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Vanishing twins: a predictor of small-for-gestational age in IVF singletons

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BACKGROUND: The purpose of this study was to assess the effect of a vanishing twin on the risk of being small-for-gestational age (SGA) in *in vitro* fertilization (IVF) singletons. METHODS: The study included 642 survivors of a vanished co-twin, 5237 primary singletons and 3678 primary twins. The survivor cohort was subdivided according to gestational age at the time of vanishing to give groups of early (<8 weeks), intermediate (8–22 weeks) and late (>22 weeks) survivors. RESULTS: The rate of SGA infants was significantly higher in survivors than in singletons (OR: 1.50, 95%CI: 1.03–2.20) and a significant inverse correlation was observed between SGA and the gestational age at the time of vanishing ($r = -0.10$, $P < 0.02$). Also in term infants, the risk of birthweight <2500 g was higher in survivors than in singletons (OR: 1.71, 95%CI: 1.06–2.74). A similar increase in the rate of low birthweight in term survivors was seen with increasing gestational age at the time of vanishing ($r = -0.12$; $P < 0.01$). In multiple logistic regression analysis adjusting for maternal age, parity, child gender and pre-eclampsia, the vanishing of a co-twin (OR: 1.56, 95%CI: 1.06–2.27) and gestational age at the time of vanishing (OR: 2.08, 95%CI: 1.00–4.35) were the only significant predictors of being SGA. CONCULSIONS: IVF singletons with a vanished co-twin had a higher rate of SGA than singletons from a single gestation and the risk of SGA is increased with increasing gestational age at the time of vanishing.

Keywords: intrauterine growth restriction; IVF; perinatal outcome; small-for-gestational age; vanishing twin

Introduction

Perinatal health problems after *in vitro* fertilization (IVF) are primarily due to multiple gestations, but even in IVF singletons low birthweight and prematurity rates are higher than that of spontaneously conceived singletons (Schieve *et al.*, 2002; Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004). Additionally, congenital malformations are seen at an increased rate (Hansen *et al.*, 2005; Källén *et al.*, 2005; Klemetti *et al.*, 2005), and the number of hospitalizations is higher (Ericson *et al.*, 2002). Concerning long-term morbidity and rare outcomes such as cerebral palsy (CP), large studies are needed to clarify potential health problems in IVF children. Studies on long-term health of IVF children are few, however Swedish and Danish nationwide register studies have shown a higher risk of CP than in naturally conceived singletons (Strömberg *et al.*, 2002; Lidegaard *et al.*, 2005). In a recent Finish national register-based survey with 4 years of follow-up, the authors concluded that IVF singletons had increased overall long-term morbidity, which was not attributable to any specific disease, but rather to small increases in many groups of diseases (Klemetti *et al.*, 2006). In Denmark, 4% of infants are born as a result of IVF. Hence, health problems in these children are of major clinical and social importance (Nyboe Andersen *et al.*, 2006).

It is known that one of the potential reasons for the poorer perinatal health of IVF singletons is the infertility itself (Pandian *et al.*, 2001; Basso and Baird, 2003; Basso and Olsen, 2005; Zhu *et al.*, 2006). Although elective single embryo transfer (eSET) is being implemented in an increasing number of European countries, double embryo transfer is still the routine in the vast majority. Thus, a sizeable number of IVF singletons still origin from a twin gestation. We have recently shown that a potential reason for the adverse perinatal outcome in IVF singletons is the vanishing twin phenomenon occurring in 10% of live born IVF singletons (Pinborg *et al.*, 2005). Further, we showed that the gestational age at the time of vanishing was inversely correlated to poor outcome, i.e. the higher gestational age at the time of vanishing, the poorer outcome.

The purpose of this study was to use nationwide registers to assess the effect of a vanishing twin on the risk of being small-for-gestational age (SGA) in IVF singletons and to evaluate the effect of gestational age at the time of vanishing on the risk of being SGA.

