

Original Research

Vanishing twin after 12 gestational weeks is associated with adverse perinatal outcomes in *in vitro* fertilization cycles

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Abstract

Background: To assess the associations between vanishing gestational age and the adverse perinatal outcomes following *in vitro* fertilization cycles. **Methods:** This is a retrospective cohort study conducted in Guangzhou Women and children hospital from January 2011 to January 2019. We identified 350 vanishing twin pregnancies including 54 secondary fetal deaths and 296 singleton deliveries compared to 2080 infants from single gestations. The children detected by transvaginal ultrasonography in gestational week 6. By linking with the hospital's registries, the main endpoints were gestational age (weeks), birth weight (g), low birth weight (LBW), preterm birth and neonatal malformation rate. Perinatal outcomes were compared among study groups and controls. **Results:** Of the 350 vanishing twin, there were 15.4% (54) secondary fetal death. Of the 2376 singleton deliveries, 12.5% (296) originated from a twin gestation. In the assessment of perinatal outcome, there were no significant differences between the singleton cohort and vanishing twin before the presence of fetal cardiac activity cohort (VT before CA) and early vanishing cohort. The late vanishing twin group had a higher risk of preterm delivery ($P < 0.001$) and LBW ($P < 0.001$). There were no significant differences between all cohorts in assessing neonatal malformation rate ($P > 0.05$). **Conclusion:** Vanishing twin after 12 weeks is a predictor of adverse perinatal outcome. The couples with a viable fetus or a non-viable fetus and an empty gestational sac in early pregnancy (< 12 weeks) have a similar outcome as the baby from a singleton delivery without vanishing.

Keywords: *In vitro* fertilization; IVF; Perinatal outcome; Vanishing twin; VT

1. Introduction

The increasing burden of infertility has led to an expanded rate of use of *in vitro* fertilization (IVF) [1]. In an increasing number of countries, elective single embryo transfer is being implemented, however in the majority of countries double embryo transfer is still the routine which has in turn increased twin conception rates and the incidence of vanishing twin (VT) pregnancies. With regard to IVF pregnancies, it is estimated to occur in 10–30% of pregnancies [2–6]. The etiology of the VT phenomenon is still obscure although, placental degeneration confirmed pathologically [7] and chromosomal abnormality in the vanishing embryo [8] have been documented. Other possible causes, include chronic maternal diseases, placental “crowding”, intrauterine bleeding, and inappropriate site for implantation have been proposed [9–12].

As to the VT, it is clinically important to understand the effects of VT on the survival fetal. An aspect that the patients care about mostly is whether the survivor would be secondary fetal death or not affected by the vanishing one. However, seldom study did such a research and none can answer the rates of secondary fetal death after the first vanishing one. Another aspect they are concerned about is whether the aborted embryo will affect the growth and de-

velopment of the surviving fetus and whether it is healthy at birth. The pathologic implications of the VT on the surviving twin have been debated. Some studies indicating a higher incidence of adverse obstetric and perinatal outcomes [2–5,13] compared with singleton pregnancies. Adverse outcomes include higher risk of preterm birth, low birth weight(LBW), small for gestational age (SGA) and birth defects. What's more, some papers reported some serious complications, such as brain damage or disseminated intravascular coagulation in the surviving one. However, other studies demonstrated that vanishing twin syndrome(VTS) were associated with slightly higher risks of preterm birth and LBW [14] or had similar outcomes with singleton pregnancies [15,16], or even a protective effect [17]. The main reason for these conflicting results may be that the definition of VTS varies among different authors. Gestational sacs can “vanish” at several time points, from empty gestational sac to gestational sac with evidence of cardiac activity [18,19] or from the first trimester to all three trimesters [2].

