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 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 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/ICSI were not compared so far.
What does this study add?

Older (>39) or younger (<25) maternal age, multiple pregnancy, placenta previa, and embryo 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 worldwide¹. Furthermore, those infants who do survive have higher rates of long-term morbidities, including cardiovascular diseases² 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 tract³-⁷. 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 studies⁸ analyzed maternal and offspring outcomes after ART, whereas larger sized studies focusing specifically on the risk factors for preterm birth and 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 was 30 (27,33) years old. The median BMI of the women was 21.94(20.08,23.76)kg/m². The study cohort consisted mostly of Han Chinese women (87.8%). Caused for infertility in the entire study populations were distributed as follows: primary infertility was present in 42.9% and secondary infertility in 57.1%. The duration of infertility was 4 (2.6) years. Median birthweight was 3100(2700, 3500)g, Median gestational age was 38.4(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, body mass index.

| Variables                      | Preterm birth (n=675) | Early-preterm birth (n=66) | Full-term birth (n=3653) | \( p^a \) | \( p^b \) |
|-------------------------------|-----------------------|-----------------------------|--------------------------|----------|----------|
| Maternal age (year)           |                       |                             |                          |          |          |
| 25~39                         | 599(88.7%)            | 55(83.3%)                   | 3339(91.4%)              | 0.026    | 0.021    |
| ≥40 or 20~24                  | 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 |
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<sup>a</sup> | p<sup>b</sup> |
|--------------------------------|-----------------------|----------------------------|--------------------------|--------------|--------------|
| 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        |
| Serum globulin (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)          | 3.3(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<sup>a</sup> | P<sup>b</sup> |
|-----------------------------------------|-----------------------|---------------------------|--------------------------|---------------|---------------|
| 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 were compared between preterm birth group and full-term birth group. \textsuperscript{a}: Preterm birth group vs. full-term birth; \textsuperscript{b}: Early-preterm birth group vs. full-term birth. \textsuperscript{b}: In the multiple pregnancy, one is female infant, another is male infant.

| Variables                          | Preterm birth (n=675) | Early-preterm birth (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  |
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), 3 factors 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 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<0.001) and early preterm birth ratio by 1.13 times (OR=2.125, 95%CI: 1.049-4.304, P<0.001). 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 and a shorter activated 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. 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, multiple pregnancies, placenta previa, gestational hypertension, high triglycerides and hypercoagulability, 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. Both younger 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. 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. 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.

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. 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. 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. 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\textsuperscript{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\textsuperscript{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\textsuperscript{21,22}. Hypercoagulability 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\textsuperscript{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\textsuperscript{23}.

With regard to triglycerides it was demonstrated that high maternal triglycerides levels during pregnancy are related to an increased risk for preterm birth in both obese women and in women with normal BMI in the general population conceiving naturally\textsuperscript{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\textsuperscript{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\textsuperscript{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 embryos simultaneously to achieve a high pregnancy rate\textsuperscript{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. An effective 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\textsuperscript{27}.

In conclusion, our study demonstrated that maternal age, multiple pregnancy, embryo reduction and placenta previa could increase the risk of preterm birth in women undergoing 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\textsuperscript{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\textsuperscript{\textsuperscript{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\textsuperscript{st}, 2016 to December 30\textsuperscript{th}, 2017 were collected.

Inclusion criteria were as follows:

- a) Women treated with super-ovulation protocols exactly as described previously\textsuperscript{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 (>42\textsuperscript{nd} 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\textsuperscript{30}. Note: Gestational age was determined as the gestational 17\textsuperscript{th} day when 6-8 cell embryo was transferred into the uterus, and gestational 19\textsuperscript{th} when blastocyst was transferred into the uterus after 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 \(\leq\) gestational age<42 weeks). Preterm birth was defined as a live birth with a gestational age of at least 20 but not over 37 weeks (20 weeks \(\leq\) gestational age<37 weeks)\textsuperscript{31}. Early-preterm birth was defined
as a live birth with a gestational age between 20 but not over 32 weeks (20 weeks \( \leq \) gestational age < 32 weeks)\(^3\).

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, transferred embryo count, 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. Jinhua Shen and Yangqin Peng performed the statistical analysis. Jinhua Shen 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: II-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.

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