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Prediction of miscarriage and stillbirth at 11–13 weeks and the contribution of chorionic villus sampling

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Objectives To derive models for estimating risk of miscarriage and stillbirth from maternal characteristics and findings of first-trimester screening for aneuploidies and to define the procedure-related risk of chorionic villus sampling (CVS) after adjusting for these factors.

Method We examined 33 856 singleton pregnancies at 11^{+0} to 13^{+6} weeks, and in 2396 CVS was carried out. Logistic regression analysis was used to examine the factors contributing to miscarriage and stillbirth.

Results There were 33 310 (98.4%) livebirths, 404 (1.2%) miscarriages and 142 (0.4%) stillbirths. Models combining maternal characteristics, nuchal translucency, pregnancy-associated plasma protein-A (PAPP-A) and flow in the ductus venosus detected 36.9% of miscarriages and 35.2% of stillbirths, at a 10% false-positive rate. The risk of miscarriage and stillbirth increased with maternal age and weight, in women of African racial origin, in those with previous miscarriages or stillbirths and in those with low serum PAPP-A and reversed A-wave in the ductus venosus. The risk of miscarriage increased in women with pre-existing diabetes mellitus, in those conceiving on ovulation-induction drugs and in those with high fetal nuchal translucency, and the risk of stillbirth increased in women with chronic hypertension and in cigarette smokers. The expected number of miscarriages and stillbirths in the CVS group and the models derived from the non-CVS group were 45 (95% prediction intervals 32–58) and 18 (95% prediction intervals 9–26), respectively. These expected numbers were not significantly different from the observed 44 and 15 cases ($p = 0.881$ and 0.480), respectively.

Conclusion A high proportion of fetal losses can be predicted at 11 to 13 weeks. A model for such predictions can be used to assess the procedure-related risks from CVS. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

KEY WORDS: first-trimester screening; chorionic villus sampling; miscarriage; stillbirth; nuchal translucency; ductus venosus; pregnancy-associated plasma protein-A

INTRODUCTION

Increased risk of miscarriage of pregnancy before 24 weeks of gestation and stillbirth at or after 24 weeks are associated with certain maternal characteristics, including increasing maternal age and maternal weight, previous miscarriage and African racial origin (Spencer *et al*., 2006; Smith and Fretts 2007; Willinger *et al*., 2009). These pregnancy complications are also associated with abnormal results of first-trimester screening for aneuploidies, including increased fetal nuchal translucency (NT) thickness, reversed A-wave in the fetal ductus venosus and low maternal serum pregnancy-associated plasma protein-A (PAPP-A) (Souka *et al*., 2001, 2005; Spencer *et al*., 2006; Bilardo *et al*., 2007; Smith *et al*., 2007; Dugoff *et al*., 2008; Maiz *et al*., 2008).

Studies investigating the procedure-related risk of miscarriage and stillbirth associated with chorionic villus sampling (CVS) have essentially compared these complications in pregnancies undergoing first-trimester CVS with those having first- or second-trimester amniocentesis and reported that the risks with transabdominal CVS are similar to those of second-trimester amniocentesis and lower than with first-trimester amniocentesis (Nicolaides *et al*., 1994; Sundberg *et al*., 1997; Tabor and Alfirevic, 2010). A randomized trial reported that the fetal loss rate in patients having second-trimester amniocentesis was 1% higher than in controls who did not have an invasive test, and it is therefore assumed that the risk of fetal loss from transabdominal CVS is also 1% (Tabor *et al*., 1986).

The procedure-related risk of miscarriage and stillbirth associated with CVS could be derived by comparing pregnancy outcome in women undergoing CVS with those who do not have an invasive test. However, the CVS-related risks derived from such comparisons are likely to be overestimated because the same components of screening leading to increased risk for chromosomal defects and therefore the uptake of CVS, such as high

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fetal NT, reversed A-wave in the fetal ductus venosus and decreased serum PAPP-A, are also associated with increased risk for miscarriage and stillbirth. The ideal method of deriving the procedure-related risk of miscarriage and stillbirth associated with CVS would be randomized studies comparing pregnancies undergoing CVS with controls who have not undergone an invasive test. However, such studies are unlikely to be acceptable to pregnant women.

