Prevalences and pregnancy outcome of vanishing twin pregnancies achieved by in vitro fertilization versus natural conception

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Objective: To evaluate whether vanishing twin (VT) pregnancies achieved by in vitro fertilization and intracytoplasmic sperm injection (IVF–ICSI) had a more adverse perinatal outcome than those after natural conception.
Design: Longitudinal, retrospective cohort study.
Setting: Tertiary university hospital.
Patient(s): Three hundred and six (78 after IVF–ICSI and 228 after natural conception) VT pregnancies over a 22-year period, with VT cases matched to primarily singleton controls.
Intervention(s): None.
Main Outcome Measure(s): Obstetric and neonatal outcome data.
Result(s): The incidence of VT was statistically significantly higher after natural conception (18.2% of twins) than after IVF–ICSI (12.6% of twins). The odds of VT in pregnancies complicated with pregestational or gestational diabetes were disproportionally higher in IVF–ICSI cases than in spontaneously conceived VT pregnancies (adjusted odds ratio [AOR]: 0.80 vs. 3.10 and 1.00 vs. 1.07, respectively). Previous induced abortion (AOR 1.34) or second-trimester fetal loss (AOR 3.3) increased the risk of VT pregnancies after spontaneous conception. Gestational diabetes mellitus in both the previous (AOR 5.41) and the present (AOR 2.3) pregnancy as well as chronic maternal diseases (AOR 3.5) and placentation anomalies all represented independent risk factors for VT after IVF–ICSI.
Conclusion(s): Vanishing twin pregnancies had a lower prevalence and a worse perinatal outcome after IVF–ICSI as compared with those of their spontaneously conceived counterparts. (Fertil Steril® 2016;106:1399–406. ©2016 by American Society for Reproductive Medicine.)
Key Words: IVF–ICSI, natural conception, perinatal outcome, singleton pregnancy, vanishing twin

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The increasing burden of infertility has led to an expanded rate of use of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (1), which has in turn enhanced twin conception rates and, presumably, the incidence of vanishing twin (VT) pregnancies (2). Double-embryo transfer is still routine in a number of countries (3) even though the benefit of a single-embryo transfer policy has already become evident (4). Some studies have reported that the obstetric outcome of the survivors of VT pregnancies after the IVF–ICSI technique is in between those of singletons and twins (5–9), whereas La Sala et al. (10) demonstrated that it is comparable to those of singletons.

The incidence of VT pregnancies varies between 10% and 39% in pregnancies resulting from IVF–ICSI (2,5,7,8,10–13), but the exact prevalence of VT after spontaneous conception has remained unclear (6, 14, 15). The etiology of the VT phenomenon is still obscure; however, placental degeneration (16) and chromosomal abnormality in the vanishing embryo (17) have been confirmed pathologically. Other possible causes, including inappropriate site for implantation, placental "crowding," intrauterine bleeding, and chronic maternal diseases have been proposed (6,18–21).

We hypothesized that there is a difference in perinatal outcomes between VT pregnancies after artificial conception and those after natural conception,

Received April 1, 2016; revised July 7, 2016; accepted July 18, 2016; published online August 24, 2016.
V.M. has nothing to disclose. J.Z. has nothing to disclose. Z.K. has nothing to disclose. A.K. has nothing to disclose.
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Fertility and Sterility® Vol. 106, No. 6, November 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.07.1098

possibly reflecting the potential differences between underlying pathomechanisms. We compared obstetric and neonatal outcomes between the survivors of VT pregnancies and matched singletons originating from single gestations after an IVF–ICSI procedure and after natural conception. Our purpose was to demonstrate the effect of the IVF–ICSI technique on the VT phenomenon and to determine the prevalence of VT pregnancies in iatrogenic and spontaneous twins.

MATERIALS AND METHODS

A retrospective cohort study was conducted at the Department of Obstetrics and Gynecology, University of Szeged. The study included data abstracted from the medical database on VT pregnancies and matched controls between January 1, 1994, and November 30, 2014, that were subsequently delivered at our department. All IVF–ICSI treatments were performed at a single IVF center. In Hungary, sonographic confirmation of all pregnancies is obtained at 5 to 8 weeks with a repeat examination at 11 to 14 weeks to scan nuchal translucency systematically, thus providing a unique opportunity to identify all VT cases.

A total of 65,237 deliveries were singletons, 1,563 were twins, and 306 pregnancies were identified as VT syndrome cases, based on a review of all routine first-trimester scans, thus demonstrating a total rate of 4.69 out of 1,000 total pregnancies. Vanishing twin was diagnosed in cases where either [1] two viable embryos with cardiac activity with spontaneous reduction of one embryo or [2] a single viable embryo and an additional gestational sac were established before 14 weeks of gestation (14). A total of 78 VT cases resulting from 617 IVF with or without ICSI dichorionic twins (12.6%) and 228 VT cases were naturally conceived for 1,252 spontaneous dichorionic twin pregnancies (18.2%) ($P=.002$). We found that 36.8 VTs occurred out of 1,000 IVF–ICSI live births compared with a rate of 3.6/1,000 live births after natural conception ($P<.001$). All the women with VT pregnancies after IVF–ICSI underwent a double-embryo transfer procedure. All cases of single fetal loss at later gestation (>14 weeks) or pregnancies after ovulation induction/insemination were excluded from the analyses. Monochorionic twins (determined through the absence of a "twin peak" sign in sonography) and women undergoing iatrogenic reduction were also excluded.