Materials and Methods

We recorded 8542 ongoing pregnancies and 9557 live born children detected by transvaginal sonography in gestational week 8 with date of embryo replacement consecutively registered during a 7-year period from 1 January 1995 to 31 December 2001. Pregnancy outcome was obtained in 96.7% of the pregnancies, whereas 3.4% were lost to follow-up due to foreigners receiving treatment in Denmark and emigration. Eleven Danish fertility clinics participated, which, according to unpublished data from the Danish Fertility Society, represent ~70% of all IVF/ICSI cycles performed in Denmark during the period.

Only pregnancies, which fulfilled the following three inclusion criteria in gestational week 8, were eligible for the study: (i) one viable fetus plus an empty gestational sac or a fetus with no fetal heart beat, (ii) one viable fetus or (ii) two viable fetuses. Pregnancies with more than two fetal heartbeats and gestations with no viable fetuses were excluded. The key variables in each record of pregnancy were the date of embryo replacement and the personal identification number (PIN) code of the woman.

The term 'vanished twin' has been interpreted in many different ways (Landy and Keith, 1998; Pinborg *et al.*, 2006) ranging from first trimester missed abortion of one of the twins (La Sala *et al.*, 2006) to vanishing of later gestational ages. In its pure sense, the vanishing twin syndrome is a first-trimester missed abortion, however to account for fetal death throughout the whole pregnancy, we decided to characterize 'vanished twin' as an empty sac or a first, second and third trimester intrauterine fetal demise, although in the latter the dead fetus does not vanish. Retrieval of register data was conducted in three steps. First, a cross-linkage with the National IVF Registry to identify the outcome of each pregnancy as a spontaneous abortion, a singleton or a twin delivery, and whether the infants were live born or stillborn was assured. The second step was to separate the children into three study cohorts. Group I: 'survivor cohort' consisted of singleton born infants diagnosed as twin gestations according to either inclusion criteria 'A' or 'C' in early pregnancy ($n = 642$). Hence, the 'survivor cohort~study cohort' comprised babies from pregnancies with twin conceptions in early pregnancy, with one fetus either vanishing or with fetal intrauterine demise in first, second or third trimester. Group II: 'singleton cohort' was singleton born infants and registered as a singleton gestation (inclusion criteria 'B') in early pregnancy ($n = 5237$). Group III: 'twin cohort' was gestations with two fetal hearts beat in early ultrasound (inclusion criteria 'C') and registered as two live born twins ($n = 3678$). The survivor cohort was further subdivided according to gestational age at onset of spontaneous reduction (SR), so that there were 424 'earlyrsquo; (SR before week 8), 187 'intermediate' (SR in weeks 8–22) and 31 'late' (SR after 22 weeks including stillborn co-twin) survivors. In the third step, we collected data on birthweight and gestational age from the Danish National Patient Registry to assess the number of SGA children. Neonatal outcome and neurological sequelae in the same cohorts have previously been published (Pinborg *et al.*, 2005).

Outcome measures

The main outcome measures for SGA were the number of children with birthweight below the lower 10th percentile for each gestational age and the proportion of term children (gestational age >37 weeks) with birthweight <2500 g.

Diagnoses according to the ICD-10 diagnosis system on pre-existing hypertension (DO10.0–11.9), pregnancy induced hypertension (PIH) (DO12.0–13.9, DO16–16.9), pre-eclampsia (PE) and HELLP (DO14.0–15.9), placenta praevia (DO44.0–44.9), placental abruption (DO45.0–45.9), bleeding in first trimester (DO20.0–20.9) and bleeding in third trimester (DO46.0–46.9) were drawn from The National Patient Registry to adjust for co-morbidity in pregnancy. Diagnoses were limited to the time interval 280 days prior to and 60 days after the date of delivery.

Statistics

Statistical analysis was performed using SPSS for Windows (Statistical Packages for Social Sciences) version 10.0. A probability value of $P < 0.05$ was considered statistically significant. Differences of means of continuous parametric data were analyzed with the use of Student's *t*-test. In univariate analyses regarding distributions between groups, risk estimates were calculated as ORs with 95% confidence intervals and differences were compared with Pearson's chi-square test. Regarding early, intermediate and late vanishing, Spearman correlation coefficients (r) for ordinal data were calculated. By multiple logistic regression analysis, we searched for specific predictors of being SGA. Multiple logistic regression analysis was performed as backward stepwise regression (likelihood ratio) with 'SGA' as the dependent variable and the following covariates as independent variables; maternal age (<35 or >35 years), parity (0 or ≥1 previous delivery), child gender, cohort (singleton or survivors) and gestational age at the time of vanishing (early, intermediate or late). Adjustment was also made for potential co-morbidity confounders; pre-existing hypertension, PIH and PE.

