Obstetric and perinatal outcomes of dizygotic twin pregnancies resulting from In Vitro Fertilization versus spontaneous conception: a retrospective study

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This study was designed to to assess perinatal and neonatal outcomes of dizygotic twin pregnancies conceived naturally or by IVF. After strict selection, the study included 470 dizygotic twin pregnancies. There were 249 resulting from IVF treatments and 221 conceiving spontaneously. After adjusting maternal age and primiparity, the results showed that there were no significant differences between the two groups (*P* > 0.05) in terms of maternal antenatal complications and neonatal outcomes. In conclusion, our study does not reveal increased risks for pregnancy-related complications and adverse neonatal outcomes in dizygotic twin pregnancies following IVF treatments. With these fundamental data, this study could provide a reference for perinatal care and clinical ART treatment and help to inform infertile parents about the potential risks of IVF treatments.

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30 **Abstract:**

31   This study was designed to to assess perinatal and neonatal outcomes of dizygotic twin

32   pregnancies conceived naturally or by IVF. After strict selection, the study included 470

33   dizygotic twin pregnancies. There were 249 resulting from IVF treatments and 221 conceiving

34   spontaneously. After adjusting maternal age and primiparity, the results showed that there were

35   no significant differences between the two groups ($P > 0.05$) in terms of maternal antenatal

36   complications and neonatal outcomes. In conclusion, our study does not reveal increased risks

37   for pregnancy-related complications and adverse neonatal outcomes in dizygotic twin

38   pregnancies following IVF treatments. With these fundamental data, this study could provide a

39   reference for perinatal care and clinical ART treatment and help to inform infertile parents about

40   the potential risks of IVF treatments.

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47 **Introduction**

48 The rapid progress and wide application of Assisted Reproductive Technology (ART), especially

49 in vitro fertilization and embryo transfer (IVF-ET), make it possible for infertile couples to

50 conceive a baby successfully. Unlike spontaneous conception, however, to maximize the

51 possibility of obtaining a live birth, two or more embryos are routinely transferred into the uterus

52 *(Vasario et al., 2010)*. Consequently, the incidence of multiple gestation rapidly rises *(Farhi et*

53 *al., 2013)*, which in turn raises perinatal and neonatal risks *(Qin et al., 2015)*.

54 In spontaneous-conceived (SC) pregnancy, the incidence of multiple gestation is about 2%,

55 while this rate is up to 40%-50% in IVF-conceived pregnancies *(Reynolds et al., 2003)*. Notably,

56 twin pregnancy accounts for the majority of multiple gestations. Although single embryo transfer

57 has been suggested, however, the implementation of single embryo transfer may reduce success

58 rate. So how to successfully carrying it out without compromising the clinical outcomes is yet a

59 technological challenge. To date, the strategy of at least two embryo for transfer is routinely

60 performed in most reproductive centers.

61 Actually, twin pregnancies are known to be related to higher risks of maternal and fetal

62 complications than singleton pregnancies in natural conception *(Qin et al., 2015; Rao et al.,*

63 *2004)*, it is yet unclear whether twin pregnancies following IVF treatments are associated with

64 higher risks of obstetric and neonatal conditions when compared with SC twin pregnancies.

65 Several studies have been performed to explore the exact role of IVF-ET in obstetric and

66 neonatal outcomes of twin pregnancies, but the results remain conflicting *(Geisler et al., 2014;*

67 *Helmerhorst et al., 2007; McDonald et al., 2005; Pourali et al., 2016; Qin et al., 2015; Sun et al.,*

68 *2016; Vasario et al., 2010)*. One of the confounding factors in some studies is that

69 monochorionicity among IVF-conceived twin pregnancies is quite rare, when compared with that

70 in SC twin pregnancies (about 2% and 22%, respectively), and monochorionic pregnancies have

71 more adverse effects on obstetric outcomes *(Penava et al., 2004)*. As a result, the adverse impact

72 of IVF treatments on the outcomes of IVF-conceived twins may be compensated by the lower

73 incidence of monochorionicity. Accordingly, it is necessary to exclude monozygotic twins and

74 merely consider dizygotic twins. In addition, other factors responsible for the conflicting

75 outcomes can be ascribed to the differences in study populations and the management

76 approaches in twin pregnancies related studies.

