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Ultrasound findings and detection of fetal abnormalities before 11 weeks of gestation

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CONTRIBUTION

What is already known about this topic?

- Many fetal structural abnormalities are detectable in the antenatal period using obstetric ultrasound
- Fetal structural abnormalities are usually detected at the mid-trimester morphology ultrasound (performed at 18-24 weeks' gestation) or the first trimester morphology ultrasound (11-14 weeks' gestation)
- Abnormalities are more likely to be diagnosed at a later gestation

What does this study add?

- There has been little study to date on the detection of fetal structural abnormalities before 11 weeks of gestation
- This study investigates the proportion of fetal structural abnormalities detected at less than 11 weeks of gestation as compared with detection at a later gestation
- It is demonstrated that some fetal structural abnormalities can be detected at a very early gestation (less than 11 weeks of gestation), and this is more likely to occur with major abnormalities

Data availability statement

To ensure patient privacy, the data supporting this study are not publicly available. For enquiries regarding the data represented in this study, please contact the corresponding author SM.

ABSTRACT

Objective: To determine the proportion of major fetal structural abnormalities that can be detected before 11 gestational weeks.

Methods: We conducted a retrospective study of individual patient files at a tertiary provider of obstetric and gynecological ultrasound in Melbourne, Australia. All patients who had a pre-cell-free DNA (pre-cfDNA) ultrasound with a crown-rump length (CRL) of less than 45mm and had one or more ultrasounds at a later gestation were included in the analysis.. The primary outcome was the incidence of a fetal structural abnormality.

Results: A total of 3333 cases were included in the final analysis. Overall, 316 fetuses (9.5%) had a structural abnormality detected at any point throughout gestation, of which 86 were major structural abnormalities (2.6%). Sixteen fetal abnormalities were detected before 11 weeks of gestation, including fifteen major abnormalities (17.4% of the major anomalies). All major fetal abnormalities detected before 11 gestational weeks were confirmed at later ultrasound examinations or the pregnancy did not continue (in four cases due to termination of pregnancy and in one case spontaneous miscarriage before first trimester morphology ultrasound).

Conclusion: Detection of fetal abnormalities is possible before 11 weeks of gestation. Early suspicion is more likely in cases of major structural abnormalities.

INTRODUCTION

Historically, prenatal diagnosis of fetal abnormalities was made at the mid-trimester morphology ultrasound, performed at 18-24 weeks of gestation.[1] Since the relationship between increased nuchal translucency and Down syndrome was established,[2] an ultrasound examination at 11-14 weeks of gestation has become commonplace in many countries for the purpose of screening for chromosomal abnormalities. With time, the utility of this first-trimester ultrasound has extended beyond the nuchal translucency measurement and has become an opportunity for early fetal anatomical survey, as it is recognized that a significant proportion of fetal abnormalities can be detected at this early stage of pregnancy.[3-5]

The advent of cell-free DNA (cfDNA) screening for fetal chromosomal abnormalities, which can be performed from 10 weeks of gestation, has prompted a more frequent use of ultrasound to examine pregnancy viability and other parameters of fetal health prior to blood sampling (pre-cfDNA ultrasound).[6,7] Recent evidence has shown that findings at this pre-cfDNA ultrasound render cfDNA screening inappropriate in approximately one in ten pregnancies.[6] Contraindications to cfDNA screening detected on pre-cfDNA ultrasound span from gestation less than estimated by last normal menstrual period to major fetal abnormalities including acrania, holoprosencephaly and major heart defects.[6] With increasing uptake of cfDNA from 10 weeks and ultrasound examinations performed before 11 weeks of gestation, as well as the improvements in imaging resolution, ultrasonography in early pregnancy is a topic of growing interest and, as the boundaries of sonographic detection of pregnancy complications are pushed to earlier gestational ages, the knowledge of sonoembryology becomes essential to deliver high-quality ultrasound.[7]

The purpose of this study is to investigate the proportion of fetal structural abnormalities detected before 11 weeks of gestation and to determine how many of those with a suspected structural abnormality had a confirmed diagnosis in later ultrasound examinations or postnatally.

