Challenges in prenatal screening and diagnosis in the Netherlands

Bakker, Merel

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Total pregnancy loss after chorionic villus sampling and amniocentesis

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Objective:
To identify maternal-, operator-, and procedure-related variables affecting the procedure related pregnancy loss after transcervical (TC) and transabdominal (TA) chorion villus sampling (CVS) and amniocentesis (AC). To estimate the spontaneous and procedure-related fetal losses in comparable subgroups of women.

Methods:
A retrospective cohort study conducted at the University Medical Centre Groningen and the Academic Medical Centre Amsterdam, the Netherlands. The databases of both centers were searched for singleton pregnancies that had undergone a combined test (CT) and/or an anomaly scan at around 20 weeks' gestation, or an invasive procedure (CVS and/or AC) between January 2001 and December 2011. Maternal characteristics, obstetric history, technical aspects of the invasive procedure, ultrasound examinations and fetal and neonatal outcomes were available in 29.201 cases.

Results:
Variables significantly associated with a higher fetal loss rate (FLR) were: for CVS (TC or TA) repeated attempts during a procedure, the use of a TC-cannula instead of a biopsy forceps, gestational age of 13 weeks or more and pregnancies after assisted reproduction; for AC if the indication was a fetal anomaly or a family history of anomalies and repeated attempts during the procedure. In the group of women aged 36 years or older who did not undergo an invasive procedure the total spontaneous FLR after a first trimester scan was 1.40%, whereas after a TC or TA CVS the total FLR was 2.76% and 2.43%, respectively. Therefore, the additional risk of a TC - CVS was 1.36% (1:74) and this varied according to the instrument used: 0.27% for the forceps and 3.12% for the cannula. After a TA - CVS the risk was 1.03% (1:97).

In women aged 36 years and older undergoing a 20 weeks scan the spontaneous FLR was .63%. In the group women undergoing an AC performed solely for advanced maternal age the FLR was 1.11%. Therefore the additional risk of an AC was .48% (1:208).

Conclusion:
The procedure related FLRs after a TA-CV, TC-CVS and AC appear lower than the current risks women are counselled on. All risks decreased when the level of experience increased.

Introduction

The wish of both women and doctors to avoid unnecessary procedures during pregnancy is the driving force behind the search for optimal screening strategies and, more recently, non-invasive prenatal testing (cell free fetal DNA). The most important factor influencing uptake of invasive procedures (chorionic villus sampling (CVS) and amniocentesis (AC)) is the procedure related fetal loss rate (FLR)[1]; which is the loss rate attributable to the invasive procedure minus the spontaneous FLR. The latter is influenced by the maternal risk profile (age, weight, parity and obstetric history)[2] and the pregnancy related risk profile (gestational age and presence of fetal anomalies).[3-5]

In contrast, the procedure related risk depends on the technique and instruments used, possible technical difficulties and operator experience.[4,6,7] As a result of interaction between these variables the total FLR varies greatly.[8]

In literature procedure related FLRs range from 0.06% to 1.0% or even higher, for both CVS and AC.[4,5,8-20] Most studies are observational and include mixed populations. Of the few randomized (controlled) trials Tabor et al compared women undergoing an AC with a control group, showing a 1% higher FLR in women undergoing amniocentesis.[10] Literature suggests that transabdominal CVS and AC seem to have smaller and comparable risks.[11,12,14,21]

It remains a challenge to report realistic risk figures, taking into account all variables that influence the procedure related FLR. Furthermore, some of the above mentioned studies were performed a while ago at a time when ultrasound systems were less advanced and techniques and training in invasive procedures less standardized. A recent meta-analysis showed that accurate estimates of current procedure-related risks following invasive procedures are lacking.[5] Many experts believe that procedure related risks need reevaluation.[22] A new consensus should be reached to avoid discrepancies in information given among centers.

The aim of this study was to identify maternal-, operator-, and procedure-related risk factors that modify the overall estimated risk of fetal loss and to create comparable subgroups in order to calculate the total spontaneous FLR and the procedure-related FLR after CVS and AC.

Methods

DESIGN

This retrospective cohort study on spontaneous as well as procedure related FLRS in women undergoing CVS and AC was conducted in two University Medical Centres: the UMCG in Groningen, and the AMC in Amsterdam, the Netherlands. Data on all consecutive singleton pregnancies that had undergone CVS and/or AC, performed in the period between January1st, 2001 and December 31st, 2011, were retrieved from the databases of both hospitals. The same data were retrieved for women who had only undergone a combined test (CT) and/or an anomaly scan at 20 weeks of gestation.

Both academic hospitals act as referral centers, but also have their own 'low risk' popu-

lation. Prenatal screening is offered to women in the form of the combined test and/or the 18-20 week anomaly scan. According to the national guideline women in the Netherlands undergoing a CVS and AC are always referred to a tertiary hospital. As a consequence this study includes both 'low risk' and referred women.

