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Fetal loss after chorionic villus sampling in twin pregnancy

T. ELGER[1], R. AKOLEKAR[2,3], A. SYNGELAKI[1], C. DE PACO MATALLANA[4], F. S. MOLINA[4], M. GALLARDO AROZENA[5], P. CHAVEEVA[6], N. PERSICO[7,8], V. ACCURTI[7], K. O. KAGAN[9], N. PRODAN[9], J. CRUZ[10] and K. H. NICOLAIDES[1]

[1]*Fetal Medicine Research Institute, King's College Hospital, London, UK;* [2]*Medway Maritime Hospital, Gillingham, UK;* [3]*Institute of Medical Sciences, Canterbury Christ Church University, Chatham, UK;* [4]*Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain;* [5]*Hospital Universitario San Cecilio, Granada, Spain;* [6]*Shterev Hospital, Sofia, Bulgaria;* [7]*Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy;* [8]*Department of Clinical Science and Community Health, University of Milan, Milan, Italy;* [9]*University Women's Hospital, Tuebingen, Germany;* [10]*Centro Hospitalar Universitário de Lisboa Central, Lisbon, Portugal*

KEYWORDS: first-trimester screening; invasive testing; miscarriage; stillbirth

CONTRIBUTION

What are the novel findings of this work?
In twin pregnancies undergoing chorionic villus sampling (CVS), compared to those not undergoing CVS, there is a 2-fold increased risk of fetal loss at < 24 weeks' gestation and loss at any stage in pregnancy. The factors providing a significant independent contribution to the prediction of miscarriage or fetal loss in twin pregnancy are increased maternal weight, black racial origin, monochorionicity, large intertwin discordance in crown–rump length and increased fetal nuchal translucency thickness.

What are the clinical implications of this work?
The 2-fold increased risk of fetal loss following CVS in twin pregnancy can, to a great extent, be explained by maternal and pregnancy characteristics rather than the invasive procedure itself.

ABSTRACT

Objective *To estimate the chorionic villus sampling (CVS)-related risk of fetal loss in twin pregnancy after adjustment for chorionicity, nuchal translucency thickness (NT), intertwin discordance in crown–rump length (CRL), maternal demographic characteristics and serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG).*

Methods *This was a multicenter study from eight fetal medicine units in which the leadership were trained at the Harris Birthright Research Centre for Fetal Medicine in London, UK, and in which the protocols for screening, invasive testing and pregnancy management are similar. Data were obtained prospectively from women with twin pregnancy undergoing routine ultrasound examination at 11–13 weeks' gestation. Multivariable logistic regression analysis with backward stepwise elimination was used to examine whether CVS provided a significant independent contribution to the prediction of risk of fetal loss after adjusting for maternal and pregnancy characteristics, including maternal age, racial origin and weight, method of conception, smoking status, parity, chorionicity, intertwin discordance in CRL, fetal NT ≥ 95th percentile and free β-hCG and PAPP-A multiples of the median. Similarly, within the CVS group, multivariable logistic regression analysis was used to investigate the effect of the number of intrauterine needle insertions and size of the needle on the risk of fetal loss.*

Results *The study population of 8581 twin pregnancies undergoing ultrasound examination at 11–13 weeks' gestation included 316 dichorionic and 129 monochorionic twins that had CVS. First, in twin pregnancies undergoing CVS, compared to those not undergoing CVS, there was a 2-fold increased risk of fetal loss at < 24 weeks' gestation and of loss at any stage in pregnancy. Second, the factors providing a significant independent contribution to the prediction of miscarriage or fetal loss in twin pregnancy were increased maternal weight, black racial origin, monochorionicity, and more so monoamnionicity, large intertwin discordance in CRL and increased fetal NT, and, in the case of fetal loss at any stage, there was also a contribution from assisted conception and low serum PAPP-A. Third, after adjustment for maternal and pregnancy characteristics, CVS did not provide a significant contribution to the risk of fetal loss. Fourth, in twin pregnancies that had CVS, there was no significant contribution to fetal loss from the number of intrauterine needle insertions or needle size.*

Correspondence to: Prof. K. H. Nicolaides, Fetal Medicine Research Institute, King's College Hospital, 16–20 Windsor Walk, Denmark Hill, London SE5 8BB, UK (e-mail: kypros@fetalmedicine.com)

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Conclusion *The 2-fold increased risk of fetal loss following CVS in twin pregnancy can, to a great extent, be explained by maternal and pregnancy characteristics rather than the invasive procedure itself.* © *2021 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

The ideal method of deriving the procedure-related risk of miscarriage and stillbirth associated with prenatal invasive testing is to compare in a randomized study pregnancies undergoing such tests with controls who have not undergone an invasive test. There is only one such study which reported that, in singleton pregnancies, the rate of fetal loss after second-trimester amniocentesis was 1% higher than that in controls who did not have an invasive test[1]. Another trial reported that the rate of fetal loss after first-trimester transabdominal chorionic villus sampling (CVS) was similar to that after second-trimester amniocentesis[2] and it was therefore assumed that the procedure-related risk of fetal loss after CVS in singleton pregnancy is also 1%[2]. Recent non-randomized studies have compared the outcome of pregnancies undergoing CVS with that of large cohorts which did not have invasive testing and, after adjustment for factors that contribute to miscarriage, such as increased nuchal translucency thickness (NT) and low pregnancy-associated plasma protein-A (PAPP-A), they reported that the risk from CVS may be considerably lower than thought previously[3–7].

