RISK OF FETAL LOSS AFTER CHORIONIC VILLUS SAMPLING IN TWIN PREGNANCIES DERIVED FROM PROPENSITY SCORE MATCHING ANALYSIS

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Contribution:

A. What are the novel findings of this work?

In women with twin pregnancies the risk of fetal loss following CVS depends on a series of maternal and pregnancy characteristics and to a lesser extent on the procedure itself. The risk factors for spontaneous fetal loss are similar to those that make CVS necessary and in women at high background risk of fetal loss the risk of fetal loss following the invasive test could paradoxically be lower than if they did not have the invasive test, for the simple reason that prenatal diagnosis often converts a spontaneous loss of a chromosomally abnormal fetus into pregnancy termination. The true procedure-related risk of fetal loss from CVS in twin pregnancies can only be derived by examining women at low background risk of fetal loss, and in such women, the risk of fetal loss may increase by about 3.5% after CVS.

B. What are the clinical implications of this work?

The true procedure-related risk of fetal loss from CVS in twin pregnancies can only be derived by examining women at low background risk of fetal loss, and in such women, the risk of fetal loss may increase by about 3.5% after CVS.

ABSTRACT

Objective: To estimate the risk of fetal loss associated to chorionic villus sampling (CVS) in twin pregnancies using propensity score analysis.

Methods: This was a multicenter cohort study performed in eight fetal-medicine units in which the leadership were trained at the Harris Birthright Research Centre for fetal medicine in London and the protocols for screening, invasive testing and pregnancy management are similar. The study population of 8581 twin pregnancies undergoing ultrasound examination at 11-13 weeks' gestation, included 445 twin pregnancies that had CVS. The risk of death of at least one fetus was compared between the CVS and non-CVS groups after propensity score matching (1:1 ratio). This procedure creates two comparable groups balancing the maternal and pregnancy characteristics that lead to CVS, in a similar way in which randomization operates in a randomized clinical trial.

Results: Death of 1 or 2 fetuses at any stage during pregnancy occurred in 11.5% (51/445) of pregnancies in the CVS group and in 6.3% (515/8136) in the non-CVS group (p<0.001). The propensity score algorithm matched 258 cases that had CVS with 258 non-CVS cases; there was at least one fetal loss in 29 (11.2%) in the CVS group and 35 (13.6%) in the matched non-CVS group (OR 0.81, 95% CI 0.48 to 1.35; p=0.415). However, there was a significant interaction between the risk of fetal loss after CVS and the background risk of feal loss; when the background risk was higher, the risk of fetal loss after CVS decreased (OR 0.46, 95% CI 0.23 to 0.90) while in pregnancies with lower background risk of fetal loss the risk of fetal loss after CVS increased (OR 2.45, 95% CI 0.95 to 7.13). The effects were statistically different (p value of the interaction = 0.005). For a pregnancy where the background risk of fetal loss was about 6% (same as in our non-CVS population) there was no change in risk after CVS, but, when the background risk was more than 6% the posterior risk was paradoxically reduced and when the background risk was less than 6% the posterior risk increased exponentially; for example, if the background risk was 2.0%, the relative risk was 2.8 and the posterior risk was 5.6%.

Conclusions: After accounting for the risk factors that lead to both CVS and spontaneous fetal loss and confining the analysis to pregnancies at lower prior risk, CVS seems to increase the risk of fetal loss by about 3.5% above the patient's background-risk.

Key words: first-trimester screening; chorionic villus sampling; miscarriage; pregnancy complications; adverse pregnancy outcome; invasive testing; invasive procedures; prenatal diagnosis.

INTRODUCTION

The procedure related risk of fetal loss following chorionic villous sampling (CVS) in twin pregnancies has not been investigated in a randomized controlled trial. Four small studies reported contradictory results concerning the risk of CVS-related miscarriage compared to controls that did not undergo invasive testing.[1-4] The issue of CVS-related risk of fetal loss was addressed by a recent multicentre study of 8581 twin pregnancies undergoing ultrasound examination at 11-13 weeks' gestation, including 445 twin pregnancies that had CVS.[5] Multivariable logistic regression analysis with backward stepwise elimination was used to examine whether CVS provided a significant independent contribution in the prediction of risk of fetal loss after adjusting for maternal and pregnancy characteristics. The study reported that in twin pregnancies undergoing CVS, compared to those that did not have CVS, there was a 2-fold increased risk of fetal loss at any stage in pregnancy and the factors providing a significant independent contribution in the prediction of fetal loss were increased maternal weight, black racial origin, monochorionicity, large intertwin discordance in crown-rump length (CRL), high fetal nuchal translucency thickness (NT), and low serum pregnancy associated plasma protein-A (PAPP-A); there was a trend for an increased risk of fetal loss from CVS after adjustment for maternal and pregnancy characteristics but this did not reach statistical significance.[5]

