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Infant morbidity following amniocentesis and chorionic villus sampling for prenatal karyotyping

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Objective To investigate whether amniocentesis and chorionic villus sampling increase the risk of postural deformities, limb reduction defects, respiratory problems in the newborn, fetal and infant mortality, prematurity, low birthweight and fetal distress, and to investigate the impact of gestational length at the time of the procedure.

Design A population-based cohort study.

Setting Sweden, 1991–1996.

Population All women, 35 to 49 years old, with single births ($n = 71{,}586$). The women were classified as exposed to amniocentesis ($n = 21{,}748$) or chorionic villus sampling ($n = 1984$) or not exposed ($n = 47{,}854$).

Methods Infant outcomes were collected from the Swedish Medical Birth Register, the Swedish Hospital Discharge Register, the Swedish Malformation Register and the Swedish Cause of Death Register. Odds ratios were calculated with logistic regression analyses.

Main outcome measures Crude and adjusted odds ratios of postural deformities, limb reduction defects, respiratory problems in the newborn, fetal and infant mortality, prematurity, low birthweight and fetal distress. Women exposed to amniocentesis or chorionic villus sampling were compared with non-exposed women.

Results An increased risk of musculoskeletal deformities (OR = 1.32, 95% CI 1.11–1.57) including club foot and hip dislocation was found in the amniocentesis group, especially for amniocentesis prior to 14 weeks of gestation. Respiratory disturbances such as neonatal pneumonia, meconium aspiration, atelectasis and tachypnea were found more often in the amniocentesis group (OR = 1.12, 95% CI 1.02–1.24), with the greatest risk at 14 and 15 weeks of gestation. For the chorionic villus sampling group, no significant associations were found. No increase regarding limb reduction defects, fetal and infant mortality, prematurity, low birthweight and fetal distress was found in either the amniocentesis or the chorionic villus sampling group.

Conclusions Among women aged 35–49 years, amniocentesis before 14 weeks of gestation increases the risk of postural deformities. Amniocentesis at 14 and 15 weeks increases the risk of respiratory disturbances. For chorionic villus sampling, a larger study group is needed before such risks can be ruled out.

INTRODUCTION

Invasive techniques such as amniocentesis and chorionic villus sampling have become established in obstetric care for prenatal karyotyping and are offered to pregnant women for low risk indications like maternal age, anxiety or chromosomal abnormality in a previous pregnancy. The extent to which these procedures constitute a risk for the pre-procedural healthy fetus and the infant still remains unclear. It is well known that amniocentesis and chorionic villus sampling increase the risk of miscarriage.[1,2] Studies on first trimester amniocentesis compared with chorionic villus sampling or second trimester amniocentesis have found the risk of fetal loss to be higher after early amniocentesis.[3–5] A British study suggested that amniocentesis in the second trimester may, apart from the risk of fetal loss, also be associated with major orthopaedic postural deformities such as club foot and hip dislocation.[6] An increased risk of club foot after early amniocentesis associated with leakage of amniotic fluid has been reported.[5,7] Other studies have not, however, found a correlation between orthopaedic deformities and amniocentesis.[8–10] Firth *et al.*[11] reported an association between very early chorionic villus sampling performed at less than 90 days of gestation and limb reductions, while other publications have not found such a connection.[12]

An increased risk of respiratory problems in the neonate related to amniocentesis[1,6,13] as well as to chorionic villus sampling[14–16] have been reported. Again, other studies have not found such a relationship.[5,7,17,18] Studies in animal

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Table 1. The study population.

	Exposed	Non-exposed	Total
Women 35–49 years old with single births in Sweden 1991–1996			81,930
Exclusions			
Giving birth outside study area			8899
Incorrect identification number			453
Uncertain exposure status			211
Left to follow up	24,091	48,276	72,367
Exclusions			
Chromosomal abnormalities	30	274	304
Incorrectly registered data	102	43	145
Missing data on gestational length or an invasive procedure before 9 or after 20 weeks of gestation	63	105	168
Both types of invasive procedure	164		164
Final study population	23,732	47,854	71,586
Live births	23,634	47,616	71,250

models have reported lung hypoplasia caused by induced oligohydramniosis, with the greatest effects during the canalicular stage of lung development.[19] Amniocentesis performed on pregnant monkeys caused a reduction in the number of alveoli and respiratory airways regardless of the time of amniocentesis, the amount of fluid removed and even if the membranes were punctured with no fluid removal.[20] Whether invasive procedures influence the risk of preterm birth, small for gestational age and infant death remains to be investigated in a larger population.