In twin pregnancies, there is a small number of non-randomized studies that reported on fetal loss after CVS, and, in only four of the studies, there was a control group that did not undergo invasive testing[8–11]. A meta-analysis of such studies reported that it was not possible to draw any conclusion on the excess risk after CVS because of the heterogeneity of the published data, but recommended that patients should be counseled that the procedure-related risk of miscarriage after CVS in twin pregnancy is approximately 1%[12]. Another meta-analysis combined the data from two of the CVS studies which included controls that did not undergo invasive testing[10,11] and another two without such controls[13,14] and compared the outcome from the total of 349 cases of CVS to those of 218 controls; there was no significant difference in the rate of fetal loss between the CVS and non-CVS groups[15].

The objectives of this multicenter study of 8581 twin pregnancies undergoing ultrasound examination at 11–13 weeks' gestation, including 316 dichorionic (DC) and 129 monochorionic (MC) twins that had CVS, was to estimate the CVS-related risk of fetal loss after adjustment for chorionicity, NT, intertwin discordance in crown–rump length (CRL), maternal demographic characteristics and serum PAPP-A and free β-human chorionic gonadotropin (β-hCG); these factors are known to be associated with the risk of fetal loss[3,16–18].

METHODS

Study population and design

This was a multicenter study from eight fetal medicine units in which the leadership were trained at the Harris Birthright Research Centre for Fetal Medicine in London, UK, and in which the protocols for screening, invasive testing and pregnancy management are similar. This was a retrospective analysis of prospectively collected data obtained from women with twin pregnancy undergoing routine ultrasound examination at 11–13 weeks' gestation at King's College Hospital, London, UK (March 2006 to August 2020), Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain (December 2008 to April 2019), Medway Maritime Hospital, Gillingham, UK (February 2007 to August 2020), Hospital Universitario San Cecilio, Granada, Spain (February 2007 to August 2020), Shterev Hospital, Sofia, Bulgaria (September 2011 to August 2020), Ospedale Maggiore Policlinico, University of Milan, Milan, Italy (January 2012 to August 2020), University Women's Hospital, Tuebingen, Germany (January 2010 to July 2020) and Centro Hospitalar Universitário de Lisboa Central, Lisbon, Portugal (April 2012 to August 2020).

At 11–13 weeks, we recorded maternal demographic characteristics, including age, racial origin (white, black, South Asian, East Asian or mixed), weight, method of conception (natural or use of assisted reproductive technologies, including *in-vitro* fertilization or use of ovulation drugs), smoking status and parity (nulliparous or parous if a previous pregnancy resulted in birth at ≥ 24 weeks' gestation). Ultrasound examination was used for, first, determination of gestational age from the measurement of CRL of the larger twin[19], second, determination of chorionicity from the number of placentae and the presence or absence of the lambda sign at the intertwin membrane–placenta junction[20], third, exclusion of a vanishing twin[21], fourth, diagnosis of major fetal abnormalities[22], fifth, assessment of intertwin discordance in CRL (difference between the two fetuses expressed as a percentage of the larger one), because large discordance is associated with adverse pregnancy outcome[17], and, sixth, measurement of fetal NT in each fetus for assessment of risk for trisomy[23] and determination of whether the NT in one or both fetuses was ≥ 95th or ≥ 99th percentile of our reference range for CRL[23], because increased NT is associated with adverse pregnancy outcome[18]. All ultrasound examinations were carried out according to standardized protocols by sonographers who had obtained the Fetal Medicine Foundation Certificate of Competence in ultrasound examination for fetal abnormalities or by trainees under the supervision of certified sonographers. In most, but not all, pregnancies, maternal serum free β-hCG and PAPP-A were measured using automated machines (DelfiaXpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA; Brahms Kryptor system, Thermo Fisher Scientific, Berlin, Germany; or Cobas e411

system, Roche Diagnostics, Penzberg, Germany) and the values were expressed as multiples of the median (MoM) after adjustment for maternal weight, height, racial origin, parity, smoking status, method of conception and machine used for the measurement[24,25].

During the study period, the general policy was, first, to manage all pregnancies on an outpatient basis, unless there was a specific pregnancy complication such as pre-eclampsia, second, in addition to the 11–13-week scan, to carry out ultrasound assessment every 4 weeks from 20 weeks' gestation until delivery in DC twins and every 1–2 weeks from 16 weeks' gestation until delivery in MC twins, and, third, to recommend delivery at around 37 weeks' gestation for DC twins, 36 weeks for MC diamniotic (DA) twins and 32–33 weeks for MC monoamniotic (MA) twins, if there were no pregnancy complications necessitating earlier delivery.

In each center, details of maternal characteristics and the findings of the 11–13-week assessment were recorded in a fetal database. Data on pregnancy outcome were obtained from the maternity computerized records or the general medical practitioners of the women and were also recorded in the database. Anonymized data from each center were provided to K.H.N. for further analysis. This study constitutes a retrospective analysis of data derived from a routine clinical service and did not require ethics committee approval.

Chorionic villus sampling

In each center, women choosing to have CVS were assessed by a fetal medicine expert who then decided if the procedure should be performed by an expert or a trainee in fetal medicine under the direct supervision of an expert. All procedures were carried out transabdominally under ultrasound guidance. In cases of MCDA or MCMA twins, only one sample was obtained, whereas, in cases of DC twins, it was generally aimed to obtain a sample from both placentae. Most operators prefer to use separate needle entries to sample each placenta, but a few use a double needle system; the outer needle with a stylet is inserted across both placentae, the stylet is removed and an inner needle is used to sample the most distant placenta, then the stylet is reinserted into the outer needle which is withdrawn to within the proximal placenta and, after removal of the stylet, a sample is obtained through the outer needle.

Inclusion and exclusion criteria

The inclusion criteria for this study were DC, MCDA or MCMA twin pregnancy with two live fetuses at 11–13 weeks' gestation and known pregnancy outcome. In cases in which CVS was carried out, only those with a normal result were included. We excluded pregnancies with a chromosomal abnormality or major defect diagnosed prenatally or postnatally, those with twin reversed arterial perfusion sequence or conjoined twins and those in which amniocentesis or embryo reduction or termination was carried out.

