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Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147 987 singleton pregnancies

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KEYWORDS: amniocentesis; chorionic villus sampling; combined first-trimester screening; fetal loss; invasive prenatal testing; miscarriage; procedure-related risk; stillbirth

ABSTRACT

Objective *To assess prospectively the risk of fetal loss associated with chorionic villus sampling (CVS) and amniocentesis (AC) following combined first-trimester screening (cFTS) for Down syndrome.*

Methods *This was a nationwide population-based study (Danish Fetal Medicine Database, 2008–2010) including 147 987 women with singleton pregnancy who underwent cFTS. Propensity score stratification was used to assess the risk of fetal loss with and without invasive testing. Analyses were performed between 3 and 21 days after cFTS for CVS and between 28 and 42 days after cFTS for AC. Results are reported as average risk differences with 95% CIs.*

Results *The risks of miscarriage and stillbirth were not higher in women exposed to CVS or AC compared with unexposed women, independent of the analysis time-point. The average effect of CVS on risk of miscarriage was −0.08% (95% CI, −0.64; 0.47) at 3 days and −0.21% (95% CI, −0.58; 0.15) at 21 days after cFTS, while the effect on risk of stillbirth was −0.18% (95% CI, −0.50; 0.13) at 3 days and −0.27% (95% CI, −0.58; 0.04) at 21 days after cFTS. Regarding the effect of AC on risk of miscarriage, the analysis at 28 days after cFTS showed an average effect of 0.56% (95% CI, −0.21; 1.33), while the effect on risk of stillbirth was 0.09% (95% CI, −0.39; 0.58) at 42 days after cFTS.*

Conclusion *Neither CVS nor AC was associated with increased risk of miscarriage or stillbirth. These findings indicate that the procedure-related risk of CVS and AC is very low.* Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

The risk of miscarriage following chorionic villus sampling (CVS) and amniocentesis (AC) was investigated in randomized clinical trials in the 1980s and 1990s. One study found that the risk of miscarriage after AC was increased by 1.0% (95% CI, 0.3–1.5%) compared to the risk with no invasive procedure[1]. Studies have compared CVS with AC and found comparable risk of miscarriage following the two procedures[2]. Since then, prenatal screening has changed from being based on maternal age to combined first-trimester screening (cFTS) for trisomy 21. This has lowered the number of women who screen false positive, reduced the number of women offered invasive testing, and changed the procedure of choice from AC to CVS[3–5]. The techniques have been improved, and the magnitude of the procedure-related risk of fetal loss has been questioned. A recent meta-analysis estimated procedure-related risks of CVS and AC to be as low as 0.2% and 0.1%, respectively[6].

The results of randomized clinical trials have limited value for counseling, because a trial can include only a selected part of the population and the timing of the invasive test cannot be randomized. Prospective risk assessment based on observational data requires advanced statistical modeling in order to minimize the selection bias due to women at increased risk of fetal loss being more likely to have an invasive test. There is evidence that high maternal age, smoking, increased nuchal translucency thickness (NT), and low levels of pregnancy-associated plasma protein A (PAPP-A) are associated with increased risk of miscarriage and stillbirth[7–9]. These factors are also associated with chromosomal abnormalities and may increase the likelihood of being offered CVS[10–13]; they

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should be taken into account when estimating the effect of invasive procedures on the risk of fetal loss.

In Denmark, all pregnant women are offered cFTS, and the uptake is more than 90%[3]. Individual pregnancy data, including dates of the invasive tests, are available through national Danish registries, thus providing the possibility to investigate the procedure-related risks in an unselected national cohort.

The aim of this study was to assess prospectively the risk of fetal loss associated with CVS and AC following cFTS.

METHODS

This national population-based study included women with singleton pregnancy with a live fetus with crown–rump length (CRL) 45–84 mm, a NT measurement and complete data on first-trimester biomarkers (free beta-human chorionic gonadotropin (β-hCG) and PAPP-A), who underwent cFTS between 1 January 2008 and 31 December 2010. Women with a CVS performed before the cFTS were excluded, as were women with invalid levels of biomarkers, defined as multiples of the median (MOM) values ≥ 20. The study was approved by the Danish Data Protection Agency (j.nr.2012-41-0727). According to Danish legislation, studies based solely on registry data, with no personal involvement of the participants, do not require approval from an ethics committee.

