Risk of miscarriage after chorionic villus sampling

Short version of article title: Risk of miscarriage after chorionic villus sampling.

M. M. GIL[1,2], F. S. MOLINA[3], M. RODRÍGUEZ-FERNÁNDEZ[1], J. L. DELGADO[4], M. P. CARRILLO[5], J. JANI[6], W. PLASENCIA[7], V. STRATIEVA[8,9], N. MAÍZ[10*], P. CARRETERO[3], A. LISMONDE[6], P. CHAVEEVA[8], J. BURGOS[10], B. SANTACRUZ[1,2], J. ZAMORA [11,12], C. DE PACO MATALLANA[4].

1. Department of Obstetrics and Gynecology, Hospital Universitario de Torrejón, Torrejón de Ardoz, Madrid, Spain.

2. School of Health Sciences. Universidad Francisco de Vitoria, Pozuelo de Alarcón, Madrid, Spain.

3. Department of Obstetrics and Gynecology, Hospital Clínico San Cecilio, Instituto de Investigación Biosanitaria IBS, Granada, Spain.

4. Department of Obstetrics and Gynecology, Hospital Clínico Universitario 'Virgen de la Arrixaca', El Palmar, Murcia, Spain; Institute for Biomedical Research of Murcia, IMIB-Arrixaca, El Palmar, Murcia, Spain.

5. Department of Obstetrics and Gynecology, Hospital Universitario 'Virgen de las Nieves', Granada, Spain.

6. Department of Obstetrics and Gynecology, University Hospital Brugmann, Université Libre de Bruxelles, Brussels, Belgium.

7. Hospiten Group, Tenerife, Canary Islands, Spain.

8. Obs / Gyn Dr Shterev Hospital, Sofia, Bulgaria.

9. OSCAR Clinic, Sofia, Bulgaria.

10. Fetal Medicine Unit. Department of Obstetrics and Gynecology, BioCruces Health Research Institute, Hospital Universitario Cruces. Universidad del País Vasco (UPV/EHU), Barakaldo, País Vasco, Spain.

11. CIBER Epidemiology and Public Health; Clinical Biostatistics Unit, Hospital Ramón y Cajal, Madrid, Spain.

12. Barts Research Centre for Women's Health, WHO Collaborating Centre, Queen Mary University of London, London, UK

* Present address: Maternal-Fetal Medicine Department. Hospital Universitari Vall d'Hebron, Barcelona, Spain. Universitat Autónoma de Barcelona, Spain.

Conflict of interest statement: The authors report no conflict of interest.

Correspondence:

María del Mar Gil Mira,

Vicerrectorado de Investigación. Facultad de Medicina. Universidad Francisco de Vitoria, Carretera Pozuelo a Majadahonda, Km 1.800, 28223 Pozuelo de Alarcón, Madrid, Spain.

Telephone: +34 91 709 14 00, email: mariadelmar.gil@ufv.es

Abstract word count: 396, **Text word count:** 3,375

Contribution:

A. What are the novel findings of this work?

The rate of miscarriage after chorionic villous sampling (CVS) is highly dependent on the patient-specific background risk of miscarriage without CVS. Because the several factors that lead to CVS are also associated with spontaneous miscarriage, in women at low-risk of aneuploidies, CVS is associated with a significant increase in the miscarriage rate while, paradoxically, when the risk is high, the risk of miscarriage after CVS is reduced, presumably due to prenatal diagnosis and termination of major aneuploidies that would have otherwise resulted in spontaneous miscarriage.

B. What are the clinical implications of this work?

The true procedure-related risk of miscarriage from CVS can only be derived by examining women at low-risk of aneuploidies and in such women their risk of miscarriage increases by about three times after CVS. Although this is a substantial increase in relative terms, in pregnancies without prior risk factors the risk of miscarriage after CVS will still remain low and similar to or slightly higher than that of the general population.

ABSTRACT

Objective: To estimate the risk of miscarriage associated to chorionic villus sampling (CVS).