For each case, three controls were matched according to the following criteria: singleton pregnancies started as singleton gestations achieved by natural conception or IVF–ICSI, which were equal in maternal age, previous gravidity, parity, and prepregnancy body mass index (BMI) and delivered after VT pregnancies in chronologic order. Demographic data, maternal characteristics, and obstetric and neonatal outcomes were retrieved from obstetric and neonatal databases and were exported to Microsoft Excel.

Vanishing twin cases were gathered from the sonography database at our department. Gestational age was established by the day of embryo transfer for the IVF–ICSI group and by sonographic measurement of the embryo in the first trimester for the spontaneous conception group. Intrauterine growth restriction (IUGR) was diagnosed when the growth curve had declined significantly below 10th percentile, resulting in a small fetus for pathological reasons. We defined birth weight as <2,500 grams for low birth weight and <1,500 grams for very low birth weight. Prematurity was defined as delivery at <37 weeks of gestation, and very premature birth was classified as birth at <32 weeks. The prepregnancy BMI registered at the initial visit during prenatal care was calculated as the body weight (kg) per height (m^2). Chronic maternal diseases (i.e., essential hypertension, cardiac, autoimmune and endocrine diseases, preexisting diabetes, and thromboembolic diseases) as risk factors for miscarriage were categorized into one variable to better interpret the statistical analyses. The demographic and obstetric history data and prevalence of chronic maternal diseases were analyzed. The following antepartum and intrapartum complications were examined: gestational diabetes mellitus (GDM), preeclampsia, placental abruption, placenta previa, retained placenta, and surgical delivery. Neonatal outcome measurements were as follows: gestational age, prematurity, birth weight, fetal growth disturbances (IUGR, very low birth weight, low birth weight), neonatal intensive care unit (NICU) admission, male sex, and congenital abnormalities.

All statistics were calculated using SPSS 22 (SPSS Inc.). The nonparametric design of the continuous variables was verified with the Shapiro-Wilk test. Vanishing twin was the major exposure variable. Univariate comparisons between VT pregnancies and controls both for IVF–ICSI and spontaneously conceived pregnancies were assessed with the Mann-Whitney *U* probe for continuous variables. Categorical variables were compared between the subgroups using chi-square tests, and odds ratios (OR) and Cornfield 95% confidence intervals (CIs) were also calculated. The resultant ORs for IVF and spontaneous pregnancies were compared with Mantel–Haenszel tests, providing an estimate of the effect of assisted reproductive techniques on the VT phenomenon. Multivariable logistic regression was performed to evaluate the factors determining VT pregnancies in both the IVF–ICSI and the spontaneous groups separately. Non-VT pregnancies were used as the reference group.

The multivariable dependence of the target variable on both categorical and continuous data were analyzed using logistic regression with stepwise (forward) model selection based on the likelihood ratio criterion ($P_{in}=.05$; $P_{out}=.10$). All the prepregnancy, maternal characteristics, and perinatal outcome variables represented independent factors. All the variables were adjusted for maternal age. Body mass index, parity, chronic maternal diseases, and obstetric history data, including miscarriage, termination of pregnancy, fetal loss in the second trimester, hypertensive disorder, prematurity, and IUGR in previous pregnancies constituted confounding risk factors.

The adjusted OR was also calculated with 95% CI. The two-tailed statistical significance level was set at 5%, and *P* values were adjusted using a Holm–Bonferroni correction for multiple comparisons (Mann-Whitney *U* tests and logistic regression analyses). The study was approved by the ethics committee of the University of Szeged, and the need for informed consent was waived.

TABLE 1

Baseline maternal characteristics and obstetric history in pregnancies complicated with vanishing twins and matched controls presented at the Department of Obstetrics and Gynecology, University of Szeged, between January 1, 1994, and November 30, 2014.