The database was divided into 5 groups, depending on the examination(s) that women had undergone. The first group consisted of women who had undergone the CT (and 20 week anomaly scan), the second group of women who had only undergone a 20 week anomaly scan, the third of women who had undergone a TC or TA CVS, the fourth of women who had undergone an AC, and the last group consisted of women who had an AC after a (unsuccessful) CVS. In order to calculate the crude procedure related FLR, the spontaneous FLRS after 11-14 weeks and after the second trimester of pregnancy were calculated in two groups of women aged 36 years and older, who had undergone the CT and/or the 20 weeks anomaly scan. These spontaneous FLRS were then compared with the FLRS in three groups of women, who had undergone a TC CVS, TA CVS or AC on maternal age indication only, without known a-priori risk factors.

In all cases information was available on procedure related characteristics (indication, operator, technique) as well as on maternal characteristics, obstetric history and pregnancy outcome.

DEFINITIONS

Outcome was classified into the following categories; alive, miscarriage, preterm labor, intra-uterine fetal demise (IUD), termination of pregnancy (TOP), fetal loss during labor or neonatal death. Alive was defined as a newborn showing signs of life after delivery. Miscarriage was defined as spontaneous delivery of the fetus before 24 weeks of gestation. Preterm labor was defined as spontaneous delivery between 24 to 37 weeks of gestation. Intra-uterine fetal demise (IUD) was defined as fetal death from 24 weeks onwards, prior to delivery. Fetal loss during labor was defined as intra-partum death from 24 weeks onwards. Neonatal death (NND) was defined as infant death before 28 days of age.

The total spontaneous FLR was defined as the total of losses before 24 weeks of gestation in the group women of 36 years and older who did not underwent an invasive procedure. After 24 weeks of gestation this was the sum of preterm labor followed by loss and IUD.

Procedure related FLR was defined as the total of fetal loss before 24 weeks of gestation based on the group of women undergoing an invasive procedure because of advanced maternal age, minus the background risk.

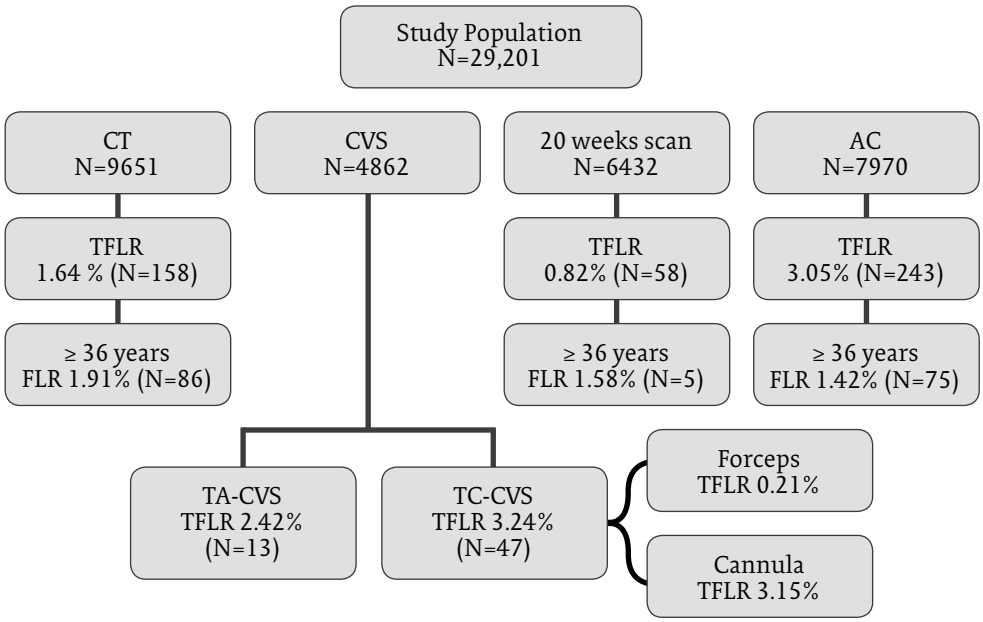
Operator's experience with performing a CVS was classified as; level 1: <50, level 2: between 50-150 and level 3: >150 procedures performed in total. For AC, operator's experience was classified as; level 1: <50, level 2: 50-150, level 3: >150-500, level 4: >500 procedures performed in total.

The number of procedures and attempts were classified as: (1) one procedure with one attempt, (2) one procedure with more than one attempt and (3) more than one procedure.

STATISTICAL ANALYSIS

To compare differences in categorical variables between women who did or did not undergo CVS and/or AC, the X^2 test (or Fisher's Exact Test, if appropriate) was used.

■ Figure 1 – Flowchart (crude data, no adjustment for background risk). Because of small numbers the group women who underwent a CVS and AC are not included in this flowchart.



CT = combined test. CVS = chorion villus sampling. AC = amniocentesis. TA-CVS: transabdominal chorion villus sampling. TC-CVS: transcervical chorion villus sampling. TFLR = total fetal loss rate.