The aim of the present study was to investigate whether amniocentesis and chorionic villus sampling in routine obstetric care performed for low risk indications increase the risk of foot, hip and other limb deformities, limb reduction defects or respiratory problems in the newborn, and whether the risk for fetal and infant mortality, prematurity, low birthweight and fetal distress increases. A second aim was to study if the gestational length at the procedure had any impact.

Table 2. Diagnostic codes used as exclusion criteria and as outcome variables according to the Swedish version of the International Classification of Diseases, Ninth Revision (ICD-9) and Tenth Revision (ICD-10).

ICD-9 code	ICD-10 code	Definition	Comments
Chromosomal aberrations			
758A	Q90.0–Q90.9	Trisomy 21	
758B	Q91.4–Q91.7	Trisomy 13	
758C	Q91.0–Q91.3	Trisomy 18	
758D	Q93.0–Q93.9	Cri-du-chat syndrome	
758F	Q92.0–Q92.9	Autosomal aberration	
758G	Q96.0–Q96.9	Turner syndrome/46 X0	
758X	Q99.9	Non-specified chromosomal aberration	
Musculoskeletal malformations			
754D	Q65.0–Q65.9	Hip dislocation	including positive Ortolani test
754E	Q68.2–Q68.5	Curving of femur, tibia, fibula and knee dislocation	
754F,G,H	Q66.0–Q66.9	Club foot	
755W	Q74.3	Arthrogryphosis multiplex	
Limb reduction malformations			
755C	Q71.0–Q71.9	Limb reductions of upper limb	
755D	Q72.0–Q72.9	Limb reductions of lower limb	
755E	Q73.0–Q73.8	Limb reductions of non-specified limb	
Respiratory disorders			
769	P22.0	Idiopathic respiratory distress syndrome	
770		Respiratory disturbances	
770A	P23.0–P23.9	Neonatal pneumonia	
770B	P24.0–P24.9	Meconium aspiration	
770C	P25.0–P25.8	Pneumothorax	
770D	P26.0–P26.9	Lung bleeding	
770EF	P28.0–P28.1	Atelectasis	
770H	P27.0–P27.9	Bronchopulmonary dysplasia	
770GX	P22.1+22.8+28.9	Other respiratory problems	e.g. wet lung syndrome, tachypnea, unspecified symptoms
770W	P28.2–P28.8+22.9	Other specified disturbances	e.g. apnea, cyanosis
Perinatal conditions			
779A	P90	Neonatal convulsions	

Table 3. The number of amniocentesis and chorionic villus samplings performed 1991–1996 and gestational age for the procedures. Values are presented as *n* (%).

Gestation at procedure (weeks + days)	Amniocentesis	Chorionic villus sampling
9 + 0 to 9 + 6	7 (0)	168 (8.5)
10 + 0 to 10 + 6	22 (0.1)	748 (37.7)
11 + 0 to 11 + 6	142 (0.7)	700 (35.3)
12 + 0 to 12 + 6	1108 (5.1)	254 (12.8)
13 + 0 to 13 + 6	5628 (25.9)	61 (3.1)
14 + 0 to 14 + 6	7421 (34.1)	19 (0.9)
15 + 0 to 15 + 6	4797 (22)	6 (0.3)
16 + 0 to 16 + 6	1937 (8.9)	3 (0.1)
17 + 0 to 17 + 6	497 (2.3)	8 (0.4)
18 + 0 to 18 + 6	125 (0.6)	7 (0.4)
19 + 0 to 19 + 6	48 (0.2)	7 (0.4)
20 + 0 to 20 + 6	16 (0.1)	3 (0.1)
Total number	21,748 (100)	1984 (100)

METHODS

The study population consisted of women 35 to 49 years old with single births in Sweden during the period 1991–1996. The study population appears from Table 1, and has been described in detail previously.[21] Infant outcomes during the first year of life were collected from the Swedish Medical Birth Register, the Swedish Hospital Discharge Register, the Swedish Malformation Register and the Swedish Cause of Death Register held by the National Board of Health and Welfare. These registers contain data including demographic data, reproductive history, complications during pregnancy, delivery and the neonatal period, all inpatient care in Sweden and major malformations, respectively. The registers have been described in detail.[21,22]

Women exposed to amniocentesis or chorionic villus sampling were identified by records from the seven genetic laboratories in Sweden. Data collected were the women's

Table 4. Maternal characteristics for women in the study population. Values are presented as *n* (%).