METHODS

Study Design and Population

This was a single-center retrospective study of all consecutive patients attending for a pre-cfDNA ultrasound at Monash Ultrasound for Women, a tertiary provider of obstetric and gynecological ultrasound in Melbourne, Australia, from January 2017 to December 2018. Routinely, a transvaginal ultrasound is performed prior to blood sampling for cell-free DNA screening for fetal chromosomal abnormalities (pre-cfDNA ultrasound) at this center. The primary purpose of this ultrasound examination is to determine pregnancy viability, gestational age, and number of fetuses as a precursor for appropriate use of cfDNA screening. In addition, fetal structural abnormalities are occasionally incidentally detected at this early gestation. Following a normal pre-cfDNA ultrasound, cfDNA screening is performed and it is recommended that women attend for a first trimester anatomy ultrasound at 12-13 weeks of gestation and a mid-trimester morphology ultrasound at 20-22 weeks of gestation.

Ethical Approval

This study was approved by Monash Health Human Research Ethics Committee (Reference Number: HREC/46571/MonH-2018-152982(v1), Monash Health Ref: RES-18-0000-567L). As this study was a retrospective analysis of data that was originally collected for routine clinical care, individual patient consent was not required.

Data Collection

We conducted a review of individual files of all pregnancies undergoing pre-cfDNA ultrasound between January 2017 to December 2018. Maternal demographic characteristics (including age, height, weight, and parity) were collected, in addition to method of conception of the current pregnancy and history of a chromosomal abnormality in a previous pregnancy. All obstetric ultrasound examination reports for each woman were individually reviewed (including pre-cfDNA, first trimester 11-14 weeks ultrasound, mid-trimester anatomy scan and any additional ultrasounds). Genetic counselling notes and the results of any diagnostic testing were also reviewed.

All pregnancies with a viable fetus with a crown-rump length (CRL) less than 45 mm at pre-cfDNA ultrasound who also had at least one subsequent ultrasound examination performed at our center were eligible for inclusion. Women were considered ineligible if they were under the age of 18 complete years on the date of pre-cfDNA ultrasound, had no further ultrasounds at the center or had a CRL of 45 mm or more at pre-cfDNA ultrasound (as the fetus was therefore of appropriate gestation for an 11-14-week anatomy ultrasound). Following this, we excluded women with a miscarriage detected at pre-cfDNA ultrasound (Figure 1). We did not exclude, however, patients who had a termination of pregnancy based on findings at the pre-

cfDNA ultrasound examination, those who had a known genetic diagnosis, or those for whom the postnatal outcome was available.

All cases of a fetal abnormality (including chromosomal abnormalities) that were detected at any point in pregnancy or postnatally were recorded, if a pre-cfDNA ultrasound had been performed. We then classified fetal abnormalities as “major” or “minor” depending upon the severity of the structural abnormality and the likely impact on future quality of life and recorded the incidence of each (Table 1). If a fetus had both major and minor abnormalities, we recorded this as a case of a major abnormality for the purposes of analysis. Cases of isolated increased nuchal thickness or subcutaneous edema where no evidence of other structural defects were classified as minor abnormalities when this was present at 11-14 weeks, and were not classified as an abnormality in fetuses less than 11 weeks.

Although an increased nuchal translucency is often associated with fetal structural abnormalities (usually in the context of chromosomal syndromes), we did not classify this as a structural abnormality as it is widely accepted as a marker, rather than a true structural defect. In addition, if the fetuses examined had a structural abnormality in the context of a chromosomal abnormality, this would already be reflected in our results.

Outcome Measures and Statistical Analysis

The primary outcome was the presence of a major fetal structural abnormality. This was recorded if an ultrasound abnormality was identified on the report of any obstetric

ultrasound performed at the center or postnatally. The classification of “major” or “minor” structural abnormality was determined based on consensus among the study authors, who routinely perform and report high-risk obstetric ultrasound examinations.

Descriptive statistics were obtained. Continuous variables were expressed as mean and standard deviation and categorical variables were expressed as frequency and proportion. The frequency of the primary outcome is reported within the total, and detection rates for major and all abnormalities are given with their respective 95% confidence interval (CI). Analyses were performed using the statistical package software SPSS® (IBM Corp. Released 2019. IBM SPSS Statistics, Version 26.0).