However, only few of these studies could rule out the possibility of confounding by vanishing gestational age and the conclusion of the existing literatures were also conflicting. Furthermore, seldom study did the research about the



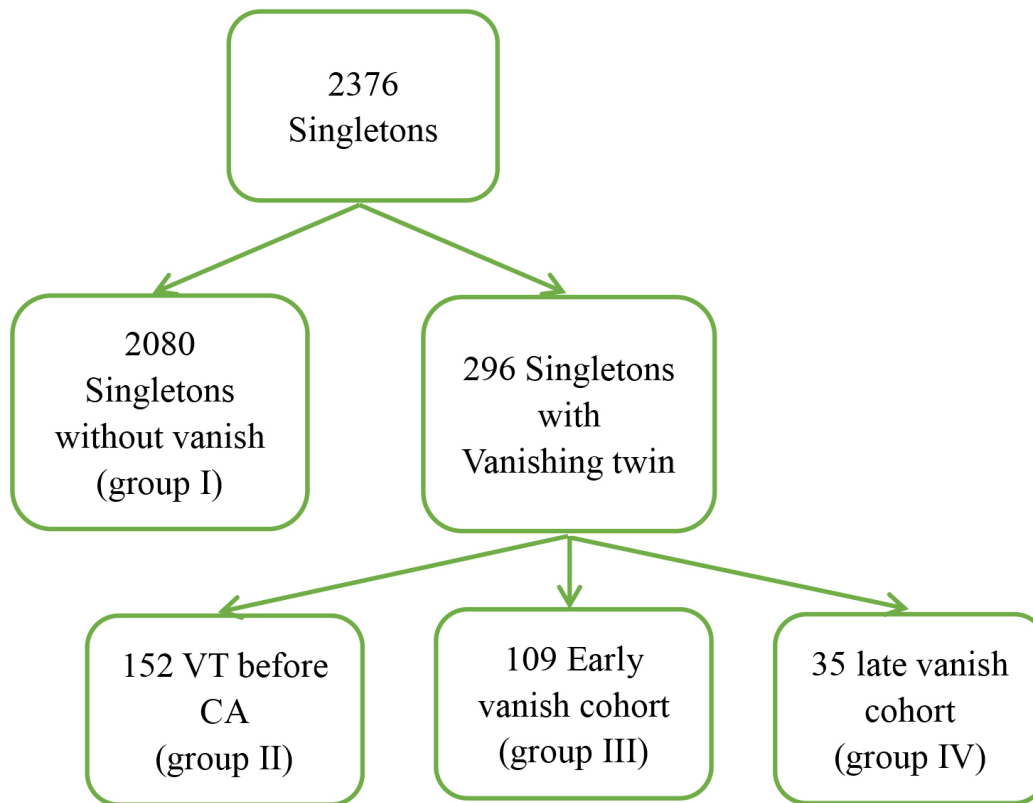


Fig. 1. Singletons grouping outcome according to vanishing gestational age.

rates of secondary fetal death after the first vanishing one. A sibling comparison design is useful to study the association between vanishing gestational age and the obstetric and neonatal outcomes for accounting for fetal loss during the whole pregnancy. The objective of the current study was therefore to estimate the rates of secondary fetal death of a vanishing twin and differential impact on perinatal outcomes according to vanishing gestational age.

2. Materials and methods

This retrospective cohort study was conducted on the clinical data obtained from IVF/ICSI performed in assisted Reproductive center of Guangzhou Women and children hospital from January 2011 to January 2019.

All the patients should sign a follow up agreement before IVF. Gestational age was calculated by the date of embryo transfer. The date of oocyte egg retrieval is day 0 (D0). If day 3 embryo is transferred, the first day of pregnancy is 17 days ahead of transfer date. If the blastocyst is transferred, the first day of pregnancy is 19 days ahead of transfer date. If pregnant, on gestational week 6, women need to return to our hospital for the first ultrasound and then they were followed by biweekly ultrasounds, gestational age of fetuses lost and the gestational sac number were calculated at that time. All the follow up data were registered in our IVF system. All the patients' information and clinical data were obtained from our IVF system.