Maternal characteristics	Non-exposed	Amniocentesis	Chorionic villus sampling
Age			
35–39	43,291 (90.5)	15,768 (72.5)	1190 (60)
40–44	4404 (9.2)	5771 (26.5)	756 (38.1)
45–49	159 (0.3)	209 (1)	38 (1.9)
Body mass index			
<18.5	553 (1.2)	308 (1.4)	8 (0.4)
18.5–<25	19,404 (40.5)	10,803 (49.7)	781 (39.4)
25–<30	8070 (16.9)	3116 (14.3)	269 (13.6)
≥30	2828 (5.9)	796 (3.7)	47 (2.3)
No information	16,999 (35.5)	6725 (30.9)	879 (44.3)
Smoking			
Non-smoker	36,007 (75.2)	16,951 (78.0)	1583 (79.8)
1–9 cigarettes/day	5484 (11.5)	2268 (10.4)	199 (10)
≥10 cigarettes/day	3967 (8.3)	1501 (6.9)	108 (5.5)
No information	2396 (5)	1028 (4.7)	94 (4.7)
Parity			
para 0	8994 (18.8)	5010 (23)	303 (15.3)
para 1 or 2	27,517 (57.5)	13,134 (60.4)	1225 (61.7)
para ≥3	11,343 (23.7)	3604 (16.6)	456 (23)
Previous miscarriage			
0	33,410 (69.8)	15,005 (69)	1334 (67.3)
1	9913 (20.7)	4706 (21.6)	465 (23.4)
>1	4531 (9.5)	2037 (9.4)	185 (9.3)
Previous stillbirth	495 (1)	200 (0.9)	21 (1)
Previous neonatal death	450 (0.9)	222 (1)	26 (1.3)
Previous postnatal death	279 (0.6)	135 (0.6)	28 (1.4)
Previous birthweight <2500	2879 (6.0)	1097 (5)	123 (6.2)
Total	47,854	21,748	1984
Women with live births	47,616	21,654	1980

personal identification number, the date for the invasive procedure, type of procedure and the karyotype. In one of the genetic laboratories, the registration regarding women's exposure to an invasive procedure and the dates for the procedures were incomplete. Therefore, all women giving birth during the study period in this region were excluded.

To exclude women with high risk indications, the study cohort was limited to women aged 35 or more.[21] Major chromosomal abnormalities were found to be under-reported to the registers. Therefore, we excluded from the study population only cases of major chromosomal abnormality (Table 2) reported to the Medical Birth Register, the Swedish Malformation Register or the Swedish Hospital Discharge Register in order to obtain comparable groups of exposed and not exposed.

Table 3 gives the number of women exposed to amniocentesis or chorionic villus sampling and the number of invasive procedures for each gestational week. The gestational length at which the invasive procedures were performed was recorded as completed weeks and calculated according to the registered date for the invasive procedure and the information from the Medical Birth Register on the estimated date of delivery.

Maternal characteristics for age, pre-pregnancy body mass index, smoking and parity were collected.[21] Information about previous miscarriage was collected from the Medical Birth Register and categorised into none, one or two and more previous miscarriages, respectively. Information about previous late fetal, neonatal and postnatal death and previous low birthweight (<2500 g) infants was extracted from the Medical Birth Register. Late fetal death was defined as intrauterine fetal death from gestational week 28 until birth. Neonatal death was defined as death occurring during the first 27 days of life, and postnatal death as death occurring from 28 days of life until the age of 12 months. Small for gestational age was defined as a birthweight of more than two standard deviations below the mean for the gestational age and gender according to a Swedish reference curve.[23] In Sweden, an ultrasound scan in the second trimester is offered to all pregnant women and 97% attend. The estimated date of delivery is primarily based on fetal biometry from that ultrasound scan. Preterm birth was categorised as extremely preterm (gestational length <196 days), very preterm (<224 days) and preterm (<259 days). As outcome measures for fetal distress, Apgar scores at 5 minutes and neonatal convulsions (ICD-9 code 779A) were used. The rates of Apgar scores at 5 minutes below 5 or below 7 were recorded.