RESULTS

Overall, 3509 patients met inclusion criteria. One patient (<0.1%) was excluded as she was under the age of 18 at the time of pre-cfDNA ultrasound and 175 (5.0%) were excluded due to miscarriage. Therefore, 3333 patients were included in final analysis. Baseline characteristics of the study population are given in Table 2 In total, 316 fetuses (9.5%) had a structural abnormality detected at any point throughout gestation. Of these, 86 fetuses had major abnormalities (2.6% of total) and 230 fetuses (6.9% of total) had minor abnormalities. Fetal abnormalities were suspected at the pre-cfDNA ultrasound with a CRL < 45 mm in 16 cases (5.0% of all abnormalities, 95% CI 2.9% to 8.1%). Fifteen were major structural abnormalities and one was a minor abnormality. As such, 16 of 85 major structural abnormalities were detected at pre-cfDNA ultrasound, giving a detection rate of 17.4% (95% CI 10.2% to 27.4%). The gestational period at diagnosis of each structural abnormality is shown in Table 3.

The mean CRL at pre-cfDNA ultrasound for those fetuses with a structural abnormality was 38.5 mm, equating to a gestational age of 10 weeks and five days. The details of each case of a structural abnormality are presented in Table 4. Of the 16 women with a detected structural abnormality at pre-cfDNA scan, 10 eventually had a termination of pregnancy, one patient had a selective cord ligation of one twin with eventual demise of the other fetus and two had a miscarriage (Table 4). Three women underwent termination of pregnancy performed due to significant, life-limiting findings detected at the pre-cfDNA ultrasound without further ultrasound examinations (one case of Pentalogy of Cantrell with *ectopia cordis*, one case of large omphalocele, spina bifida, bladder exstrophy and limb defects, and one case with alobar holoprosencephaly later diagnosed with trisomy 13 – Table 4. In addition, one woman had chorionic villous sampling (CVS) performed before a formal first trimester anatomy ultrasound which revealed trisomy 18. Eleven of the fifteen women with major structural abnormalities (68.8%) diagnosed at pre-cfDNA ultrasound returned for first trimester morphology ultrasound. Nine of these patients (82% of those with major structural abnormalities suspected at pre-cfDNA ultrasound) had major structural abnormalities confirmed, and one had a missed miscarriage.

All major fetal abnormalities detected before 11 weeks of gestation were confirmed at later ultrasound examinations, or the pregnancy was not continued due to miscarriage or termination of pregnancy prior to first trimester morphology ultrasound due to severe abnormalities. Notably, one case of a minor fetal structural abnormality (dilated fourth ventricle) detected at before 11 weeks had normal further scans. There were no other false positive results (overall false positive rate 0.03%, 95% CI 0 to 0.16%). Thirty-five fetuses (1.0%) had major fetal structural abnormalities detected at the 11-14-week ultrasound. Therefore, 42.9% (95% CI 26.3% to 60.6%) of major abnormalities that

were detectable at first trimester anatomy scan were also identified before 11 weeks of gestation.

Eight of the 16 cases of fetal structural abnormality before 11 weeks had genetic testing performed, using CVS, amniocentesis or after miscarriage on products of conception. Five of these cases (31.2% of fetuses with fetal structural abnormality detected before 11 weeks) had an abnormal karyotype of trisomy 13 or trisomy 18 (Table 4).

DISCUSSION

Main findings and clinical implications

In this study, approximately one sixth of all major abnormalities and approximately 40% of the major fetal structural abnormalities that were detected at 11-14 weeks detected were identified before 11 weeks of gestation. All cases of major fetal abnormalities with early diagnosis were confirmed later in pregnancy, had a miscarriage, or underwent termination of pregnancy based on severe abnormalities identified before 11 weeks of gestation. The three cases that resulted in termination of pregnancy prior to further sonographic assessment were of severe life-limiting multisystem anomalies in which no doubt existed about diagnosis. However, due to the nature of embryological development, detection of every fetal structural abnormality at the early gestation of less than 11 weeks is currently not feasible. Therefore, performing an ultrasound before 11 weeks of gestation should neither replace the fetal morphological assessment at 11-14 weeks nor the mid-trimester anatomy ultrasound.