77 Given current evidence on this topic is contradictory, and the results in medical literatures

might vary greatly due to study populations, management approaches, and sample size. This study is thus designed to assess perinatal and neonatal outcomes of dizygotic twin pregnancies conceived naturally or by IVF.

Materials and Methods

This retrospective study was performed at the Second Affiliated Hospital of Wenzhou Medical University during the period from July 2015 to April 2016, and was approved by the Ethics Committee of the hospital. Informed consent was not needed for this retrospective investigation. The subjects in this study were mainly from Wenzhou area and its surrounding area, South-East Zhejiang province, China.

Patients who had antenatal care and delivered at Obstetric department of the Second Affiliated Hospital of Wenzhou Medical University during the period from July 2015 to April 2016 were taken into consideration. Only dizygotic twin pregnancies delivered after 28 weeks' gestation were included. In the IVF-conceived group, only those conceived following IVF/ICSI treatments were included, those conceived by other forms of Assisted Reproduction Technology were excluded. Twin gestations obtained after natural abortion or fetal reduction in multiple pregnancies were excluded. After selection, 470 patients were included, 249 dizygotic pregnancies conceived by IVF and 221 conceived spontaneously, as described in Figure 1.

Data were obtained from medical records, which include maternal age, gestational age, delivery mode, duration of hospitalization, perinatal complications, neonatal birth defects, birthweight, and neonatal intensive care unit (NICU) admission. The medical diagnosis was categorized according to the criteria proposed by Sun et al. *(Sun et al., 2016)*.

The diagnosis of dichorionic twin pregnancies was based on ultrasounds between 6 and 10 weeks' gestation by the observation of two gestational sacs on vaginal sonography in the first trimester. Chorionicity was confirmed by examination of the placenta following delivery. Gestational age was calculated from 14 days before the embryo transfer for IVF-conceived women, and from the first day of the last menstrual cycle for SC women. Length of hospitalization was calculated from the day of admission to the day of discharge after delivery.

Gestational hypertension is the development of new hypertension in a pregnant woman after 20 weeks' gestation without the presence of protein in the urine or other signs of preeclampsia. Hypertension is defined as having a blood pressure greater than 140/90 mmHg. GDM was diagnosed by oral 75g glucose tolerance test (OGTT) at between24 and 28 weeks' gestation.

109   According to the International association for research on gestational diabetes 2010. The glucose

110   threshold of diagnosing GDM is 5.1mmol/L for fasting blood test, 10.0mmol/L at 1 hour after

111   OGTT, 8.5mmol/L at 2 hours after OGTT. Premature rupture of membranes was documented as

112   rupture of membranes prior to the woman goes into labor. Maternal anemia was determined as a

113   decrease in whole-blood hemoglobin concentration of more than standard deviations below the

114   mean of an age-matched reference range. Placenta previa was recorded as after 28 weeks of

115   gestation, the placenta attaches to the lower segment of the uterus, and even the lower margin of

116   the placenta reaches or covers the cervical orifice, and its position is lower than the first exposed

117   part of the fetus. Placental acrete is defined as the abnormal adherence to or invasion of the

118   villous tissue into the myometrium secondary to damage to the endometrium-myometrial

119   interface of the uterine wall. Postpartum hemorrhage was defined as vaginal blood loss of ≥ 500

120   ml within 24 hours after delivery, and when blood loss during cesarean delivery exceeds 1000ml.

121   Intrahepatic cholestasis of pregnancies was based on: 1) onset of generalized pruritus in the

122   second or third trimester of pregnancy; 2) bile acid level > 10 μ mol/L; and 3) spontaneous relief

123   within 3 weeks after delivery. The standard of diagnosing polyhydramnios by B-ultrasound is

124   amniotic fluid index (AFI) ≥25cm or amniotic fluid volume (AFV)≥ 8cm. Placental

125   abruption is defined as after 20 weeks of gestation or during childbirth, the placenta in its normal

126   position is partially or completely removed from the wall of the uterus before the fetus is

127   delivered.