In Table 2, the ICD codes used as outcome variables are listed. Subcategories were used in the outcome analysis but due to geographical and individual differences regarding the use of diagnostic codes, we sometimes chose to use the three-character categories. Musculoskeletal deformities were identified using the ICD-9 code 754 (D, E, F, G, H), together with the code for congenital arthrogryphosis (ICD-9 code 755W), which more naturally belongs to the group of musculoskeletal deformities. The corresponding ICD-10 codes were used for outcome analyses for infants born at the end of the study period.

Logistic regression analyses were performed to calculate crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) using the SAS programme package, version 8 (SAS Institute, Cary, North Carolina). Comparisons were made between the amniocentesis group *versus* non-exposed and the chorionic villus sampling group *versus* non-exposed. Except for the outcomes late fetal, neonatal and post-neonatal death, infant outcomes were given for the population of women giving birth to a live infant. Maternal age, parity, body mass index, smoking and delivery hospital were regarded as possible confounders and controlled for in all calculations. A previous infant with low birthweight (<2500 g) was used in the model as a possible confounder for small for gestational age and preterm delivery. Preterm rupture of membranes and gestational length were regarded as possible intermediate variables for respiratory disturbances. The gestational length was used in the model in a third degree polynomial. The Ethical Committee of the Medical Faculty at Uppsala University and the Swedish Data Inspection Board gave consent to the study.

RESULTS

The study population consisted of 71,586 women 35 to 49 years old who gave birth in Sweden during 1991 to 1996 and of these were 21,748 (30%) exposed to amniocentesis and 1984 (3%) to chorionic villus sampling. A total of 71,250 women had live births. Maternal characteristics are described in Table 4. The amniocentesis procedures were mainly performed between 13 and 16 weeks and the chorionic villus sampling procedures between 10 and 12 weeks (Table 3).

The risk of musculoskeletal malformations was increased in the amniocentesis group (OR = 1.32, 95% CI 1.11–1.57) compared with the non-exposed group (Table 5). The elevated risk was found when amniocentesis was performed before 14 weeks of gestation. For amniocentesis performed at 14 weeks of gestation or later, the ORs remained above one although not significantly (Table 6). The OR for hip dislocation after amniocentesis was 1.22 (95% CI 0.99–1.50) (Table 5) and ORs were above one for each gestational week (Table 6). The risk of club foot was higher in the amniocentesis group (OR = 1.45, 95% CI 1.06–1.99) compared with the non-exposed group (Table 5), and the elevated risk was found to be associated with amniocentesis at less than 14 weeks of gestation and the highest risk at less than 13 weeks (Table 6). No association was found between club foot and premature rupture of membranes. The rate of congenital arthrogryphosis and curving of the femoral, tibial and fibular bone and knee dislocation was higher in the amniocentesis group, although the reported numbers were few (Table 5). No increased risk

Table 5. Infant outcome. Outcome for infants to mothers exposed to amniocentesis or chorionic villus sampling *versus* non-exposed women expressed as crude OR and adjusted OR with 95% CI. Each event is given in absolute numbers and numbers per thousand.