The inclusion criteria includes: pregnancies that meet the inclusion criteria in gestational week 6 (A) one viable fetus; (B) one fetus with cardiac activity and a gestational sac/fetus without fetal heart beat; or (C) two viable fetuses. The following were excluded: women who received pre-implantation genetic diagnosis or fetal reduction; pregnancies in which more than two fetal hearts were beating, gestations with no viable fetuses and monozygotic twins (Either of two twins developed from the same fertilized ovum). In this research, the VTS was defined as: two gestational sacs were demonstrated at gestational weeks 6 and thereafter, only one fetal pulse was demonstrated, the cases were included as the study group.

For a better understanding of the rates of secondary fetal death after the first vanishing one, we compared the pregnancy outcomes of the co-twin in VT pregnancies and determine the rates of secondary fetal death after the first vanishing one according to gestational week at the time of determination of VT.

Subsequently, we selected patients that singletons gestation without vanishing and the vanishing twin who delivered a singletons gestation into a study group. We separate the study group into four study cohorts. Singleton cohort (group I) consisted of born singletons registered with only a gestational sac/fetus and one heart beat in early pregnancy in accordance with 'inclusion criteria A'; Vanish before the presence of fetal cardiac activity cohort (VT before CA)

Table 1. Pregnancy outcomes of the co-twin in VT pregnancies according to gestational age at time of vanishing.

| Pregnancy outcome | Vanishing twin (350) | | | | | |
|--------------------|----------------------|------------------------------|-------------|------------------------------|------------|------------------------------|
| | 6 w | <i>P</i> -value ^a | 7–12 w | <i>P</i> -value ^b | >12 w | <i>P</i> -value ^c |
| Secondary abortion | 29 (16.0%) | 0.86 | 2 (16.8%) | 0.27 | 3 (7.9%) | 0.30 |
| Delivered | 152 (84.0%) | \ | 109 (83.2%) | \ | 35 (92.1%) | \ |

^aComparison of 6 w versus 7–12 w of co-twin. ^bComparison of 7–12 w versus >12 w of co-twin.

^cComparison of 6 w versus >12 w of co-twin.

Table 2. Patient and IVF/ICSI treatment characteristics comparing VT cohorts with singleton pregnancies.

| Maternal characteristics | Singletons | <i>P</i> -value | VT before CA | <i>P</i> -value | Early vanish | <i>P</i> -value | Late vanish |
|--------------------------------|--------------|-----------------------|--------------|------------------------|--------------|-----------------------|--------------|
| | (Group I) | (Group I vs Group II) | (Group II) | (Group I vs Group III) | (Group III) | (Group I vs Group IV) | (Group IV) |
| Women age at delivery (years) | 31.43 ± 4.26 | 0.612 | 31.54 ± 3.91 | 0.256 | 32.00 ± 4.54 | 0.06 | 32.68 ± 3.92 |
| Women BMI (kg/m ²) | 21.31 ± 2.80 | 0.509 | 21.22 ± 2.99 | 0.184 | 21.67 ± 2.87 | 0.008 | 20.01 ± 1.90 |
| Type of infertility | | | | | | | |
| Primary | 1092 | 0.275 | 87 | 0.844 | 56 | 0.734 | 17 |
| Secondary | 988 | 0.275 | 65 | 0.844 | 53 | 0.734 | 18 |
| Treatment characteristics | | | | | | | |
| Cycle type | | | | | | | |
| Fresh | 757 | 0.862 | 54 | 0.004 | 25 | 0.289 | 16 |
| Frozen | 1323 | 0.862 | 98 | 0.004 | 84 | 0.289 | 19 |
| Mode of insemination | | | | | | | |
| IVF | 1407 | 0.419 | 108 | 0.142 | 66 | 0.364 | 21 |
| ICSI | 673 | 0.419 | 44 | 0.142 | 43 | 0.364 | 14 |
| Embryo transfer stage | | | | | | | |
| Day 3 | 1759 | 0.643 | 131 | 1.00 | 92 | 0.641 | 31 |
| Blastocyst | 321 | 0.643 | 21 | 1.00 | 17 | 0.641 | 4 |
| Sex of surviving offspring | | | | | | | |
| Boy | 1081 | 1.0 | 79 | 0.118 | 44 | 0.690 | 17 |
| Girl | 999 | 1.0 | 73 | 0.118 | 65 | 0.690 | 18 |