Methods: This was a retrospective cohort study performed in eight fetal-medicine units in Spain, Belgium and Bulgaria. Two populations were included: first, all singleton pregnancies attending to their first-trimester assessment in Murcia, Spain, and second, all singleton pregnancies having a CVS following first-trimester assessment at any of the participating centers. We used propensity score matching analysis to estimate the association between CVS and miscarriage. We compared risks of miscarriage of 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: The study population consisted of 22,250 participants in the non-CVS group and 3,613 in the CVS group. The incidence of miscarriage in the CVS group was 2.1% (77/3,613), which was significantly higher than the 0.9% (207/22,250) in the non-CVS group ($p < 0.001$). The propensity score algorithm matched 2,122 CVS cases with 2,122 non-CVS cases including 40 (1.9%) and 55 (2.6%) miscarriages in the CVS and non-CVS groups, respectively (OR 0.72 [95% CI 0.48 to 1.10]; $p = 0.146$). However, we found a significant interaction between the CVS risk of miscarriage and the risk of aneuploidies, suggesting a different effect of the CVS for different baseline characteristics in such a way that, when the risk of aneuploidies is low, the risk after CVS increases (OR 2.87 [95% CI 1.13 to 7.30]) but when the risk

is high, the risk after CVS is paradoxically reduced (OR 0.47 [95% CI 0.28 to 0.76]), presumably due to prenatal diagnosis and termination of major aneuploidies that would have otherwise resulted in spontaneous miscarriage.

Conclusions: The risk of miscarriage in women having a CVS is about 1% higher than in women without CVS, although this excess risk is not entirely due to the invasive procedure but to some extent the demographic and pregnancy characteristics of the patient undergoing CVS. After accounting for these risk factors and confining the analysis to low-risk pregnancies, CVS seems to increase the risk of miscarriage about three times above the patient's background-risk. Although this is a substantial increase in relative terms, in pregnancies without risk factors, the risk of miscarriage after CVS will still remain low and similar to or slightly higher than that of the general population. For example, if her risk of aneuploidy is 1 in a 1,000 (0.1%), her risk of miscarriage after CVS will increase to 0.3% (0.2% higher).

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

INTRODUCTION

Chorionic villous sampling (CVS), which was first described in 1975[1] and introduced into widespread practice in the 1980's, is a useful invasive test for early prenatal diagnosis of chromosomal and genetic abnormalities. The procedure related risk of miscarriage was not investigated in studies that randomized women into CVS vs. non-invasive testing groups. However, the risk was derived indirectly through first, randomized control trials (RCTs) comparing CVS with first or second trimester amniocentesis, and second, comparison of rates of miscarriage in groups with similar risk factors that had CVS with those that did not have invasive testing. The results of trials established that first, the risk of miscarriage following CVS was lower than that of early amniocentesis but similar to that of mid-trimester amniocentesis, and second, the risk of transabdominal and transcervical CVS was similar.[2-7] Consequently, since the only trial comparing mid-trimester amniocentesis to expectant management reported a 1% higher risk of miscarriage in the amniocentesis group,[8] it was assumed that the risk of miscarriage from CVS was also about 1%.

Another approach for estimating the procedure-related risk of miscarriage from CVS is to compare rates of miscarriage in groups that had CVS with those that did not have invasive testing. However, such an approach is likely to provide a bias against CVS because several of the factors that lead to CVS are also risk factors for miscarriage, i.e. increased maternal age, increased fetal nuchal translucency (NT), low serum pregnancy associated plasma protein-A (PAPP-A), and abnormal flow in the fetal ductus venosus.[9-13] One possible approach to overcome this problem, is to

carry out logistic regression analysis to identify predictors of miscarriage in women who did not have CVS and then apply the model to women who had CVS and compare the observed to the expected number of miscarriages in the latter group.[13-15] A second approach is to perform a propensity score (PS) analysis that creates two homogeneous groups suitable for comparisons.[16] PS analysis 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 multivariate analysis could adjust for.[17,18] Studies utilizing these approaches reported that the procedure-related risk of miscarriage from CVS may be considerably lower than 1%.[13-16] A recent meta-analysis included 7 studies comparing 13,011 women who had a CVS with 232,680 women who did not have the procedure and estimated the risk of miscarriage following CVS at 0.20% (95% CI, −0.13 to 0.52%).[19] However, the results from the different studies were heterogeneous and the value of pooled estimates from meta-analyses in such cases is questionable.[20]

The main objective of this multicenter study was to estimate the CVS-related risk of miscarriage after accounting for the effect of maternal and pregnancy characteristics which could have driven the decision around performing or not a CVS.