The aims of our study are firstly to derive a method for estimating the risk of miscarriage and stillbirth from maternal characteristics and the findings of first-trimester screening for aneuploidies and secondly to define the procedure-related risk of CVS after adjusting for such maternal and pregnancy characteristics.

METHODS

Screening study population

The data for this study were derived from a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy. In this visit, which is held at 11^{+0} to 13^{+6} weeks of gestation, we record maternal characteristics and medical history, measure their weight and height and perform an ultrasound scan to firstly determine gestational age from the fetal crown–rump length, and secondly, diagnose any major fetal abnormalities and thirdly, measure fetal NT thickness as part of screening for chromosomal abnormalities (Robinson and Fleming 1975; Snijders *et al*., 1998). In addition, the maternal serum PAPP-A and free β-human chorionic gonadotrophin (free β-hCG) are determined and the results are combined with the fetal NT to calculate the patient-specific risk for trisomy 21 (Kagan *et al*., 2008). In addition, we perform transabdominal Doppler assessment of the flow in the fetal ductus venosus as recommended by the Fetal Medicine Foundation (Maiz *et al*., 2009). Waveforms are assessed qualitatively and considered to be abnormal if the A-wave is reversed. Women choosing to have CVS are assessed by a fetal medicine expert who then decides if the procedure should be performed by an expert or a trainee in fetal medicine under the direct supervision by an expert. Trainees perform a total of 100 such procedures. More than 99% of procedures are carried out transabdominally.

Women were asked to complete a questionnaire on age, racial origin (Caucasian, African, South Asian, East Asian or Mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous or assisted by ovulation drugs), parity (nulliparous with no previous pregnancies, nulliparous with previous miscarriage before 16 weeks, parous or nulliparous with previous miscarriage at $16–23^{+6}$ weeks, parous with no previous stillbirths at or after 24 weeks, parous with previous stillbirths at or after 24 weeks). The questionnaire was then reviewed by a doctor together with the woman.

Details of maternal characteristics and the findings of the 11 to 13 weeks assessment were recorded in our 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 our database. The entry criteria for this study were singleton pregnancies with live fetus at 11^{+0} to 13^{+6} weeks and first-trimester combined screening for aneuploidies. We excluded pregnancies with known major defects and chromosomal or genetic abnormalities diagnosed prenatally or postnatally, those resulting in termination and those with no known outcome.

Statistical analysis

The patients were subdivided into three groups according to pregnancy outcome: miscarriage, stillbirth and live birth. Comparison of the maternal and fetal characteristics in the three outcome groups was by the χ^2-square test and Fisher exact test for categorical variables and Mann–Whitney U-test for continuous variables, respectively.

Logistic regression analysis was used to develop a model for prediction of miscarriage and stillbirth derived from factors in the maternal history and characteristics, the components of first-trimester screening and CVS. Firstly, we performed univariate analysis to examine the independent contribution of individual predictor variables with the occurrence of miscarriage and stillbirth by assessing their odds ratios (ORs) and 95% confidence intervals (CIs). Secondly, we performed multivariate logistic regression analysis with backward stepwise elimination to develop the model for prediction. Prior to fitting the regression model, we assessed whether in case of continuous variables such as maternal age, weight and height, fetal NT, serum PAPP-A and free ß-hCG, the association with miscarriage and stillbirth was linear or nonlinear. If the association was found to be nonlinear, then the variable was transformed by truncation of values to approach linearity. Thirdly, to assess the predictive accuracy of our model, we calculated the shrinkage factor using the equation $[\chi^2 - (df - 1)]/\chi^2$ where χ^2 is the model chi-square derived from the -2 log likelihood statistic in the multivariate logistic regression analysis. This shrinkage factor was then applied to all the parameters in the model. Finally, the patient-specific risks for miscarriage and stillbirth were calculated from the formula: odds/(1 + odds), where odds $= e^Y$ and Y was derived from the respective logistic regression analysis. The distribution of risks was then used to calculate detection and false-positive rates (FPR) at different risk cut-offs and the performance of screening was determined by receiver operating characteristic (ROC) curves analysis.

The procedure-related risk of miscarriage and stillbirth was investigated in two ways. Firstly, CVS was included as one of the variables in the logistic regression analysis to determine if this had a significant contribution in the prediction of miscarriage or stillbirth. Secondly, logistic regression models for miscarriage and stillbirth were developed from the study of patients who did not have CVS. These models were then applied in the patients who had CVS to derive the expected number

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of miscarriages and stillbirths with 95% prediction intervals. The significance of difference between the observed and expected rates was determined by the χ^2 test.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and Medcalc for windows, version 9.6.2.0 (MedCalc Software, Mariakerke, Belgium) were used for data analyses.