Associations between single co-variables and outcome were assessed by univariate binary logistic regression and expressed in odds ratios (ORs, 95% confidence intervals). Correlations were used to check for confounders and interactions. Multiple binary logistic regression analysis (backward stepwise elimination method) was performed to evaluate the adjusted impact of co-variables. Statistical significance was defined as $p < 0.05$ (two-sided). All statistical analyses were conducted using SPSS 17.0.0.

Results

In total 36.350 cases were available for analysis, however only cases with known outcome (N=29.201, 80.3%) were used. The population was divided into five main groups; 9.651 women had undergone a CT, 6.432 had undergone a 20 week anomaly scan, 4.862 had undergone a CVS (TA-CVS: N=1341 and TC-CVS: N=2833; of which 1787 by forceps and 1046 by a cannula and 7970 cases had undergone an AC. In 286 cases both a CVS and an AC were performed (Figure 1).

Table 1 shows the baseline characteristics in all groups. Women opting for CVS or AC were on average older (<.01), and more frequently multiparous (<.01) and smokers (<.01).

The results of the multiple regression analyses are presented in Table 2a and 2b. In the group of women undergoing a CVS (TC + TA) the variables significantly associated with a

■ Table 1 – Baseline characteristics

	Groups					
	CT (+20 weeks scan) N(%)	20 weeks scan N(%)	CVS N(%)	AC N(%)	CVS + AC N(%)	*p*
Age:						<.01**
<36 years	5140 (53.26)	2695 (89.51)	1652 (33.98)	1857 (23.30)	86 (30.07)	
≥36 years	4511 (46.74)	316 (10.49)	3210 (66.02)	6113 (76.70)	200 (69.93)	
Gravida - Para:						<.01
Primiparous	3284 (34.03)	2401 (37.33)	1428 (29.37)	2580 (32.37)	88 (30.77)	
Multiparous	4999 (51.80)	2520 (39.18)	3149 (64.77)	4861 (60.99)	188 (65.73)	
Unknown	1368 (14.17)	1511 (23.49)	285 (5.86)	529 (6.64)	10 (3.50)	
Conception:						<.01
Spontaneous	3876 (40.16)	728 (11.32)	2689 (55.31)	4705 (59.03)	192 (67.13)	
Medication, IUI, IVF, ICSI, KID or Egg Donation	611 (6.33)	58 (.90)	134 (2.76)	301 (3.78)	5 (1.75)	
Unknown	5164 (53.51)	5646 (87.78)	2039 (41.94)	2964 (37.19)	89 (31.12)	
Smoking:						<.01**
Yes	574 (5.95)	273 (4.24)	399 (8.21)	641 (8.04)	17 (5.94)	
Stopped	57 (.59)	67 (1.04)	11 (.23)	11 (.14)	-	
No	5632 (58.36)	1743 (27.10)	3428 (70.51)	5998 (75.26)	229 (80.07)	
Unknown	3388 (35.11)	4349 (67.62)	1024 (21.06)	1320 (16.56)	40 (13.99)	
BMI:						<.01**
Underweight (<18.50)	148 (1.53)	103 (1.60)	80 (1.65)	97 (1.22)	5 (1.75)	
Normal range (18.50 – 24.99)	3428 (35.52)	1688 (26.24)	2360 (48.54)	2253 (28.27)	150 (52.45)	
Overweight (25.00 – 29.99)	1205 (12.49)	630 (9.79)	754 (15.51)	706 (8.86)	44 (15.38)	
Obese (≥30.00)	520 (5.39)	416 (6.47)	254 (5.22)	287 (3.60)	23 (8.04)	
Unknown	4350 (45.07)	3595 (55.89)	1414 (29.08)	4627 (58.06)	64 (22.38)	
Year:						<.01**
2001	545 (5.6)	39 (0.6)	352 (7.24)	858 (10.77)	22 (7.7)	
2002	547 (5.7)	31 (0.5)	407 (8.37)	824 (10.34)	23 (8.0)	
2003	745 (7.7)	32 (0.5)	394 (8.10)	787 (9.87)	15 (5.2)	
2004	981 (10.2)	113 (1.8)	400 (8.23)	739 (9.27)	13 (4.5)	
2005	822 (8.5)	83 (1.3)	375 (7.71)	611 (7.67)	11 (3.8)	
2006	760 (7.9)	260 (4.0)	350 (7.20)	844 (10.59)	11 (3.8)	
2007	1384 (14.3)	1187 (18.5)	598 (12.30)	776 (9.74)	31 (10.8)	
2008	1183 (12.3)	1273 (19.8)	584 (12.01)	741 (9.30)	29 (10.1)	
2009	1077 (11.2)	1063 (16.5)	508 (10.45)	682 (8.56)	52 (18.2)	
2010	1096 (11.4)	1143 (17.8)	487 (10.02)	658 (8.26)	50 (17.5)	
2011	511 (5.3)	1208 (18.8	407 (8.37)	450 (5.65)	29 (10.1)	

* Due to rounding the numbers do not add up to 100%.
** Fisher Exact Test was used.
CT = combined test. CVS = chorion villus sampling. AC = amniocentesis.

higher risk of fetal loss were repeated attempts during a procedure, the use of a TC cannula, gestational age of 13 weeks or beyond and pregnancies after assisted reproduction In the group of women undergoing an AC variables significantly associated with a higher risk of spontaneous fetal loss were repeated attempts during a procedure, the presence of an anomaly and a past history for congenital anomalies in the family.