Results

According to the ultrasonographic inclusion criteria, we identified 8542 ongoing pregnancies in gestational week 8. Of the pregnancies diagnosed as early vanishing (one live and one fetus with no fetal heartbeat at ultrasound in week 8), 6.2% ended as a spontaneous abortion, whereas 93.8% resulted in a singleton birth. Baseline characteristics showed no significant differences in maternal age, parity, boy/girl ratio and treatment method between survivor and control populations (Table 1).

The proportion of SGA children in the three cohorts is reported in Figs 1 and 2. The risk of SGA infants was 50% higher in singleton pregnancies with a vanishing twin than in the primary singleton cohort (OR: 1.50, 95%CI: 1.03–2.20), however the risk of SGA babies was highest in the twin cohort (Table 2). There was a significant inverse correlation between gestational age at the time of onset of SR and the proportion of SGA babies in the survivor cohort ($r = -0.10$, $P < 0.02$). Mean birthweight differed significantly between early survivors (3365 ± 695 g) and singletons (3442 ± 662 g) with a difference of 77 g and $P = 0.02$. When comparing mean birthweight in early and intermediate survivors (3309 ± 756 g) with singletons, we found a difference of 133 g ($P < 0.0001$).

Term children (born after 37 completed weeks)

As another outcome measure of intrauterine growth retardation and to avoid the influence of premature born children, we excluded all infants born before 37 completed weeks. The frequencies of term children with birthweight <2500 g [low birthweight (LBW)] are reported in Figs 3 and 4. For term infants, mean birthweight was significantly lower among survivors (3454 ± 575 g) than singletons (3539 ± 552 g) ($P < 0.001$). Term survivors had significantly higher mean birthweights than term twins (3454 ± 575 g versus 2813.90 ± 420 g) ($P < 0.001$). Further, we compared mean birthweight

Table 1: Maternal, pregnancy and infant characteristics data in the survivor, singleton and twin cohort

Cohort	Survivor	Survivor versus singletons		Survivors versus twins	
		Singleton	*P*-value	Twin	*P*-value
Live born children (*n*)	642	5237		3678	
Boy/girl	53.1%	51.8%	0.6[a]	52.8%	0.9[a]
Maternal age (mean ± SD)	33.3 ± 3.9	33.3 ± 3.7	0.7[b]	32.6 ± 3.6	<0.0001[b]
Age < 30 years (%)	20.3	18.8	0.4[a]	23.4	0.09[a]
Nulliparity	82.7%	81.2%	0.4[a]	81.6%	0.5[a]
Treatment method (%)					
IVF	65.9	62.8	0.1[a]	65.8	0.3[a]
ICSI	26.4	26.7		26.8	
FER	6.6	8.9		5.4	
50%IVF/50%ICSI	1.1	1.6		2.0	

The survivor cohort consisted of 424 'early survivors' (one twin was reduced <8 weeks), 187 'intermediate survivors' (one twin vanished >8 but <22 weeks) and 31 'late survivors', surviving a stillborn co-twin. FER, frozen embryo replacement.
[a]Proportions were calculated by Pearson's chi-square test.
[b]Differences between mean values were calculated by Student's *t*-test.