RESULTS

Study population

During the study period (March 2006–September 2009) first-trimester combined screening for aneuploidies was carried out in 36 743 singleton pregnancies. We excluded 2887 cases because they had missing outcome data ($n = 2005$), the pregnancies resulted in termination or the birth of babies with major defects ($n = 726$) or they had amniocentesis ($n = 156$).

The 33 856 singleton pregnancies fulfilling the entry criteria included 31 460 (92.9%) that did not have CVS and 2396 (7.1%) who had CVS. The procedure of CVS was carried out by either one of 15 fetal medicine experts ($n = 546$) or one of 48 trainees under the direct supervision by an expert ($n = 1850$). In the 33 856 cases there were 33 310 (98.4%) livebirths, 404 (1.2%) miscarriages and 142 (0.4%) stillbirths. The maternal and pregnancy characteristics of the three outcome groups are compared in Table 1. The characteristics of the CVS and non-CVS groups are compared in Table S1, Supporting information.

Prediction of miscarriage and stillbirth in the screened population

The results of the univariate and multivariate regression analysis for the prediction of miscarriage and stillbirth in the 33 856 pregnancies are shown in Tables 2 and 3, respectively. The shrinkage coefficients for the models of miscarriage and stillbirth were 0.97 and 0.91, respectively, and all parameters in the model were adjusted accordingly.

The risk of miscarriage increased with maternal weight and with maternal age, above and below 28 years (J-shaped relation); it was higher in women of African and mixed racial origin, in women with pre-existing diabetes mellitus, in those with previous miscarriages or stillbirths, in those conceiving on ovulation-induction drugs and in those with high fetal NT, low serum PAPP-A and reversed A-wave in the ductus venosus (Table 2). The risk of stillbirth increased with maternal age and weight and decreased with height, it was

Table 1—Maternal and pregnancy characteristics in the screening population according to pregnancy outcome

Maternal and pregnancy characteristics	Live birth ($n = 33\,310$)	Miscarriage ($n = 404$)	Stillbirth ($n = 142$)
Maternal age in years, median (IQR)	32.3 (27.9–36.0)	32.7 (27.6–36.9)	33.2 (27.0–36.9)
Maternal weight in kg, median (IQR)	65.7 (59.0–75.0)	70.0 (60.0–83.0)*	71.0 (60.0–83.3)*
Racial origin			
Caucasian, *n* (%)	23 967 (72.0)	199 (49.3)	81 (57.0)
African, *n* (%)	6271 (18.8)	170 (42.1)*	48 (33.8)*
South Asian, *n* (%)	1458 (4.4)	14 (3.5)	6 (4.2)
East Asian, *n* (%)	654 (2.0)	5 (1.2)	2 (1.4)
Mixed, *n* (%)	960 (2.9)	16 (4.0)	5 (3.5)
History of pre-existing diabetes, *n* (%)	254 (0.8)	10 (2.5)	3 (2.1)
History of chronic hypertension, *n* (%)	374 (1.1)	10 (2.5)	8 (5.6)
Parity			
Nulliparous (reference)	10 749 (32.3)	93 (23.0)	48 (33.8)
Nulliparous with previous miscarriage <16 weeks	5156 (15.5)	83 (20.5)*	28 (19.7)
Nulliparous with previous miscarriage 16–23 weeks	383 (1.1)	36 (8.9)*	1 (0.7)
Parous with previous stillbirth	264 (0.8)	11 (2.7)*	3 (2.1)
Parous with no previous stillbirths	16 758 (50.3)	181 (44.8)	62 (43.7)
Cigarette smoker, *n* (%)	2711 (8.1)	37 (9.2)	18 (12.7)
History of substance abuse, *n* (%)	230 (0.7)	6 (1.5)	1 (0.7)
History of alcohol abuse, *n* (%)	333 (1.0)	2 (0.5)	2 (1.4)
Conception			
Spontaneous, *n* (%)	32 062 (96.3)	363 (89.9)	132 (93.0)
Ovulation-induction drugs, *n* (%)	1248 (3.7)	41 (10.1)*	10 (7.0)
Crown–rump length in mm, median (IQR)	64.0 (59.2–69.5)	63.9 (58.5–69.0)	64.0 (59.2–71.4)
Fetal delta nuchal translucency in mm, median (IQR)	0.12 (−0.08–0.34)	0.09 (−0.12–0.32)	0.13 (−0.05–0.38)
Maternal serum PAPP-A MoM, median (IQR)	1.02 (0.70–1.44)	0.91 (0.59–1.43)*	0.88 (0.57–1.29)*
Maternal serum-free β-hCG MoM, median (IQR)	1.00 (0.68–1.51)	0.98 (0.67–1.50)	0.90 (0.64–1.57)
Reversed A-wave in ductus venosus, *n* (%)	864 (2.6)	33 (8.2)*	11 (7.7)*