(group II) consisted of singletons detected as twin pregnancies on the basis of ‘criteria B’ by ultrasound during early pregnancy; Early vanish cohort (group III) consisted of singletons detected as twin pregnancies on the basis of ‘criteria C’ by ultrasound and co-twin perishing after 6 gestational weeks but prior to 12 weeks gestational; and late vanish cohort (group IV) consisted of singletons detected as twin pregnancies on the basis of ‘criteria C’ by ultrasound and co-twin disappeared after 12 weeks gestational. We compared the perinatal outcomes of the co-twin in VT pregnancies with IVF/ICSI singletons without vanishing.

Preterm delivery was defined as child born before 37 weeks’ gestational age. LBW was defined as birth weight under 2500 g [20]. Perinatal malformation and birth weight data were acquired by telephone interview within 1 week of birth. Perinatal outcomes were compared in terms of mean maternal age, patient Body Mass Index(BMI), type of infertility, cycle type, mode of insemination (IVF/ICSI), embryo transfer stage and sex of the offspring, gestational age, birth weight among the groups. Data are reported using the mean SD or n (%).

Statistics: Chi-squared test was used to evaluated the differences between proportions. Differences between parameters in the specific patient groups were evaluated us-

ing the *t*-test where appropriate. Then, multivariate analysis was performed using binary logistic regression to adjusted for all factors. A result was considered statistically significant if a two-tailed *P*-value was less than 0.05. All data analyses were performed using SPSS for windows 20.0 (IBM, Armonk, NY, USA).

3. Results

3.1 Demographic data

The study involved 350 vanishing twin including 54 (15.4%) secondary fetal death thereafter and 296 (84.6%) singleton deliveries as shown in Table 1. when the vanishing happened after 12 weeks gestation, the rate of secondary fetal death (7.9%) was much lower than vanishing at 6 w (16%) and 6–12 w (16.8%) but there were no significant statistical differences between the groups.

As shown in Fig. 1, of the 2376 singleton deliveries, there were 296 (12.5%) cases of vanishing twin and 2080 (87.5%) singletons without a vanishing (group I). As a result, 152 (51.4%) cases belonged to VT before CA (group II), 109 (36.8%) cases belong to early vanish cohort (group III) and 35 (11.8%) cases belong to late vanish cohort (group IV).

Table 3. Neonatal outcome in the vanishing cohort according to time of vanish of co-twin.

| Neonatal outcome | Singletons cohort | P-value ^a | VT before CA | | Early vanish | | Late vanish | |
|----------------------------|-------------------|----------------------|--------------|----------------------|--------------|----------------------|-------------|--|
| | | | Group I | P-value ^b | Group II | P-value ^c | Group III | |
| Live-born children (n) | 2080 | \ | 152 | \ | 109 | \ | 35 | |
| Birth weight (g) | 3.23 ± 0.49 | 0.240 | 3.19 ± 0.55 | 0.356 | 3.22 ± 0.54 | 0.03 | 2.68 ± 0.67 | |
| Gestational age (weeks) | 38.5 ± 1.59 | 0.692 | 38.4 ± 1.74 | 0.242 | 38.3 ± 1.99 | 0.279 | 37.0 ± 3.65 | |
| LBW | 114 (5.5%) | 0.359 | 11 (7.2%) | 0.39 | 8 (7.3%) | <0.001 | 10 (28.6%) | |
| Preterm birth | 219 (10.5%) | 0.785 | 17 (11.2%) | 0.632 | 13 (11.9%) | <0.001 | 10 (28.6%) | |
| Neonatal malformation rate | 20 (2.4%) | 0.107 | 3 (2.0%) | 0.758 | 4 (3.7%) | 0.825 | 1 (2.9%) | |

Values are mean ± SD or n (%).