An alternative to multivariable logistic regression analysis for the assessment of CVS-related risk of fetal loss is use of propensity score analysis, whereby an attempt is made to emulate a randomized controlled trial by matching every CVS case to a similar non-CVS control, adjusting for those maternal and pregnancy characteristics that are known to be risk factors for subsequent fetal loss.[5-11] This approach of propensity score analysis was carried out in singleton pregnancies to estimate the CVS-related risk of miscarriage.[12] The study reported that although there was no significant differences in the rate of miscarriage between the CVS and non-CVS groups, there was an interaction between the estimated risk of aneuploidies and the risk of miscarriage; the risk of miscarriage following the procedure in patients at higher risk of aneuploidies and therefore, presenting the worst profile for spontaneous pregnancy loss, was reduced, whilst the opposite effect was seen in the group of patients at lower risk of aneuploidies, who increased their risk following CVS.[12] The authors concluded that the true effect of CVS could only be examined in low-risk pregnancies; in the high-risk

group there were many aneuploid pregnancies leading to terminations thereby masking potential spontaneous miscarriages that would have happened had the pregnancies not been terminated.

The objective of this study is to investigate the risk of CVS-related fetal loss in twin pregnancies using propensity score analysis in the same dataset that we previously examined using multivariable logistic regression analysis.[5]

METHODS

Study design and population

This was a multicentre cohort study from eight fetal medicine units in the UK, Spain, Italy, Bulgaria and Portugal, in which the leadership were trained at the Harris Birthright Research Centre for fetal medicine in London and the protocols for screening, invasive testing and pregnancy management are similar.[5] At 11-13 weeks we recorded maternal demographic characteristics, and carried out ultrasound examination for first, determination of gestational age from the measurement of CRL of the larger twin,[13] second, determination of chorionicity from the number of placentas and the presence or absence of the lambda sign at the inter-twin membrane-placental junction,[14] third, exclusion of vanishing twin,[15] fourth, diagnosis of major fetal abnormalities,[16] fifth, assessment of the intertwin discordance in CRL (difference between the two fetuses expressed as a percentage of the larger one), because a large discordance is associated with adverse pregnancy outcome,[10] sixth, measurement of fetal NT in each fetus for assessment of risk for trisomies and determination whether the NT in one or both fetuses was ≥95th percentile of our reference range for CRL,[17] because high NT is associated with adverse pregnancy outcome.[11] In most, but not all pregnancies, maternal serum free ß-hCG and PAPP-A were measured by automated machines (DelfiaXpress system, PerkinElmer Life and Analytical Sciences, Waltham, 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.[18,19]

In each centre details of maternal characteristics and the findings of the 11-13 weeks assessment were recorded in a fetal database. Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were also recorded in the database. Anonymized data from each centre were provided to KN for further analysis. These studies constitute retrospective analysis of data derived from a routine clinical service and did not require ethics committee approval.

Chorionic villous sampling

All procedures were carried out transabdominally under ultrasound guidance. In the case of monochorionic twins only one sample was obtained, whereas in the case of dichorionic twins it was generally aimed to obtain a sample from both placentas. Most operators used separate needle entries to sample each placenta, but a few used a double needle system; the outer needle with a stylet was inserted across both placentas, the stylet was removed and an inner needle was used to sample the most distant placenta, then the stylet was again inserted into the outer needle which was withdrawn to within the proximal placenta and after removal of the stylet a sample was obtained through the outer needle.

Inclusion and exclusion criteria

The inclusion criteria for this study were dichorionic, monochorionic-diamniotic and monochorionic-monoamniotic twin pregnancies with two live fetuses at 11-13 weeks' gestation and known pregnancy outcome. In cases where CVS was carried out only those with a normal result were included. We excluded pregnancies with chromosomal abnormalities or major defects diagnosed prenatally or postnatally, those with twin reversed arterial perfusion (TRAP) sequence or conjoined twins and those in which amniocentsis or embryo reduction or termination was carried out.

Outcome measures

The primary outcome was the rate of fetal loss (pregnancies with one or two miscarriages or fetal deaths) at any stage following CVS or first-trimester scan.