	IVF/ICSI pregnancies							Spontaneously conceived pregnancies						
	Vanishing twin (n = 78)		Matched singleton (n = 234)					Vanishing twin (n = 228)		Matched singleton (n = 684)				
Characteristics	n	%	n	%	*P* value[a]	Unadjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]	n	%	n	%	*P* value[a]	Unadjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]	*P* value[b]
Age (mean ± SD) (y)[c]	34.8 ± 3.71		35.4 ± 3.74		.46	0.96 (0.90–1.03)	0.89 (0.86–0.94)	33.20 ± 4.38		33.30 ± 4.46		.85	0.99 (0.96–1.03)	0.91 (0.78–1.14)	
No. of children (mean ± SD)[c]	0.15 ± 0.37		0.15 ± 0.36		1.00	1.00 (0.49–2.04)	1.00 (0.65–1.81)	0.71 ± 0.83		0.74 ± 0.85		.86	0.96 (0.81–1.15)	0.87 (0.77–1.34)	
Prepregnancy BMI (kg/m^2)[c]	23.22 ± 3.10		23.54 ± 3.96		.53	0.94 (0.87–1.01)	0.93 (0.85–1.06)	24.27 ± 3.44		24.53 ± 4.90		.30	1.01 (0.98–1.05)	1.00 (0.95–1.09)	
Weight gain during pregnancy	12.54 ± 3.95		11.85 ± 4.87		.38	0.99 (0.96–1.01)	1.00 (0.91–1.08)	12.34 ± 5.08		13.24 ± 5.29		.39	0.97 (0.96–1.02)	0.95 (0.91–1.05)	
Smoking during pregnancy	0	0	0	0	–[d]	–[d]	–[d]	6	2.6	36	5.3	.67	0.49 (0.20–1.17)	0.33 (0.11–1.45)	.92
Chronic maternal diseases[e]	15	19.2	18	7.7	.009	2.86 (1.36–6.00)	1.10 (1.10–2.20)	54	23.7	90	13.2	<.001	2.05 (1.41–2.99)	2.01 (1.56–2.67)	.97
	Total no. multiparous IVF							**Total no. multiparous spontaneously conceived**						
	Vanishing twin (n = 18)		Matched singleton (n = 54)					Vanishing twin (n = 144)		Matched singleton (n = 414)				
Previous pregnancy[f]	n	%	n	%				n	%	n	%				
Miscarriage in first trimester	18	100	54	100	–[d]	–[d]	–[d]	66	45.8	174	42	.44	1.17 (0.80–1.71)	1.34 (0.80–2.82)	.98
Termination of pregnancies in first trimester	0	0	0	0	–[d]	–[d]	–[d]	66	45.8	144	34.8	.02	1.59 (1.08–2.33)	1.34 (1.10–2.82)	.51
Fetal loss in second trimester	0	0	0	0	–[d]	–[d]	–[d]	18	12.5	18	4.3	.001	3.14 (1.59–6.23)	3.32 (1.80–7.82)	.72
Preterm birth	0	0	6	11.1	.33	0.89 (0.81–0.97)	(0.66) (0.54–0.79)	12	8.3	24	5.8	.32	1.48 (0.72–3.04)	1.77 (0.90–2.24)	.98
IUGR	0	0	0	0	–[d]	–[d]	–[d]	18	12.5	0	0	<.001	1.14 (1.07–1.21)	1.22 (1.20–1.34)	.59
Hypertensive disorders	0	0	0	0	–[d]	–[d]	–[d]	0	0	24	5.8	.001	0.94 (0.92–0.97)	0.12 (0.10–0.44)	.82
GDM	6	33.3	6	11.1	.06	4.00 (1.09–14.6)	3.1 (1.88–10.1)	6	4.2	22	5.3	.66	0.78 (0.31–1.95)	1.22 (0.91–1.29)	.98

Note: All variables were adjusted for age, body mass index, parity, chronic maternal diseases, and obstetric history data: miscarriage, termination of pregnancy, fetal loss in the second trimester, hypertensive disorder, prematurity, and IUGR in previous pregnancies. BMI = body mass index; CI = confidence interval; GDM = gestational diabetes mellitus; ICSI = intracytoplasmic sperm injection; IUGR = intrauterine growth restriction; IVF = in vitro fertilization; OR = odds ratio; SD = standard deviation.

[a] *P* value, odds ratio, and 95% confidence interval for comparison of categorical data with Fisher's exact test or chi-square test.

[b] *P* value for the Mantel-Haenszel test.

[c] Continuous variables displayed as mean ± standard deviation (SD). Comparison of continuous data with Mann-Whitney *U* test.

[d] Statistical analysis was not meaningful.

[e] Chronic maternal diseases (i.e., essential hypertension, cardiac, autoimmune or endocrine diseases, pregestational diabetes, and thromboembolic diseases) were grouped together into one variable because they are all risk factors of miscarriage.

[f] Only women with previous pregnancies were counted.

Márton. Pregnancy outcome of vanishing twin. Fertil Steril 2016.

RESULTS

Table 1 presents the patterns for maternal characteristics in the VT pregnancies and their matched singleton pregnancies after IVF–ICSI and natural conception. The two IVF–ICSI subgroups were comparable with regard to maternal age; similar BMI and weight gain at delivery were observed in those two groups. The number of children did not statistically significantly differ between the two IVF–ICSI study groups. Maternal age was higher for the IVF–ICSI pregnancies compared with the spontaneously conceived pregnancies. The BMI at delivery was statistically significantly lower among the spontaneous VT pregnant women ($P=.049$) than their matched controls, whereas weight gain during pregnancy was similar in the naturally conceived subgroups. All the women in the IVF–ICSI subgroups were nonsmokers, and negligibly small proportions smoked in the spontaneously conceived subgroups.

All chronic maternal diseases categorized as a single variable were statistically significantly more prevalent among VT pregnancies even after controlling for relevant cofactors; however, mode of conception was not a distinguishable determinant. Previous miscarriage in the first trimester was not different in the VT and non-VT pregnancies due to the matching of our cohorts, but termination of pregnancy and second-trimester fetal loss previously came into prominence as risk factors for single fetal loss. Intrauterine growth restriction in a previous pregnancy was associated with a statistically significantly higher risk of VT in the spontaneous group. In contrast, the occurrence of hypertensive disorders during any previous pregnancy was statistically significantly higher in the non-VT pregnancies in the spontaneous group. There were no recorded IUGR or hypertensive disorders in the previous IVF–ICSI pregnancies. A history of diabetes during a previous gestation was an independent contributing factor to VT pregnancy in IVF–ICSI pregnancies after controlling for confounders by multivariate analysis, but it was not a statistically significant determinant in the spontaneous conception group. Preterm birth in the previous pregnancy did not influence the occurrence of VT.