128   Low birth weight was defined as birthweight below 2500g at delivery. Growth discordance

129   was defined as a birthweight difference greater than 25% between twins. Neonatal asphyxia was

130   classified according to Hjalmarson (*Hjalmarson O, 1981*). Transient tachypnea, respiratory

131   distress, and other types of respiratory disturbances were included in the analysis. Stillbirth is the

132   death of a fetus in the uterine cavity after 20 weeks of gestation. Malformation was mainly

133   defined whether the defecti was fatal or potentially life-threatening or likely to lead to serious

134   handicap or major cosmetic defect if not surgically corrected. As to the rate of stillbirth and

135   malformation, the numerator is the number of stillbirth and malformation, while the denominator

136   is the number of twin deliveries. NICU admission and birth weight discordance (BWD)>25%

137   calculated by the following formula: ((birth weight of larger twin–birth weight of smaller twin)/

138   birth weight of larger twin) × 100%.

139   Statistical analysis was performed using the Statistical Package for the Social Sciences,

140   version 22.0 (SPSS Inc., Chicago, IL). Differences between groups were tested statistically using

141   the Chi-square test for categorical data and the independent-sample *t*-test for continuous

142   variables. A multiple logistic regression model was established to assess the association between

143   the selected variables and the chance of pregnancy complications. $P < 0.05$ was considered

144   statistically significant.

145

146   **Results**

147   The maternal age and the proportion of primiparity in the IVF-conceived group were

148   significantly higher than in the SC group (30.66 ± 3.80 versus 28.44 ± 4.27 years old, $P < 0.05$,

149   and 85.5% versus 62.4%, $P < 0.05$, respectively). The average duration of hospitalization in IVF-

150   conceived population was significantly longer than in SC women (7.34 ± 4.99 versus 6.98 ± 4.92

151   days, $P < 0.05$). In contrast, the proportion of cesarean section and were similar (96% versus

152   93.2%, $P > 0.05$) in both groups. When the comparison of first cesarean section between the two

153   groups was restricted to women with at least one previous delivery, the proportion of first

154   cesarean section were similar (27.8% versus 29.3%, $P > 0.05$). The average gestational age in the

155   SC group was 36.41 ± 1.65 weeks, which was significantly longer than 36.07 ± 1.71 weeks in the

156   IVF-conceived group (Table 1). The birth weight between the two groups were similar

157   (2490.57±432.37kg versus 2484.05±428.03kg, $P > 0.05$).

158   After adjustment for maternal age and primiparity, regression analysis revealed there were

159   no significant differences between the two groups ($P > 0.05$) in terms of maternal complications,

160   with one exception of anemia ($P =0.05$), as described in Table 2.

161   Neonatal outcomes are shown in Table 3. There were no significant differences in terms of

162   the incidence of low birth weight, growth discordance, neonatal asphyxia and admission for

163   NICU ($P > 0.05$). The stillbirth and malformation were described in Table 4, two cases in two

164   different twins were included in SC group. Specifically, one is intrauterine fetal death infant, and

165   the other is cystic mass of chest wall infant. In the IVF group there were four stillbirths and three

166   malformed infants, including four intrauterine fetal death infants, two congenital heart

167   malformation infants and one congenital high jejunal atresia infant. Moreover, the seven cases

168   came from seven different pairs. However, statistical analysis was not performed on stillbirth and

169   malformation due to the small number and rare conditions.

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Discussion

     In the past few years, growing interests has been paid to the role of IVF-ET treatments in obstetric and neonatal outcomes of twin pregnancies, but the results remain to be inconsistent *(Geisler et al., 2014; Helmerhorst et al., 2007; McDonald et al., 2005; Moini et al., 2012; Pourali et al., 2016; Qin et al., 2015; Sun et al., 2016; Vasario et al., 2010)*. The current study compared the obstetric complications and neonatal outcomes of dizygotic twin gestations conceived via IVF with those from SC twin pregnancies.