Statistical analysis

Descriptive data were expressed as median and interquartile range (IQR) and in proportions (absolute and relative frequencies). Comparisons between treatment groups were performed by Mann-Whitney U-test or Fisher's exact test as appropriate. Analyses were run on a complete case basis, and the number of pregnancies included in each analysis were reported wherever necessary. Level of significance was set at 0.05.

Propensity score matching analysis was performed to assess the effect of CVS on the risk of fetal loss adjusting for the confounding bias caused by the different maternal and pregnancy characteristics in the two study groups. The propensity score was defined as the conditional probability of having a CVS given the measured covariates in order to balance covariates in the two groups. To obtain the propensity score, we fitted a logistic regression model with CVS as dependent variable and then we modelled the conditional probability of having a CVS as a function of baseline and those clinical characteristics associated with having a CVS. We used the propensity score to match, without replacement, each complete CVS case with the non-CVS case with the closest propensity score in a 1:1 ratio, to optimise the precision of the estimate of association and limit bias. Additionally, we only accepted cases if the difference in propensity score between matched cases was small (calliper of 0.1), resulting in excellent balance between the CVS and the non-CVS cases as matched samples.[20] We computed standardized differences for all variables included in the propensity score before and after matching to assess the effect of matching on the imbalance. We deemed a 10% standardized difference as the limit for a correct balance. After matching, we compared fetal loss rate between the CVS cases and those without CVS as matched groups. Finally, we calculated an odds ratio (OR) to quantify the association between CVS and fetal loss using a univariable logistic regression fitted by generalized estimating equations to account for matched data. To assess a possible interaction between propensity score and CVS (different effect in risk when the CVS is performed in different propensity score profiles) we divided the matched cases into those with a propensity score lower than its median (50% of cases) and those with a propensity score higher than its median (50%). We then calculated the OR for each group by logistic regression analysis and assessed the significance of their difference by calculating the p value of the interaction.

The statistical software package R was used for data analyses.[21] The R package MatchIt[22] was used for matching with propensity score and calculating the standardized differences. Analysis of matched cases was performed using the R package Geepack.[23]

RESULTS

Study population

The study population that fulfilled the inclusion criteria comprised of 445 twin pregnancies that had undergone CVS and 8136 that did not have CVS; patient and pregnancy characteristics of the two groups are summarized in Table 1.[5] Measurement of fetal CRL, NT and heart rate in each fetus was carried out in all cases but serum free ß-hCG and PAPP-A was measured in only 90.6% (7776/8581) of the pregnancies. In the CVS group, compared to the non-CVS group, median maternal age, discrepancy in CRL, fetal NT and serum free β-hCG MoM were significantly higher and maternal weight and PAPP-A MoM were significantly lower. The incidence of black racial origin, conception by assisted reproductive techniques and dichorionic twins was lower in the CVS group, compared to the non-CVS group. The only parameters not significantly different between groups were smoking status, parity and gestational age at the time of the ultrasound assessment.

In the monochorionic twin pregnancies an 18G or 20G needle was used to sample one of the placentas. In the dichorionic twin pregnancies either a 17/19G double needle system was used to obtain a sample from both placentas through a single uterine entry, or two separate 18G or 20G needles were introduced twice into the uterus to obtain a sample from each placenta, or an 18G or 20G needle was used to sample only one of the placentas.

Death of 1 or 2 fetuses at any stage during pregnancy occurred in 11.5% (51/445) of pregnancies in the CVS group and in 6.3% (515/8136) in the non-CVS group ($p<0.001$).

Propensity score matching

We calculated the propensity score for each case in the study population based on their probability of having a CVS. The predictive model included maternal age, method of

conception, maternal weight, smoking status, race and parity, chorionicity, gestational age at the time of the ultrasound assessment, CRL discrepancy, maximum NT, minimum fetal heart rate and serum free ß-hCG and PAPP-A as shown in Table 2. The propensity score algorithm matched 258 CVS cases with 258 non-CVS pregnancies, largely reducing the initial imbalance between women with and without CVS, with between-group standardized differences for all instances lower than the recommended 10% limit (Figure 1, Table 3). The number of cases with any fetal loss was 29 (11.2%) in the CVS group and 35 (13.6%) in the matched non-CVS group. Overall, propensity score analysis did not find any significant association between CVS and fetal loss (OR 0.81, 95% CI 0.48 to 1.35; p=0.415).