Table 3 shows the pregnancy outcome for each group, including all variables and indications. The total FLR before 24 week' gestation in the CT group was 1.21% and in the CVS group (TC + TA) 3.12%; giving a procedure related risk of 1.91% or 1:52 for CVS. This per-

■ Table 2a – CVS <24 weeks of gestational age

	Univariate Logistic Regression		Multiple Logistic Regression	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Maternal Characteristics				
Age:				
<36 years	1.347	.083		
>36 years	-			
Parity:				
Primiparous	1.104	.597		
Multiparous	-			
Unknown	1.660	.095		
Conception:				
Spontaneous	.405	.013	.352	.011
Assisted	-		-	-
Unknown	.421	.019	.243	.002
Smoking:				
Yes	1.570	.104		
Stopped	.000	.999		
No	-			
Unknown	1.523	.030		
BMI:				
Underweight (<18.50)	1.815	.298		
Normal range (18.50 – 24.99)	.747	.422		
Overweight (25.00 – 29.99)	.857	.699		
Obese (≥30.00)	-			
Unknown	.897	.769		
CVS Characteristics				
Number of CVS procedures:				
1 procedure – 1 attempt	-		-	
1 procedure – >1 attempt	2.359	.001	2.476	.001
>1 procedure	.000	.998	.000	.999
GA when procedure takes place:				
≤10 weeks	-		-	-
11 weeks	1.112	.795	1.469	.400
12 weeks	1.385	.425	1.845	.192
13 weeks	1.955	.116	3.203	.018
≥14 weeks	3.407	.012	4.047	.027
Indication:				
Maternal age	-			
Increased risk CT	1.180	.458		
(Suspicion) Anomaly	2.726	.001		
Obstetric history	1.261	.504		
Family history	.747	.626		
DNA examination	.924	.821		
Different reason	.000	.998		
Operator Characteristics				
Experience	.891	.371		
Instrument:				
TA 17-19G Needle	-		-	

Table 2a (continued)

TA 20 G Needle	1.663	.266	1.836	.287
TA 22G Needle	1.815	.274	2.708	.085
TV Forceps	.983	.942	1.584	.114
TV Cannula	1.556	.067	3.018	.002

CVS = chorionvillus biopsy. GA = gestational age.

■ Table 2b – AC <24 weeks of gestational age

	Univariate Logistic Regression		Multiple Logistic Regression	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Maternal Characteristics				
Age:				
<36 years	.350	.001		
>36 years	-			
Parity:				
Primiparous	1.137	.504		
Multiparous	-			
Unknown	.880	.749		
Conception:				
Spontaneous	1.589	.434		
Assisted	-			
Unknown	1.602	.431		
Smoking:				
Yes	.613	.247		
Stopped	14.410	.001		
No	-			
Unknown	1.253	.323		
BMI (WHO):				
Underweight (<18.50)	.986	.983		
Normal range (18.50 – 24.99)	.502	.068		
Overweight (25.00 – 29.99)	.625	.278		
Obese (≥30.00)	-			
Unknown	.420	.017		
AC Characteristics				
Number of AC procedures:				
1 procedure – 1 attempt	-			
1 procedure – >1 attempt	2.220	.045	2.901	.017
>1 procedure – 1 attempt	.000	1.000	4.185	.192
>1 procedure – >1 attempt	6.238	.014		
GA when procedure takes place:				
≤15 weeks	-		-	
16 weeks	.717	.172	.707	.281
17 weeks	1.938	.035	1.431	.442
18 weeks	2.331	.075	1.405	.607
19 weeks	1.844	.309	.624	.578
20 weeks	4.236	.001	.727	.593
21 weeks	1.033	.950	.078	.021
22 weeks	1.591	.439	.185	.130

Table 2b (continued)

	Univariate Logistic Regression		Multiple Logistic Regression	
	OR (95% CI)	*p*	OR (95% CI)	*p*
23 weeks	1.616	.638	.000	.998
≥24 weeks	.000	.994	.000	.995
Indication:				
Maternal age	-		-	
(Suspicion) Anomaly	4.017	.001	8.531	.001
Increased risk CT	1.499	.158	1.558	.229
AC after CVS	.000	.999	.000	.999
Obstetric history	1.970	.195	2.713	.104
Family history	2.065	.319	4.964	.032
DNA examination	3.032	.279	5.153	.128
Different reason	1.866	.297	2.665	.191
Approach:				
Via amniotic fluid	-			
Via placenta	.924	.745		
Amount of amniotic fluid	.999	.852		
Operator Characteristics				
Experience	.858	.088		
Instrument:				
TA 17-19G Needle	-			
TA 20G Needle	.499	.189		
TA 21G Needle	.000	.999		
TA 22G Needle	.395	.080		

AC = amniocentesis. GA = gestational age.

centage includes all indications for an invasive irrespective of CVS technique. The total spontaneous FLR before 24 weeks' gestation in the 20 weeks anomaly scan group was 0.31% and in the AC group 1.56%; giving a procedure related risk of 1.25% or 1:80 for AC. Univariately there was a trend between an increased level of experience and a lower fetal loss rate. These estimates included all indications.