METHODS

Study design and population

This is a retrospective cohort study performed at eight fetal-medicine units in Spain (Hospital Clínico Universitario Virgen de la Arrixaca in Murcia, Hospital Clínico Universitario San Cecilio and Hospital Universtario Virgen de las Nieves in Granada, Hospiten de Tenerife in Tenerife and Hospital Universitario de Cruces in Bilbao), Belgium (Brugmann University Hospital in Brussels) and Bulgaria (Shterev Hospital and OSCAR Clinic in Sofia). In the participating centers women attended for a routine ultrasound examination at 11^{+0}-13^{+6} weeks' gestation. During this visit patient characteristics and medical history were recorded, ultrasound examination was carried out to assess viability, diagnose major defects and measure fetal crown-rump length (CRL) and fetal NT thickness and assess ductus venosus a-wave as positive or negative / reversed. Blood was also collected in the same visit (n = 651 [2.5%]) or 1-2 weeks previously (n= 25,212 [97.5%]) for measurement of serum free β-human chorionic gonadotropin (β-hCG) and PAPP-A. Screening for trisomies 21, 18 and 13 was carried out using The Fetal Medicine Foundation algorithm, which combines maternal age, fetal NT, ductus venosus flow and multiple of the median (MoM) values of free β-hCG and PAPP-A.[21] The estimated risk for trisomies was then used to counsel women and in those choosing invasive testing CVS was performed by the same transabdominal technique by or under the supervision of a fetal medicine expert trained at King's College Hospital, London, UK. Pregnancies were dated according to the fetal CRL at the time of screening if they were naturally conceived[22] and according to conception date if they were conceived by *in-vitro* fertilization.

We recorded the following patient characteristics: maternal age, weight, height, racial origin (White, Black, South Asian, East Asian and mixed), method of

conception (natural or assisted conception requiring the use of ovulation drugs or *in-vitro* fertilization), cigarette smoking during pregnancy (yes or no) and parity (parous or nulliparous if no previous pregnancy at ≥ 24 weeks' gestation), and medical history of diabetes mellitus and chronic hypertension (yes or no).

Two populations were included in this study; first, all singleton pregnancies attending to their first-trimester assessment in Murcia (Spain) who did not have CVS, and second, all singleton pregnancies having a CVS following first-trimester assessment at any of the participating centers. In the control group there were 21,873 (98.3%) pregnancies with a low-risk from the first-trimester combined test, 345 (1.6%) with a high-risk and 32 (0.1%) who declined risk assessment. Indication for CVS was mainly increased risk for aneuploidies but also increased NT, history of genetic disease in the family, previous aneuploidy or even maternal request. The patients were examined between July 2007 and June 2018. The eligibility criteria were singleton pregnancy with a live fetus at 11^{+0} to 13^{+6} weeks without genetic anomalies or major fetal defects (such as acrania, holoprosencephaly, megacystis, exomphalos, congenital heart defects) diagnosed before or after birth. We excluded pregnancies resulting in termination for any reason, pregnancies without follow up and pregnancies having an amniocentesis later on in pregnancy.

The primary outcome measure was miscarriage, defined as pregnancy loss occurring before 24 weeks' gestation regardless of the interval between CVS and fetal demise. Results of the investigations and pregnancy outcome were recorded in computer databases. Approval for the study and waiver of consent was obtained

from the relevant research ethics committee in each center in which the study was conducted.

Statistical analyses

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 two-tailed $\chi 2$-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.

Because we noted important differences in baseline clinical characteristics between the CVS and the non-CVS groups, we performed a propensity score matching analysis to assess the effect of CVS in the risk of miscarriage adjusting for the confounding bias caused by this imbalance. Compared with classic multivariate adjustments, the PS permits finer adjustments for wider sets of covariates. The PS 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 PS, 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 use the PS to match, without replacement, each complete CVS case with the non-CVS case with the closest PS in a 1:1 ratio, to optimise the precision of the estimate of association and limit bias. We also accepted cases only if the difference in PS between matched cases was small (calliper of 0.1), resulting in excellent balance between the CVS

and the non-CVS cases as matched samples.[23] We computed standardised differences for all variables included in the PS 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 miscarriage 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 miscarriage using a univariate logistic regression fitted by generalised estimating equations to account for matched data.