The detection of fetal abnormalities is dependent upon the severity and the organ system involved,[8] as well as the skill level of the ultrasound operator.[9] A review by Weisz *et al.* showed that detection of fetal head and brain abnormalities is possible before 14 weeks, but that the presence of complex fetal cardiac abnormalities cannot be reliably determined.[4] Syngelaki *et al.* have shown that approximately one third of all fetal abnormalities can be identified at 11-14 weeks, and that a high detection rate exists for certain fetal abnormalities in the first trimester including acrania and body stalk anomaly, however other abnormalities are more difficult to diagnose at this early gestation (such as agenesis of the corpus callosum and many renal defects).[3,8]

Despite these significant advances in first trimester ultrasonography, there is little literature focusing on the evolving detection of fetal structural abnormalities at very early gestations. Very early detection of fetal abnormalities (before 11 weeks gestational age) can be challenging to implement in the clinical setting and depends on adequate extensive training of ultrasound operators. However, with the increasing uptake of cell-free DNA testing from 10 weeks of gestation, a window of opportunity to assess fetal anatomy will inevitably evolve. Early detection of lethal and major fetal abnormalities that are not compatible with normal postnatal life, such as anencephaly and alobar holoprosencephaly, is certainly of benefit both for pregnancy management and from a psychological perspective, as it has been shown that more advanced gestational age at diagnosis and management is associated with a greater likelihood of adverse psychological sequalae.[10] Therefore, a fine balance must be sought between ensuring that women are informed and have the opportunity to participate in their own care and creating unnecessary anxiety about a fetal abnormality. A quagmire exists in the context of a potential fetal structural abnormality that may or may not be

confirmed later in the pregnancy. In this case, the healthcare team, together with the family, must make a decision regarding the most appropriate management.

While detecting critical fetal abnormalities in early pregnancy may mean that early termination of pregnancy is possible for patients, it is important that such major clinical decisions are not made on the basis of a structural abnormality that may evolve or resolve throughout pregnancy, or without follow-up imaging in cases of uncertain diagnosis. The limitations to the implementation of ultrasound examination in early pregnancy lies mainly in the borderline cases and in those where the diagnosis cannot be precisely affirmed, where further assessment of fetal anatomy will be always required. In these cases, detection of a potential structural abnormality will likely result in significant anxiety and, in the worst case, lead to termination of a normal pregnancy. The presence of fetal structural abnormalities may also change diagnostic and management strategies for these patients, which often involves withholding cfDNA testing and proceeding in preference with genetic counselling and later invasive testing.

Comparison with previous studies

A pictorial study by Rolnik *et al.* recently illustrated the detection of fetal structural abnormalities with the aid of three-dimensional imaging and high-resolution transvaginal ultrasonography.[7] There is, however, very limited literature focusing on the detection of fetal abnormalities at a very early gestation. No study has been published in setting of a routine clinical cfDNA service. A study by Votino *et al.* described the association between skin edema and hydrothorax at less than 11 weeks

of gestation with abnormal ultrasounds later in pregnancy. The presence of skin edema and hydrothorax were associated with miscarriage, fetal chromosomal or structural abnormality (odds ratio of 13.457 for skin edema and 19.965 for hydrothorax). However, no fetal structural abnormalities were recorded at this early gestation.[11] Twenty-seven of the 1318 cases (2.0%) eventually had a fetal malformation diagnosed in their cohort.[11]

We observed a relatively high rate of abnormalities in our study (9.5%), which is likely due to a number of factors. First, there were higher proportions of women who were of advanced maternal age, pregnancies conceived using IVF and of twin pregnancies compared to the general population. As these are recognized risk factors for fetal chromosomal and structural abnormalities, there may be a degree of selection bias present. Second, as we included minor abnormalities (such as mild renal pelvis dilatation, bicuspid aortic valve, and solitary renal cysts) and abnormalities that are not true structural defects (such as hypoplastic nasal bone and increased nuchal translucency) we expect a higher incidence of ultrasound findings than reported for major structural abnormalities alone. Finally, the study of fetal malformations before 11 weeks is still relatively limited and we believe that there are significantly more abnormalities than are present at 11-14 weeks.