χ^2 test for categorical variables and Mann–Whitney *U*-test with *post hoc* Bonferroni correction for continuous variables): adjusted significance level * $p < 0.025$.

IQR, inter-quartile range; OR, odds ratio, CI, confidence interval, PAPP-A, pregnancy-associated plasma protein-A; β-hCG, β-human chorionic gonadotrophin.

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Table 2—Univariate and multivariate logistic regression analysis for the prediction of miscarriage by factors in maternal history, pregnancy characteristics and compenents of first-trimester screening in the total screening population

	Univariate analysis			Multivariate analysis[a]		
Variables	OR	95% CI	*p*	OR	95% CI	*p*
Age (per year)	0.796	0.704–0.900	<0.0001	0.870	0.766–0.988	0.032
Age^2	1.004	1.002–1.006	<0.0001	1.002	1.000–1.005	0.016
Weight (per kg)	1.021	1.015–1.026	<0.0001	1.011	1.005–1.017	0.001
Height (per cm)	1.000	0.986–1.015	0.966	—	—	—
Racial origin			<0.0001			
Caucasian (reference)						
African	3.265	2.265–4.014	<0.0001	2.963	2.379–3.690	<0.0001
South Asian	1.156	0.671–1.993	0.601	—	—	—
East Asian	0.921	0.378–2.244	0.921	—	—	—
Mixed	2.007	1.201–3.354	0.008	2.199	1.309–3.693	0.003
History of pre-existing diabetes mellitus	3.303	1.742–6.262	<0.0001	2.081	1.066–4.065	0.032
History of chronic hypertension	2.235	1.183–4.221	0.013	—	—	—
Parity			<0.0001			
Nulliparous (reference)						
Nulliparous with previous miscarriage <16 weeks	1.861	1.381–2.506	<0.0001	1.632	1.271–2.096	<0.0001
Nulliparous with previous miscarriage 16–23 weeks	10.864	7.297–16.175	<0.0001	6.361	4.369–9.262	<0.0001
Parous with previous stillbirth	4.816	2.548–9.103	<0.0001	2.954	1.570–5.557	0.001
Parous with no previous stillbirths	1.248	0.971–1.605	0.084	—	—	—
Use of ovulation-induction drugs	2.902	2.091–4.027	<0.0001	2.954	2.093–4.171	<0.0001
Cigarette smoking	1.138	0.810–1.599	0.457	—	—	—
History of substance abuse	2.168	0.958–4.906	0.063	—	—	—
History of alcohol abuse	0.493	0.122–1.985	0.319	—	—	—
Delta nuchal translucency	1.696	1.437–2.001	<0.0001	1.778	1.496–2.114	<0.0001
Reversed flow in A-wave of ductus venosus	3.340	2.325–4.799	<0.0001	2.208	1.508–3.232	<0.0001
Log_{10} PAPP-A MoM	0.343	0.223–0.530	<0.0001	0.356	0.233–0.543	<0.0001
Log_{10} free β-hCG MoM	0.945	0.650–1.374	0.767	—	—	—
Chorionic villus sampling	1.620	1.181–2.221	0.003	—	—	—

OR, odds ratio, CI, confidence interval, PAPP-A, pregnancy-associated plasma protein-A; β-hCG, β-human chorionic gonadotrophin.
[a] Only the OR found significant at multivariate analyses are reported.

higher in women of African racial origin, in women with chronic hypertension and in cigarette smokers and lower in parous women without previous stillbirth and the risk was increased in those with low serum PAPP-A and reversed A-wave in the ductus venosus (Table 3). The association between fetal NT and serum PAPP-A with fetal miscarriage and stillbirth was not linear but linearity was approached by truncation of values at 1.2 for delta NT (the risks increased for higher values of NT) and 1.5 MoM for PAPP-A (the risks increased for lower values of PAPP-A).