The statistical software package R was used for data analyses. [24] The R package MatchIt[25] was used for matching with PS. Analysis of matched cases was performed using the R package Geepack.[26]

RESULTS

Study population

The study population consisted of 22,250 participants in the non-CVS group and 3,613 in the CVS group (figure 1). Maternal and pregnancy characteristics are shown in Table 1. In the CVS group, compared to the non-CVS group, median maternal age, gestational age, fetal NT and serum free β-hCG MoM were significantly higher and maternal weight and PAPP-A MoM were lower, and the incidence of parous women, Black or South Asian racial origin, chronic hypertension and conception by assisted reproductive techniques and abnormal flow in the fetal ductus venosus was higher. The only parameter not significantly different was the frequency of pre-existing diabetes mellitus.

The incidence of miscarriage in the CVS group was 2.1% (77/3,613), which was significantly higher than the 0.9% (207/22,250) in the non-CVS group (p <0.001).

Procedure-related risk of miscarriage

We calculated PS for each case in the study population based on their probability of having a CVS. Multivariable regression analysis demonstrated that significant predictors associated to having a CVS were increasing maternal age, decreasing maternal weight, assisted conception, chronic hypertension, increasing gestational age, high fetal NT, abnormal flow in the ductus venosus, high free β-hCG and low PAPP-A (Table S1).

The PS algorithm matched 2,122 of our CVS cases with 2,122 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 2, tables 1 and 2). The number of miscarriages was 40 (1.9%) in the CVS group and 55 (2.6%) in the matched non-CVS group. PS analysis did not find any significant association between CVS and miscarriage (OR 0.72 [95% CI 0.48 to 1.10]; p=0.146). We hypothesized that the most likely explanation for this paradoxical effect of CVS “decreasing” the risk of miscarriage was that many of the cases that would have resulted in spontaneous miscarriage had the pregnancy continued, were converted into elective pregnancy terminations following an abnormal genetic diagnosis. If this was true, this “protective” effect should be higher in cases at high-risk of having a genetic anomaly and lower in cases at low-risk.

Therefore, we aimed to investigate whether the effect of having a CVS was the same in women at higher risk of aneuploidies as compared to those at lower risk. Thus, we investigated a possible interaction between the risk of aneuploidy and CVS. Since the risk factors associated to having a CVS are the same factors that increase the risk of aneuploidies, we divided our 4,244 matched cases in two equal groups by the median of the PS. The median PS was 0.402 (IQR 0.331-0.490) in the high-risk subgroup (n=2,122) and 0.131 (IQR 0.057, 0.197) in the low-risk subgroup (n=2,122). In the high-risk subgroup there were 1,062 cases having a CVS, including 23 (2.2%) miscarriages and 1,060 non-CVS cases, including 49 (4.6%) miscarriages (OR 0.47 [95% CI 0.28 to 0.76]); in contrast, in the low-risk subgroup we found 17 (1.6%) miscarriages in the CVS (n = 1,060) group compared to 6 (0.6%) miscarriages in the non-CVS (n= 1,062) group (OR 2.87 [95% CI 1.13 to 7.30]. Both effects were statistically different (p value of the interaction = 0.0003) (figure 3). These results suggest that there is something which makes the CVS behave differently when the risk of aneuploidies is high compared to when it is low. Thus, using the PS as a proxy of the risk of aneuploidies, for a patient with a 10% probability of aneuploidy based on her pregnancy characteristics, the risk of miscarriage after the procedure is still very high but halved to about 5%, suggesting that in such case CVS is highly “protective” of miscarriage. However, for a patient with a low probability of aneuploidy, her risk of miscarriage will increase. For example, if her risk of aneuploidy is 1 in a 1,000 (0.1%), her risk of miscarriage after CVS will increase to 0.3% (0.2% higher) or, in other words, we would need to perform

500 CVSs to cause a miscarriage. Further analysis on this interaction is provided in Appendix 1.

DISCUSSION

Principal findings

In this study we found that: first, following a first trimester scan demonstrating a structurally normal fetus, the risk of subsequent miscarriage for the general population is about 1%; second, in women having CVS the risk of miscarriage is about 1% higher than in women without CVS although this excess risk is not entirely due to the invasive procedure but to some extent the demographic and pregnancy characteristics of the patient undergoing CVS; and third, the actual procedure-related risk of the CVS may only become apparent in patients at low risk of aneuploidies and, in these cases, the risk of miscarriage after CVS increases by about three times.