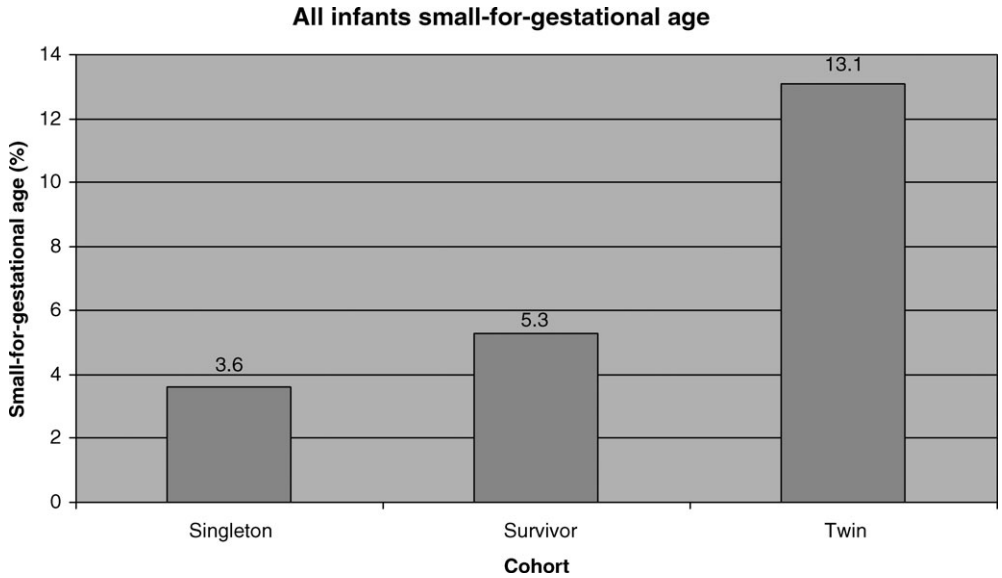

Figure 1: The percentage of infants in the three cohorts, who were born as SGA

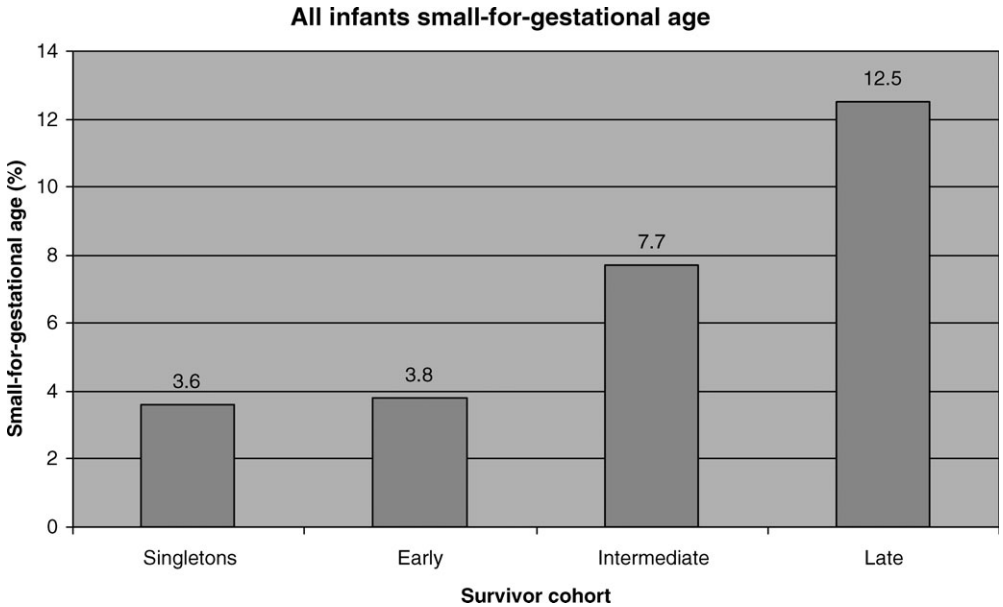

Figure 2: The percentage of infants in the survivor cohort being SGA according to the gestational age at the time of the co-twin vanishing

Table 2: The risk of being SGA in all infants and among term infants (i.e. BW > 2500 g), calculated in univariate analyses as crude odds ratios (OR) and 95% confidence intervals (CI)

OR (95%CI)	Survivor ($n = 642$)	Singleton ($n = 5237$)	Twin ($n = 3678$)
The risk of SGA			
All infants	1.00	0.67 (0.45–0.97)	2.70 (1.87–3.88)
Term infants (>37.wks)	1.00	0.58 (0.36–0.94)	5.88 (3.70–9.09)
	Early (≤8 weeks) ($n = 424$)	Intermediate (8–22 weeks) ($n = 187$)	Late (>22 weeks) ($n = 31$)
The risk of SGA			
All infants	1.00	2.08 (0.99–4.36)	3.59 (0.97–13.3)
Term infants (>37.wks)	1.00	2.78 (1.11–7.14)	9.09 (1.72–50.0)

Crude ORs with 95% CIs were calculated in univariate analyses and no adjustments were made in the table.