The performance of screening for miscarriage and stillbirth is shown in Table 4. At a 10% FPR the detection rate of miscarriage in screening by maternal factors was 34.2% and this increased to 36.9% by the addition of the results of first trimester screening for aneuploidies. The respective values in screening for stillbirth were 28.9 and 35.2%, respectively.

The stillbirths were subdivided firstly, according to the gestation of death into less than 34 weeks ($n = 74$) and at or greater than 34 weeks ($n = 68$) and secondly, according to whether the birth weight was below ($n = 55$) or at or above ($n = 87$) the 10th percentile for gestation (Poon *et al.*, 2011). At an FRP of 10% the detection rate for stillbirths before 34 weeks was 44.6% and for those at or after weeks it was 25.0%. The detection rate for stillbirths with birth weight below the

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Table 3—Univariate and multivariate logistic regression analysis for the prediction of stillbirth by factors in maternal history, pregnancy characteristics and compenents of first-trimester screening in the total screening population

	Univariate analysis			Multivariate analysis[a]		
Variables	OR	95% CI	*p*	OR	95% CI	*p*
Age (per year)	1.026	0.988–1.066	0.187	1.041	1.002–1.082	0.040
Weight (per kg)	1.021	1.011–1.031	<0.0001	1.019	1.008–1.029	<0.0001
Height (per cm)	0.973	0.949–0.997	0.025	0.962	0.937–0.987	0.003
Racial origin			<0.0001			
Caucasian (reference)						
African	2.265	1.583–3.240	<0.0001	1.962	1.356–2.841	<0.0001
South Asian	1.218	0.530–2.795	0.642	—	—	—
East Asian	0.905	0.222–3.688	0.889	—	—	—
Mixed	1.541	0.623–3.811	0.349	—	—	—
History of pre-existing diabetes mellitus	2.809	0.889–8.874	0.078	—	—	—
History of chronic hypertension	5.258	2.558–10.808	<0.0001	2.886	1.346–6.188	0.006
Parity			0.256			
Nulliparous (reference)						
Nulliparous with previous miscarriage <16 weeks	1.216	0.762–1.940	0.412	—	—	—
Nulliparous with previous miscarriage 16–23 weeks	0.585	0.080–4.247	0.596	—	—	—
Parous with previous stillbirth	2.545	0.788–8.222	0.119	—	—	—
Parous with no previous stillbirths	0.829	0.568–1.209	0.329	0.612	0.433–0.866	0.006
Use of ovulation-induction drugs	1.946	1.021–3.711	0.043	—	—	—
Cigarette smoking	1.638	0.998–2.690	0.051	1.857	1.123–3.072	0.016
History of substance abuse	1.020	0.142–7.323	0.984	—	—	—
History of alcohol abuse	1.415	0.349–5.737	0.627	—	—	—
Delta nuchal translucency	1.302	0.961–1.763	0.089	—	—	—
Reversed flow in A-wave of ductus venosus	3.153	1.698–5.856	<0.0001	2.468	1.315–4.631	0.005
Log_{10} PAPP-A MoM	0.184	0.095–0.358	<0.0001	0.213	0.111–0.409	<0.0001
Log_{10} free β-hCG MoM	0.716	0.381–1.345	0.299	—	—	—
Chorionic villus sampling	1.565	0.915–2.677	0.102	—	—	—

OR, odds ratio, CI, confidence interval, PAPP-A, pregnancy-associated plasma protein-A; β-hCG, β-human chorionic gonadotrophin.
[a] Only the OR found significant at multivariate analyses are reported.

10th percentile was 43.6% and for those at or above the 10th percentile it was 29.9%.

Miscarriage and stillbirth in the CVS group

In the CVS group, the rate of miscarriage was 1.8% (44 of 2396) which was significantly higher than that of 1.1% (360 of 31 460) in the non-CVS group ($p = 0.004$). In contrast, the rate of stillbirth in the CVS group was 0.6% (15 of 2396) which was not significantly different than the 0.4% (127 of 31 460) in the non-CVS group ($p = 0.144$).