We have demonstrated that, although in women at high-risk of aneuploidies CVS appears to be "protective" against miscarriage, the most likely explanation for this observation is that CVS leads to the diagnosis of major aneuploidies followed by elective pregnancy termination in cases that would have otherwise resulted in spontaneous miscarriage. In the CVS group we excluded 22.2% (1,135/5,112) of cases because of termination of pregnancy or fetal defects, compared to only 4.2% (1,070/25,519) in the non-CVS group (figure 1). Had these cases been included and the pregnancy had continued, many would have resulted in miscarriage and then the rate of miscarriage in the CVS group would have been considerably higher than in

the non-CVS group. To try to avoid this selection bias, we studied separately the effect of the CVS in cases with a low 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 aneuploidies, the procedure significantly increases this risk by about three times.

Comparison with findings of previous studies

Our results offer an explanation for the contradictory findings of previous studies that showed that CVS did not significantly modify the risk of miscarriage, and a meta-analysis that reported a non-significant "protective" effect of CVS against miscarriage[17].

First, one large study examined 31,460 pregnancies undergoing first-trimester combined screening for aneuploidies without CVS and identified risk factors for miscarriage.[13] They then applied this model in 2,396 pregnancies with CVS and found that the estimated number of miscarriages was 45 (95% CI 32 to 58) which was similar to the observed number of 44.[13] Two subsequent studies following a similar methodology did not find significant differences between groups.[14,15]

Second, a large national registry-based study assessing 147,987 singleton pregnancies that had first-trimester combined screening for aneuploidies, including 5,072 that had CVS, reported that the average effect of CVS on risk of miscarriage was -0.21% (95% CI, -0.58 to 0.15).[16] In this study the CVS-related risk of miscarriage was assessed by a dynamic PS stratification approach.[16] The advantage of this approach is that it allows use of the whole sample but the major disadvantage is that the higher the number of cases per stratum the greater is the

difference in baseline characteristics of the patients even within the same stratum. In our matching approach we used a 1:1 ratio and a small difference in PS between matched cases (calliper of 0.1) to ensure that the CVS and non-CVS groups had a very similar risk-profile.

Third, a recent RCT randomized women at high-risk of aneuploidies into cell-free DNA testing (n = 1,015) or invasive testing, both amniocentesis or CVS (n = 982), and found not significant differences in the risk of miscarriage between the two groups (0.8% vs. 0.8%, for a risk difference of −0.03% (1-sided 95%CI, −0.68% to ∞; P = 0.47).[25]

Clinical implications

In those cases where there is a clear indication to perform prenatal genetic testing, we can reassure women that their risk of miscarriage mainly depends on the results from genetic diagnosis and the conditions that lead to it more than the procedure itself. However, in the absence of any major fetal defect or other additional risk factors for chromosomal abnormalities, we should report an individualized procedure-related risk based on women clinical characteristics.

Strengths and limitations

The main limitations of the study derive from its observational and retrospective nature with the immediate consequence of the heterogeneity between comparison groups (figure 2). Although we tried to mitigate these differences, we were able to balance only those maternal and pregnancy characteristics that had been recorded, therefore, we cannot disregard the possibility of some residual confounding.

Additionally, we could not assess the influence of technical factors or experience of operators since they are not routinely recorded in any of the participating centers; however, its influence in the risk of miscarriage is well studied[26,27]. Fetal karyotype was not available in most cases miscarrying spontaneously and therefore our assumption on increased rate of aneuploidies among them remains hypothetical. We chose to exclude aneuploidies and fetal defects from the analysis because these would overestimate the risk of miscarriage in the CVS group, since they are the cases most likely to miscarry. However, this exclusion inevitably leads to the opposite effect as shown in our results: underestimation of the procedure-related risk due to lack of knowledge about karyotype in most of the miscarriages in the non-CVS group while the CVS sample is “clean” of aneuploidies.

The main strength of our study relates to the large sample of both, CVS and non-CVS cases, which were selected after matching women of both groups but with identical propensity of CVS. Since the matching was indirectly based on known risk-factors for aneuploidies, we were able to perform subgroup analysis to demonstrate the interaction between the risk of aneuploidies and CVS by comparing patients with a very similar risk-profile.