Table 4 shows the pregnancy outcome for each group, stratified for indication. The total spontaneous FLR in women of 36 years and older undergoing a CT was 1.40% (N=63). For women undergoing a TC or TA CVS, performed on advanced maternal age indication, was 2.76% (N=40) and 2.43% (N=13), respectively (Chi Square <.01). The additional risk of a TC CVS was therefore 1.36% or 1:74; the additional risk of a TA CVS was 1.03% or 1:97 (Chi Square .06), respectively. When TC CVS was performed by forceps the additional risk was 0.27% (1.67 - 1.40%); and when performed by cannula 3.12% (4.52 - 1.40%).

When the number of procedures and attempts was taken into account for CVS (TC and TA) the risk was 1.07% (2.47 - 1.40%) when only one procedure and attempt had taken place, 4.48% (5.88 - 1.40%) when during one procedure more than one attempt was performed. No risk could be calculated for the group where more than one procedure and attempt were performed because the group was too small (N=8, 0 fetal loss).

In women aged 36 years and older undergoing the 20 weeks scan the total FLR was .63% (N=2) and in women undergoing an AC 1.11% (N=60)(Chi Square .325). Therefore

■ Table 3 – Outcome per group

		Alive	Miscarriage	IUVD 24-37 weeks	IUVD >=37 weeks	Partus prematurus and deceased 24-37 weeks	TOP	Deceased durante partu >=37 weeks	Neonatal Death
CT (+20 weeks scan)	N	9,403	117	18	11	6	71	3	15
	%	97.50	1.21	.19	.11	.06	.74	.03	.16
18-20 weeks scan	N	6,361	20	23	5	6	9	-	8
	%	98.90	.31	.36	.08	.09	.14	-	.12
TA-CVS	N	1,001	40	8	2	-	283	-	6
	%	74.70	2.99	.60	.15	-	21.12	-	.45
TC-CVS Forceps	N	1,445	47	4	2	3	278	-	4
	%	81.04	2.64	.22	.11	.17	15.59	-	.22
TC-CVS Cannula	N	822	43	6	-	-	146	-	2
	%	80.67	4.22	.59	-	-	14.33	-	.20
AC	N	7,184	124	91	21	3	449	2	89
	%	90.22	1.56	1.14	.26	.04	5.64	.03	1.12
CVS + AC	N	226	11	-	-	-	48	-	1
	%	79.02	3.85	-	-	-	16.78	-	.35
Total	N	26,442	402	150	41	18	1284	5	125
	%	92.89	1.41	.53	.14	.06	4.51	.02	.44

All indications were included.
CT = combined test. CVS = chorion villus sampling. AC = amniocentesis. TA-CVS = transabdominal chorion villus sampling.
TC-CVS: transcervical chorion villus sampling. IUD = intra-uterine death. TOP = termination of pregnancy.

the additional risk of an AC was .48% or 1:208. In women younger than 36 years undergoing a midtrimester scan the spontaneous FLR decreased to .52% (p .79).

When the number of procedures and attempts was taken into account for the AC, the risk was .34 (.97 - .63%), the additional risk was 1.34% (1.97 - .63%) when during one procedure more than one attempt was performed, and 5.25% (5.88 - .63%) when more than one procedure and more than one attempt were performed.

With increasing operator experience the FLR after a TA-CVS decreased. At level 1 the risk was 2.24% (3.64 - 1.40%), at level 2 1.65% (3.05% - 1.40%) and at level 3 0.42 (1.82 - 1.40%) (Figure 2a). The risk after TC CVS by forceps could not be calculated for level 1 and 2 due to a relative low number of women and no fetal losses. At level 3 the risk was 0.44% (1.84 - 1.40%) (Figure 2b). The risk when using the cannula was 0.68% (2.08 - 1.40%) at level 1, 1.57% (2.97 - 1.40%), at level 2, and 6.85% (8.25 - 1.40%) at level 3 (Figure 2c).

The influence of operator's experience on fetal loss rate after an AC was examined according to four levels of experience. At level 1 the additional risk was 0.82% (1.45 - .63%), at level 2 1.00% (1.63 - .63%), at level 3 .17% (0.80 - .63%) and at level 4 .52% (1.15 - .63%) (Figure 2d).