     During the past two decades, the rate of cesarean section in China mainland has risen rapidly. Elective and emergency caesarean section may contribute to this situation *(Geisler et al., 2014)*. Moreover, there is no exception for the twin pregnancy population. Consistent with the study by Moini et al. *(Moini et al., 2012)*, the rate of caesarean deliveries in the present study was similarly high in both twin pregnancy groups. Twin pregnancy has been known to be associated with increased morbidity and mortality for the mother and neonate *(Rao et al., 2004)*. This intrinsic risk may be responsible for the high prevalence of cesarean section in this study. In contrast, some studies have shown similar, but lower rates of caesarean delivery. Lambalk et al. reported a much lower rate of caesarean delivery (30% and 37%) in both twin gestations groups *(Lambalk et al., 2001)*. However, several other studies have demonstrated higher rates of caesarean delivery among IVF-conceived twin pregnancies *(Domingues et al., 2014; Geisler et al., 2014; Helmerhorst et al., 2007; Pourali et al., 2016; Vasario et al., 2010)*. These results indicate that differences in study population and methodology for twin pregnancies related studies likely lead to the conflicting results.

     In this study, the gestational age in the IVF-conceived group was significantly shorter than that in the SC group. However, the gap between the two groups was as small as a difference of 0.34 weeks, which means a little less than two and a half days. Meanwhile, 2∼5 days' in vitro culture time before embryo transfer should also be added into the patients' pregnancy duration of IVF-conceived group. Accordingly, the difference between the two groups may be meaningless though it is statistically significant.

     Actually, the difference in gestational time is the indication of the incidence of prematurity. Significantly shorter gestational time in IVF-conceived population has been shown in similar studies done by Nassar *(Nassar et al., 2003),* Kallen *(Kallen et al., 2010)* and Caserta *(Caserta et al., 2014)*. On one hand, this condition may be attributable to the infertility history and the

202 importance of childbirth in the IVF group, obstetricians hence prefer to perform cesarean section
203 ahead of schedule *(Pourali et al., 2016; Vannuccini et al., 2016)*. On the other hand, families
204 facing infertility usually believe that a trial of vaginal birth is more risky than cesarean section.
205 Therefore, pregnant women are more inclined to choose cesarean section earlier than the
206 expected date of childbirth.

207 Several previous studies have demonstrated that, in contrast to natural singleton pregnancy,
208 IVF-conceived singleton gestation is independently associated with increased obstetric
209 complications *(Farhi et al., 2013; Pinborg et al., 2013)*. But does this effect also occurs in twin
210 pregnancies when compared with SC twin pregnancies? The findings are conflicting. Some
211 studies have shown that IVF treatments in dizygotic twin gestations are related to increased risks
212 of obstetric complications, specifically preeclampsia, intrahepatic cholestasis of pregnancies
213 (ICP) *(Sun et al., 2016)*, and GDM *(Moini et al., 2012; Pourali et al., 2016)*. In contrast, this
214 relationship was not observed in several other studies *(Anbazhagan et al., 2014; Geisler et al.,*
215 *2014; Moini et al., 2012; Vasario et al., 2010)*. In addition, a systematic review demonstrated
216 that perinatal mortality in twins conceived via ART was 40% lower compared with SC twins
217 *(Helmerhorst et al., 2004)*. When comparing the obstetric outcomes of IVF-conceived
218 dichorionic twin pregnancies with those of SC dizygotic twin pregnancies, this study showed
219 similar incidences of maternal and perinatal complications in terms of gestational diabetes
220 mellitus (GDM), hypertensive disorder, premature rupture of membranes, maternal anemia,
221 placenta previa, placental accrete, postpartum hemorrhage, drug use, ICP, and polyhydramnios.
222 The only exception was maternal anemia, which was more frequently observed in the IVF twins
223 group. However, the marginal P value (p= 0.05) is inadequate to allow valid estimation of the
224 real prevalence of maternal anemia in the present study population, and the exact conclusion
225 warrants further assessment based on larger sample size.

226 It has been suggested that some infertility factors and IVF treatment itself may lead to the
227 increased risks of perinatal complications. Unfortunately, because of the inadequate information
228 obtained during the primary study, we could not address the effects of different infertility factors
229 on perinatal outcomes. Thereby, our study still leaves open the question of whether one or a
230 combination of infertility factors results in adverse perinatal outcomes.