Outcome measures

The primary outcome was the rate of fetal loss (defined as a pregnancy with one or two miscarriages or fetal deaths) at any stage following CVS or the first-trimester scan. Secondary outcomes were fetal loss occurring at < 24 and ≥ 24 weeks' gestation.

Statistical analysis

Data from categorical variables are presented as *n* (%) and those from continuous variables as median and interquartile range. Comparisons of outcome measures between DC, MCDA and MCMA twin pregnancies were carried out using Fisher's exact test for categorical variables and Mann–Whitney *U*-test for continuous variables. Significance was assumed at 5%, and *post-hoc* Bonferroni correction was used to adjust for multiple comparisons when necessary.

Univariable logistic regression analysis was used to examine the significance of the association between CVS and the risk of fetal loss. Multivariable logistic regression analysis with backward stepwise elimination was used to examine whether CVS provided a significant independent contribution to the prediction of risk of fetal loss after adjusting for maternal and pregnancy characteristics, including maternal age, racial origin, weight, method of conception, smoking status, parity, chorionicity, intertwin discordance in CRL, fetal NT $\geq 95^{th}$ percentile and free β-hCG and PAPP-A MoM. Similarly, within the CVS group, multivariable logistic regression analysis was used to investigate the effect of the number of intrauterine needle insertions and the size of the needles on the risk of fetal loss. The effect size of characteristics associated with the risk of fetal loss was expressed as odds ratio (OR) (95% CI) and presented graphically using forest plots.

The statistical package SPSS version 24.0 for Windows (IBM Corp., Armonk, NY, USA; 2016) was used for data analyses.

RESULTS

Study population

The total number of pregnancies undergoing CVS was 690, but 245 were excluded either due to loss to follow-up ($n = 8$) or because aneuploidy or a major defect was diagnosed and the parents chose embryo reduction ($n = 195$) or to continue with the pregnancy ($n = 42$). The total number of pregnancies that did not have CVS was 8542, but 406 were excluded either due to loss to follow-up ($n = 250$) or because a major defect was diagnosed and the parents chose embryo reduction ($n = 85$) or to continue with the pregnancy ($n = 71$).

Patient and pregnancy characteristics of the study population of 445 twin pregnancies that had CVS and the 8136 that did not have CVS are summarized in Table 1. Measurements of CRL and NT in each fetus were carried out in all cases, but serum free β-hCG and PAPP-A were measured in only 90.6% (7776/8581) of the pregnancies.

Chorionic villus sampling

The indications for CVS were high-risk result on the first-trimester combined test or increased NT ($n = 341$), parental carriership of a genetic abnormality ($n = 16$, including β-thalassemia, sickle-cell disease, hemophilia A, fragile X syndrome, spinal muscular atrophy and Duchenne muscular dystrophy), parental balanced translocation ($n = 9$), previous pregnancy affected by aneuploidy ($n = 9$), maternal request ($n = 24$), increased maternal age ($n = 31$), large intertwin discordance in CRL ($n = 6$) and ultrasound marker of fetal aneuploidy ($n = 9$).

The CVS procedures were carried out by one of 58 operators. In the 122 MCDA and seven MCMA twin pregnancies, an 18-G ($n = 103$) or 20-G ($n = 26$) needle was used to sample one of the placentae. In the 316 DC twin pregnancies, either a 17-/19-G double needle system was used to obtain a sample from both placentae through a single uterine insertion ($n = 49$), two separate 18-G ($n = 172$) or 20-G ($n = 60$) needles were introduced twice into the uterus to obtain a sample from each placenta, or an 18-G ($n = 32$) or 20-G ($n = 3$) needle was used to sample only one of the placentae.

Fetal loss

Pregnancy outcome in the three types of twin pregnancies, according to whether CVS was performed, is shown in Table 2. In both DC and MCDA twin pregnancies undergoing CVS, compared to those not undergoing CVS, there was a higher incidence of death of one or both fetuses at any stage in pregnancy.

Risk of fetal loss at any stage after chorionic villus sampling in twin pregnancy

In the study population of 8581 twin pregnancies, including 445 (5.2%) that had CVS, there were 566 (6.6%) cases with one or two fetal deaths at any stage during pregnancy. The risk of fetal loss was significantly higher in the CVS group (51/445; 11.5%) compared to the non-CVS group (515/8136; 6.3%) ($P < 0.0001$). Univariable logistic regression analysis demonstrated that the risk of fetal loss in the CVS group was nearly 2-fold higher compared with that in the non-CVS group (OR, 1.92; 95% CI, 1.41–2.60; $P < 0.0001$).

Multivariable logistic regression analysis demonstrated that, after adjustment for maternal and pregnancy

Table 1 Demographic and pregnancy characteristics of the study population of 8581 twin pregnancies with two live fetuses at 11–13 weeks' gestation, according to chorionicity and whether chorionic villus sampling (CVS) was performed