^aComparison of singletons versus group I of co-twin. ^bComparison of singletons versus group II of co-twin. ^cComparison of singletons versus group III of co-twin.

3.2 Patient and IVF treatment characteristics

Patient characteristics and treatment data between groups, such as mean maternal age, BMI, type of infertility, cycle type, mode of insemination, embryo transfer stage and sex of the offspring are summarized in Table 2. Compared with singletons without vanishing (group I), BMI of the late vanish cohort (group IV) was much lower ($P = 0.008$), the proportion of frozen embryo transfer (77.1%) was much higher in the early vanish cohort (group III) with a statistically significant difference ($P = 0.004$). There were no statistically significant differences for mean maternal age, type of infertility, mode of insemination, embryo transfer stage ($P > 0.05$).

3.3 Neonatal outcome

As shown in Table 3, gestational week, preterm delivery and LBW, in the group of singletons without vanishing (group I), VT before CA (group II), early vanish cohort (group III) and late vanish cohort (group IV) were evaluated and listed. There were no significant differences between the singletons without vanishing (group I), VT before CA (group II) and early vanish cohort (group III). On the contrary, when singletons without vanishing (group I) were compared with late vanish cohort (group IV), late vanish cohort (group IV) had higher risk of preterm delivery ($P < 0.001$) and LBW ($P < 0.001$). Multivariate analysis performed to adjust for maternal age, women BMI, and Cycle type (fresh/frozen) as shown in Table 4. and confirmed the significant association of time of vanish of co-twin with LBW and preterm birth. There were no significant differences between any of these cohorts for the rate of neonatal malformation rate ($P > 0.05$).

4. Discussion

Given the rising rates of assisted reproductive technologies (ART) pregnancies internationally, pregnancy and perinatal outcomes in ART pregnancies are very important basis for assessing ART safety. This study showed that VT pregnancy after 12 weeks gestation did not have a higher rate of secondary fetal death but was identified as a factor for determining the risk of adverse perinatal outcome

to the surviving singleton. From our knowledge, it's the first study to evaluate the secondary fetal death rates of vanishing co-twin. We elucidated the rates of secondary fetal death of a vanishing twin and differential impact on perinatal outcomes according to vanishing gestational age at the time of vanishing. In our study, the rate of secondary fetal death of vanishing twin is 15.4% with the surviving twin following VTS happening prior to 12 weeks gestation having similar secondary fetal death rates. With late vanishing twin (>12 weeks), the surviving singleton had a higher risk of preterm delivery ($P < 0.001$) and LBW ($P < 0.001$) compared to a singleton without vanishing.

In our study, 12.5% of singleton deliveries after IVF/ICSI were originated from a VT pregnancy. The result was consistent with some other studies describing approximately 10% [2,21]. When compared the perinatal outcome data between very early vanish or early vanish and singleton group, we find no significant differences on the term of mean gestational week, mean birth weight, rates of preterm delivery and LBW. This is different from one study which reported that with vanish before 12 weeks gestation, survivors showed a higher frequency of lower birth weight and smaller gestational age when compared with a singleton cohort [3] In comparing late vanish and singleton controls, the late vanish group showed smaller gestational age, a higher rate of preterm delivery and LBW. In addition to the adverse perinatal outcomes, studies have demonstrated higher neonatal mortality and birth defects in singleton deliveries of late VT pregnancies [4,21,22]. In our study, those differences did not reach statistical significance.