Discussion

This study shows that the FLR in women aged 36 years or older undergoing a transcervical CVS on maternal age indication was 0.27% or 3.12%, depending on whether a forceps or a cannula was used, respectively. For the transabdominal approach the risk was 1.36%. Factors influencing the fetal loss rate after CVS were the use of a TC cannula (OR 3.0),

■ Table 4 – Outcome according to indication

	Outcome								
	Alive	Miscarriage	IUVD 24-37 weeks	IUVD >=37 weeks	Partus prematurus and deceased 24-37 weeks	TOP	Deceased durante partu >=37 weeks	Neonatal Death	Total
CT									
<36 years	5,010 (97.55)	54 (1.05)	8 (.16)	2 (.04)	4 (.08)	47 (.92)	2 (.04)	9 (1.18)	5,136
≥36 years	4,393 (97.45)	63 (1.40)	10 (.22)	9 (.20)	2 (.04)	24 (.53)	1 (.02)	6 (.13)	4,508
18-20 wk scan									
<36 years	2,656 (98.55)	14 (.52)	12 (.45)	1 (.04)	6 (.22)	4 (.15)	-	2 (.07)	2,695
≥36 years	307 (97.15)	2 (.63)	1 (.32)	1 (.32)	-	3 (.95)	-	2 (.63)	316
CVS (TA)									
Maternal Age	508 (94.78)	13 (2.43)	-	1 (.19)	-	13 (2.43)	-	1 (.19)	536
Increased risk CT	318 (67.80)	14 (2.99)	2 (.43)	-	-	134 (28.57)	-	1 (.21)	469
Anomaly	60 (31.58)	10 (5.26)	4 (2.11)	1 (.53)	-	111 (58.42)	-	4 (2.11)	190
Anomaly in obstetric history	68 (89.47)	3 (3.95)	1 (1.32)	-	-	4 (5.26)	-	-	76
Anomaly present in family member or parents	13 (59.09)	-	1 (4.55)	-	-	8 (36.36)	-	-	22
DNA-research	24 (66.67)	-	-	-	-	12 (33.33)	-	-	36
Other	9 (90.00)	-	-	-	-	1 (10.00)	-	-	10
CVS Forceps									
Maternal Age	845 (94.31)	15 (1.67)	2 (.22)	1 (.11)	-	31 (3.46)	-	2 (.22)	896
Increased risk CT	194 (71.85)	9 (3.33)	1 (.37)	-	2 (.74)	64 (23.70)	-	-	270
Anomaly	52 (33.77)	12 (7.79)	1 (.65)	-	-	89 (57.79)	-	-	154
Anomaly in previous pregnancy	105 (92.11)	3 (2.63)	-	-	-	6 (5.26)	-	-	114
Anomaly in one of the parents or family member	53 (77.94)	-	-	1 (1.47)	-	14 (20.59)	-	-	68
DNA-investigation	186 (68.63)	8 (2.95)	-	-	1 (.37)	74 (27.31)	-	2 (.74)	271
Other	10 (100)	-	-	-	-	-	-	-	10
CVS Cannula									
Maternal Age	507 (91.68)	25 (4.52)	2 (.36)	-	-	18 (3.25)	-	1 (.18)	553
Increased risk CT	111 (69.81)	5 (3.14)	2 (1.26)	-	-	40 (25.16)	-	1 (.63)	159
Anomaly	28 (35.90)	4 (5.13)	1 (1.28)	-	-	45 (57.69)	-	-	78

Table 4 (continued)

	Outcome								
	Alive	Miscarriage	IUVD 24-37 weeks	IUVD >=37 weeks	Partus prematurus and deceased 24-37 weeks	TOP	Deceased durante partu >=37 weeks	Neonatal Death	Total
Anomaly in previous pregnancy	78 (87.64)	4 (4.49)	-	-	-	7 (7.87)	-	-	89
Anomaly in one of the parents or family member	45 (73.77)	3 (4.92)	1 (1.64)	-	-	12 (19.67)	-	-	61
DNA-investigation	70 (72.92)	2 (2.08)	-	-	-	24 (25.00)	-	-	96
Other	10 (100)	-	-	-	-	-	-	-	10
AC									
Maternal Age	5,218 (96.74)	60 (1.11)	13 (.24)	4 (.07)	-	83 (1.54)	1 (.02)	15 (.28)	5,394
Anomaly	625 (54.82)	47 (4.12)	66 (5.79)	14 (1.23)	3 (.26)	313 (27.46)	1 (.09)	71 (6.23)	1,140
Increased risk CT	1,011 (90.92)	17 (1.53)	7 (.63)	2 (.18)	-	74 (6.65)	-	1 (.09)	1,112
AC after CVS	66 (85.71)	1 (1.30)	-	-	-	10 (12.99)	-	-	77
Anomaly in previous pregnancy	197 (94.71)	4 (1.92)	-	-	-	6 (2.88)	-	1 (.48)	208
Anomaly in one of the parents or family member	94 (93.07)	2 (1.98)	2 (1.98)	1 (.99)	-	2 (1.98)	-	-	101
DNA-investigation	33 (78.57)	1 (2.38)	1 (2.38)	-	-	7 (16.67)	-	-	42
Other	161 (96.41)	3 (1.80)	-	-	-	2 (1.20)	-	1 (0.60)	167

*Total fetal loss includes intra-uterine death, preterm labor and miscarriage.
CT = combined test. CVS = chorion villus sampling. AC = amniocentesis. TA-CVS: transabdominal chorion villus sampling. TC-CVS = transcervical chorion villus sampling.