All invasive procedures were performed by the same technique and by fetal medicine experts or their trainees at the end of such training. This represents both an advantage, because this reduces the variability between operators, and a disadvantage, since the results might not be valid for different approaches and level of expertise.

Conclusions

The risk of miscarriage in women having a CVS is about 1% higher than in women without CVS, although this excess risk is not entirely due to the invasive procedure but to some extent to the demographic and pregnancy characteristics of the patient undergoing CVS. After adjusting for these risk factors and confining the analysis to low-risk pregnancies, CVS seems to increase the risk of miscarriage about three times above the patient's background-risk. Although this is a substantial increase in relative terms, in pregnancies without risk factors, the risk of miscarriage after CVS will remain low and similar to or slightly higher than that of the general population.

Acknowledgment

This study was supported by Fundación para la Investigación y el Desarrollo de la Medicina Materno-Fetal y Neonatal, *iMaterna* (Registry No: 2148). The authors are grateful to Prof José Luis Brita-Paja Segoviano and Prof Teresa Pérez Pérez from Department of Statistics and Data Science at Universidad Complutense de Madrid, Spain, to Borja Manuel Fernández Félix from CIBER Epidemiology and Public Health, Clinical Biostatistics Unit, Hospital Universitario Ramón y Cajal, Madrid, Spain, and to Valeria Rolle Sóñora from Bioestatistics and Epidemiology Platform at Fundación para la Investigación e Innovación Biosanitaria del Principado de Asturias, Oviedo, Spain, for statistics and data handling support. Their contribution was neither funded nor compensated in any form.

References

1. Department of Obstetrics and Gynecology, Tietung Hospital, Anshan C. Fetal sex prediction by sex chromatin of chorionic villi cells during early pregnancy. *Chin Med J* 1975; **1**: 117–126.

2. Nicolaides K, Brizot Mde L, Patel F, Snijders R. Comparison of chorionic villus sampling and amniocentesis for fetal karyotyping at 10-13 weeks' gestation. *Lancet* 1994; **344**: 435-439.

3. Sundberg K, Bang J, Smidt-Jensen S, Brocks V, Lundsteen C, Parner J, Keiding N, Philip J. Randomised study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling. *Lancet* **1997**; 350: 697-703.

4. Multicentre randomised clinical trial of chorion villus sampling and amniocentesis. First report. Canadian Collaborative CVS-Amniocentesis Clinical Trial Group. *Lancet* 1989; **1**: 1–6.

5. Medical Research Council European trial of chorion villus sampling. MRC working party on the evaluation of chorion villus sampling. *Lancet* **1991**; 337:1491–1499.

6. Jackson LG, Zachary JM, Fowler SE, Desnick RJ, Golbus MS, Ledbetter DH, Mahoney MJ, Pergament E, Simpson JL, Black S, et al. A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National

Institute of Child Health and Human Development Chorionic-Villus Sampling and Amniocentesis Study Group. *N Engl J Med* 1992; **327**: 594-598.

7. Alfirevic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane database Syst Rev* 2017; **9**: CD003252.

8. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986; **1**: 1287–1293.

9. Souka AP, Krampl E, Bakalis S, Heath V, Nicolaides KH. Outcome of pregnancy in chromosomally normal fetuses with increased nuchal translucency in the first trimester. *Ultrasound Obstet Gynecol* 2001; **18**: 9–17.

10. Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol* 2005; **192**: 1005–1021.

11. Spencer K, Cowans NJ, Avgidou K, Nicolaides KH. First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of impending fetal death. *Ultrasound Obstet Gynecol* 2006; **28**: 637–643.

12. Maiz N, Valencia C, Emmanuel EE, Staboulidou I, Nicolaides KH. Screening for adverse pregnancy outcome by ductus venosus Doppler at 11-13+6 weeks of gestation. *Obstet Gynecol* 2008; **112**: 598–605.

13. Akolekar R, Bower S, Flack N, Bilardo CM, Nicolaides KH. Prediction of miscarriage and stillbirth at 11-13 weeks and the contribution of chorionic villus sampling. *Prenat Diagn* 2011; **31**: 38–45.

14. Wah YM, Leung TY, Cheng YKY, Sahota DS. Procedure-Related Fetal Loss following Chorionic Villus Sampling after First-Trimester Aneuploidy Screening. *Fetal Diagn Ther* 2017; **41**: 184–190.