Women were identified from the Danish Fetal Medicine Database (DFMD)[14], which is based on data from all departments of obstetrics and gynecology in Denmark. Since 1 January 2008, data on maternal characteristics, first-trimester levels of PAPP-A and free β-hCG, NT scans, first-trimester risk of trisomy 21 based on maternal age, biochemistry and NT as well as anomaly scans have been entered, as part of routine practice and in accordance with national standards, in 20 local Astraia databases, (Astraia Software GmbH, Munich, Germany). Assessment of gestational age is based on CRL at cFTS[15]. These data are transferred to the DFMD server daily. The DFMD also receives and merges pregnancy outcome data from the Danish Cytogenetic Central Register (prenatal, termination and postnatal chromosomal analyses), the Danish National Patient Register (miscarriages and terminations) and the Danish National Birth Register (information about pregnancy complications, deliveries and newborns) by using a unique personal identification number (CPR-number).

We identified additional data on fertility treatment by cross-linkage with the Danish IVF-Register, including *in-vitro* fertilization/intracytoplasmic sperm injection and intrauterine insemination. Data on maternal characteristics such as previous miscarriage, previous stillbirth/intrauterine death (from 22 weeks until delivery), previous preterm birth (between 22 and 33 + 6 weeks of gestation) and parity were obtained by cross-linkage to the Danish National Birth Register.

In Denmark, the two major indications for invasive testing are cFTS risk > 1:300 at the time of testing (based on maternal age, NT thickness and the biomarkers PAPP-A and free β-hCG) and presence of structural anomalies. Other indications include family history of genetic disease or known chromosomal structural rearrangement, but these women are normally offered CVS before the NT scan and thus were not included in the study population.

CVS and AC procedures were performed mainly by fetal medicine experts; fewer than 10% were carried out by trainees under the supervision of a fetal medicine expert. The standard procedure at all departments in Denmark uses an ultrasound-guided transabdominal approach using a guide needle. For CVS, a double-needle technique with an 18-G guide needle and a 20-G blunt aspiration needle is used; for AC, a 20-G needle is used in all cases. Since AC is often performed when intrauterine death has been diagnosed, all patient files of cases with fetal loss within 7 days after AC were assessed to determine fetal viability at the time of the procedure. The group of normal karyotypes included: 46,XX, 46,XY, tetraploidy and the common pericentric inversion on chromosome 9.

Pregnancy outcome was classified as: live birth, miscarriage (fetal loss before 22 weeks of gestation), stillbirth (fetal loss at or after 22 weeks of gestation) and termination. Cases in which the parents emigrated and those with unknown outcome were included until the point of last contact.

The primary endpoint of the study was the risk of miscarriage and of stillbirth associated with CVS or AC. Analyses were repeated at different time intervals following cFTS and included all women who were still pregnant at the time.

Statistical analysis

Maternal and pregnancy characteristics at cFTS were grouped according to NT thickness (increased NT: ≥ 95th percentile; normal NT: < 95th percentile) and compared using Pearson's chi-square test for categorical variables and *t*-tests and Wilcoxon's rank sum tests for continuous variables, as appropriate. The level of significance was set at 5%.

The procedure-related risks of fetal loss were assessed by a dynamic propensity score stratification approach. The propensity score approach allows one to design and analyze an observational (non-randomized) study so that it mimics some of the particular characteristics of a randomized controlled trial. This statistical method estimates the effect of intervention by accounting for the individualized probabilities of receiving treatment. In this study, the propensity score is the probability of receiving an invasive test until the time-point of analysis, conditional on the baseline characteristics at cFTS. By conditioning on the propensity score, the distribution of observed baseline characteristics would be similar between women exposed to CVS or AC and those unexposed until the time-point of analysis[16–19].