	Dichorionic		*Monochorionic diamniotic*		*Monochorionic monoamniotic*	
Characteristic	*CVS (n = 316)*	*No CVS (n = 6314)*	*CVS (n = 122)*	*No CVS (n = 1749)*	*CVS (n = 7)*	*No CVS (n = 73)*
Maternal age (years)	35.9 (32.5–38.7)	33.9 (30.4–37.1)	35.0 (30.9–37.6)	32.3 (28.5–36.0)	39.4 (37.4–40.6)	31.9 (28.7–35.3)
Maternal weight (kg)	64.5 (58.0–72.0)	66.3 (59.0–77.0)	63.0 (56.1–70.8)	65.0 (57.4–75.7)	62.0 (58.1–69.0)	65.1 (58.0–77.0)
Racial origin						
White	302 (95.6)	5550 (87.9)	110 (90.2)	1549 (88.6)	5 (71.4)	67 (91.8)
Black	7 (2.2)	555 (8.8)	6 (4.9)	120 (6.9)	0 (0)	3 (4.1)
South Asian	6 (1.9)	114 (1.8)	2 (1.6)	44 (2.5)	1 (14.3)	1 (1.4)
East Asian	1 (0.3)	32 (0.5)	4 (3.3)	19 (1.1)	1 (14.3)	2 (2.7)
Mixed	0 (0)	63 (1.0)	0 (0)	17 (1.0)	0 (0)	0 (0)
Conception						
Natural	196 (62.0)	3445 (54.6)	106 (86.9)	1567 (89.6)	6 (85.7)	65 (89.0)
Assisted reproduction	120 (38.0)	2869 (45.4)	16 (13.1)	182 (10.4)	1 (14.3)	8 (11.0)
Parity						
Nulliparous	179 (56.6)	3533 (56.0)	57 (46.7)	869 (49.7)	6 (85.7)	40 (54.8)
Parous	137 (43.4)	2781 (44.0)	65 (53.3)	880 (50.3)	1 (14.3)	33 (45.2)
Smoking	20 (6.3)	531 (8.4)	7 (5.7)	149 (8.5)	0 (0)	8 (11.0)
GA (weeks)	12.9 (12.5–13.4)	12.9 (12.5–13.3)	12.8 (12.3–13.2)	12.9 (12.4–13.3)	12.9 (12.5–13.0)	12.9 (12.5–13.2)
CRL discordance (%)	4.3 (1.9–8.1)	3.5 (1.6–6.5)	5.6 (2.6–8.9)	3.8 (1.7–6.5)	5.4 (0.8–9.0)	3.1 (1.6–5.5)
CRL discordance ≥ 10%	47 (14.9)	592 (9.4)	25 (20.5)	199 (11.4)	2 (28.6)	7 (9.6)
NT						
One or both ≥ p95	92 (29.1)	350 (5.5)	35 (28.7)	103 (5.9)	0 (0)	5 (6.8)
One or both ≥ p99	73 (23.1)	35 (0.6)	34 (27.9)	23 (1.3)	4 (57.1)	1 (1.4)
β-hCG MoM*	1.15 (0.73–1.73)	1.01 (0.71–1.46)	1.17 (0.81–1.81)	0.99 (0.69–1.48)	1.00 (0.57–1.44)	1.17 (0.66–1.75)
PAPP-A MoM*	0.90 (0.57–1.22)	1.10 (0.78–1.50)	0.68 (0.46–1.09)	1.11 (0.77–1.51)	0.33 (0.25–0.68)	1.00 (0.60–1.28)

Data are given as median (interquartile range) or *n* (%). *Measurements of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) available for only 90.6% (7776/8581) of pregnancies. CRL, crown–rump length; GA, gestational age; MoM, multiples of the median; NT, nuchal translucency thickness; p95, 95th percentile; p99, 99th percentile.

characteristics that were associated with the risk of fetal loss, CVS did not provide a significant contribution to the risk of fetal loss (OR, 1.14; 95% CI, 0.78–1.66). There was a significant independent contribution from maternal weight, black racial origin, assisted conception, chorionicity, intertwin discordance in CRL and fetal NT $\geq 95^{th}$ percentile but not from maternal age, cigarette smoking or parity ($R^2 = 0.116$; $P < 0.0001$) (Table 3). The risk of fetal loss was associated significantly with chorionicity, with a 3.8-fold increased risk in MCDA twins (OR, 3.81; 95% CI, 3.13–4.64) and an 8.1-fold increased risk in MCMA twins (OR, 8.08; 95% CI, 4.66–14.02), compared to DC twins. The risk of fetal loss was 1.6- and 2.9-fold higher in pregnancies with fetal NT $\geq 95^{th}$ percentile (OR, 1.61; 95% CI, 1.19–2.19) and in those with fetal NT $\geq 99^{th}$ percentile (OR, 2.88; 95% CI, 1.80–4.60), respectively, compared to those with fetal NT $< 95^{th}$ percentile.

In the group of 7776 pregnancies with measurements of free β-hCG and PAPP-A, multivariable logistic regression analysis demonstrated that a significant independent contribution to fetal loss was provided by maternal weight, black racial origin, cigarette smoking, assisted conception, chorionicity, intertwin discordance in CRL, increased fetal NT and low PAPP-A, but not by maternal age, parity, serum free β-hCG or CVS ($R^2 = 0.112$; $P < 0.0001$) (Table S1).

In the study population, there were 6630 DC twin pregnancies (including 316 (4.8%) that had CVS), of which 288 (4.3%) had one or two fetal deaths at any stage during pregnancy. Univariable logistic regression analysis demonstrated that the risk of fetal loss in DC pregnancies in the CVS group was 1.7-fold higher compared with that in the non-CVS group (OR, 1.70; 95% CI, 1.09–2.67; $P = 0.021$). Multivariable logistic regression analysis demonstrated that, after adjustment for maternal and pregnancy characteristics that were associated with the risk of fetal loss, CVS did not provide a significant contribution to the risk of fetal loss (OR, 1.15;

Table 3 Multivariable regression analysis for prediction of loss of one or both fetuses at any stage after the first-trimester scan in 8581 twin pregnancies, from maternal and pregnancy characteristics