Table 2 provides an overview of the pregnancy characteristics and intrapartum complications in the subgroups. The average gestational length at the time of vanishing in the IVF–ICSI group was 9.86 ± 2.06 weeks, whereas VT was confirmed at 8.86 ± 2.70 weeks in the spontaneous conception group ($P=.057$) (data not shown). A substantially higher percentage of women with VT pregnancies (OR 3.0; 95% CI, 1.6–5.6) developed GDM compared with the non-VT pregnancies after assisted reproduction ($P=.01$); it was inversely less common among their naturally conceived counterparts (OR 0.46; 95% CI, 0.2–1.1). Even after adjusting for confounders, IVF–ICSI was statistically significantly more associated with GDM, and this was more pronounced for VT cases ($P<.001$) due to its very low occurrence in the spontaneous conception group.

Similarly, pregestational diabetes mellitus represented an increased risk for VT in IVF–ICSI (adjusted OR [AOR] 1.07; 95% CI, 1.04–1.2), but it was not a distinguishable

TABLE 2

Pregnancy characteristics and intrapartum complications in pregnancies complicated with vanishing twins and matched controls presented at the Department of Obstetrics and Gynecology, University of Szeged, between January 1, 1994, and November 30, 2014.

	IVF/ICSI pregnancies							Spontaneously conceived pregnancies						
	Vanishing twin (n = 78)		Matched singleton (n = 234)					Vanishing twin (n = 228)		Matched singleton (n = 684)				
Variable	n	%	n	%	*P* value[a]	Unadjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]	n	%	n	%	*P* value[a]	Unadjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]	*P* value[b]
Primiparity	72	92.3	198	84.6	.124	2.18 (0.88–5.41)	1.12 (0.79–3.42)	108	47.4	330	48.2	.88	0.97 (0.72–1.30)	0.99 (0.72–1.44)	.88
Primigravidity	60	76.9	180	76.9	1.00	1.00 (0.54–1.84)	1.00 (0.68–2.1)	84	36.8	270	39.5	.53	0.85 (0.66–1.22)	0.89 (0.45–1.43)	.85
Gestational DM	24	30.8	30	12.8	.01	3.02 (1.63–5.59)	3.1 (1.68–5.7)	6	2.6	38	5.6	.08	0.46 (0.19–1.10)	0.80 (0.64–1.7)	< .001
Pregestational DM	6	7.7	0	0	< .001	1.08 (1.02–1.15)	1.07 (1.04–1.11)	0	0	6	0.9	.35	0.99 (0.99–1.01)	1.00 (0.94–1.04)	< .001
Preeclampsia	5	6.4	10	4.3	.24	1.87 (0.66–5.32)	1.6 (0.69–6.1)	14	6.2	42	6.1	1.00	1.00 (0.54–1.87)	1.0 (0.78–1.77)	.78
Placenta previa	6	7.7	6	2.6	.08	3.17 (0.99–10.12)	3.8 (1.01–9.3)	0	0.0	6	0.9	.35	0.99 (0.99–1.01)	0.8 (0.71–1.3)	.19
Placental abruption	6	7.7	6	2.6	.08	3.17 (0.99–10.12)	3.60 (1.5–9.2)	6	2.6	2	0.4	.004	9.22 (1.85–45.99)	10.6 (2.5–39.2)	.47
Retained placenta	12	15.4	6	2.6	< .001	6.91 (2.50–19.11)	7.2 (3.1–19.2)	6	2.6	36	5.3	.14	0.49 (0.20–1.17)	0.67 (0.30–1.2)	< .001
Operative delivery	30	38.5	102	43.6	.51	0.81 (0.48–1.37)	0.89 (0.41–1.2)	114	50.0	366	53.5	.36	0.87 (0.64–1.17)	0.99 (0.73–1.1)	.82

Note: All variables were adjusted for age, body mass index, parity, chronic maternal diseases, and obstetric history data: miscarriage, termination of pregnancy, fetal loss in the second trimester, hypertensive disorder, prematurity, and IUGR in previous pregnancies. CI = confidence interval; DM = diabetes mellitus; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; OR = odds ratio; SD = standard deviation.

[a] *P* value, odds ratio, and 95% confidence interval for comparison of categorical data with Fisher's exact test or chi-square test.

[b] *P* value for the Mantel-Haenszel test.

Márton. Pregnancy outcome of vanishing twin. Fertil Steril 2016.

factor for the spontaneously conceived subgroups (AOR 1.00; 95% CI, 0.94–1.00), thus exhibiting a statistically significant difference between the IVF–ICSI and non-IVF pregnancies ($P<.001$). Preeclamptic pregnancies in VT subgroups after IVF–ICSI (AOR 1.6; 95% CI, 0.7–6.1) and spontaneous conception (AOR 1.00; 95% CI, 0.8–1.8) showed similar proportions compared with matched controls. Chronic hypertension was largely infrequent in all subgroups (data not shown). Placenta previa was almost statistically significantly more prevalent in VT pregnancies in the IVF–ICSI group ($P=.08$, AOR 3.8; 95% CI, 1.0–9.3) compared with the non-VT subgroup.

Although the spontaneous VT pregnancies were statistically significantly more likely to be complicated by placental abruption than the matched controls (AOR 10.6; 95% CI, 2.5–39), this was less marked after IVF–ICSI (AOR 3.6; 95% CI, 1.5–9). This difference became more pronounced after controlling for confounders. Retained placenta exhibited a markedly higher rate in IVF–ICSI VT pregnancies (AOR 7.2; 95% CI, 3.1–19) compared with matched controls or spontaneous pregnancies (AOR 0.67; 95% CI, 0.3–1.2). The incidence of surgical delivery was lower among the IVF–ICSI pregnancies (though not statistically significant) compared with the spontaneously conceived counterparts.