Outcome	Non-exposed (N = 47,854; Liveborn = 47,616)		Amniocentesis (N = 21,748; Liveborn = 21,654)				
	n	*n*/1000	*n*	*n*/1000	OR		95% CI Adjusted
					Crude	Adjusted	
Musculoskeletal malformations	432	9.1	257	11.9	1.31	1.32	1.11–1.57
Hip dislocation	314	6.6	172	7.9	1.21	1.22	0.99–1.50
Club foot	119	2.5	80	3.7	1.48	1.45	1.06–1.99
Curving of femur, tibia, fibula; knee dislocation	4	0.1	16	0.7	8.80	6.44	1.99–20.81
Arthrogryphosis	3	0.1	5	0.2	3.67	10.02	1.88–53.48
Limb reduction defects	22	0.5	9	0.4	0.90	0.90	0.38–2.09
Respiratory disturbances	1735	36.4	894	41.3	1.14	1.12	1.02–1.24
Neonatal pneumonia	206	4.3	137	6.3	1.46	1.29	1.02–1.65
Meconium aspiration	99	2.1	64	3.0	1.42	1.29	0.91–1.84
Pneumothorax	147	3.1	70	3.2	1.05	1.04	0.76–1.44
Lung bleeding	7	0.1	1	0.0	0.31	0.16	0.02–1.42
Atelectasis	8	0.2	8	0.4	2.20	3.36	1.11–10.18
Bronchopulmonary dysplasia	56	1.2	26	1.2	1.02	1.27	0.71–2.28
Other respiratory problems	1120	23.5	542	25.0	1.07	1.11	0.99–1.25
Other specified disturbances	278	5.8	144	6.7	1.14	0.98	0.77–1.23
Idiopatic respiratory distress syndrome	350	7.4	139	6.4	0.87	0.85	0.65–1.11
Fetal distress							
5-minute Apgar ≤4	200	4.2	80	3.7	0.88	0.82	0.62–1.09
5-minute Apgar ≤7	521	11.0	221	10.3	0.93	0.90	0.75–1.07
Neonatal convulsions	112	2.4	48	2.2	0.94	1.03	0.71–1.50
Preterm birth							
Delivery ≤28 weeks of gestation	167	3.5	61	2.8	0.80	0.72	0.52–0.99
Delivery ≤32 weeks of gestation	466	9.8	209	9.7	0.99	0.90	0.75–1.08
Delivery ≤37 weeks of gestation	2959	62.1	1275	58.9	0.94	0.90	0.83–0.97
Small for gestational age	1445	30.4	690	32.0	1.05	0.89	0.80–0.99
Fetal and infant death							
Late fetal death	238	5.0	94	4.3	0.87	0.80	0.62–1.04
Neonatal death	143	3.0	56	2.6	0.86	0.82	0.58–1.15
Post-neonatal death	69	1.4	25	1.1	0.80	0.90	0.54–1.48

Outcome	Chorionic villus sampling (N = 1984; Liveborn = 1980)				
	n	*n*/1000	OR		95% CI Adjusted
			Crude	Adjusted	
Musculoskeletal malformations	14	7.1	0.78	0.84	0.49–1.45
Hip dislocation	8	4.0	0.61	0.65	0.32–1.32
Club foot	6	3.0	1.21	1.36	0.58–3.19
Curving of femur, tibia, fibula; knee dislocation	1	0.5	6.01	4.07	0.40–41.25
Arthrogryphosis	–	–	–	–	–
Limb reduction defects	1	0.5	1.09	1.27	0.16–10.14

Table 5. (*continued*)

Outcome	Chorionic villus sampling ($N = 1984$; Liveborn = 1980)				
	n	*n*/1000	OR		95% CI Adjusted
			Crude	Adjusted	
Respiratory disturbances	77	38.9	1.07	1.17	0.91–1.50
Neonatal pneumonia	9	4.5	1.05	1.29	0.64–2.57
Meconium aspiration	5	2.5	1.22	1.14	0.45–2.89
Pneumothorax	2	1.0	0.33	0.33	0.08–1.37
Lung bleeding	–	–	–	–	–
Atelectasis	–	–	–	–	–
Bronchopulmonary dysplasia	3	1.5	1.29	1.43	0.35–5.91
Other respiratory problems	56	28.3	1.21	1.40	1.04–1.86
Other specified disturbances	10	5.1	0.86	0.82	0.42–1.61
Idiopatic respiratory distress syndrome	16	8.1	1.10	1.31	0.68–2.53
Fetal distress					
5-minute Apgar ≤4	9	4.6	1.08	1.00	0.50–1.99
5-minute Apgar ≤7	16	8.1	0.74	0.68	0.41–1.14
Neonatal convulsions	6	3.0	1.29	1.51	0.64–3.57
Preterm birth					
Delivery ≤28 weeks of gestation	9	4.5	1.30	0.79	0.39–1.58
Delivery ≤32 weeks of gestation	16	8.1	0.82	0.58	0.35–0.97
Delivery ≤37 weeks of gestation	107	54.0	0.86	0.73	0.60–0.90
Small for gestational age	45	22.8	0.74	0.70	0.52–0.96
Fetal and infant death					
Late fetal death	4	2.0	0.40	0.36	0.13–0.99
Neonatal death	4	2.0	0.67	0.52	0.19–1.44
Post-neonatal death	3	1.5	1.04	0.93	0.28–3.05