The gestational age distribution at miscarriage or stillbirth is shown in Figure 1. The median gestation at miscarriage in the CVS group was not significantly different from that in the non-CVS group (18.3 vs 18.6 weeks, $p = 0.469$). The proportion of miscarriages before 14 weeks was 4.6% (2 of 44) in the CVS group and 9.2% (33 of 360) in the non-CVS group ($p = 0.562$).

In the logistic regression analysis for prediction of miscarriage and stillbirth there was no significant contribution from CVS after adjusting for maternal characteristics and results of first-trimester screening for aneuploidies ($p = 0.541$ and 0.846, respectively; Tables 2 and 3).

Logistic regression models for miscarriage and stillbirth in patients who did not have CVS are given in Tables S2 and S3, Supporting information. The expected number of miscarriages and stillbirths in the CVS

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Table 4—Performance of screening for all miscarriages less than 24 weeks and stillbirths at or after 24 weeks by maternal factors and components of first-trimester screening at fixed FPRs

Outcome	AUROC (95% CI)	Detection rates	
		5% FPR	10% FPR
Miscarriage			
Maternal factors	0.709 (0.682–0.736)	23.8	34.2
Maternal factors and results of screening at 11–13 weeks	0.724 (0.698–0.750)	27.2	36.9
Stillbirth			
Maternal factors	0.658 (0.611–0.706)	21.1	28.9
Maternal factors and results of screening at 11–13 weeks	0.676 (0.629–0.724)	23.2	35.2

AUROC, area under receiver operating characteristic curves; CI, confidence interval; FPR, false-positive rate.

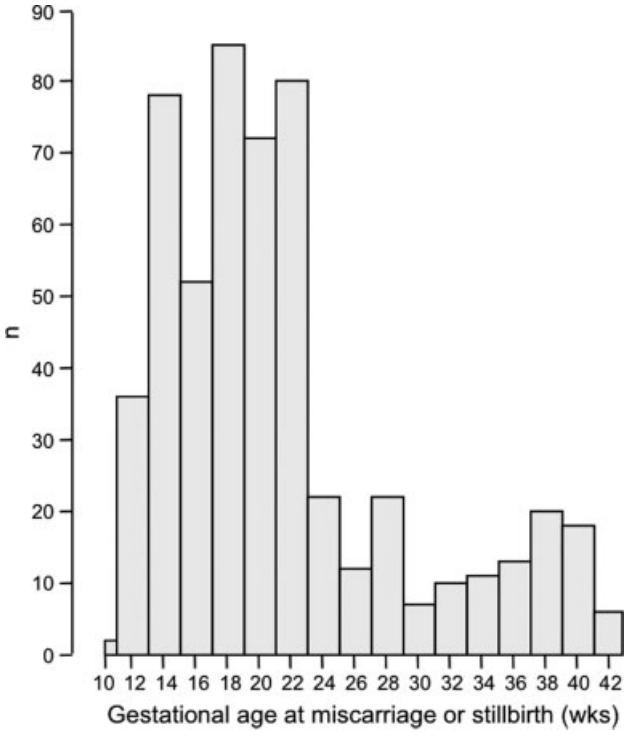

Figure 1—Gestational age at miscarriage or stillbirth

group from the models derived from the non-CVS group were 45 (95% prediction intervals 32–58) and 18 (95% prediction intervals 9–26), respectively. These expected numbers were not significantly different from the observed 44 and 15 cases ($p = 0.881$ and 0.480), respectively. In the subgroup of CVS performed by fetal medicine experts ($n = 546$), the expected number of miscarriages and stillbirths were 12 (95% prediction intervals 5–19) and 5 (95% prediction intervals 1–9), respectively, and the respective observed numbers were not significantly different ($n = 15$, $p = 0.386$ and $n = 6$, $p = 0.655$). Similarly, in the subgroup of CVS performed by trainees ($n = 1850$), the expected number of miscarriages and stillbirths were 33 (95% prediction intervals 22–44) and 13 (95% prediction intervals 6–20), respectively, and the respective observed numbers were not significantly different ($n = 29$, $p = 0.486$ and $n = 9$, $p = 0.267$).