15. Beta J, Zhang W, Geris S, Kostiv V, Akolekar R. Procedure-related risk of miscarriage following chorionic villus sampling and amniocentesis. *Ultrasound Obstet Gynecol* 2019; **54**: 452–457.

16. Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, Tabor A. Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: A national cohort of 147 987 singleton pregnancies. *Ultrasound Obstet Gynecol* 2016; **47**: 38–44.

17. Shah BR, Laupacis A, Hux JE, Austin PC. Propensity score methods gave similar results to traditional regression modelling in observational studies: a systematic review. *J Clin Epidemiol* 2005; **58**: 550–559.

18. Glynn, R.J., Schneeweiss, S. and Stürmer, T. Indications for Propensity Scores and Review of their Use in Pharmacoepidemiology. *Basic Clinic Pharmacol Toxicol* 2006; **98**: 253-259.

19. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. *Ultrasound Obstet Gynecol* 2019; **54**: 442-451.

20. Navaratnam K, Alfirevic Z. Trials and tribulations of meta-analyzing procedure-related risks of amniocentesis and chorionic villus sampling. *Ultrasound Obstet Gynecol* 2019; **54**: 437-441.

21. Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. *Fetal Diagn Ther* 2014; **35**: 118–26.

22. Robinson HP, Fleming JE. A critical evaluation of sonar “crown-rump length” measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.

23. Austin PC. Optimal caliper widths for propensity-score matching when estimating differences in means and differences in proportions in observational studies. *Pharm Stat* 2011; **10**: 150–161.

24. R Development Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2011.

25. Ho DE, Imai K, King G, Stuart EA. Matching as Nonparametric Preprocessing for Reducing Model Dependence in Parametric Causal Inference. *Polit Anal* 2007; **15**: 199–236.

26. Halekoh U, Højsgaard S, Yan J. “The R Package geepack for Generalized Estimating Equations.” *Journal of Statistical Software* 2006, **15**: 1–11.

27. Malan V, Bussières L, Winer N, Jais JP, Baptiste A, Le Lorc'h M, Elie C, O'Gorman N, Fries N, Houfflin-Debarge V, et al. Effect of Cell-Free DNA screening vs direct invasive diagnosis on miscarriage rates in women with pregnancies at high risk of trisomy 21: A Randomized Clinical Trial. *JAMA* 2018; **320**: 557–565.

28. Odibo AO, Dicke JM, Gray DL, Oberle B, Stamilio DM, Macones GA, Crane JP. Evaluating the rate and risk factors for fetal loss after chorionic villus sampling. *Obstet Gynecol* 2008; **112**: 813–819.

29. Bakker M, Birnie E, Robles de Medina P, Sollie KM, Pajkrt E, Bilardo CM. Total pregnancy loss after chorionic villus sampling and amniocentesis: a cohort study. *Ultrasound Obstet Gynecol* 2017; **49**: 599–606.

Table 1. Maternal and pregnancy characteristics of the study population.

Variable	Non-chorionic villus sampling* (n = 22,250)	Chorionic villus sampling (n = 3,613)	*P* value	*Standardized difference (%)*
Maternal age, y	32.5 (28.4, 35.8)	35.2 (31.4, 38.3)	<0.0001	49.0
Maternal weight, kg	64.0 (57.3, 73.0)	63.5 (57.0, 72.0)	0.0014	-5.4
Maternal height, cm	163 (160, 168)	163 (159, 167)	0.0281	-3.4
Racial origin				6.0
White	21937 (98.6)	3526 (97.6)	<0.0001	
Black	221 (1.0)	52 (1.4)	0.0190	
South Asian	21 (0.1)	13 (0.4)	0.0001	
East Asian	71 (0.3)	22 (0.6)	0.0108	
Method of conception			0.0048	4.9
Natural	21258 (95.5)	3413 (94.5)		
Assisted	992 (4.5)	200 (5.5)		
Parity				18.0
Nulliparous	10246 (46.0)	1345 (37.2)	<0.0001	
Parous	12004 (54.0)	2268 (62.8)	<0.0001	
Cigarette smoking	3137 (14.1)	467 (12.9)	0.0625	3.4
Medical history				
Diabetes mellitus	223 (1.0)	40 (1.1)	0.4240	1.7
Not known	1846 (8.3)	469 (13.0)	<0.0001	
Chronic hypertension	157 (0.7)	46 (1.3)	<0.0001	7.4
Not known	66 (0.3)	510 (14.1)	<0.0001	
Gestational age, wk	12.6 (12.2, 13.1)	13.0 (12.5, 13.5)	<0.0001	50.4
Delta nuchal translucency, mm	0.16 (-0.06, 0.40)	0.32 (‘-0.01, 0.85)	<0.0001	43.4
Ductus venosus				
Abnormal flow	1059 (4.8)	384 (10.6)	<0.0001	26.6
Not known	907 (4.1)	511 (14.1)	<0.0001	
Free β-hCG, MoM	1.05 (0.69, 1.63)	1.29 (0.77, 2.12)	<0.0001	28.9
PAPP-A, MoM	0.94 (0.67, 1.34)	0.52 (0.32, 0.86)	<0.0001	-69.1
Miscarriage, n (%)	207 (0.9)	77 (2.1)	<0.0001	