The initial analysis to assess the risk of fetal loss used the data available 3 days after cFTS. Women who were still pregnant 3 days after cFTS were divided into two

groups: those who had CVS within 3 days after cFTS and those who did not have CVS within 3 days after cFTS. To avoid selection bias, the two groups were further subdivided into strata such that the propensity to have CVS within 3 days and to still be at risk of fetal loss 3 days after cFTS was approximately equal within strata. The propensity score was based on logistic regression and included the following predictors available at cFTS: NT (mm), PAPP-A (MoM) and free β-hCG (MoM), maternal age and gestational age at cFTS. The models allowed for a flexible functional relationship between the propensity score and the predictors[20]. Analyses were repeated at analysis time-points 3–21 days after cFTS for CVS and 28–42 days after cFTS for AC. The analyses for AC included only AC performed up to 42 days after cFTS in order to evaluate the risk of fetal loss for AC performed for indications more comparable to those for CVS i.e. AC performed before the 18–20-week anomaly scan.

Risk differences between the groups were reported as Mantel–Haenszel weighted averages across propensity score strata with 95% CIs[21].

RESULTS

We identified 147 987 singleton pregnancies with a live fetus at cFTS who fulfilled the entry criteria; 105 with a CVS performed before the cFTS and 25 with invalid levels of biomarkers were not included. The mean gestational age at entry to the study (gestational age at cFTS) was 88.9 (interquartile range (IQR), 86–92) days. The mean maternal age was 29.9 (range, 14–54; IQR, 27–33) years, and 45.8% of the women for whom parity was known were nulliparous and 54.2% were parous. The maternal and pregnancy characteristics at cFTS are summarized in Table 1.

The numbers of women, invasive tests and fetal losses included at five specific analysis time-points are detailed in Figure 1.

Pregnancy outcome included: 144 429 (97.60%) live births, 820 (0.55%) miscarriages and 452 (0.31%) stillbirths, 1163 (0.79%) terminations and six non-obstetric maternal deaths (Figure 1). Outcome of pregnancy was unknown in 1117 cases (0.75%), of which 854 were because of emigration. Gestational age at miscarriage is shown in Figure S1.

The population included 890 (0.6%) pregnancies with abnormal karyotype identified pre- or postnatally. Among the 717 chromosomal abnormalities identified prenatally there were 336 (46.86%) cases of trisomy 21, 53 (7.39%) of trisomy 13, 95 (13.25%) of trisomy 18, 47 (6.56%) with karyotype 45,X, 38 (5.30%) with other sex chromosome abnormalities and 148 (20.64%) with other chromosomal abnormalities. Among the 173 chromosomal abnormalities identified postnatally, there were 45 (26.01%) cases of trisomy 21, one (0.58%) of trisomy 13, five (2.89%) of trisomy 18, eight (4.62%) with karyotype 45,X, 21 (12.14%) with other sex chromosome abnormalities and 93 (53.76%) with other chromosomal abnormalities.

Invasive procedures were performed in a total of 6881 pregnancies (4.7%), including 5072 (3.4%) CVS and 1809 (1.2%) AC. The median gestational ages for the performance of CVS and AC were 92 (IQR, 89–96) days and 117 (IQR, 111–140) days, respectively. More than half of the CVS procedures were performed within 3 days (2900/5072) and 91.3% (4629/5072) within 7 days after cFTS. As regards AC, 46.4% (840/1809) were performed within 28 days and 60.6% (1097/1809) within 42 days after cFTS (Figure S2).

Risk of fetal loss associated with CVS

The analyses for the effect of CVS on the risk of miscarriage are summarized in Figure 2a. Women who underwent CVS ('exposed') were compared with women who did not ('unexposed') at analysis time-points between 3 and 21 days after cFTS; the figure shows the average risk differences across the propensity score strata. There was no significant difference in risk of miscarriage when comparing women exposed to CVS with unexposed women, independent of analysis time-point. The average effect of CVS on risk of miscarriage was −0.08% (95% CI, −0.64; 0.47) for the day-3 analysis time-point and did not become statistically significant at any later time-points, being −0.41% (95% CI, −0.86; 0.05) at 7 days, −0.40% (95% CI, −0.80; 0.01) at 14 days and −0.21% (95% CI, −0.58; 0.15) at 21 days after cFTS. Likewise, the analyses for the effect of CVS on the risk of stillbirth (Figure 2b) showed no significant difference in risk of stillbirth for women exposed to CVS compared with unexposed women, independent of the analysis time-point, the average effect of CVS on risk of stillbirth ranging from −0.18% (95% CI, −0.50; 0.13) at 3 days to −0.27% (95% CI, −0.58; 0.04) at 21 days after cFTS. The effect of CVS on the probability of termination showed a significantly higher risk for women exposed to CVS, with an average risk difference of 8.66% (95% CI, 7.21; 10.10) for the day-3 analysis time-point, decreasing to 0.99% (95% CI, 0.43; 1.55) for the latest analysis time-point, at 21 days (Table S1).