Table 3 shows the differences in neonatal outcomes in the case and control subgroups. Similar gestational age at time of delivery was noted in all of the study groups. The incidence of preterm birth was equally low among cases and controls in both the IVF–ICSI and spontaneous subgroups. Furthermore, there were no very preterm deliveries in the study groups. The rate of IUGR was statistically significantly higher in the VT pregnancies in the spontaneous group (AOR 3.0; 95% CI, 1.8–5.2) and even more so among the IVF–ICSI cases (AOR 9.2; 95% CI, 5–22). The incidence of low birth weight was threefold higher among the IVF–ICSI VT pregnancies and two times higher in the naturally conceived VT pregnancies compared with the control groups. No IVF–ICSI pregnancies were registered as very low birth weight, and very low birth weight was extremely scant in the spontaneous matched controls.

The proportion of macrosomia was slightly lower in the VT pregnancies than in the controls in the IVF–ICSI group, but VT was associated with a statistically significantly lower rate of macrosomia after spontaneous conception. The VT phenomenon resulted in a statistically significantly higher male sex rate in spontaneous pregnancies (AOR 1.4; 95% CI, 1.2–1.9) but not in the IVF–ICSI group (AOR 1.1; 95% CI, 0.8–1.9). The frequencies of neonatal intensive care unit (NICU) admission and congenital malformation were not more common in the VT cases.

Table 4 displays the results of the multiple logistic regression analyses, reflecting a different risk factor structure for VT pregnancies achieved by IVF and natural conception. Previous and present GDM influenced the occurrence of VT in the IVF–ICSI group with AORs of 5.41 and 2.33, respectively. Chronic maternal disease was also a predictor, with an AOR of 3.48. The women with IVF–ICSI VT pregnancies had an overall 4.35-fold higher risk of placental abruption. Within the IVF–ICSI VT group, there was a 8.00-fold higher risk of retained placenta and 28.2-fold higher risk of an IUGR neonate. The risk of VT rose to 2.10-fold when a chronic maternal disease was present, and the surviving fetus had 3.65-fold higher odds for IUGR in spontaneous VT pregnancies.

DISCUSSION

The principal finding of the present study is that VT carries a higher risk of various pregnancy complications, particularly after the IVF–ICSI procedure. Embryonic/fetal loss at ≤ 14 weeks has a statistically significant effect on more twin pregnancies after natural implantation (18.2%) than after the iatrogenic transfer of two embryos (12.6%). Early pregnancy loss and the VT phenomenon both share a chromosomal defect in the conceptus (22, 23), and this explains the idea that the artificial selection procedure for morphologically normal embryos decreases the rate of VT after IVF–ICSI. Further, transfer of only an intermediate-quality embryo increases the chance of VT (23). Other possible explanations could be the fresh embryo transfer, which is associated with a higher perinatal risk (24). However, the VT rates for twins after IVF–ICSI reported in the literature are between 10.8% and 39.0% (2,7,11–13).

In total, 5.2% of all singleton deliveries originated from a VT pregnancy after assisted conception, and 0.37% did so after spontaneous single gestation. The observed difference in the incidences of VT supports the idea that an artificial selection procedure favors embryos with high developmental potential (22, 23). This raises the possible higher risk of the implantation of a genetically impaired vanishing embryo in naturally conceived pregnancies, as suggested earlier (22). Further, transfer of an intermediate-quality embryo increases the chance of VT (23). Other possible explanations for this difference are the technology itself (9) or the artificially modified endometrium (25) in assisted reproductive cycles. Although a moderately thick endometrium with a triple-line pattern is more likely linked to a good clinical outcome, the decidualized endometrium acts as a biosensor of embryo quality, and the interaction between the mucosa and the embryo with inadequate quality might coordinate the VT phenomenon (26).

Another major finding of our study is that chronic maternal diseases and a history of certain high-risk pregnancies (e.g., IUGR and GDM) might contribute to the absorption of a single embryo in a twin pregnancy, particularly in IVF–ICSI pregnancies. This further emphasizes the detrimental effect of the greater prepregnancy susceptibility pertaining directly to the inherent factors in the pathomechanism of the VT phenomenon. Furthermore, this is in line with findings in the literature that an unfavorable perinatal outcome is related mostly to the infertility background in IVF–ICSI pregnancies, with more frequent adverse maternal characteristics than their noninfertile counterparts (9, 27). An adverse obstetric history comprising induced abortion and second-trimester fetal loss as classic miscarriage-related factors is also strongly associated with single loss in twins in spontaneous pregnancies.

We still noted VT as an independent risk factor for adverse perinatal outcome after controlling for all possible

TABLE 3

Neonatal outcome in pregnancies complicated with vanishing twins and matched controls presented at the Department of Obstetrics and Gynecology, University of Szeged, between January 1, 1994, and November 30, 2014.