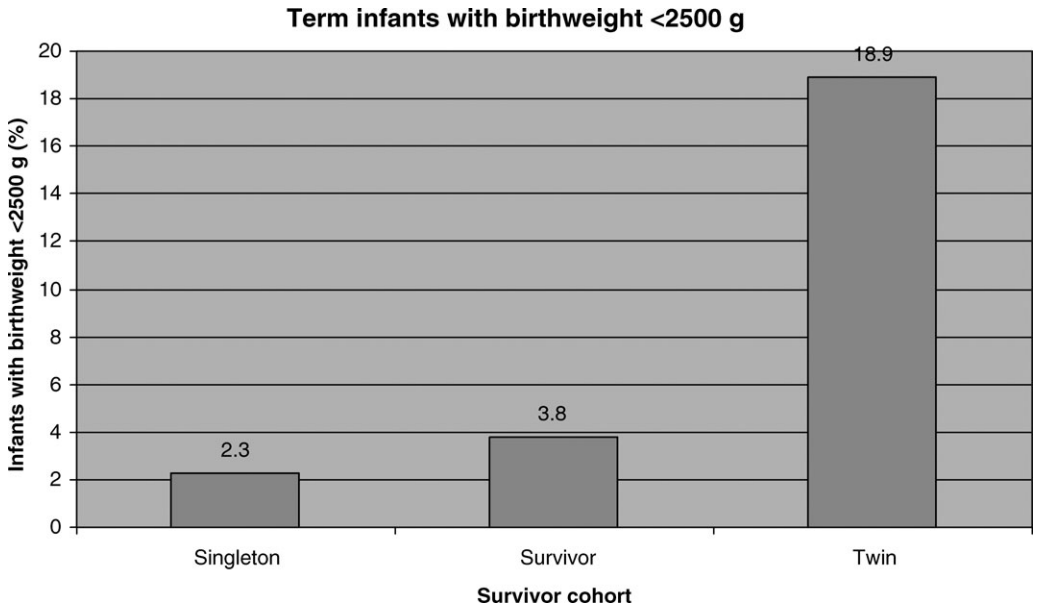

Figure 3: The percentage of term infants who were born with birthweight <2500 g (LBW)

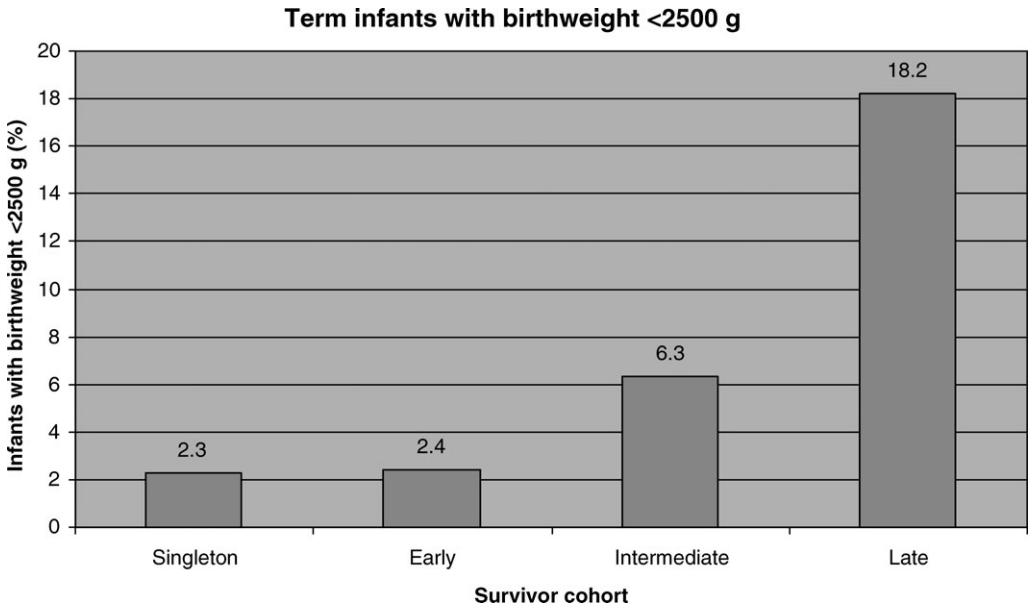

Figure 4: The percentage of term survivors born with LBW according to the gestational age at the time of the co-twin vanishing