Risk of fetal loss associated with AC

The results of the analyses for the effect of AC on the risk of miscarriage are summarized in Figure 3a. Women who underwent AC (exposed) were compared with those who did not (unexposed) at analysis time-points between 28 and 42 days after cFTS; the risk differences across the propensity score strata are shown in the figure. There was no significant effect of AC on the risk of miscarriage independent of analysis time-point. The average risk differences for analysis time-points at 28, 35 and 42 days were 0.56% (95% CI, −0.21; 1.33), 0.42% (95% CI, −0.22; 1.05) and 0.52% (95% CI, −0.06; 1.10), respectively. The analyses for the effect of AC on the risk of stillbirth (Figure 3b) showed no significant difference in risk of stillbirth for women exposed to AC compared with unexposed women independent of the analysis time-point up to 42 days after cFTS, the average risk

Table 1 Maternal and pregnancy characteristics and first-trimester markers at combined first-trimester screening according to nuchal translucency thickness (NT) in 147 987 singleton pregnancies

	NT ≥ 95th percentile (n = 3833)	*NT < 95th percentile (n = 144 154)*	P
Maternal age			< 0.0001
< 25 years	657 (17.1)	26 497 (18.4)	
25–35 years	2546 (66.4)	99 390 (68.9)	
35–40 years	528 (13.8)	16 470 (11.4)	
> 40 years	102 (2.7)	1797 (1.2)	
Body mass index*			0.11
< 18.5 kg/m^2	2585 (67.4)	96 693 (67.1)	
18.5–24.9 kg/m^2	213 (5.6)	8793 (6.1)	
25–29.9 kg/m^2	686 (17.9)	24 458 (17.0)	
≥ 30 kg/m^2	349 (9.1)	14 210 (9.9)	
Ethnicity			0.049
Afro-Caribbean	24 (0.6)	1147 (0.8)	
Asian	79 (2.1)	2936 (2.1)	
Caucasian	3542 (93.8)	134 296 (94.1)	
Mixed	70 (1.9)	2694 (1.9)	
Oriental	63 (1.7)	1653 (1.2)	
Unknown	55	1428	
Smoking status			< 0.0001
No	3237 (85.0)	124 537 (86.8)	
Quit	81 (2.1)	3600 (2.5)	
Yes	490 (12.9)	15 263 (10.6)	
Unknown	25	754	
Parity			0.030
Parous	1972 (52.4)	76 286 (54.2)	
Nulliparous	1789 (47.6)	64 366 (45.8)	
Unknown	72	3502	
Obstetric history			
Previous early miscarriage ≤ 16 weeks			0.12
No	3206 (83.6)	121 935 (84.6)	
Yes	627 (16.4)	22 219 (15.4)	
Previous late miscarriage > 16 weeks			0.38
No	3818 (99.6)	143 427 (99.5)	
Yes	15 (0.4)	727 (0.5)	
Previous preterm birth ≤ 34 weeks			0.21
No	3802 (99.2)	142 672 (99.0)	
Yes	31 (0.8)	1482 (1.0)	
Previous stillbirth ≥ 22 weeks			0.23
No	3803 (99.2)	143 269 (99.4)	
Yes	30 (0.8)	885 (0.6)	
Method of conception			0.15
Spontaneous	3414 (93.7)	129 294 (94.2)	
IVF/ICSI	152 (4.2)	4892 (3.6)	
IUI	79 (2.2)	3043 (2.2)	
Unknown	188	6925	
Biomarkers			
PAPP-A MoM			< 0.0001
< 5th percentile	333 (8.7)	7067 (4.9)	
5–95th percentile	3313 (86.4)	129 874 (90.1)	
≥ 95th percentile	187 (4.9)	7213 (5.0)	
β-hCG MoM			< 0.0001
< 5th percentile	278 (7.3)	7122 (4.9)	
5–95th percentile	3362 (87.7)	129 825 (90.1)	
≥ 95th percentile	193 (5.0)	7207 (5.0)	