DISCUSSION

In this study, we have developed an algorithm for the estimation of patient-specific risks for miscarriage and stillbirth based on maternal characteristics and results from first-trimester screening for aneuploidies. This algorithm can be applied in women undergoing CVS to defined the expected number of miscarriages and stillbirths and therefore assess the invasive procedure-related risk in producing these pregnancy complications. The algorithm is freely accessible at www.fetalmedicine.com.

In our study, the rates of miscarriage and stillbirth after demonstration of a live fetus at 11 to 13 weeks were 1.2 and 0.43%, respectively. These rates were higher than the 0.9 and 0.29%, respectively, reported in a USA multicentre study of first-trimester screening for aneuploidies in 36 014 singleton pregnancies (Dugoff *et al.*, 2008), possibly because in our population the mean maternal age was higher (31.7 vs 30.0 years) and there was a higher proportion of women of African racial origin (19.2 vs 5.0%). The rate of stillbirth in our population was similar to the UK national rate of 0.46% (CMACE, 2010).

There was a J-shaped relation between maternal age and risk of miscarriage which was about 2.3% at 15 years of age declining to a nadir of 1.0% at the 28 years and increasing thereafter to 2.3% at 45 years. Such J-shaped relationship has also been observed in a population-based register linkage study of more than 600 000 pregnancies, where the rate of miscarriage was about 15% at 15 years, declined to 12% at 25 years and increased to 70% at 45 years (Nybo Andersen *et al.*, 2000). The much higher rates of miscarriage in this report than in our study is because they included fetal losses before 11 weeks, pregnancy viability was not ascertained by ultrasonography and they did not exclude fetal abnormalities.

The increase in both miscarriage and stillbirth with maternal weight is compatible with the results of large population-based studies (Sebire *et al.*, 2000; Stephansson *et al.*, 2001; Metwally *et al.*, 2008). Similarly, in women of African racial origin, compared to Caucasians, there is a threefold increase in risk for miscarriage and a doubling in risk for stillbirth. A study of 5 138 122 singleton pregnancies from the US National Center of Health Statistics reported that in women of African racial

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origin, compared to Caucasian women, the risk of stillbirth at 20 to 23 weeks was 2.8 times higher and the risk of death at 39 to 40 weeks was 1.6 times higher (Willinger *et al*., 2009). In the report of confidential enquiries into maternal and child health in the UK, the rate of stillbirth in women of African racial origin was 2.3 times higher than in Caucasians (CMACE, 2010).

The risk of stillbirth was inversely related to maternal height and increased from about 0.2% for a woman of 170 cm in height to 0.6% for one of 140 cm. A population-based study of 952 630 singleton pregnancies also reported that the risk of stillbirth after 28 weeks was increased by a factor of 1.4 in women less than 160 cm compared to those more than 160 cm (Zhang *et al*., 2010).

Cigarette smoking doubled the risk of stillbirth. A population-based cohort study of 1 224 133 singleton pregnancies, including 240 247 (19.6%) smokers, reported that after adjustment for other confounders smoking was associated with an increased risk for stillbirth by a factor of 1.2 (Aliyu *et al*., 2010). Similarly, a causal association between smoking and stillbirth was suggested by the findings of a population-based cohort study of 526 691 singleton pregnancies which reported that the risk of stillbirth in women who smoked during pregnancy was 1.35 times higher than in non-smokers, whereas the risk in smokers who did not smoke during the index pregnancy was not increased (Högberg and Cnattingius, 2007).

The use of ovulation-induction drugs tripled the risk of miscarriage. In a previous study, the risk of miscarriage in 1945 pregnancies after assisted conception was significantly higher than in 4814 naturally conceived pregnancies (21 vs 15%; Wang *et al*., 2004). Similarly, increased risk of miscarriage was reported in a study of 2143 pregnancies conceived by use of ovulation-induction drugs compared to 3484 spontaneous pregnancies (25.1 vs 20.1%; Dickey *et al*., 1996).

Chronic hypertension tripled the risk of stillbirth and pre-existing diabetes mellitus doubled the risk of miscarriage. A systematic review examining the causes of stillbirth reported that chronic hypertension is associated with an increased risk with odds ratios of 1.5 to 2.7 (Fretts, 2005). A UK national population-based cohort study of 2359 pregnancies in women with type 1 and 2 diabetes reported that the rate of stillbirth was 4.7 times higher than in non-diabetics but after exclusion of congenital defects the increased rate was reduced to 2.1 (Macintosh *et al*., 2006). In our study, we excluded major defects and the finding of increased rate of miscarriage, but not stillbirth, in women with diabetes suggests that glycemic control in early pregnancy may have been poor but this was subsequently improved (Mills *et al*., 1988). An alternative explanation is that there is a selection bias, whereby women with poorly controlled diabetes at conception end up miscarrying or having fetal anomalies, thus being excluded from the risk calculation for stillbirth.