of musculoskeletal malformations was found in the chorionic villus sampling group (Table 5).

The occurrence of limb reduction defects was similar in the amniocentesis (0.4/01000), chorionic villus sampling (0.5/1000) and non-exposed group (0.5/1000) (Table 5).

Respiratory disturbances (ICD-9 code group 770) were more frequent in infants born after amniocentesis (OR = 1.12, 95% CI 1.02–1.24) compared with the non-exposed group (Table 5). Amniocentesis performed at 14 and 15 weeks of gestation constituted the highest risk (Table 6). There was an elevated risk of neonatal pneumonia in the amniocentesis group (OR = 1.29, 95% CI 1.02–1.65) (Table 5). The highest risk was found for amniocentesis at less than 13 weeks of gestation (OR = 2.76, 95% CI 1.61–4.72) (Table 6). Due to geographical and individual differences in the use of diagnostic codes, the occurrence of acquired pneumonia (ICD-9 codes 480–82, 485, 486) was also studied. No differences were found. The risk of meconium aspiration was not significantly elevated in the total amniocentesis group (OR = 1.29, 95% CI 0.91–1.84) but for amniocentesis at 13 weeks of gestation a significant risk increase (OR = 1.77, 95% CI 1.06–2.93) was found. No correlation was found between neonatal pneumonia or meconium aspiration and chorioamnionitis (ICD-9 code 658E). In the chorionic villus sampling group, the corresponding ORs were on the same level above one, but the differences were not significant (Table 5). The risk of atelectasis was higher in the amniocentesis group (OR 3.36) although the number of cases were low (Table 5). The occurrence of 'other respiratory problems' was significantly more frequent in the chorionic villus sampling group (OR = 1.40, 95% CI 1.04–1.86) but not in the amniocentesis group (OR = 1.11, 95% CI 0.99–1.25) (Table 5). The ORs for idiopathic respiratory distress syndrome were 0.85 in the amniocentesis and 1.31 in the chorionic villus sampling group, but not significant (Table 5). No association between respiratory disturbances and preterm rupture of membranes after 28 weeks of gestation was found.

The rates of low Apgar scores at 5 minutes, classified as ≤4 or ≤7, were similar between the amniocentesis (OR = 0.82 and 0.90, respectively), chorionic villus sampling (OR = 1.0 and 0.68, respectively) and non-exposed groups. No significant difference was found in neonatal convulsions between exposed and non-exposed, although the OR was 1.51 in the chorionic villus sampling group (Table 5).

The occurrence of a preterm birth was less frequent among women exposed to an invasive procedure (Table 5).

Table 6. The risk of infant complications according to the gestation (weeks) for the amniocentesis calculated as OR with 95% CI *versus* no invasive test. Subcategories are written in italics. All subcategories are not presented.

	≤12 weeks				13 weeks				14 weeks			
	n	*n*/1000	Adjusted		*n*	*n*/1000	Adjusted		*n*	*n*/1000	Adjusted	
			OR	95% CI			OR	95% CI			OR	95% CI
Musculoskeletal malformations	31	24.4	2.63	1.78–3.88	68	12.1	1.34	1.02–1.76	75	10.2	1.13	0.88–1.47
Hip dislocation	13	10.2	1.52	0.86–2.71	43	7.7	1.18	0.84–1.65	54	7.3	1.13	0.83–1.53
Club foot	17	13.4	5.14	2.93–9.00	25	4.5	1.76	1.11–2.80	17	2.3	0.92	0.54–1.56
Respiratory disturbances	59	46.4	1.10	0.82–1.48	212	37.8	0.98	0.84–1.16	321	43.5	1.21	1.06–1.39
Neonatal pneumonia	17	13.4	2.76	1.61–4.72	31	5.5	1.06	0.71–1.58	49	6.6	1.33	0.95–1.85
Meconium aspiration	2	1.6	0.96	0.23–4.00	21	3.7	1.77	1.06–2.93	22	3	1.28	0.78–2.09
Bronchopulmonary dysplasia	1	0.8	0.72	0.08–6.12	9	1.6	1.28	0.54–3.02	9	1.2	1.72	0.73–4.08
Other respiratory problems	32	25.2	0.90	0.62–1.31	127	22.6	0.96	0.79–1.18	191	25.9	1.17	0.99–1.39