To investigate whether the effect of CVS on fetal loss was the same in women at higher risk of having CVS as compared to those at lower risk we divided our 516 matched cases into two equal groups by the median of the propensity score, considering the propensity score as a proxy for the prior risk of fetal loss (the variables increasing the risk of receiving a CVS are those increasing the risk of spontaneous fetal loss). The median propensity score was 0.209 (IQR 0.141-0.358) in the higher-risk group (n=258) and 0.037 (IQR 0.019, 0.061) in the lower-risk group (n=258). In the higher-risk group there were 11.7% (15/128) fetal losses in the CVS group and 22.3% (29/130) fetal losses in the non-CVS group (OR 0.46, 95% CI 0.23 to 0.90). In contrast, in the lower risk group there were 10.8% (14/130) fetal losses in the CVS group and 4.7% (6/128) fetal losses in the non-CVS group (OR 2.45, 95% CI 0.95 to 7.13); both effects were statistically different (p value of the interaction = 0.005) (Figure 2). These results suggest that there is something which makes CVS behave differently, in relation to the risk of fetal loss, when the risk of aneuploidies is high compared to when it is low.

To assess the increase in risk of fetal loss after CVS according to patient and pregnancy characteristics, we used our previously published model (supplementary Table 1[5]) to calculate the background risk of pregnancy loss for each one of our cases. We then calculated the relative risk after CVS by our propensity score analysis (Figure 3). For a pregnancy where the background risk of fetal loss was about 6% (same as in our non-CVS population[5]) there was no change in risk after CVS, however, when the background risk was more than 6% the posterior risk was paradoxically reduced and when the background risk was less than 6% the posterior risk increased exponentially; for example, if the background

risk was 5.0%, 4.0%, 3.0% or 2.0%, the relative risks were 1.2, 1.5, 1.9 and 2.8 and the posterior risks were 6.0%, 6.0%, 5.7% and 5.6%, respectively (Figure 3).

DISCUSSION

Principal findings

The main finding of this study is that the CVS-related risk of fetal loss in twin pregnancies is not constant, but it mainly depends on the prior risk of fetal loss. In women with patient and pregnancy characteristics suggesting a high risk of fetal loss, the posterior risk after CVS is paradoxically reduced, whereas in women at low background risk of fetal loss, there may be a 3.5% increase in risk following CVS.

Interpretation of results and comparison with findings of previous studies

In our previous study attempting to estimate the CVS-related risk of fetal loss in twin pregnancies we used multivariable logistic regression analysis to adjust for maternal and pregnancy characteristics and found that after such adjustment CVS did not provide a significant independent contribution in the prediction of risk of fetal loss.[5]

Propensity score analysis creates homogeneous groups suitable for comparisons and has emerged as a robust methodology well suited to estimate causal effects from observational data while accounting for a greater number of confounder effects than classical multivariable analysis could adjust for.[24,25] In our matching approach we used a 1:1 ratio and a small difference in propensity score between matched cases to ensure that the CVS and non-CVS groups had a very similar risk-profile. The most likely explanation for the finding that CVS appears to be protective against fetal loss is that invasive testing leads to the diagnosis of major aneuploidies followed by elective pregnancy termination in cases that would have otherwise resulted in spontaneous miscarriage. To try to avoid this selection bias, we studied separately the effect of the CVS on fetal loss in cases with a lower probability of having a CVS and in those with a higher probability. Contrary to what happens in high risk cases, in women at low risk of fetal loss, CVS increases the risk by about 3.5%.

Our findings in twin pregnancies are consistent with those of a previous study investigating the risk of miscarriage after CVS in singleton pregnancies in which propensity score was used to match 2122 CVS with 2122 non-CVS cases.[12] Overall, there was no significant difference between groups in the risk of miscarriage following CVS (OR 0.72, 95% CI, 0.48–1.10), but, after dividing the matched population into two equal groups by the median of the propensity score (one group having a higher risk of aneuploidies than the other) there was a significant decrease in risk of miscarriage after CVS in the higher risk group (OR 0.47, 95%CI, 0.28–0.76) and a significant increase in risk after CVS in the lower risk group (OR 2.87, 95% CI, 1.13–7.30).[12]

Strengths and limitations

The main strength of the study is the large study population which made it possible to match 258 CVS cases with 258 controls with a very similar risk profile allowing fair comparisons between groups and even subgroup analysis. Moreover, the multicentre and multi-operator nature of the study makes the results generalizable for other experienced fetal medicine units. The main limitation of the study is the non-randomized design. Although propensity score analysis is a well-accepted method to emulate randomized trials when they are not feasible, we could only balance those maternal and pregnancy characteristics that had been recorded, therefore, we cannot disregard the possibility of some residual confounding. Finally, since it is impossible to define all the potential factors that contribute to fetal loss it is likely that the inclusion and exclusion criteria of the study may have introduced a bias resulting in a higher rate of fetal loss in pregnancies that did not have CVS. For example, 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 undiagnosed chromosomal abnormalities.