	IVF/ICSI pregnancies							Spontaneously conceived pregnancies						
	Vanishing twin (n = 78)		Matched singleton (n = 234)					Vanishing twin (n = 228)		Matched singleton (n = 684)				
Outcome	n	%	n	%	*P* value[a]	Unadjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]	n	%	n	%	*P* value[a]	Unadjusted OR (95% CI)[a]	Adjusted OR (95% CI)[a]	*P* value[b]
Gestational age (mean ± SD) (wk)[c]	38.4±1.10		38.4±1.32		.66	0.98 (0.80–1.20)	0.91 (0.71–1.31)	38.80±1.59		38.80 ± 1.44		.91	1.01 (0.92–1.12)	1.00 (0.89–1.2)	
Preterm birth	0	0	2	2.6	1.00	1.01 (1.00–1.02)	0.99 (1.00–1.03)	12	5.3	30	4.4.	.59	1.21 (0.61–2.41)	1.32 (0.55–2.2)	.27
Birth weight (mean ± SD) (g)[c]	3,178.5 ± 562.2		3,302.8 ± 542.5		.38	0.99 (0.98–1.0)	0.99 (0.98–1.0)	3,321.4 ± 524.8		3,391.1 ± 565.4		.29	1.0 (1.0–1.0)	1.0 (0.9–1.1)	
IUGR	18	23.1	6	2.6	<.001	11.4 (4.34–30.0)	9.2 (4.99–21.9)	12	5.3	12	1.8	.007	3.11 (1.38–7.03)	3.0 (1.77–5.2)	.04
Low birth weight (<2,500 g)	12	15.4	12	5.1	.006	3.36 (1.44–7.84)	3.99 (1.78–7.11)	18	7.9	24	3.5	.01	2.36 (1.26–4.43)	2.1 (1.55–4.0)	.69
Very low birth weight (<1,500 g)	0	0	0	0	–[d]	–[d]	–[d]	0	0	6	0.9	.35	1.00 (1.00–1.02)	0.89 (0.78–1.2)	–[d]
NICU admission	0	0.00	18	7.7	.009	0.92 (0.89–0.96)	0.34 (0.11–0.61)	12	5.3	24	3.5	.24	1.53 (0.75–3.11)	1.9 (0.66–2.81)	.10
Male sex	42	53.8	114	48.7	.51	1.23 (0.74–2.05)	1.1 (0.81–1.89)	132	57.9	282	41.2	<.001	1.96 (1.45–2.66)	1.4 (1.2–1.9)	.12
Congenital malformations	6	7.7	18	7.7	1.00	1.00 (0.38–2.62)	1.00 (0.55–1.5)	6	2.6	18	2.6	1.00	1.00 (0.39–2.55)	1.00 (0.22–2.90)	1.00

Note: All variables were adjusted for age, body mass index, parity, chronic maternal diseases and obstetric history data: miscarriage, termination of pregnancy, fetal loss in the second trimester, hypertensive disorder, prematurity, and IUGR fetus in previous pregnancies.
CI = confidence interval; ICSI = intracytoplasmic sperm injection; IUGR = intrauterine growth restriction; IVF = in vitro fertilization; NICU = neonatal intensive care unit; OR = odds ratio; SD = standard deviation.

[a] *P* value, odds ratio, and 95% CI for comparison of categorical data with Fisher's exact test or chi-square test.

[b] *P* value for the Mantel-Haenszel test.

[c] Continuous variables displayed as mean ± SD. Comparison of continuous data in two distinct years with Mann-Whitney *U* test.

[d] Statistical analysis was not meaningful.

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TABLE 4

Logistic regression models on presenting risk factors of vanishing twin pregnancies achieved by in vitro fertilization or natural conception presented at the Department of Obstetrics and Gynecology, University of Szeged, between January 1, 1994, and November 30, 2014.

Logistic regression model of vanishing twin pregnancies	AOR (95% CI)[a]	*P* value[a]
By in vitro fertilization (n = 312)		
GDM in the previous pregnancy	5.41 (1.36–21.7)	.017
Chronic maternal diseases	3.48 (1.27–9.60)	.016
GDM in the present pregnancy	2.33 (1.14–4.55)	.021
Placental abruption	4.35 (1.31–22.8)	.020
Retained placenta	8.00 (2.74–23.3)	< .001
IUGR	28.2 (2.18–14.5)	< .001
By natural conception (n = 912)		
Chronic maternal diseases	2.10 (1.39–3.34)	< .001
IUGR	3.65 (1.43–8.5)	.006

Note: Both logistic regression models were adjusted for maternal age and body mass index, parity, chronic maternal diseases, and obstetric history data: miscarriage, termination of pregnancy, fetal loss in the second trimester, hypertensive disorder, prematurity, and small-for-gestational-age/growth retarded fetus in the previous pregnancies. GDM = gestational diabetes mellitus; IUGR = intrauterine growth restriction.

[a] *P* value, adjusted odds ratio (AOR), and 95% confidence interval (CI) for comparison of multiple logistic regression.

Márton. Pregnancy outcome of vanishing twin. Fertil Steril 2016.

confounders. Our most striking result is that the resorption of an embryo induces growth restriction in the remaining twin, particularly after IVF–ICSI, as compared with age- and previous gestation-matched singletons. This observation has been a consistent finding in other reports (2,8,9,28–30). After IVF–ICSI, the odds of a birth weight <10th percentile greater than 9 times and 4-fold odds for low birth weight were observed, whereas the respective figures for natural conception were only 3.1 and 2.4. One reason for this difference may be that the VT phenomenon in IVF–ICSI pregnancies was detected at a greater gestational length, suggesting that reduction occurs at a later stage. Later timing of the demise of a cotwin in IVF–ICSI might indicate that it is not the quality of the vanished embryo that is the dominant factor but the uterine environment (7, 10) or the impaired utero-fetal interaction of the developing cotwin with possibly normal growth potential (23).