at term for early survivors (SR before 8 weeks)(3490 ± 551 g) with singletons and found a mean birthweight difference of 49 g, but this was not statistical significant ($P = 0.1$). However, a significant difference was found between early and intermediate survivors (3464 ± 572 g) compared with singletons ($P = 0.003$). The mean birthweight in term late survivors is 2966g ± 488 SD.

Also for term infants, the risk of LBW <2500 g was significantly higher in the survivor versus the singleton cohort (OR: 1.71, 95%CI: 1.06–2.74) (Table 2). Similar to the finding for all infants, we found a significant inverse correlation between gestational age at the time of vanishing and LBW in term survivors ($r = -0.12$, $P < 0.01$). Hence, the higher the gestational age at the time of vanishing, the higher the rate of LBW in term infant survivors.

Pregnancy complications

Figures and percentages of pregnancy complications in the three cohorts are shown in Table 3. There were no statistical significant differences between the survivor and the singleton and twin cohort according to the risk of bleeding in pregnancy [8.4%; 7.9%; 9.2%] and placenta praevia [1.7%; 0.9%; 1.3%]. Although not statistical significant, the risk of placental abruption was almost twice as high in the survivor cohort (11/642) compared with the singleton (48/5237) cohort (OR: 1.89, 95%CI: 0.97–3.70) (Table 3). Of the 11 cases of placental abruption in the survivor cohort, one was among late survivors, two in the intermediate survivors and eight in the early survivor cohort. We observed no significant differences between the three cohorts in univariate statistical analyses of the risk of PE or gestational proteinuria/hypertension (Table 3).

Predictors of SGA children

In multiple logistic regression analysis with the following covariates: maternal age (<35 or ≥35 years), parity (0 or ≥1 previous delivery), child gender, cohort (singleton/survivors) and gestational age at the time of vanishing (early, intermediate and late), vanishing of a co-twin (OR: 1.56, 95%CI: 1.06–2.27) and late gestational age at vanishing (OR: 2.08, 95%CI: 1.00–4.35) were the only significant predictors of being SGA.

As PE should be considered as a potential confounder due to the association between PE and intrauterine growth retardation, we also adjusted for PE, eclampsia and hypertension in pregnancy, but this did not alter the above results on predicting factors for SGA.

Discussion

This study is the first to show that IVF singletons from vanishing twin gestations have a higher risk of being SGA than singletons from a single gestation in early pregnancy, and that the higher the gestational age at the time of vanishing, the higher the risk that the surviving infant is being SGA. Further, the only independent predictors of SGA were vanished co-twin and late gestational ages at the time of vanishing. One reassuring finding was that only 6.2% of the early (<8 week) vanishing group ended-up with a spontaneous abortion. Hence, we can inform the concerned couples with a viable fetus and an empty gestational sac or a non-viable fetus in early pregnancy that more than 93% are likely to deliver a live born baby. In a recent paper, we found that vanishing twins occur in 10% of IVF singletons and have a higher risk of short and long-term morbidity (Pinborg *et al.*, 2005). Together with our earlier publication, the present study provides evidence that vanishing twins from multiple embryo transfer is one of the reasons for the poorer outcome in IVF singletons.