231 With regard to the neonatal outcomes of twin pregnancies from IVF and natural conception,
232 the literature provides contradictory findings. One meta-analysis *(Hansen et al., 2013)* and one

233   well-designed study *(Qin et al., 2015)* concluded that multiple pregnancies resulting from IVF-

234   ET were at significantly greater risks for adverse pregnancy outcomes than SC multiple

235   pregnancies. Similarly, Saccone et al. performed a study including 668 women and showed that

236   IVF-conceived oligohydramnios pregnancies had a higher rate of spontaneous preterm birth than

237   SC dichorionic pregnancies *(Saccone et al., 2017)*. It is hypothesized that underlying infertility

238   factors and invasive IVF-ET manipulations contribute to this condition *(Hansen et al., 2013;*

239   *Nassar et al., 2003; Saccone et al., 2017)*. However, in the current study, IVF treatments did not

240   show any adverse effects on neonatal outcomes. The conclusion was in agreement with that in

241   several previous studies, which also have failed to find the increased risks when comparing the

242   neonatal outcomes according to mode of conception *(Anbazhagan et al., 2014; Pourali et al.,*

243   *2016; Sun et al., 2016; Vasario et al., 2010)*. Differences in study population and methodology

244   may account for the conflicting data.

245   Although the present study used strict inclusion criteria, limitations do exist. First, some

246   patients conceived by ovulation induction and artificial insemination may have been categorized

247   into the SC group, and it has been reported that negative perinatal outcomes are more frequently

248   observed in multiple pregnancies resulting from induction and artificial insemination compared

249   with those conceived naturally *(Qin et al., 2015)*. This may underestimate the relationship

250   between IVF-ET and adverse outcomes. Second, the retrospective nature of data collection and

251   the single-center study may restrict the strength and the quality of evidence. A well-controlled,

252   multicenter study may be more convincing to address the issue. In addition, low number of cases

253   may also lead to insufficient power of this kind of study.

254   **Conclusion**

255   Although older age and shorter gestational age were more frequent in the IVF-conceived twin

256   pregnancy population, this study did not show any increased risks for pregnancy-related

257   complications and adverse neonatal outcomes in dizygotic twin pregnancies following IVF. With

258   these fundamental data, this study could provide a reference for perinatal care and clinical ART

259   treatment and help to inform infertile parents about the potential risks of IVF.

260   **Acknowledgements**

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262   2nd Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University, for their

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345

A workflow to show inclusion criteria in the study

Figure 1 A workflow to show inclusion criteia
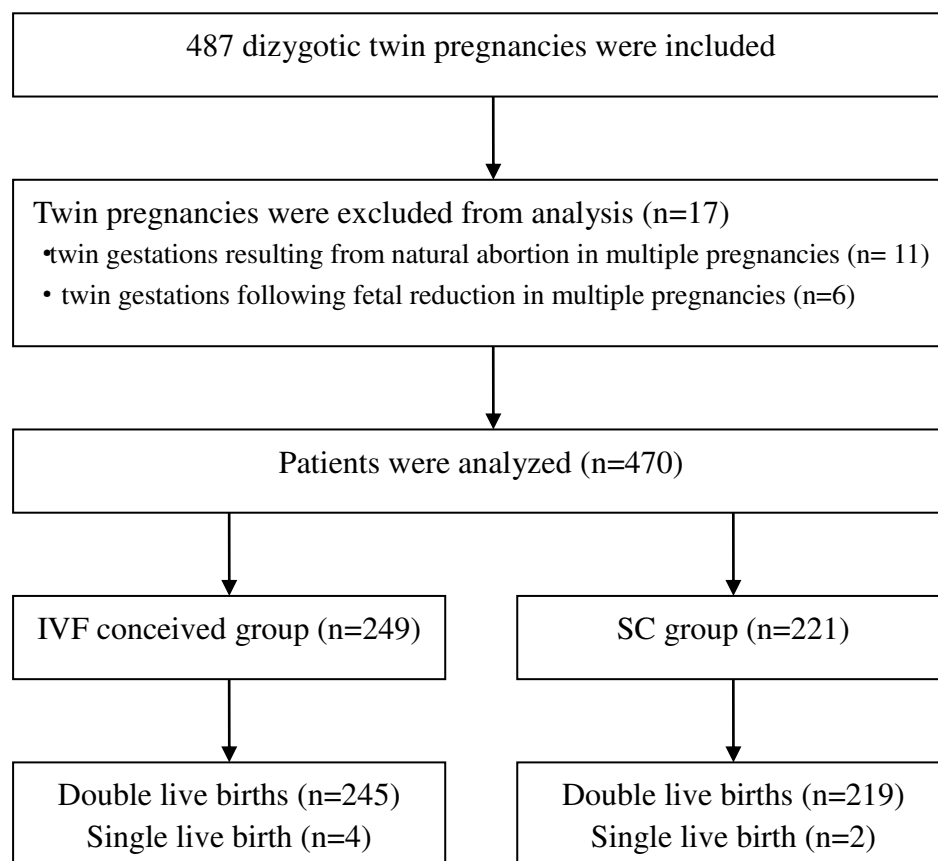
Figure 1. A workflow to show inclusion criteria in the study