Increased NT defined as ≥ 95th percentile and normal NT as < 95th percentile. *Missing body mass index values were imputed as 'normal' (BMI, 18.5–24.9 kg/m^2). β-hCG, beta-human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, *in-vitro* fertilization; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A.

difference of stillbirth being −0.26% (95% CI, −0.63; 0.12) at 28 days and 0.09% (95% CI, −0.39; 0.58) at 42 days after cFTS. The probability for termination was significantly higher for women exposed to AC compared with those unexposed, with an average risk difference of 4.03% (95% CI, 2.27; 5.49) for the 28-day analysis time-point, decreasing to 2.36% (95% CI, 1.37; 3.34) for the latest analysis time-point at 42 days.

Table S1 gives corresponding risk estimates for total fetal loss.

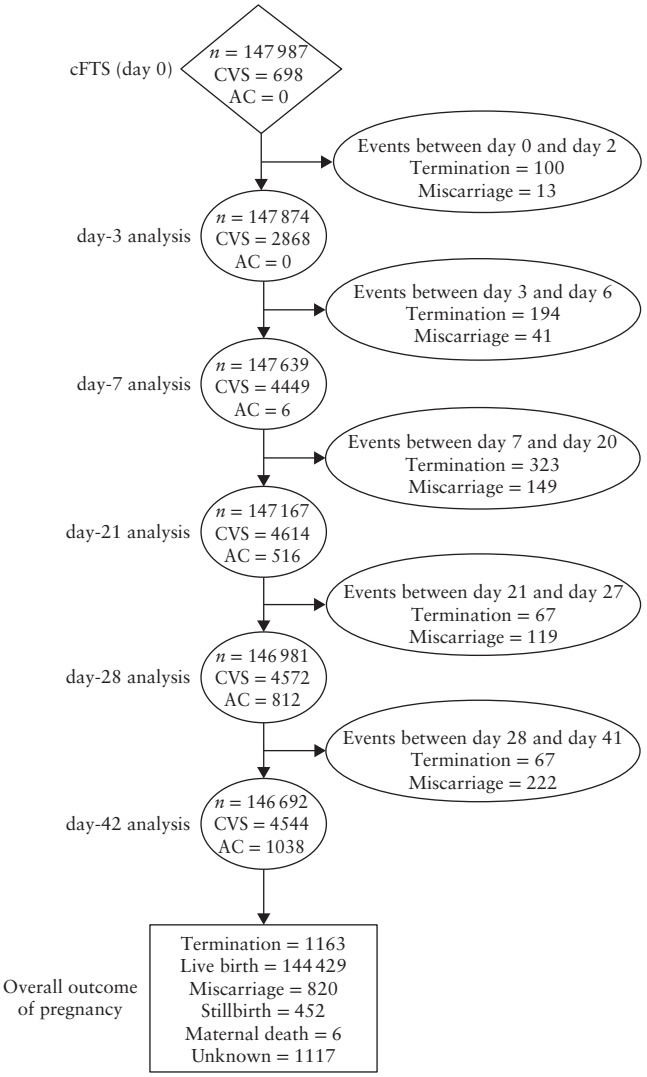

Figure 1 Flowchart showing numbers of singleton pregnancies which underwent combined first-trimester screening (cFTS) included in the different time-point analyses. Circles show numbers of pregnant women at that time-point and numbers of chorionic villus sampling (CVS) and amniocentesis (AC) procedures performed up to the time-point. Ellipsoids show fetal losses between adjacent time-points. Overall outcome of pregnancy presents all events including those occurring after analysis time-point at day 42.