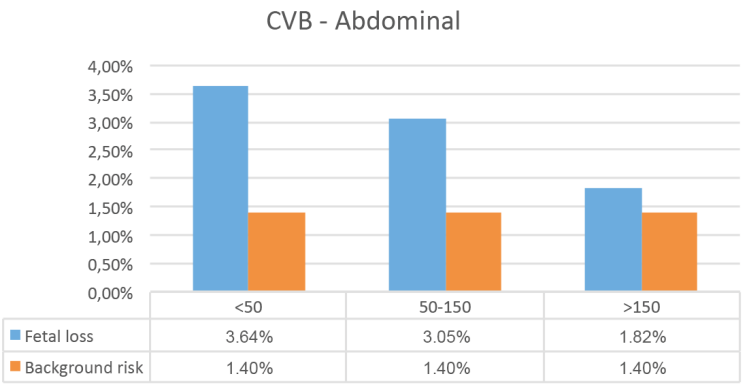
repeated attempts (OR 2.5), gestational age of 13 weeks or beyond (OR 3.2-4.0) and pregnancies after assisted reproduction.

Pregnancy loss after an AC was 1.11%. Factors affecting fetal losses after AC were repeated attempts (OR 2.9), the presence of fetal anomalies (OR 8.5) and a family history of anomalies (OR 5.0). Furthermore, the influence of operator's experience on iatrogenic fetal losses was confirmed.[4,23,24]

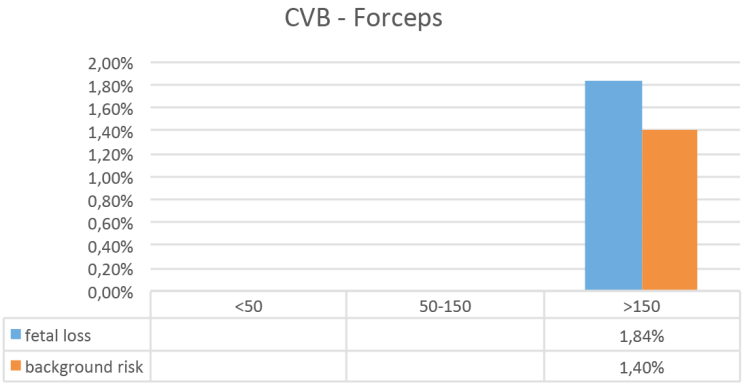
This study confirms the negative effect of variables such as an enlarged NT or structural anomalies on the total FLR (including TOP) after CVS (TC or TA) and AC. These factors are by far more predisposing to pregnancy losses than advanced maternal age.[5,9,25]

This retrospective study shows that the previously quoted risk of fetal loss after AC of 1.0% does not reflect current practice. The risk after invasive procedures performed by experienced operators seems to be lower (.17 - .52% for the most experienced operators (level 3 for CVS and level 4 for AC)), which is in line with the meta-analysis of Akolekar et al.[4,5,9,10,23,26]

Strength of this study is that it includes a large population of women undergoing proce-



■ Figure 2a – Fetal loss in percentage (x-axis) in relation to level of experience when performing a transabdominal CVS (y-axis): (level 1) <50, (level 2) between 50-150 and (level 3) >150 procedures performed in total.



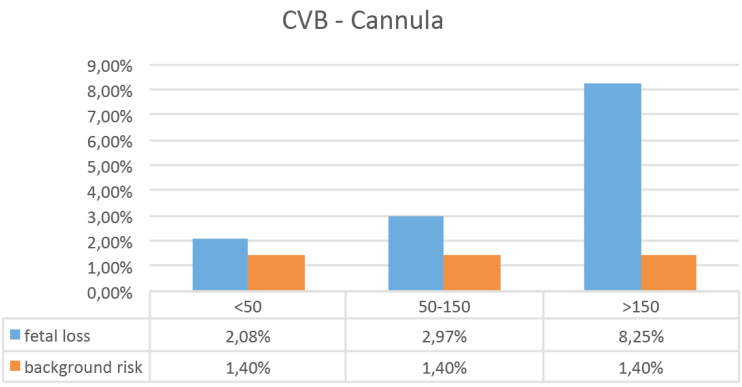
■ Figure 2b – Fetal loss in percentage (x-axis) in relation to level of experience when performing a transcervical CVS using a forceps (y-axis): (level 1) <50, (level 2) between 50-150 and (level 3) >150 procedures performed in total.

dures for different indications and two control groups. A limitation is its retrospective nature and, in the attempt of creating similar groups and excluding possible biases, some groups had only few patients or losses. Although the only way of confirming the conclusions of this study would be to perform a prospective randomized study, this is nowadays ethically unfeasible.[22,27]