Variable	*OR (95% CI)*	P
MA (in years) − 33	0.99 (0.96–1.00)	0.112
MW (in kg) − 69	1.01 (1.00–1.01)	0.014
Racial origin		< 0.0001
White	1.00 (reference)	
Black	2.49 (1.89–3.28)	< 0.0001
South Asian	1.58 (0.90–2.76)	0.108
East Asian	0.76 (0.27–2.19)	0.613
Mixed	0.96 (0.34–2.67)	0.934
Cigarette smoking	1.26 (0.93–1.72)	0.134
Assisted conception	1.29 (1.02–1.63)	0.035
Nulliparous	1.18 (0.97–1.43)	0.095
Chorionicity		< 0.0001
Dichorionic	1.00 (reference)	
MCDA	3.81 (3.13–4.64)	< 0.0001
MCMA	8.08 (4.66–14.02)	< 0.0001
CRL discordance (in %)	1.08 (1.07–1.10)	< 0.0001
NT		< 0.0001
Both < p95	1.00 (reference)	
One or both ≥ p95	1.61 (1.19–2.19)	0.002
One or both ≥ p99	2.88 (1.80–4.60)	< 0.0001
CVS	1.14 (0.78–1.66)	0.498

CRL, crown–rump length; CVS, chorionic villus sampling; MA, maternal age; MCDA, monochorionic diamniotic; MCMA, monochorionic monoamniotic; MW, maternal weight; NT, nuchal translucency thickness; OR, odds ratio; p95, 95^{th} percentile; p99, 99^{th} percentile.

Table 2 Pregnancy outcome in 8581 twin pregnancies with two live fetuses at 11–13 weeks' gestation, according to chorionicity and whether chorionic villus sampling (CVS) was performed

	Dichorionic			*Monochorionic diamniotic*			*Monochorionic monoamniotic*		
Outcome	*CVS (n = 316)*	*No CVS (n = 6314)*	P	*CVS (n = 122)*	*No CVS (n = 1749)*	P	*CVS (n = 7)*	*No CVS (n = 73)*	P
Fetal loss at any stage									
One or both fetuses	22 (7.0)	266 (4.2)	0.032	26 (21.3)	233 (13.3)	0.020	3 (42.9)	16 (21.9)	0.348
One fetus	18 (5.7)	129 (2.0)	0.0002	13 (10.7)	97 (5.5)	0.028	0 (0)	1 (1.4)	1.000
Both fetuses	4 (1.3)	137 (2.2)	0.415	13 (10.7)	136 (7.8)	0.296	3 (42.9)	15 (20.5)	0.185
Overall fetuses*	26/632 (4.1)	403/12 628 (3.2)	0.197	39/244 (16.0)	369/3498 (10.5)	0.019	6/14 (42.9)	31/146 (21.2)	0.045
Fetal loss at < 24 weeks									
One or both fetuses	13 (4.1)	202 (3.2)	0.331	22 (18.0)	183 (10.5)	0.015	3 (42.9)	13 (17.8)	0.139
One fetus	10 (3.2)	72 (1.1)	0.005	10 (8.2)	60 (3.4)	0.022	0 (0)	0 (0)	1.000
Both fetuses	3 (0.9)	130 (2.1)	0.217	12 (9.8)	123 (7.0)	0.274	3 (42.9)	13 (17.8)	0.139
Overall fetuses*	16/632 (2.5)	332/12 628 (2.6)	1.000	34/244 (13.9)	306/3498 (8.7)	0.007	6/14 (42.9)	26/146 (17.8)	0.015
Fetal loss at ≥ 24 weeks									
One or both fetuses	9 (2.8)	66 (1.0)	0.009	4 (3.3)	50 (2.9)	0.777	0 (0)	3 (4.1)	1.000
One fetus	8 (2.5)	61 (1.0)	0.016	3 (2.5)	37 (2.1)	0.743	0 (0)	1 (1.4)	1.000
Both fetuses	1 (0.3)	5 (0.1)	0.254	1 (0.8)	13 (0.7)	0.612	0 (0)	2 (2.7)	1.000
Overall fetuses*	10/632 (1.6)	71/12 628 (0.6)	0.005	5/244 (2.0)	63/3498 (1.8)	0.800	0/14 (0)	5/146 (3.4)	1.000

Data are given as *n* (%) or *n*/*N* (%). Numbers are expressed as percentage of pregnancies, unless indicated otherwise. *Numbers expressed as percentage of fetuses.

95% CI, 0.67–1.98). There was a significant independent contribution from maternal weight, black racial origin, assisted conception, intertwin discordance in CRL and fetal NT $\geq 95^{th}$ percentile but not from maternal age, cigarette smoking or parity ($R^2 = 0.059$; $P < 0.0001$) (Table S2). The risk of fetal loss was 1.6- and 3.6-fold higher in pregnancies with fetal NT $\geq 95^{th}$ percentile (OR, 1.59; 95% CI, 1.04–2.42) and in those with fetal NT $\geq 99^{th}$ percentile (OR, 3.61; 95% CI, 1.86–7.02), respectively, compared to those with fetal NT $< 95^{th}$ percentile.

Risk of miscarriage from chorionic villus sampling in twin pregnancy

In the study population of 8581 twin pregnancies, there were 436 (5.1%) with one or two fetal deaths at < 24 weeks' gestation. The risk of miscarriage was significantly higher in the CVS group (38/445; 8.5%) compared to the non-CVS group (398/8136; 4.9%) ($P = 0.001$). Univariable logistic regression analysis demonstrated that the risk of miscarriage in the CVS group was nearly 2-fold higher compared with that in the non-CVS group (OR, 1.82; 95% CI, 1.28–2.57; $P < 0.0001$).