Khurana *et al.* described a markedly higher incidence of fetal structural abnormalities than was present in our cohort before 11 weeks of gestation (53 of 600 fetuses, 8%). However, 22 fetuses (41% of those with abnormal morphology) were non-viable at the time of initial ultrasound examination, a group that was excluded from our cohort.[12]

Hartge *et al.* conducted a prospective study using 3D ultrasound to evaluate fetuses with a missed miscarriage diagnosed in the first trimester (mean gestational age 70.3

days). Of the 107 fetuses, 23 had an abnormality detected (21.7%).[13] Additionally, another study was conducted by Hartge *et al.* in 2018 to specifically evaluate neural tube defects in cases of a missed miscarriage in the first trimester. They found that 37 of 223 fetuses (16.6%) were affected by a neural tube defect. Although we did not include non-viable fetuses in our study, these references support the conclusion that there is a naturally high attrition in embryos with fetal abnormalities before 11-14 weeks.[14]

Strengths and limitations

To our knowledge, this is the largest study on the detection of fetal structural abnormalities before 11 weeks of gestation. To reduce uncertainty and bias, we aimed to include only cases with further confirmation or a later final diagnosis, and examinations followed the clinic protocol for early fetal assessment and cell-free DNA screening.

The center at which this study was conducted is a large tertiary center with a specialization in obstetric ultrasound. Therefore, the population included in the study is at higher-than-average risk, there is a high skill level of the clinicians who perform ultrasound at this center and our results may not be generalizable to all centers. Another limitation to our study is our limited access to postnatal outcomes. By not including women who did not have follow-up ultrasound examinations at our center, it is possible that some degree of selection bias is present in our study. However, our overall rates of major fetal abnormalities are relatively similar to previously published figures. Due to the rare nature of many of these abnormalities, there were relatively

small numbers of specific abnormalities, which will inevitably limit conclusions about the detection of specific organ systems abnormalities at earlier gestational ages. Therefore, larger studies are needed to determine the detection rates for major fetal defects in early pregnancy with higher precision and which fetal malformations can be reliably identified before 11 weeks of gestation.

CONCLUSION

Detection of major fetal abnormalities is possible before 11 weeks of gestation in approximately one sixth of such cases. Certain conditions that are lethal or encompass very poor prognosis, such as major brain anomalies including acrania and alobar holoprosencephaly, may be reliably identified before 11 weeks of pregnancy, modifying diagnostic and management pathways. Additionally, fetuses with trisomy 13 or 18 are more likely to have multiple, severe abnormalities that may be detected at less than 11 weeks of gestation. Caution and further assessment are required, however, in situations where the diagnosis is less certain.

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TABLES

Table 1: Classification of fetal structural abnormalities.

Classification	Description
Major	- Cardiac abnormalities (COA, HLHS, aortic stenosis, double outlet right ventricle, double aortic arch, TGA, AVSD) - Amniotic band syndrome - Significant renal abnormalities (Pelvic kidney/renal agenesis/MCDK/absent bladder/horseshoe kidney) - Polydactyly and syndactyly - Multisystem abnormalities - Major chromosomal abnormalities - Talipes - Ventriculomegaly - Congenital diaphragmatic hernia - Cerebellar cyst - Hemivertebrae and scoliosis - Hypospadias - Mesocardia - Posterior fossa cysts - Right-sided aortic arch and aberrant left subclavian artery - Encephalocele - Spina bifida/NTDs - Cleft lip and palate - Omphalocele - Retrognathia - Isomerism - Arnold-Chiari malformations - TRAP sequence - Cardiomyopathies
Minor	- Pyelectasis - Absent/hypoplastic nasal bone - Muscular VSDs - Small apical VSDs - Bicuspid aortic valves - Clenched hand with no other abnormalities - Discordance of great vessels - Echogenic and thickened interventricular septum and right ventricular wall - Single renal cyst - Asymmetry of great vessels - Mild micrognathia - Borderline ventriculomegaly - Periventricular pseudocysts

	- Echogenic foci of the spine - Apparently isolated increased NT/NF and cystic hygroma - Persistent right umbilical vein - ARSA - Echogenic bowel - Dilated and echogenic bowel - Slight enlargement of the right ventricle - Bilateral SVC - Duplex kidney
No structural abnormality	- Single umbilical artery - FGR/ SGA - Short long bones - Dolicocephaly - Demise of a fetus - Oligohydramnios/polyhydramnios - Intracardiac echogenic foci - Arrhythmias - Bifid rib - Jugular sacs - Mild-moderate mitral regurgitation - Liver calcification - Umbilical cord cyst - Umbilical vein varix - Tortuous ductus arteriosus with mild narrowing - Isolated intrathymic left brachiocephalic vein - Echogenic foci of liver