One can speculate that the larger fetoplacental tissue requires a longer elimination process (31), resulting in a poorer obstetric outcome (2, 8). During restoration, the remodeling of the fetoplacental blood flow might be driven by decomposition products from the vanishing fetus. Hence, blood flow toward the surviving twin might be decreased temporarily, inducing a relative placental insufficiency that could delay placental expansion and retard fetal growth.

It is interesting that we did not find that the vanishing fetus had any significant influence on the rate of preterm birth either in the IVF–ICSI group or in the spontaneous group, a result that contrasts with other epidemiologic and observational studies (2, 11) but confirms still other studies (18, 30). One intriguing finding was that we did not find any low birth weight or very preterm births in our VT groups. Our study provides evidence that adverse outcomes of VT pregnancies are more likely to be associated with the greater odds of lower birth weight and an increased proportion of age-related morbidities, which are usually more common in IVF pregnancies (32).

Cases of pregestational and gestational diabetes are noticeably more common among VT pregnancies achieved by IVF–ICSI. One potential reason could be that two placentas exceed the uterine capacity, resulting in "crowding" (6), because placentomegaly and relative uteroplacental insufficiency (33) develop rather early in diabetic pregnancies. Diabetes-generated teratogens might also induce miscarriage (34), which might even lead to the loss of one infant from a pair of twins possibly due to the uneven susceptibilities of the fetuses. In accordance with other studies (8, 10), we found that placental dysfunction caused by hypertensive disorders appears not to determine the outcome of VT pregnancies considerably because essential hypertension is extremely uncommon. There does not seem to be any link between VT and preeclampsia.

Placental factors may also contribute to these adverse outcomes because placental insertion abnormalities such as placenta previa and retained placenta are associated with VT. Moreover, the IVF–ICSI technique promotes higher rates of these anomalies (32). This observation corroborates the finding that impaired placentation at an inappropriate uterine site (6) might have a putative effect on the single loss in twins particularly after IVF–ICSI.

In line with the concept that twinning frequency increases with maternal age (32, 35), we found that the VT phenomenon is also associated with advanced age in both groups, as documented by others in IVF–ICSI pregnancies (7, 12). Because aging decreases oocyte quality, which is the strongest predictor of embryo potential (36), it might be assumed that genetically impaired oocytes implant particularly after natural conception. One advantage of our study is that pregnancies resulting from ovulation induction or insemination were excluded, as these procedures increase the risk of VT among twins by up to 38% (7). Miscarriage-related risk factors such as obesity and smoking do not seem to play a role in VT because of the possible effect on both conceptions. Nor does it appear that cavity deformities such as congenital uterine anomalies count for VT.

Our study has some limitations. The relatively low incidence of VT denotes the low power of the statistical analyses, particularly when confounders were considered in multiple statistical analyses. Theoretically, the rate of VT pregnancies after IVF–ICSI depends on the rate of multiple embryo transfer. Furthermore, the study spans more than two decades in which ART has improved dramatically, and the focus was not on procedure specifics (i.e., fresh vs. frozen ET or IVF vs. ICSI procedures). However, it is of note that artificial procedures have a profound effect on the splitting of the zygote (37), which represents an unavoidable bias in the comparison of spontaneous and IVF–ICSI VT-pregnancies. However, all the women with VT pregnancies after IVF–ICSI underwent a double-embryo transfer procedure, and all the monochorionic

twin pregnancies were excluded from both of the study groups. We further assume that the absorption of an embryo in a monochorionic twin pregnancy could also lead to the elimination of the remaining cotwin at a high rate.

In conclusion, it appears that different pathologic processes might cause VT and eliminate the fetoplacental unit in a different uterine environment, thus exhibiting a trend toward higher rates of perinatal complications in IVF–ICSI pregnancies compared with those conceived spontaneously. Spontaneous reduction occurs more frequently in spontaneous twin pregnancies than in conceptions after assisted reproduction. The main findings of the high rate of IUGR neonates in connection with certain maternal illnesses is related to adverse perinatal outcomes in VT pregnancies. For further consistency, prospective epidemiologic studies are necessary to investigate the role of less prevalent miscarriage-related factors.