Multivariable logistic regression analysis demonstrated that, in the prediction of miscarriage, there was no significant contribution from CVS (OR, 0.97; 95% CI, 0.63–1.49) after adjustment for maternal and pregnancy characteristics that were associated with the risk of miscarriage. There was a significant independent contribution from maternal weight, black racial origin, chorionicity, intertwin discordance in CRL and increased fetal NT but not from maternal age, cigarette smoking, assisted conception or parity ($R^2 = 0.115$; $P < 0.0001$) (Figure 1, Table S3). The risk of miscarriage was associated significantly with chorionicity, with a 4-fold increased risk in MCDA twins (OR, 3.95; 95% CI, 3.16–4.92) and an 8.9-fold increased risk in MCMA twins (OR, 8.91; 95% CI, 4.94–16.06), compared with that in DC twins. The risk of miscarriage was 1.6- and 3.6-fold higher in pregnancies with fetal NT $\geq 95^{th}$ percentile (OR, 1.60; 95% CI, 1.14–2.27) and in those with fetal NT $\geq 99^{th}$ percentile (OR, 3.64; 95% CI, 2.21–6.00) respectively, compared to those with fetal NT $< 95^{th}$ percentile.

In the group of 7776 pregnancies with measurements of free β-hCG and PAPP-A, multivariable logistic regression analysis demonstrated that a significant independent contribution to fetal loss < 24 weeks was provided by maternal weight, black racial origin, chorionicity, intertwin discordance in CRL and increased fetal NT but not by maternal age, cigarette smoking, assisted conception, parity, serum free β-hCG or PAPP-A, or CVS ($R^2 = 0.114$; $P < 0.0001$) (Table S4).

In the study population of 6630 DC twin pregnancies, there were 215 (3.2%) with one or two fetal deaths at < 24 weeks' gestation. Univariable logistic regression analysis demonstrated that the risk of miscarriage in the CVS group was 1.3-fold higher compared with that in the non-CVS group (OR, 1.30; 95% CI, 0.73–2.30) but this was not statistically significant ($P = 0.372$). Multivariable logistic regression analysis demonstrated that, in the prediction of miscarriage in DC twin pregnancies, there was a significant independent contribution from maternal weight, black racial origin, intertwin discordance in CRL and fetal NT $\geq 99^{th}$ percentile but not from maternal age, cigarette smoking, assisted conception or parity ($R^2 = 0.059$; $P < 0.0001$) (Table S5). Compared to pregnancies with fetal NT $< 95^{th}$ percentile, there was a 4.2-fold increased risk of miscarriage in those with fetal NT $\geq 99^{th}$ percentile (OR, 4.21; 95% CI, 2.23–7.96) and a 1.6-fold higher risk in those with fetal NT $\geq 95^{th}$ percentile (OR, 1.57; 95% CI, 0.97–2.54) but the latter did not reach statistical significance.

Effect of number of uterine entries and needle size on fetal loss

In the 445 twin pregnancies that had CVS, multivariable logistic regression analysis demonstrated that a significant independent contribution to fetal loss was provided

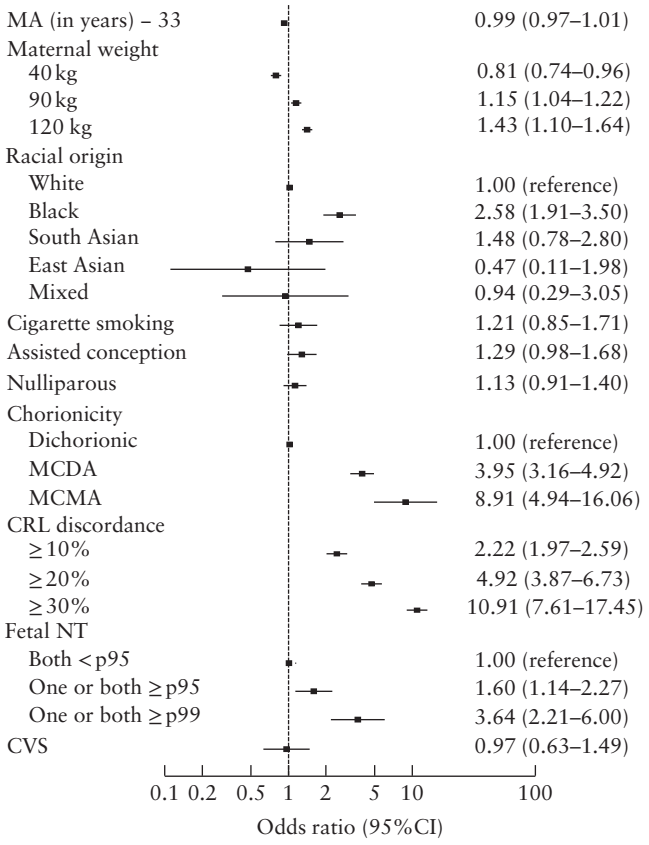

Figure 1 Forest plot showing the results of multivariable logistic regression analysis of the association of maternal and pregnancy characteristics with the risk of miscarriage of one or both fetuses after the first-trimester scan in twin pregnancy. CRL, crown–rump length; CVS, chorionic villus sampling; MA, maternal age; MCDA, monochorionic diamniotic; MCMA, monochorionic monoamniotic; NT, nuchal translucency thickness; p95, 95th percentile; p99, 99th percentile.

by chorionicity and intertwin discordance in CRL, but not increased fetal NT, number of intrauterine needle insertions or needle size (Table S6). Similarly, multivariable logistic regression analysis demonstrated that a significant independent contribution to fetal loss at < 24 weeks' gestation was provided by chorionicity and intertwin discordance in CRL, but not increased fetal NT, number of intrauterine needle insertions or needle size (Table S7).