COA: coarctation of the aorta, HLHS: hypoplastic left heart syndrome, TGA: transposition of the great arteries, AVSD: atrioventricular septal defect, MCDK: multicystic dysplastic kidney, NTD: neural tube defect, TRAP: twin reverse arterial perfusion, VSD: ventricular septal defect, NT: nuchal translucency, NF: nuchal fold, ARSA: aberrant right subclavian artery, SVC: superior vena cava, FGR: intrauterine growth restriction, SGA: small for gestational age, SD: standard deviation

Table 2: Characteristics of the study population

Maternal age (years), mean (SD)	33.46 (4.5)
Age < 35 years, n (%)	2159 (64.8)
Age 35 to 39.9 years, n (%)	923 (27.7)
Age ≥ 40 years, n (%)	251 (7.5)
Weight (Kg), mean (SD)	67.33 (16.0)
Height (cm), mean (SD)	163.85 (7.1)
BMI (Kg/m^2), mean (SD)	25.01 (5.4)
Parity	
Nulliparous, n (%)	1750 (52.5)
Parous, n (%)	1547 (46.4)
Missing	36 (1.1)
Conception	
Spontaneous, n (%)	2748 (82.4)
IVF, n (%)	585 (17.6)
Own egg, n (% of IVF)	526 (89.9)
Egg donor, n (% of IVF)	59 (10.1)
Twin pregnancy, n (%)	107 (3.2)
Crown-rump length at pre-cfDNA ultrasound, mean (SD)	38.2 (4.2)
History of fetal chromosomal abnormality	76 (2.2)

Missing data on 36 patients for parity and 38 for height, weight and BMI (excluded case-wise). SD: standard deviation, BMI: body mass index, IVF: *in vitro* fertilization, cfDNA: cell-free DNA

Table 3: Incidence of each fetal structural abnormality

Fetal abnormalities	Cases detected			
	< 11 weeks	11-14 weeks	> 14 weeks	Total
Neurological				
Acrania/anencephaly	1	-	-	1
Holoprosencephaly	2	-	-	2
Encephalocele	1	-	-	1
Dilated fourth ventricle	2	3	-	4
Ventriculomegaly	-	-	3	3
Posterior fossa cyst	-	-	1	1
Periventricular cysts	-	-	1	1
Spina bifida	1	-	-	1
Craniofacial				
Absent or hypoplastic nasal bone	-	13	2	15
Cleft lip with intact palate	-	1	-	1
Cleft palate with intact lip	-	1	-	1
Cleft lip and palate	-	4	-	4
Retrognathia	-	2	1	3
Cardiovascular				
Transposition of the great arteries with large perimembranous ventricular septal defect	-	1	-	1
Hypoplastic left heart syndrome	1	3	-	4
Polyvalvular dysplasia	-	1	-	1
Aortic stenosis	-	-	1	1
Bicuspid aortic valve	-	-	2	2
Tricuspid regurgitation	-	1	1	2
Cardiomegaly and tricuspid regurgitation	1	-	-	1
Cardiomyopathy	-	-	1	1
Coarctation of aorta	-	-	3	3
Dextrocardia with pericardial and pleural effusion	-	1	-	1
Double outlet right ventricle	-	-	1	1
Mitral regurgitation	-	1	-	1
Atrioventricular septal defect	1	2	-	3
Aortic insufficiency	-	1	-	1
Transposition of the great arteries	-	-	1	1
Mesocardia	-	-	1	1
Echogenic and thickened interventricular septum and right ventricular wall	-	-	1	1

Bilateral superior vena cava	-	-	4	4
Aberrant right subclavian artery	-	8	10	18
Pulmonary artery stenosis	-	1	-	1
Asymmetric ventricles	-	4	-	4
Abnormal ductus venosus drainage (potential portosystemic shunt)	-	1	-	1
Foramen ovale aneurysm	-	-	1	1
Isolated apical or muscular ventricular septal defect	-	2	13	15
Double aortic arch	-	1	-	1
Right-sided aortic arch	-	1	2	3
Discordance of great vessels	-	1	3	4
Right atrial isomerism	-	1	-	1
Reversed flow along ductus arteriosus	1	-	-	1
Persistent right umbilical vein	-	-	2	2
Pulmonary				
Congenital pulmonary adenomatous malformation	-	-	2	2
Congenital diaphragmatic hernia	-	-	1	1
Abdominal wall				
Omphalocele	2	2	-	4
Gastroschisis	1	-	-	1
Gastrointestinal				
Duodenal stenosis with central gallbladder	-	-	1	1
Echogenic bowel	-	-	2	2
Dilated and echogenic bowel	-	1	-	1
Genitourinary				
Unilateral renal agenesis	-	1	-	1
Bilateral renal agenesis	-	-	1	1
Horseshoe kidney	1	-	-	1
Absent bladder	-	3	-	3
Echogenic kidney	-	2	-	2
Duplex kidney	-	1	2	3
Multicystic dysplastic kidney	-	-	3	3
Pyelectasis	-	-	75	75
Pelvic kidney	-	-	1	1
Renal cyst	-	-	1	1
Hydroureter	-	-	1	1
Ureterocele	-	-	1	1
Hypospadias	-	-	1	1