The natural division of the vanishing twin group would have been according to each trimester, however, this was impossible for two reasons. First, ultrasound scans in each trimester are not obligatory in Denmark, and second, ultrasound diagnoses are not recorded in the National Patient Registry. On the other hand, ultrasound scans in week 8 are obligatory at IVF clinics and recorded in the clinical databases, from where we could draw the data. The division before or after week

Table 3: The proportion and the risk (crude OR with 95% CIs) of women in study and control populations having pregnancy complications

Cohort	Survivor ($n = 642$)	Singleton ($n = 5237$)	Twin ($n = 3678$)
N (%)			
Bleeding in first trimester	54 (8.4)	415 (7.9)	339 (9.2)
Bleeding in second or third trimester	13 (2.0)	123 (2.3)	96 (2.6)
Placenta praevia	8 (1.2)	57 (1.1)	44 (1.2)
Placental abruption	11 (1.7)	48 (0.9)	47 (1.3)
Crude OR (95%CI)			
Bleeding in first trimester	1.00	0.94 (0.70–1.26)	1.10 (0.82–1.49)
Bleeding in second or third trimester	1.00	1.10 (0.65–2.07)	1.29 (0.72–2.33)
Placenta praevia	1.00	0.87 (0.41–1.84)	0.96 (0.45–2.05)
Placental abruption	1.00	0.53 (0.27–1.03)	0.74 (0.38–1.44)
N (%)			
PE, HELLP, eclampsia	33 (5.1)	210 (4.0)	161 (4.4)
Hypertension or proteinuria without PE	13 (2.0)	92 (1.8)	52 (1.4)
Crude OR (95%CI)			
PE, HELLP, eclampsia	1.00	0.77 (0.53–1.12)	0.85 (0.57–1.23)
Hypertension or proteinuria without PE	1.00	0.86 (0.48–1.56)	0.69 (0.38–1.28)

Crude ORs were calculated in univariate analyses; no adjustments were made in the table.

22 was possible according to the National Patient Registry as before week 22, fetal loss is a spontaneous abortion, and from week 22, fetal loss is accounted as stillbirth.

The difference between the early survivor and the singleton cohort in mean birthweight was 77 g with a *P*-value of 0.02, which is statistical significant but of less clinical importance. For term children, there was a mean birthweight difference between early survivors and singletons of 49 g. Although they have a better outcome than intermediate and late survivors, early survivors still have poorer birthweight outcome than the singleton cohort. One could argue that the physiologic effect of a third trimester intrauterine demise on the surviving twin could be quite significant and not comparable to a first trimester loss. However, to give the full picture of the 'vanishing twin' contribution to the poorer outcome in IVF singletons and in the light of our findings on birthweight, early survivors do contribute, but to a much lesser extent than second and third trimester survivors. Two-third of the vanishing twin cohort is 'early vanishers' however for the risk of SGA, the intermediate and late survivors particularly contribute. The total number of vanishing twin survivors in the IVF singleton cohort is 10% (Pinborg *et al.*, 2005), of which the one-third constituted by intermediate and late survivors in particular account for pregnancies at high risk of SGA. Thus the true proportion of IVF singletons pregnancies at high risk of SGA due to a vanished twin is not 10% but closer to 3–4%.

Strengths and limitations

Since data were retrieved from the IVF clinics between 1995 and 2001 with the Danish IVF register initiated in 1994, this was the largest possible study population of vanishing twins in Denmark, including more than two-thirds of all IVF singleton and twin births on a national level. The strengths of the study were the follow-up of 97% of the cases, and that important covariates, such as maternal age, parity and PE were available for almost all participants.

The major weakness of the study was that we had no comparable data on the rate of SRs in spontaneously conceived singletons, but as the twin rate is 20-fold lower after natural conception (1/80 versus 1/4), we can anticipate that vanishing twins occur in ~0.5% of spontaneously conceived singletons. To address the specific rate of vanishing twins in spontaneously conceived singletons, a large prospective study with early ultrasound is required. As early ultrasound of spontaneously conceived gestations is not routinely performed in Denmark, a prospective study would demand considerable resources. Another limitation was the potential risk of underestimation of early vanishing twins, as an empty gestational sac can be over-looked or misinterpreted as an intrauterine hematoma.

We have no information on the number of transferred embryos in our data. However, we know from unpublished data from The Danish Fertility Society that more than 90% of embryo transfers in Denmark in 1995–2002 were double embryo transfers. In the current period, almost all single embryo transfers were performed when only one embryo was available for transfer as either only one embryo was fertilized or only one embryo was of high enough quality to transfer. Only a very limited number of couples chose a voluntary single embryo transfer, as the current clinical practice was double embryo transfer. Therefore, only a very small proportion of the singletons were from elective SET transfers and the vast majority were from SET with only one embryo available for transfer. For these reasons, the true singletons had no inherent better reproductive performance in age comparable individuals in our cohort.