	15 weeks				≥16 weeks			
	n	*n*/1000	Adjusted		*n*	*n*/1000	Adjusted	
			OR	95% CI			OR	95% CI
Musculoskeletal malformations	55	11.5	1.29	0.96–1.72	28	10.7	1.18	0.80–1.75
Hip dislocation	42	8.8	1.36	0.97–1.91	20	7.7	1.18	0.74–1.87
Club foot	12	2.5	0.99	0.54–1.82	9	3.4	1.36	0.68–2.72
Respiratory disturbances	210	44	1.24	1.05–1.45	92	35.2	1.00	0.80–1.26
Neonatal pneumonia	29	6.1	1.30	0.86–1.95	11	4.2	0.98	0.53–1.83
Meconium aspiration	15	3.1	1.31	0.74–2.30	4	1.5	0.62	0.22–1.71
Bronchopulmonary dysplasia	7	1.5	1.60	0.61–4.19	–	–	–	–
Other respiratory problems	133	27.8	1.28	1.06–1.56	59	22.6	1.05	0.80–1.38

Whatever the limit for preterm birth, the amniocentesis and chorionic villus sampling groups had ORs less than one and in the amniocentesis group the risk of preterm birth before 28 and 37 weeks was significantly decreased. In the chorionic villus sampling group, the risk of preterm birth before 32 and 37 weeks was significantly decreased. A decreased risk of a small for gestational age birth was found in women exposed to an invasive procedure (Table 5).

The risk of late fetal death did not differ between the amniocentesis and the non-exposed group, but was lower in the chorionic villus sampling (OR = 0.36) compared with the non-exposed group. The ORs for neonatal and postneonatal death were below one in the amniocentesis (0.82 and 0.90, respectively) and chorionic villus sampling (0.52 and 0.93, respectively) groups, although not significant.

Finally, we analysed whether the effect was modified by age, but no significant effect-modifying was found for any of the outcomes.

DISCUSSION

Amniocentesis performed before 14 weeks of gestation increases the risk of chromosomally normal infants being born with club foot. The risk for respiratory disturbances is increased especially after amniocentesis at 14 and 15 weeks. Moreover, an association between amniocentesis and orthopaedic postural deformities such as hip dislocation, curving of bones in the lower limbs, knee dislocation and congenital arthrogryphosis was found. Regarding chorionic villus sampling, no association to postural deformities was found, but for respiratory disturbances a possible correlation is suggested. There were no elevated risks of limb reduction defects, preterm delivery, small for gestational age births, fetal distress or fetal and infant death neither in the amniocentesis nor in the chorionic villus sampling groups.

There is no reason to believe that under-reporting of chromosomal abnormalities differed between the exposed and non-exposed groups. Likewise, there is no reason to believe that the number of records with missing or incorrectly registered data differed between the groups. Thus, we believe our study population to be representative. All women included in the study should have been offered invasive testing because that was routine procedure in Sweden. The uptake for invasive testing was 33%. During the study period, early amniocentesis was common (Table 3). Publications from the late 1990s[4,5] have shown more adverse outcomes after early amniocentesis, which has led to a policy where amniocentesis is performed at 15 weeks of gestation. Routines for offering invasive testing and the use of diagnostic codes at different hospitals could differ. Therefore, hospital was used as a confounder.[22] Maternal age, smoking and parity are well-known factors of the importance for pregnancy outcome.[24,25] Smoking and body mass index were used as proxies for socio-economic status.[26–30] The

chorionic villus sampling group was somewhat smaller than expected, which reduced the statistical power markedly.