DISCUSSION

Principal findings of study

The results from this multicenter study of 8581 twin pregnancies, including 445 that had CVS, demonstrate that, first, in pregnancies undergoing CVS, compared to those not undergoing CVS, there is a 2-fold increased risk of fetal loss at < 24 weeks' gestation and loss at any stage in pregnancy, second, the factors providing a significant independent contribution to the prediction of miscarriage or fetal loss in twin pregnancy are increased maternal weight, black racial origin, monochorionicity, and more so monoamnionicity, large intertwin discordance in CRL and increased fetal NT, and, in the case of fetal loss at any stage, there is also a contribution from assisted reproduction and low serum PAPP-A, third, after adjustment for maternal and pregnancy characteristics, CVS did not provide a significant contribution to the risk of fetal loss, and, fourth, in twin pregnancies that had CVS, there is no significant contribution to fetal loss from the number of intrauterine needle insertions or needle size.

Comparison with other studies

The finding that, in twin pregnancies, the risk of fetal loss following CVS is twice as high as that in twin pregnancies without CVS, but such excess loss is to a great extent explained by maternal and pregnancy characteristics rather than the invasive procedure *per se*, is consistent with the finding of our previous study in singleton pregnancies[3]. The study in singletons included 2396 that had CVS and 31 460 that did not; in the CVS group, compared to those that did not have invasive testing, the risk of miscarriage was significantly higher (1.8% *vs* 1.1%; $P = 0.004$)[3]. However, similar to our current study, CVS did not provide a significant independent contribution to fetal loss after adjustment for maternal and pregnancy characteristics. In singleton pregnancies, many of the factors that are associated with an increased risk for trisomy and that lead to CVS being performed are the same as those associated with an increased risk for fetal loss, such as increased maternal age and NT and low PAPP-A; consequently, these factors should be taken into account when estimating the procedure-related risk of fetal loss[3].

There are four previous studies reporting on the outcome of twin pregnancies after CVS in which there was a control group that did not have invasive testing. De Catte *et al.* reported that the number of pregnancies with one or two fetal deaths at any gestational age after 9–13 weeks was 8/104 (7.7%) in the CVS group and 5/101 (5.0%) in controls; the study did not specify chorionicity and there were no exclusion criteria[8]. Aytoz *et al.* reported that the number of pregnancies with one or two fetal deaths at any gestational age after 11–14 weeks was 6/110 (5.5%) in the CVS group and 11/175 (6.3%) in controls; the study did not specify chorionicity, and pregnancies undergoing embryo reduction were excluded[9]. Brambati *et al.* reported that the number of pregnancies with one or two fetal deaths at any gestational age after 7–12 weeks was 5/147 (3.4%) in the CVS group and 0/63 (0%) in controls; this study was confined to DC twins, and pregnancies undergoing embryo reduction or termination were excluded[10]. Kim *et al.* reported that the number of pregnancies with one or two fetal deaths between 12 and 24 weeks' gestation was 4/54 (7.4%) in the CVS group and 6/155 (3.9%) in controls; this study was confined to DC twins, and pregnancies with a chromosomal anomaly or lethal fetal defect and those undergoing embryo reduction were excluded[11]. The small number of patients in these studies and the substantial heterogeneity between the studies preclude any meaningful conclusions being drawn from meta-analyses attempting to synthesize the existing evidence.

Strengths and limitations

The main strengths of this study are, first, the large study population which made it possible to define, through multivariable regression analysis, the maternal and pregnancy characteristics with a significant contribution to fetal loss, and, second, the multicenter nature of the study which makes the results generalizable for fetal medicine units undertaking the management of twin pregnancies. The main limitation of this study is the non-randomized design. Since it is impossible to define all the potential factors that contribute to fetal loss, it is possible that the inclusion and exclusion criteria of the study may have introduced bias, resulting in a higher rate of fetal loss in pregnancies that did not have CVS. For example, pregnancies with fetal chromosomal abnormalities are at increased risk of fetal death, and, in the CVS group, all such cases were excluded, whereas, in the non-CVS group, some of the fetal losses may have been the consequence of an undiagnosed chromosomal abnormality.

Conclusion

In twin pregnancies undergoing CVS, compared to those not undergoing CVS, there is a 2-fold increased risk of fetal loss, but such an increased risk can, to a great extent, be explained by maternal and pregnancy characteristics rather than the invasive procedure itself.

ACKNOWLEDGMENT

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Multivariable regression analysis for prediction of loss of one or both fetuses at any stage after the first-trimester scan in 7776 twin pregnancies, from maternal and pregnancy characteristics including maternal serum biochemistry

Table S2 Multivariable regression analysis for prediction of loss of one or both fetuses at any stage after the first-trimester scan in 6630 dichorionic twin pregnancies, from maternal and pregnancy characteristics

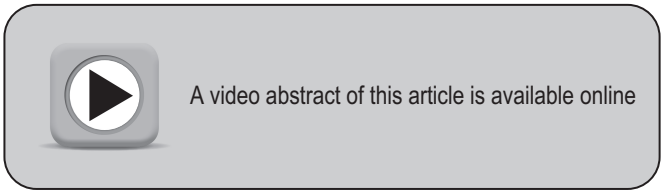
Table S3 Multivariable regression analysis for prediction of miscarriage of one or both fetuses after the first-trimester scan in 8581 twin pregnancies, from maternal and pregnancy characteristics

Table S4 Multivariable regression analysis for prediction of miscarriage of one or both fetuses after the first-trimester scan in 7776 twin pregnancies, from maternal and pregnancy characteristics including maternal serum biochemistry

Table S5 Multivariable regression analysis for prediction of miscarriage of one or both fetuses after the first-trimester scan in 6630 dichorionic twin pregnancies, from maternal and pregnancy characteristics

Table S6 Multivariable regression analysis for prediction of loss of one or both fetuses at any stage after chorionic villus sampling (CVS) in 445 twin pregnancies undergoing CVS

Table S7 Multivariable regression analysis for prediction of miscarriage of one or both fetuses after chorionic villus sampling (CVS) in 445 twin pregnancies undergoing CVS



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Muerte fetal tras la biopsia de vellosidades coriónicas en el embarazo de gemelos

RESUMEN

Objetivo Estimar el riesgo de muerte fetal relacionado con la biopsia de vellosidades coriónicas (BVC) en el embarazo de gemelos una vez hechos los ajustes para tener en cuenta la corionicidad, el grosor de la translucidez nucal (TN), la discordancia entre gemelos en la longitud céfalo-caudal (LCC), las características demográficas maternas, la proteína plasmática A asociada al embarazo (PAPP-A, por sus siglas en inglés) y la hormona gonadotrópica coriónica humana (subunidad B) libre (β-hCG).