Comparison with other studies

Late intrauterine death of one twin in naturally conceived twin pregnancies is associated with a considerably increased morbidity and mortality risk in the surviving co-twin (Pharoah and Adi, 2000; Scher *et al.*, 2002). In 2002, Dickey *et al.* (2002) found that pregnancy duration and birthweight was inversely related to the initial number of gestational sacs irrespective of the final birth number. Further, the incidence of intrauterine growth restriction (IUGR) was 4.5%, 15.7% and 14.3% in singletons from initially one, two and three gestational sacs, although no consistent relationship was revealed. This could probably be due to the limited sample size of 154 spontaneously reduced singletons. In 2006, La Sala *et al.* found that the vanishing twin syndrome (defined as vanishing embryo in the first trimester) occurred in 12.2% of all live born singletons following ART, and found no differences in obstetric outcome between the vanishing twin group and the singleton cohort, but only 44 survivors were eligible for the study. No analyses on SGA were made.

Uteroplacental insufficiency is thought to be a relatively common cause of growth restriction in multiple gestations. The etiology of the observed increase in the frequency of IUGR in the vanishing twin cohort is not certain. Depp *et al.* (1996) was the first to suggest that first trimester 'crowding' of the developing gestations or lack of appropriate implantation sites could be determining factors in placental expansion and ultimate fetal growth. They found a statistically significant trend toward increasing frequency of IUGR with increasing initial number of fetuses in multifetal reduction pregnancies, which is in accordance with our finding of 50% increased risk of IUGR in singleton pregnancies with spontaneously reduction. In coherence with our study, they also found that the pre-pregnancy risk factor hypertension did not appear to be related to IUGR in the reduced pregnancies (Depp *et al.*, 1996). A second possible explanation for the observed increase in IUGR in survivors is an interaction of fetal growth determining factors or decomposition products segregated after SR, most of which are as yet unknown.

A third causality between IUGR and SR could be that SR cause vaginal bleeding, which is predictive of IUGR. This was recently confirmed in a population-based study, where first trimester heavy vaginal bleeding was an independent risk factor for IUGR (Weiss *et al.*, 2004). On the contrary, we did not show a higher risk of bleeding during pregnancy in the survivor cohort than in the singleton cohort. Our register-based data on bleeding during pregnancy are probably underestimating the problem and we were also unable to quantify the severity of bleeding. However, our findings were in accordance with a recent study on 253 first-trimester ART pregnancies

with vaginal bleeding, where an increased risk of preterm birth existed but no IUGR difference was found between those with first-trimester bleedings and controls (De Sutter *et al.*, 2006). This could be due to the limited sample size or to the fact that no differentiation on the extent of bleeding was performed.

We found that PE or hypertension had no influence on vanishing twin as a predictor of IUGR in IVF pregnancies. This is in coherence with a recent study, which revealed that PE and unexplained growth retardation are biological independent entities, although they are often assumed to be related to placental insufficiency (Villar *et al.*, 2006).

The recording of PE diagnoses in the Danish National Patient Registry has recently been validated and found to result in seemingly correct estimates of incidences of PE including other serious diseases such as HELLP and eclampsia (Klemmensen *et al.*, in press). Therefore, our result on these diagnoses should be robust.

There is increasing evidence that slow growth during fetal life and infancy is followed by accelerated weight gain in childhood. Two disorders that predispose to coronary heart disease, type 2 diabetes and hypertension, are preceded by similar paths of growth.

Barker has proposed the hypothesis that coronary heart disease appears to be a developmental disorder that originates through two widespread biological phenomena, developmental plasticity and compensatory growth (Barker, 2002). Although it has not been shown that the Barker theory is valid for the type of IUGR in vanishing twin pregnancies, the increased risk of IUGR in vanishing twin pregnancies may according to the Barker theory have clinical long-term effects on the outcome of IVF singletons.

It is obvious that the consequences of the adverse obstetric outcome in IVF/ICSI singleton survivors of a vanishing co-twin should further facilitate the process of implementing eSET.

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