Conclusion

The risk of fetal loss following CVS depends on a series of maternal and pregnancy characteristics and to a lesser extent on the procedure itself. The risk factors for fetal loss are similar to those that make CVS necessary and in women at high prior risk of fetal loss the risk of fetal loss following the invasive test could paradoxically be lower than if they did not

have the invasive test, for the simple reason that prenatal diagnosis often converts a spontaneous loss of a chromosomally abnormal fetus into pregnancy termination. As shown in this study the CVS-related risk of fetal loss can become apparent by examining women at low risk of fetal loss and in such cases, there may be an up to about 3.5% increase in risk following CVS.

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Table 1. Maternal and pregnancy characteristics of the study population

Variable	**No CVS (n=8136)**	**CVS (n=445)**	**p-value**
Maternal age (years)	33.6 [29.9,36.8]	35.7 [32.2,38.6]	<0.001
Conception			<0.001
Spontaneous	5077 (62.4)	308 (69.2)	0.004
In vitro fertilization	2848 (35.0)	119 (26.7)	<0.001
Ovulation drugs	211 (2.6)	18 (4.0)	0.070
Weight	66.0 [59.0,76.6]	64.0 [57.8,72.0]	<0.001
Active smoker			0.078
Yes	688 (8.5)	27 (6.1)	
No	7448 (91.5)	418 (93.9)	
Racial origin			<0.001
Black	678 (8.3)	13 (2.9)	
Non-Black	7458 (91.7)	432 (97.1)	
Parity			0.961
Nulliparous	4442 (54.6)	242 (54.4)	
Parous	3694 (45.4)	203 (45.6)	
Chorionicity			0.004
Dichorionic	6314 (77.6)	316 (71.0)	0.002
Monochorionic-diamniotic	1749 (21.5)	122 (27.4)	0.004
Monochorionic-monoamniotic	73 (0.9)	7 (1.6)	0.195
Gestational age at scan (weeks)	12.9 [12.5,13.3]	12.9 [12.4,13.4]	0.545
Discrepancy in crown-rump length (%)	3.57 [1.57,6.47]	4.74 [1.98,8.45]	<0.001
Maximum nuchal translucency (mm)	1.90 [1.64,2.10]	2.60 [1.92,3.40]	<0.001
Serum free ß-hCG (MoM)	1.01[0.70,1.46] NA: 689 (8.4)	1.16 [0.74,1.77] NA: 116 (26.1)	<0.001
Serum PAPP-A (MoM)	1.10 [0.78,1.50] NA: 689 (8.4)	0.84 [0.54,1.19] NA: 116 (26.1)	<0.001
Minimum fetal heart rate (bpm)	157 [152,161]	158 [153,162]	0.012
Outcome			<0.001
Both alive	7621 (93.7)	394 (88.5)	
One or two deaths	515 (6.3)	51 (11.5)	

CVS: chorionic villus sampling; ß-hCG: ß-human chorionic gonadotropin; PAPP-A: pregnancy associated plasma protein-A; MoM: multiple of the median.

Table 2. Propensity score model used to calculate the probability of having chorionic villus sampling by logistic regression analysis.

Variable	Adjusted odds ratio	p-value
Maternal age (years)	1.096 [1.068, 1.126]	<0.001
Conception		
Spontaneous	Reference	-
In vitro fertilization	0.534 [0.381, 0.743]	<0.001
Ovulation drugs	1.438 [0.717, 2.661]	0.273
Weight (Kg)	0.984 [0.975, 0.994]	0.001
Active smoker		
No	Reference	-
Yes	0.801 [0.469, 1.293]	0.388
Racial origin		
Non-Black	Reference	-
Black	0.529 [0.266, 0.958]	0.049
Parity		
Nulliparous	Reference	-
Parous	1.068 [0.810, 1.408]	0.641
Chorionicity		
Dichorionic	Reference	-
Monochorionic-diamniotic	1.031 [0.747, 1.409]	0.851
Monochorionic-monoamniotic	1.246 [0.289, 3.686]	0.727
Gestational age at scan (weeks)	0.703 [0.550, 0.898]	0.005
Discrepancy in crown-rump length (%)	1.025 [0.998, 1.050]	0.059
Maximum nuchal translucency (mm)	7.328 [5.988, 9.030]	<0.001
Serum free ß-hCG (MoM)	1.518 [1.340, 1.712]	<0.001
Serum PAPP-A (MoM)	0.263 [0.195, 0.351]	<0.001
Minimum fetal heart rate (bpm)	1.019 [0.999, 1.041]	0.067

ß-hCG: ß-human chorionic gonadotropin; PAPP-A: pregnancy associated plasma protein-A; MoM: multiple of the median.