A population-based cohort study of 151 021 pregnancies reported that the risk of miscarriage in women with a previous miscarriage was twice as high as in women with no previous miscarriages (Bhattacharya *et al*., 2010). In our study, we found that in women with previous fetal loses the risk of miscarriage is increased and provided further details suggesting that the increase in risk is related to the gestation of previous losses, being 1.6, 6.3 and 3 times, respectively, for previous miscarriage before 16 weeks, miscarriage at 16 to 23 weeks and stillbirth. A nationwide study of 410 021 women delivering first and second consecutive infants, including 1842 in which the first pregnancy resulted in stillbirth, reported that the risk of recurrence was 2.5 times higher than in those with a previous live birth (Surkan *et al*., 2004). In our study, there were only 278 women with a previous stillbirth and could not demonstrate a significantly increased risk of recurrence. Nevertheless, we found that in women with previous successful pregnancies the risk of stillbirth was halved compared to women with no previous pregnancies.

The risk for miscarriage and stillbirth is increased by the findings of high fetal NT, low maternal serum PAPP-A and reversed A-wave in fetal ductus venosus at 11 to 13 weeks. These findings are compatible with the results of previous studies (Souka *et al*., 2001, 2005; Spencer *et al*., 2006; Bilardo *et al*., 2007; Smith *et al*., 2007; Dugoff *et al*., 2008; Maiz *et al*., 2008). We used an approach analogous to screening for aneuploidies by combining maternal characteristics with the results of biophysical and biochemical testing at 11 to 13 weeks to develop models for the estimation of patient-specific risks for miscarriage and stillbirth. The performance of combined testing for miscarriage and stillbirth, with estimated detection rates of 37 and 35%, respectively, at a FPR of 10%, is poor compared to the detection of more than 90% of aneuploid fetuses at a FPR of less than 5% (Nicolaides, 2011). However, unlike screening for aneuploidies where the end point is well defined the heterogeneous etiology of miscarriage and stillbirth will hamper efforts to develop a high-performance screening test, unless the fetal losses are subdivided according to cause and we introduce disease-oriented biophysical and biochemical testing. We have shown that the performance of combined screening for stillbirth is higher if death occurred before rather than after 34 weeks and if the birth weight was below than above the 10th percentile.

In screening for miscarriage we have shown that the model of prediction can be used for monitoring of risks from invasive prenatal interventions, such as CVS. The same risk factors leading to CVS, including increased maternal age, high fetal NT, reversed A-wave in the fetal ductus venosus and decreased serum PAPP-A, are also associated with increased risk for miscarriage and these factors should be taken into account in estimating the procedure-related risk. We have shown that in a specialist centre there is no apparent increase in risk of miscarriage from CVS over the background risk, defined by maternal characteristics and the results of first-trimester screening for aneuploidies, irrespective of whether the procedure is performed by an expert or a trainee under the direct supervision of an expert. It is possible that these results may have been influenced by factors that we have overlooked in the development

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or the application of the model of predicting miscarriage. One such factor is the higher gestational age of the CVS compared to the non-CVS group (13.1 vs 12.5 weeks) and therefore pregnancies with abnormal screening results miscarrying before planned CVS would have been included in the non-CVS group. In applying the model derived from the non-CVS group to those undergoing CVS we would have overestimated the expected number of miscarriages and consequently underestimated the procedure-related risk from CVS. However, we found that there were no significant differences between the CVS and non-CVS groups in either the median gestation at miscarriage or the proportion of miscarriages before 14 weeks.

In screening for aneuploidies, a high risk result could lead to invasive testing. In contrast, there is no direct risk to the mother or fetus from the classification of being at high risk of miscarriage or stillbirth, without wishing to underestimate the anxiety that such a classification may cause. At present, the only intervention for pregnancies at high risk for stillbirth would be higher frequency of ultrasound scans to monitor fetal growth and well-being with the beneficial consequence that at least some cases of stillbirth could be avoided by timely delivery.

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