Our study confirms an association between amniocentesis and musculoskeletal postural deformities.[6] A significantly increased risk was seen for amniocentesis before 14 weeks of gestation (Table 6). The risk figures for hip dislocation were on a similar level but not significant. Recent studies have reported a frequency of 1.3–1.7% of club foot after early amniocentesis,[3,5,7] which corresponds to the 1.3% found for amniocentesis at 12 weeks of gestation or less in this study (Table 6). For the whole amniocentesis group, of which more than half of the procedures were performed after 13 weeks of gestation, the figure for club foot was 0.37%. Under-reporting of club foot could have occurred, but the higher figure for club foot for amniocentesis at less than 13 weeks of gestation probably indicates a true biological effect. A considerably increased risk of club foot was found after early amniocentesis when leakage of amniotic fluid was reported.[5,31] Even when a reduced volume of amniotic fluid was sampled, or the amniotic fluid was recirculated back to the cavity after filtration, club foot was still found to occur in 1.6–1.7%.[7,32] In our study we could obtain information about premature rupture of membranes after 28 weeks of gestation and no correlation to club foot was found. For chorionic villus sampling, where the procedure was performed with almost the same technique except that the membranes were not punctured and amniotic fluid not withdrawn, no increased risk was found. With amniocentesis earlier in gestation, a relatively larger volume is withdrawn.[33] Before week 14 when the amnion and chorion have not yet fused, an overt or subclinical leakage of amniotic fluid is more likely and may affect the physiological development and position of the lower limbs. From our finding and others, an association between club foot and withdrawal of amniotic fluid or puncturing of the membranes, is suggested. An underlying process of a similar nature may be the cause of hip dislocation and other postural deformities.

No association between limb reduction defects and chorionic villus sampling after nine weeks of gestation was found in our study, although the small chorionic villus sampling group reduced the statistical power. The incidence of limb reductions was of the same magnitude as has been reported from population-based studies[34–36] as well as the WHO-sponsored chorionic villus sampling registry.[12]

Our results confirm a correlation between amniocentesis and infant lung dysfunction. Moreover, our report found a possible association between timing of amniocentesis and effect, with the most evident impact at 14 and 15 weeks of gestation (Table 6). Thus, the findings from previous minor studies are verified, reporting an effect of amniocentesis on infant lung function expressed in varying diagnoses such as respiratory distress and pneumonia,[1] unexplained respiratory difficulties,[6] increased respiratory morbidity[15] and findings indicating an effect on lung growth and development.[37] The chorionic villus sampling group had a significantly greater proportion of infants with tachypnea and unspecified respiratory symptoms (diagnosis 770 G, X). Also the other subcategories showed a trend towards an increased risk in the chorionic villus sampling group. Although the figures did not reach significance, probably due to the smaller number of chorionic villus sampling cases, the ORs were on the same level as found for amniocentesis. The findings are in concordance with previous studies where associations between chorionic villus sampling and neonatal respiratory distress,[14] high airway resistance[16] and an increased respiratory morbidity the first year of life were reported.[15]

The risk of fetal distress, preterm birth, small for gestational age and fetal and infant death was not increased after invasive procedures irrespective of method used. On the contrary, the reversed situation was found regarding preterm birth and small for gestational age for both the amniocentesis and chorionic villus sampling groups. Furthermore, the ORs were below one in the amniocentesis and chorionic villus sampling groups for late fetal, neonatal and postnatal death without reaching significance, except for late fetal death in the chorionic villus sampling group. There is no reason to believe that invasive procedures have a protective effect. We know that invasive procedures increase the risk of fetal loss.[1,2,4] One possible explanation is that these more vulnerable pregnancies could have ended in miscarriages after exposure to an invasive procedure but continued to a preterm birth or a late fetal death in the non-exposed group. Another explanation could be differences in socio-economic status between exposed and non-exposed groups.

To conclude, we find amniocentesis performed before 14 weeks to be associated with an increased risk of musculoskeletal deformities and amniocentesis performed at 14 and 15 weeks of gestation to be associated with respiratory disturbances in the infant. For chorionic villus sampling, we found no increased infant morbidity except for respiratory disturbances, where a possible association was found.

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