In other studies, some investigators did not distinguish vanishing time between the first and second trimesters [2], and failed to consider vanishing gestational age during pregnancy [13,23,24]. For example, Pinborg [2] has suggested that a surviving co-twin from the early vanishing times group (<8 weeks), and singletons from singleton pregnancies, have similar perinatal outcomes. However, pregnancy loss before and after the presence of fetal cardiac activity was not differentiated. Our data as that of others have concluded that vanishing gestational age was close correlated to poor pregnancy outcomes. The higher

Table 4. Multiple logistic regression analyses showing predictors of preterm birth and LBW in survivors of a vanishing co-twin.

| Neonatal outcome | Vanishing cohorts | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|------------------|-------------------------|------------------------|----------------------|
| | Singletons cohort | ReF | ReF |
| LBW | VT before CA (group I) | 1.35 (0.71, 2.56) | 1.35 (0.71, 2.56) |
| | Early vanish (group II) | 1.75 (0.88, 3.45) | 1.69 (0.85, 3.33) |
| | Late vanish (group III) | 5.10 (2.27, 11.5) | 5.56 (2.44, 12.66) |
| | Singletons cohort | ReF | ReF |
| Preterm birth | VT before CA (group I) | 0.86 (0.49, 1.52) | 0.87 (0.49, 1.54) |
| | Early vanish (group II) | 1.75 (0.72, 4.35) | 2.22 (0.90, 5.43) |
| | Late vanish (group III) | 1.92 (1.15, 3.13) | 1.82 (1.10, 3.03) |

OR, odds ratio.

Adjusted for women age at delivery, women BMI and Cycle type (fresh/frozen). Results are presented as ORs with 95% CIs.

the vanishing gestational age, the higher the risk that the surviving infant will be subjected to a poor perinatal outcome [2,21,22], especially when vanishing occurred after 12 weeks gestation. As the rates of vanishing twin happened prior to 12 weeks gestation being close to 90%, this results in most of the singleton with a vanishing twin are more likely to be born a baby as healthy as the infant from singleton delivery.

The reason for the poor perinatal outcomes when the vanishing twin occurs after 12 weeks gestation might be secondary to the increase in necrotic fetoplacental tissue needing to be absorbed and the release of more prostaglandins and cytokines which can initiate an intense inflammatory response thus influencing placental function which can affect the growth and development of the surviving fetus [25]. However, the inflammatory response caused by VT before 12 weeks gestation is too small to influence the perinatal outcomes of surviving singleton.

The limitations of our current study is as follows. First, the sample size of our study is small, large sample studies are necessary for better understanding the relationship between the VT and multiple pregnancy influences. Another limitation is that it's difficult to determine an empty gestational sac by ultrasound and it could be misinterpreted or overlooked resulting in a possibility of an underestimation of spontaneous reduction at <6 weeks gestation.

5. Conclusion

IVF/ICSI twins do carry a higher risk than IVF/ICSI singletons regarding perinatal outcome measures. Twin after 12 weeks gestation is a predictor of adverse perinatal outcome compared the singletons without VT. Thus, the adverse outcome carried in VT after 12 weeks gestation is still a major concern in ART. Hence, the elective single embryo transfer policy in IVF/ICSI patients is now highly recommended. As related to patients with VT after 12 weeks gestation, we should more carefully monitor their antenatal course. The couples with a viable fetus or a non-viable fetus and an empty gestational sac in early pregnancy (<12 weeks) have a similar outcome as the baby from a singleton delivery without vanishing.

Author contributions

LS, JL, CLL designed the research study. LS contributed to revise the article critically for important intellectual content; JL, CLL performed the research, analyzed the data, drafted and revised the whole article; QL, ZW, FL, DC and CYL contributed to collect the data, provide help and advice on this research. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was approved by the Independent Ethics Committee of Guangzhou Women and Children's Hospital (approval number: #2020-8).

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Conflict of interest

The authors declare no conflict of interest.

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