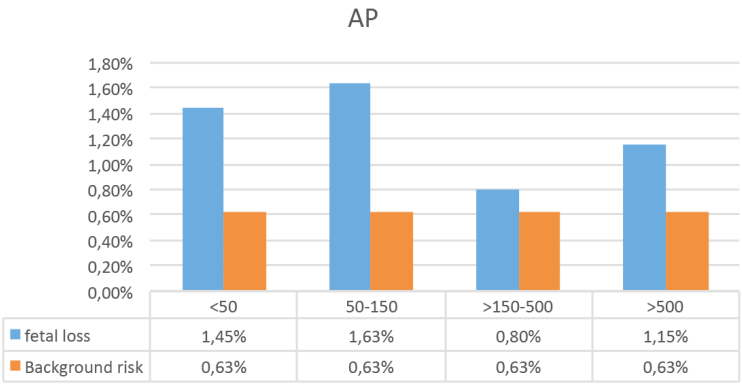
Furthermore, we were unable to retrieve the follow-up in 19% of the cases. We did not opt for the possibility of imputation, and choose to stay as close to the data as registered in first instance. In our experience frequently normal outcomes are missing as caregivers tend to report fetal losses, especially iatrogenic losses. Although we cannot be certain, it is likely that we overestimated the risk of fetal loss rather than underestimating it.

Regarding first trimester procedures, it is clear that the TC CVS by forceps and the TA technique are the methods of choice. A Cochrane review on CVS did not show convincing evidence to favor TA CVS over the TC technique.[6]
In contrast, Chueh et al and Jensen et al showed a significant difference in risk between the two techniques, prompting abandonment of the TC CVS in their centers.[11] This study shows that depending on the technique, both TA and TC CVS can have low FLR.[28]

In our study the TC-CVS was the technique in use in the early years. Only since the introduction of first trimester screening, the TA technique has become more widespread. Rationale for using TC-CVS is that it allows for a larger sample, especially useful in case of DNA analysis, and that it can be performed from 11 weeks onwards. We confirm that the



■ Figure 2c – Fetal loss in percentage (x-axis) in relation to level of experience when performing a transcervical CVS using a cannula (y-axis): (level 1) <50, (level 2) between 50-150 and (level 3) >150 procedures performed in total.



■ Figure 2d – Fetal loss in percentage (x-axis) in relation to level of experience when performing an AC (y-axis): (level 1) <50, (level 2) 50-150, (level 3) >150-500, (level 4) >500 procedures performed in total.

FLR after a TC and TA-CVS seems to be comparable, but only when the forceps is used.[11,28]

The higher FLR when using the cannula, irrespective of the operator's experience, is possibly due to the need in 2008 to abandon the previous cannula which was withdrawn from the market and using a new less flexible cannula, in one of the two centers. Since 2010 this center has adopted the forceps for TC CVS also and implemented the TA -approach. The advantage of being skilled in both techniques enhances the chance of successful sampling irrespective of placental localization or position of the uterus. Operators performing TC CVS should preferably be trained to use the forceps. As suggested in the literature, growing experience with CVS techniques has the potential to bring down the fetal losses to similar levels of the AC.[11,12,14,21]

One of the concerns of comparing women of 36 years and older undergoing an AC at around 16 weeks with a mid-trimester control group was the possible effect of the 2-4 weeks difference in gestational age on the spontaneous FLR with a possible overestimation of the procedure related loss rate at the time of the procedure.[22,27]

We were therefore surprised when, after adjustment for gestational age (≥16 - <24 weeks), both groups showed the same trend of a lower loss rate after AC. The background FLR in this study (.63% for women of 36 years and older) falls within previously reported percentages, varying between 0.2 and 1.16%.[20] Similarly, the low procedure related risk after AC performed by an experienced operator is in line with some recent studies with a high caseload and experienced operators.[5,13,16,23]

A recent large national population based study performed in Denmark, even showed that neither TA-CVS nor AC is associated with a higher FLR in comparison with the control group. Suggesting that the procedure-related FLR is very low for both CVS and AC.[29]

Appropriate training of new operators under experienced supervision or by the use of training models, can minimize the learning curve effect and increase the success rate.[28,30]

More than one needle insertion during a procedure was associated with an increased risk of pregnancy loss. Silver et al showed that there is a direct relationship between operator caseload and sampling efficiency.[31]

With the declining numbers of CVS and AC due to the widespread use of cell ff-DNA screening, a new directive on the number of operators performing invasive procedures and a minimum caseload per operator should be defined by the professional society. In the Netherlands the Dutch society for Obstetrics and Gynecology recommends a minimal number of 30 procedures per operator per year.[32]

In view of our results and declining number of invasive procedures, centralization in a few centers by experienced operators seems recommended, increasing the minimal caseload per year per operator. We suggest that in the future, similarly to the FMF audit for the NT, the development of an individual quality control program for invasive procedures should be considered, taking into account numbers, efficiency and safety.[5,7,32]

The real question is whether operator's fetal loss rate should be mentioned when counseling women regarding their choices in prenatal screening methods.

In conclusion, pregnancy losses after invasive procedures performed transabdominally (CVS and AC) or transcervically by forceps, are lower than thought and reported in the past when performed by experienced operators.

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