Data are given as median (interquartile range) or n (%). *The subset of women included in the propensity score regression analysis was taken from this group. hCG = human chorionic gonadotropin; PAPP-A = pregnancy associated plasma protein-A; Comparisons between outcome groups were by χ2-test for categoric variables and Mann-Whitney U test for continuous variables.

Table 2. Maternal and pregnancy characteristics of the chorionic villus sampling and non-chorionic villus sampling cases matched by propensity score.

Variable	Non-chorionic villus sampling (n = 2,122)	Chorionic villus sampling (n = 2,122)	*P* value	Standardized difference (%)
Maternal age, y	34.8 (31.5,37.7)	34.7 (31.1,37.9)	0.5789	2.1
Maternal weight, kg	63.0 (57.0,71.5)	63.0 (56.6,71.2)	0.9949	-0.2
Maternal height, cm	163 (159,167)	163 (159,167)	0.9582	-0.8
Racial origin, n (%)			0.8592	1.1
White	2107 (99.3)	2105 (99.2)		
Non-White	15 (0.7)	17 (0.8)		
Method of conception, n (%)			0.3681	3.0
Natural	2019 (95.1)	2005 (94.5)		
Assisted	103 (4.9)	117 (5.5)		
Parity, n (%)			1.000	0.1
Nulliparous	853 (40.2)	854 (40.2)		
Parous	1269 (59.8)	1268 (59.8)		
Cigarette smokers, n (%)	288 (13.6)	272 (12.8)	0.4963	-2.2
Medical history, n (%)				
Diabetes mellitus (n= 2367; 2450)	20 (0.9)	23 (1.1)	0.8669	1.0
Chronic hypertension	27 (1.3)	26 (1.2)	1.000	-0.4
Gestational age, weeks	13.0 (12.5,13.4)	12.9 (12.4,13.4)	0.0414	-7.3
Delta nuchal translucency, mm	0.33 (0.08,0.65)	0.26 (-0.02,0.65)	<0.0001	0.3
Abnormal flow in ductus venosus	251 (11.8)	232 (10.9)	0.3843	-2.8
Free β-hCG, MoM	1.19 (0.74,1.91)	1.22 (0.75,1.96)	0.5273	6.3
PAPP-A, MoM	0.66 (0.48,0.90)	0.52 (0.32,0.87)	<0.0001	-9,9
Miscarriage, n (%)	55 (2.6)	40 (1.9)	0.1463	

Data are given as median (interquartile range) or n (%). Comparisons between outcome groups were by chi, square test for categoric variables and Mann, Whitney U test for continuous variables.
The covariates used to identify matched women without chorionic villus sampling were maternal age, weight height and racial origin, method of conception, parity, smoking status, chronic hypertension, gestational age, nuchal translucency, free β-hCG and PAPP-A.
hCG = human chorionic gonadotropin; PAPP-A = pregnancy associated plasma protein-A.

Figure legends

Figure 1. Flow diagram of patients included in the study. CVS, chorionic villus sampling.

Figure 2. Propensity score matching of cases with chorionic villus sampling with cases without chorionic villus sampling. The grey band denotes 10% standardised difference between covariates.

Figure 3. Odds ratio for miscarriage after chorionic villus sampling in women with high and low risk of having a CVS. CVS, chorionic villus sampling.