Métodos Este fue un estudio multicéntrico de ocho unidades de medicina fetal en las que la gerencia se formó en el Harris Birthright Research Centre for Fetal Medicine de Londres (Reino Unido), en las cuales los protocolos de cribado, pruebas agresivas y la atención médica al embarazo son similares. Los datos se obtuvieron de forma prospectiva de mujeres embarazadas con gemelos que se sometieron a una ecografía rutinaria a las 11-13 semanas de gestación. Se utilizó un análisis de regresión logística multivariable con eliminación por etapas hacia atrás para examinar si la BVC contribuía de forma independiente y significativa a la predicción del riesgo de muerte fetal tras los ajustes respecto a las características maternas y del embarazo, como la edad, el origen étnico y el peso de la madre, el método de concepción, el estado de tabaquismo, la paridad, la corionicidad, la discordancia entre gemelos en la LCC, la TN fetal ≥95° percentil y varios múltiplos de la mediana de la β-hCG libre y la PAPP-A libre. Del mismo modo, dentro del grupo de BVC, se utilizó un análisis de regresión logística multivariable para investigar el efecto del número de inserciones de agujas intrauterinas y el tamaño de la aguja sobre el riesgo de muerte fetal.

Resultados La población de estudio fue de 8581 embarazos de gemelos que se sometieron a una ecografía a las 11–13 semanas de gestación e incluyó 316 (78,2%) embarazos bicoriales (BC) y 129 (21,8%) monocoriales (MC). En primer lugar, en los embarazos de gemelos sometidos a una BVC, en comparación con los que no se sometieron a ella, hubo un riesgo 2 veces mayor de muerte fetal antes de las 24 semanas de gestación y de pérdida en cualquier fase del embarazo. En segundo lugar, los factores que aportaron una contribución independiente significativa a la predicción del aborto o la muerte fetal en el embarazo de gemelos fueron un mayor peso materno, el origen étnico de raza negra, la monocorionicidad, y sobre todo la monoamnionicidad, una gran discordancia entre gemelos en la LCC y una mayor TN fetal, y, en el caso de la muerte fetal en cualquier fase, también contribuyeron la concepción asistida y la PAPP-A sérica baja. En tercer lugar, tras el ajuste respecto a las características maternas y del embarazo, la BVC no aportó una contribución significativa al riesgo de muerte fetal. En cuarto lugar, en los embarazos de gemelos sometidos a BVC, el número de inserciones de agujas intrauterinas o el tamaño de las mismas no contribuyeron de forma significativa a la muerte fetal.

Conclusión El riesgo dos veces mayor de muerte fetal después de una BVC en el embarazo de gemelos puede, en gran medida, explicarse por las características maternas y del embarazo más que por el procedimiento traumático en sí.

双胎妊娠中绒膜绒毛取样后的妊娠丢失

摘要

目的对绒毛膜、胎儿颈项透明膜厚度（NT）、双胎顶臀长（CRL）不一致、母亲的人口学特征和血清妊娠相关的血浆蛋白-A（PAPP-A）以及游离β-人绒毛膜促腺性激素（β-hCG）的调整后，评估双胎妊娠中与绒膜绒毛取样（CVS）相关的妊娠丢失的风险。

方法这是一项由八个胎儿医学单位参与的多中心研究，在英国伦敦的
Harris Birthright研究中心举办了领导力培训，其中对筛检、
侵入性测试和妊娠管理的协议均相似。从双胎妊娠的妇女处预期获得的数据，这些孕妇在妊娠11-13周进行了常规超声检查。用多变量回归分析（采用倒退阶梯式去除）来检查在调整了母亲及妊娠特征（包括母亲的年龄、种族和体重、怀孕方法、吸烟状态、胎次、绒毛膜、双胎CRL不一致、胎儿NT大于第95个百分点、游离β-hCG和PAPP-A中位倍数）之后，CVS对妊娠丢失风险的预测是否起到一个重大的独立作用。同样地，在CVS组内采用多变量回归分析研究子宫内进针针数和针的大小对妊娠丢失风险的影响。

结果研究人群为8581个双胎妊娠（包括进行了CVS的316个双绒毛膜和129个单绒毛膜双胎），在妊娠11-13周进行了常规超声检查。首先，进行了CVS的双胎妊娠与未进行过CVS的双胎妊娠对比，在妊娠24周以后妊娠丢失的风险和在妊娠期间任何阶段流产的风险增加了两倍。第二，在双胎妊娠中对流产或妊娠丢失的预测起到重大独立影响的因素有：增加的产妇体重、黑人种族、单绒毛膜（更为明显）、较大的双胎CRL不一致以及增加的胎儿NT，而在任何阶段的妊娠丢失情况中，辅助怀孕和低血清PAPP-A也起到一定的作用。第三，在对母亲和妊娠特征调整后，CVS并未对妊娠丢失的风险起到重大影响。第四，在进行了CVS的双胎妊娠中，从子宫内进针针数和针的大小来看，并没有对妊娠丢失起到任何重大影响。

结论双胎妊娠中在进行了CVS后妊娠丢失风险增加两倍在很大程度上可以用母亲和妊娠特征来解释，而不是侵入性治疗本身。