Maternal characteristics and delivery modes in twin pregnancy

1   Table 1 Maternal characteristics and delivery modes in twin pregnancy

	SC (n=221)	IVF (n=249)	P value
Age (y)	28.44±4.27	30.66±3.80	<0.001
primiparity	138 (62.4)	212 (85.1)	<0.001
gestational age (w)	36.41±1.65	36.07±1.71	0.026[*]
Cesarean section	206 (93.2)	239 (96.0)	0.081
First Cesarean section[#]	24/82 (29.3)	10/36 (27.8)	0.527
Length of hospitalization (d)	6.98±4.92	7.34±4.99	0.042
Birth weight (kg)	2490.57±432.37	2484.05±428.03	0.817

2   Notes: values are cases(%) or mean±standard deviation; * the difference in mean gestational length attributes

3   to the difference in gestational length counting methods for the two groups; # the comparison is restricted to

4   women with at least one previous delivery.

Maternal complications

1 Table 2 Maternal complications

complications	SC (n=221)	IVF (n=249)	Adjusted OR	Adjusted 95% CI	P value
gestational diabetes mellitus	32 (14.5)	56 (22.5)	1.24	0.74～2.10	0.42
hypertensive disorder	27 (12.2)	29 (11.6)	0.95	0.52～1.77	0.88
premature rupture of membranes	23 (10.4)	25 (10.0)	0.72	0.37～1.38	0.32
maternal anemia	110 (49.8)	141 (56.6)	1.51	1.01～2.26	0.05
placenta previa	5 (2.3)	12 (4.8)	2.08	0.66～6.61	0.21
placental accreta	7 (3.2)	13 (5.2)	1.33	0.48～3.69	0.58
postpartum hemorrhage	16 (7.2)	23 (9.2)	1.20	0.58～2.48	0.63
intrahepatic cholestasis of pregnancies	11 (5)	14 (5.6)	1.25	0.51～3.09	0.63
polyhydramnios	8 (3.6)	9 (3.6)	0.80	0.28～2.31	0.68

2 OR is the abbreviation of odds ratio, and CI is the abbreviation of confidence interval.

Neonatal outcomes

1   Table 3 Neonatal outcomes

	SC (n=221)	IVF (n=248)	Adjusted OR	Adjusted 95% CI	P value
Growth discordance	15 (6.8)	14 (5.7)	0.76	0.34~1.70	0.50
Low birth weight	139 (62.9)	142 (57.3)	0.97	0.65~1.44	0.86
Neonatal asphyxia	16 (7.2)	14 (5.7)	0.90	0.58~1.42	0.66
NICU admission	54 (24.4)	64 (25.8)	0.80	0.36~1.76	0.57

2   Values are cases (%) or mean (standard deviation); OR is the abbreviation of odds ratio, and CI is the
3   abbreviation of confidence interval.

Stillbirth and malformation among the two groups

1   Table 4 Stillbirth and malformation among the two groups

SC(n)	IVF(n)
Intrauterine fetal death (1)	Intrauterine fetal death (4)
Cystic mass of chest wall (1)	Congenital heart malformation (2)
	Congenital high jejunal atresia (1)