REFERENCES

1. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Barfield WD, Centres for Disease Control and Prevention (CDC). Assisted reproductive technology surveillance—United States, 2011. MMWR Surveill Summ 2014;2163:1–28.
2. Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. Hum Reprod 2005;20:2821–9.
3. Ishihara O, Adamson GD, Dyer S, de Mouzon J, Nygren KG, Sullivan EA, et al. International committee for monitoring assisted reproductive technologies: world report on assisted reproductive technologies, 2007. Fertil Steril 2015; 103:402–13.
4. Martikainen H, Tiitinen A, Tomás C, Tapanainen J, Orava M, Tuomivaara L, et al. Finnish ET Study Group. One versus two embryo transfer after IVF and ICSI: a randomized study. Hum Reprod 2001;16:1900–3.
5. Almog B, Levin I, Wagman I, Kapustiansky R, Lessing JB, Amit A, et al. Adverse obstetric outcome for the vanishing twin syndrome. Reprod Biomed Online 2010;20:256–60.
6. Depp R, Macones GA, Rosenn MF, Turzo E, Wapner RJ, Weinblatt VJ. Multifoetal pregnancy reduction: evaluation of foetal growth in the remaining twins. Am J Obstet Gynecol 1996;174:1233–8.
7. Dickey RP, Taylor SN, Lu PY, Sartor BM, Storment JM, Rye PH, et al. Spontaneous reduction of multiple pregnancy: incidence and effect on outcome. Am J Obstet Gynecol 2002;186:77–83.
8. Pinborg A, Lidegaard O, Freisleben N, Andersen AN. Vanishing twins: a predictor of small-for-gestational age in IVF singletons. Hum Reprod 2007;22: 2707–14.
9. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med 2002;346:731–7.
10. La Sala GB, Villani MT, Nicoli A, Gallinelli A, Nucera G, Blickstein I. Effect of the mode of assisted reproductive technology conception on obstetric outcomes for survivors of the vanishing twin syndrome. Fertil Steril 2006;86: 247–9.
11. Chasen ST, Luo G, Perni SC, Kalish RB. Are in vitro fertilisation pregnancies with early spontaneous reduction high risk? Am J Obstet Gynecol 2006;195: 814–7.
12. La Sala GB, Nucera G, Gallinelli A, Nicoli A, Villani MT, Blickstein I. Spontaneous embryonic loss after in vitro fertilisation with and without intracytoplasmic sperm injection. Fertil Steril 2004;82:1536–9.
13. Tummers P, De Sutter P, Dhont M. Risk of spontaneous abortion in singleton and twin pregnancies after IVF/ICSI. Hum Reprod 2003;18:1720–3.
14. Landy HJ, Keith LG. The vanishing twin: a review. Hum Reprod Update 1998; 4:177–83.
15. Sampson A, de Crespigny LC. Vanishing twins: the frequency of spontaneous foetal reduction of a twin pregnancy. Ultrasound Obstet Gynecol 1992;2:107–9.
16. Jauniaux E, Elkazen N, Leroy F, Wilkin P, Rodesch F, Hustin J. Clinical and morphologic aspects of the vanishing twin phenomenon. Obstet Gynecol 1988;72:577–81.
17. Landy HJ, Weiner S, Corson SL, Batzer FR, Bolognese RJ. The "vanishing twin" ultrasonographic assessment of foetal disappearance in the first trimester. Am J Obstet Gynecol 1986;155:14–9.
18. Barton SE, Missmer SA, Hornstein MD. Twin pregnancies with a 'vanished' embryo: a higher risk multiple gestation group? Hum Reprod 2011;26: 2750–3.
19. Greenwold N, Jauniaux E. Collection of villous tissue under ultrasound guidance to improve the cytogenetic study of early pregnancy failure. Hum Reprod 2002;17:452–6.
20. Jauniaux E, Van Oppenraaij RH, Burton GJ. Obstetric outcome after early placental complications. Curr Opin Obstet Gynecol 2010;22:452–7.
21. Oloyede OA, Iketubosin F, Bamgbopa K. Spontaneous foetal reduction and early pregnancy complications in multiple pregnancies following in vitro fertilisation. Int J Gynaecol Obstet 2012;119:57–60.
22. Jauniaux E, Ben-Ami I, Maymon R. Do assisted-reproduction twin pregnancies require additional antenatal care? Reprod Biomed Online 2013;26: 107–19.
23. La Sala GB, Nicoli A, Villani MT, Gallinelli A, Nucera G, Blickstein I. Spontaneous embryonic loss rates in twin and singleton pregnancies after transfer of top- versus intermediate-quality embryos. Fertil Steril 2005;84:1602–5.
24. Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril 2005;83:1650–8.
25. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod 2006;21:2216–22.
26. Zhao J, Zhang Q, Li Y. The effect of endometrial thickness and pattern measured by ultrasonography on pregnancy outcomes during IVF-ET cycles. Reprod Biol Endocrinol 2012;10:100.
27. Evron E, Sheiner E, Friger M, Sergienko R, Harlev A. Vanishing twin syndrome: is it associated with adverse perinatal outcome? Fertil Steril 2015; 103:1209–14.
28. Luke B, Brown MB, Grainger DA, Stern JE, Klein N, Cedars MI, Society for Assisted Reproductive Technology Writing Group. The effect of early foetal losses on singleton assisted-conception pregnancy outcomes. Fertil Steril 2009;91:2578–85.
29. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Factors affecting obstetric outcome of single- tons born after IVF. Hum Reprod 2011;26:2878–86.
30. Shebl O, Ebner T, Sommergruber M, Sir A, Tews G. Birth weight is lower for survivors of the vanishing twin syndrome: a case-control study. Fertil Steril 2008;90:310–4.
31. Bass HN, Oliver JB, Srinivasan M, Petrucha R, Ng W, Lee JE. Persistently elevated AFP and AChE in amniotic fluid from a normal foetus following demise of its twin. Prenat Diagn 1986;6:33–5.
32. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update 2013;19:87–104.
33. Rizzo G, Capponi A, Pietrolucci ME, Aiello E, Arduini D. First trimester placental volume and three dimensional power doppler ultrasonography in type I diabetic pregnancies. Prenat Diagn 2012;32:480–4.
34. Loeken MR. Advances in understanding the molecular causes of diabetes-induced birth defects. J Soc Gynecol Investig 2006;13:2–10.
35. Jones HW. Multiple births: how are we doing? Fertil Steril 2003;79:17–21.
36. Younis JS. Ovarian aging: latest thoughts on assessment and management. Curr Opin Obstet Gynecol 2011;23:427–34.
37. Khorram O, Patrizio P, Wang C, Swerdloff R. Reproductive technologies for male infertility. J Clin Endocrinol Metab 2001;86:2373–9.