DISCUSSION

This large national registry-based study estimated prospectively the effect of CVS and AC on the risk of miscarriage and stillbirth by propensity score stratification including information from cFTS. Using this method allowed a more direct comparison of the pregnancy outcomes of women who had the same propensity of having the invasive test in a similar way to that in a randomized trial.

This study demonstrated no significant difference in the risk of miscarriage or stillbirth for women who underwent CVS compared with those who did not, independent of analysis time-point after cFTS. Similarly, we found no significant difference in the risk of miscarriage or stillbirth following AC.

Our results are in accordance with a study of 33 310 singleton pregnancies[22] by The Fetal Medicine Foundation, including 2393 cases undergoing CVS, which, after adjusting for maternal characteristics and first-trimester markers, used a retrospective case–control design to show that CVS was not associated with elevated risk of miscarriage or stillbirth. Likewise, our findings are consistent with a recently published meta-analysis[6] which reported the procedure-related risk of fetal loss before 24 weeks to be as low as 0.2% for CVS and 0.1% for AC. To minimize heterogeneity, only observational studies since the year 2000 were included and controls were matched for gestational age, while cFTS markers were not taken into account.

Another meta-analysis by Mujezinovic and Alfirevic[23] showed a pooled relative risk for fetal loss before 28 weeks of 1.46 (95% CI, 0.86–2.49) for AC. This result is more comparable with findings of previous randomized trials[1,24]; nevertheless, the authors underlined that the procedure-related risk of fetal loss was difficult to estimate due to heterogeneity and a lack of comparable controls to estimate the background risk. The studies that were included in the review have limited value for counseling due to their retrospective study design.

While cases with aneuploidy and major malformation were excluded from the meta-analyses[6,23], we focused on the knowledge available at the time of the cFTS to counsel the women, and therefore did not find it appropriate to exclude cases of aneuploidy. This is in line with the randomized trials[1,24] which did not exclude pregnancies with abnormal karyotypes that continued the pregnancy or were diagnosed postnatally.

Likewise, we only evaluated the effect of AC performed up to 42 days after cFTS, since AC performed later in pregnancy would be more likely to have been carried out due to findings at the anomaly scan, i.e. due to fetal anomaly or intrauterine growth restriction rather than a high risk at cFTS. Knowledge about these abnormal findings is not available at the time of cFTS and our propensity score approach would thus be biased due to residual confounding.

Our national study population showed a slightly lower risk of overall total fetal loss of 0.86% (0.55% miscarriages and 0.31% stillbirths) when compared with more selected cohorts such as in the FASTER trial[7], a multicenter study of 36 104 singleton pregnancies (0.9% miscarriages and 0.29% stillbirths), as well as the study from The Fetal Medicine Foundation[22] (1.2% miscarriages and 0.43% stillbirths). In comparison, this single-center study had a higher mean maternal age (31.7 compared with our value of 29.9 years) and a higher proportion of women of Afro-Caribbean origin.

Within the limitations of an observational study design, our findings are valuable for assessing the causal effect of invasive testing in order to improve counseling at cFTS. It is a strength of the study that the propensity stratification score approach[16–19] made it possible to include information on potential confounding variables known at the time of cFTS[7–9] and thereby allowed us to