Table 3. Maternal and pregnancy characteristics of the cases matched by propensity score.

Variable	**No CVS (n=258)**	**CVS (n=258)**	**p-value**
Maternal age (years)	35.5 [31.7,38.5]	35.7 [31.7,38.4]	0.765
Conception			0.881
Spontaneous	172 (66.7)	167 (64.7)	0.711
In vitro fertilization	73 (28.3)	79 (30.6)	0.629
Ovulation drugs	13 (5.0)	12 (4.7)	1
Weight (kg)	63.0 [56.3,71.9]	64.0 [57.1,74.0]	0.525
Active smoker			0.712
Yes	14 (5.4)	17 (6.6)	
No	244 (94.6)	241 (93.4)	
Racial origin			0.693
Black	15 (5.8)	12 (4.7)	
Non-Black	243 (94.2)	246 (95.3)	
Parity			0.660
Nulliparous	130 (50.4)	136 (52.7)	
Parous	128 (49.6)	122 (47.3)	
Chorionicity			1
Dichorionic	189 (73.3)	188 (72.9)	1
Monochorionic-diamniotic	66 (25.6)	66 (25.6)	1
Monochorionic-monoamniotic	3 (1.2)	4 (1.6)	1
Gestational age at scan (weeks)	12.9 [12.6,13.3]	12.9 [12.4,13.4]	0.875
Discrepancy in crown-rump length (%)	4.81 [2.76,8.09]	4.16 [1.94,8.23]	0.115
Maximum nuchal translucency (mm)	2.30 [1.90,2.80]	2.30 [1.80,3.00]	0.674
Serum free ß-hCG (MoM)	1.16 [0.76,1.74]	1.21 [0.74,1.73]	0.667
Serum PAPP-A (MoM)	0.88 [0.63,1.18]	0.84 [0.50,1.26]	0.182
Minimum fetal heart rate (bpm)	158 [153,162]	157 [153,162]	0.779
Outcome			0.505
Both alive	223 (86.4)	229 (88.8)	
One or two deaths	35 (13.6)	29 (11.2)	

CVS: chorionic villus sampling; ß-hCG: ß-human chorionic gonadotropin; PAPP-A: pregnancy associated plasma protein-A; MoM: multiple of the median.

Table S1. Model for prediction of fetal loss from maternal and pregnancy characteristics [5].

Variable	Coefficient	Standard error	P value
Intercept	-3,93775	0,111	<0.0001
Maternal age >33 years*	-0,01333	0,009	0,126
Maternal weight >69 kg#	0,00710	0,003	0,015
Racial origin			<0.0001
Caucasian	Reference		-
Afro-Carribean	0,91106	0,140	<0.0001
South Asian	0,45745	0,285	0,108
East Asian	-0,26630	0,537	0,620
Mixed	-0,04958	0,523	0,925
Cigarette smoking	0,23190	0,156	0,138
Assisted conception	0,24660	0,119	0,039
Nulliparity	0,16418	0,098	0,093
Chorionicity			<0.0001
Dichorionic	Reference		
Monochorionic diamniotic	1,33705	0,100	<0.0001
Monochorionic monoamniotic	2,09025	0,281	<0.0001
CRL discordance (%)	0,07984	0,008	<0.0001
Increased nuchal translucency			<0.0001
Fetal NT < 95th centile	Reference		
1 or both > 95 th centile	0,50397	0,151	0,001
1 or both > 99 th centile	1,13346	0,211	<0.0001

CRL: crown-rump length; NT: nuchal translucency.

FIGURE LEGENDS

Figure 1. Propensity score matching of cases that had undergone chorionic villus sampling (CVS) with cases that did not have CVS. The grey band denotes 10% standardized difference between covariates.

Figure 2. Adjusted odds ratio for fetal loss after chorionic villus sampling (CVS) in women with high and low risk of having a CVS.

Figure 3. Estimated relative risk of fetal loss after chorionic villus sampling for a modelled *prior* risk of fetal loss between 2% and 10%.