal medicine*, **15**, 336–341 (2010).
26. Ledger, W. & Johnson, M. H. One plus one equals two: why fetal reduction is always a second-best solution. *Reproductive biomedicine online*, **32**, 467–468 (2016).
27. Fechner, A. J. *et al.* Effect of single embryo transfer on the risk of preterm birth associated with in vitro fertilization. *Journal of assisted reproduction and genetics*, **32**, 221–224 (2015).
28. de Vries, J. I., van Pampus, M. G., Hague, W. M., Bezemer, P. D. & Joosten, J. H. Low-molecular-weight heparin added to aspirin in the prevention of recurrent early-onset pre-eclampsia in women with inheritable thrombophilia: the FRUIT-RCT. *Journal of thrombosis and haemostasis: JTH*, **10**, 64–72 (2012).

29. Li, Y. *et al.* Cumulative Live Birth Rates in Low Prognosis Patients According to the POSEIDON Criteria: An Analysis of 26,697 Cycles of in vitro Fertilization/Intracytoplasmic Sperm Injection. *Frontiers in endocrinology*, **10**, 642 (2019).
30. Zegers-Hochschild, F. *et al.* International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. *Fertility and sterility.* **92**, 1520-1524 (2009).
31. Di Renzo, G. C. *et al.* Preterm Labor and Birth Management: Recommendations from the European Association of Perinatal Medicine. The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 30, 2011-2030(2017).
32. Tul, N. *et al.* The contribution of twins conceived by assisted reproduction technology to the very preterm birth rate: a population-based study. *European journal of obstetrics, gynecology, and reproductive biology*, **171**, 311–313 (2013).

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