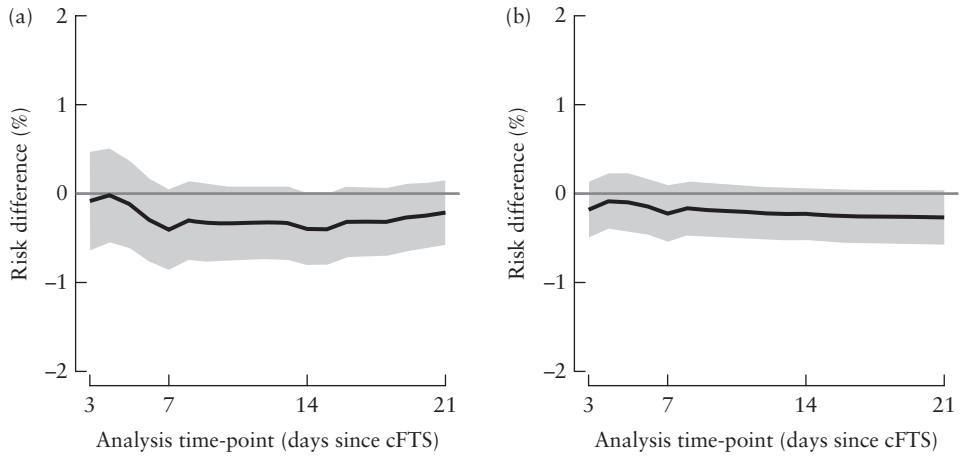

Figure 2 Effect of chorionic villus sampling (CVS) on risk of miscarriage (a) and risk of stillbirth (b) from analysis time-points 3–21 days after combined first-trimester screening (cFTS), showing average risk differences across propensity score strata.

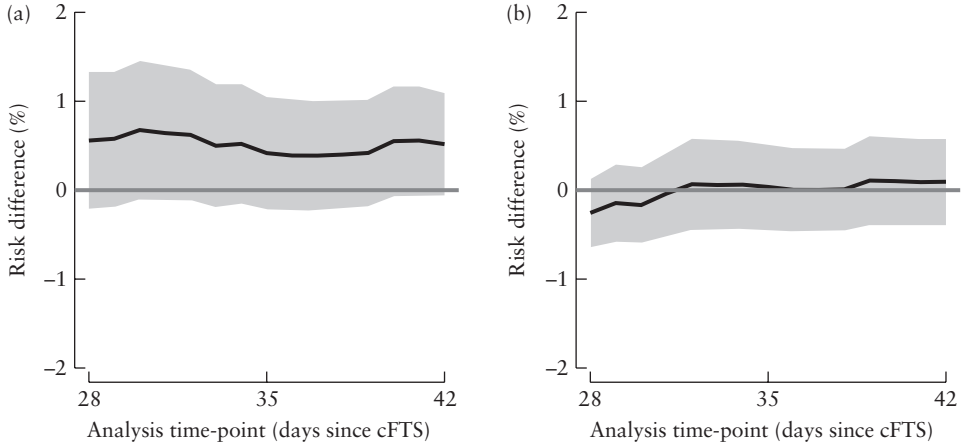

Figure 3 Effect of amniocentesis (AC) on risk of miscarriage (a) and risk of stillbirth (b) from analysis time-points 28–42 days after combined first-trimester screening (cFTS), showing average risk differences across propensity score strata.

establish comparable propensity strata for women having and not having a CVS or AC to evaluate the risk difference of fetal loss. This is of particular importance because the results of retrospective studies have been impeded by the lack of comparable control groups to estimate the background risk of fetal loss. Furthermore, the risk differences were evaluated over a series of time-points up to 3 and 6 weeks after cFTS for CVS and AC, respectively, to take into account the changes in study population over time (Figure 1).

In the present study, the invasive procedures were carried out mainly by fetal medicine experts. The risk of fetal loss following invasive procedures has been shown to be correlated inversely to the skill and experience of the operator[25–27]. The new cell-free fetal DNA techniques are likely to further reduce the number of invasive procedures and this decrease will have implications not only for training, but also for maintaining skills and expertise[4,6]. Even in a small country such as Denmark, we will have to consider centralizing invasive procedures in order to allow a smaller number of operators in a smaller number of centers to maintain competence and expertise, with the aim of optimizing patient safety.

In conclusion, in our large national cohort, neither CVS nor AC was found to have any significant effect on the risk of miscarriage or stillbirth. The findings of this study indicate that the procedure-related risk of CVS and AC is very low. This must be taken into consideration when counseling women at cFTS.

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SUPPORTING INFORMATION ON THE INTERNET

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

Figure S1 Frequency of miscarriage according to gestational age.
Figure S2 Timing and numbers of chorionic villus samplings and amniocenteses performed after combined first-trimester screening.
Table S1 Risk difference of miscarriage, stillbirth, total fetal loss and termination associated with chorionic villus sampling and amniocentesis according to time since combined first-trimester screening