Skeletal				
Hemivertebrae	-	3	-	3
Absent clavicle	-	1	-	1
Radial ray defect	2	-	-	2
Polydactyly	1	4	1	6
Syndactyly	-	-	3	3
Bilateral thumb abnormalities	-	-	1	1
Clenched hand	-	1	1	2
Abnormal hand	-	1	-	1
Talipes equinovarus	-	1	4	5
Abnormal foot (overextended with overlapping toes)	-	-	1	1
Multisystem abnormalities				
Pentalogy of Cantrell	1	-	-	1
Amniotic band syndrome	-	-	1	1
Body stalk anomaly	1	-	-	1
TRAP sequence	1	-	-	1

TRAP: twin reverse arterial perfusion sequence

Note: each abnormality was recorded as individual. Therefore, if a fetus had more than one abnormality, it may be represented in the table more than once. 86 fetuses had major abnormalities (2.6% of total) and 230 fetuses had minor abnormalities (6.9% of total).
N.B. the abnormalities are recorded at first detection

Table 4: Structural abnormalities detected below 11 weeks of gestational age.

Case Number	Abnormalities at pre-cfDNA ultrasound	CRL pre-cfDNA scan (mm)	Ultrasound 11–14 weeks	Outcome
1	Features consistent with holoprosencephaly, fourth ventricle dilatation, polydactyly	42.4	N/A	TOP before first trimester anatomy ultrasound, karyotype on products of conception indicated T13
2	Single umbilical artery, reversal of flow along ductus arteriosus and increased nuchal thickness (4.9 mm)	35.5	Miscarriage	Miscarriage. No genetic testing on POC.
3	Suspected cardiac abnormality, single umbilical artery and increased nuchal thickness (3.7 mm)	34.0	Atrioventricular septal defect, nuchal translucency > 99th centile (4.9 mm), aberrant right subclavian artery	No genetic testing. TOP
4	Suspected meningocele or meningoencephalocele	43.3	Occipital encephalocele with herniation of the cerebellum and midbrain through the posterior skull defect	No genetic testing. TOP
5	Acrania	42.0	N/A	Miscarriage before first trimester anatomy ultrasound. No genetic testing.
6	Suspected large omphalocele, fourth ventricle dilatation and increased nuchal thickness (6 mm)	41.0	Nuchal translucency > 99th centile (8.8 mm) omphalocele, ventricular septal defect	CVS indicated T18. TOP
7	Radial ray deformity with univentricular heart	40.1	N/A	CVS indicated T18. TOP before first trimester anatomy ultrasound.
8	Suspected cardiac abnormality, single umbilical artery and subcutaneous edema	32.5	Nuchal translucency > 95th centile (3 mm), single umbilical artery. At mid-trimester morphology ultrasound: abnormal thumbs, central gallbladder, dilated proximal duodenum.	Amniocentesis showed normal karyotype.
9	Suspected brain abnormality, abnormal posterior fossa (increased intracranial translucency), likely dilatation of the fetal cerebral ventricles	31.5	Severe brain abnormalities (suspected holoprosencephaly or schizencephaly), ventriculomegaly	CVS showed normal karyotype. TOP
10	Radial ray deformity and subcutaneous edema	44.0	Complex cardiac abnormality (double outlet right ventricle with sub-	CVS indicated T18, TOP

			pulmonary VSD or common arterial trunk) and radial ray deformity	
11	Suspected large omphalocele and subcutaneous edema	37.9	Omphalocele, cardiac anomaly suspected polyvalvular dysplasia, hydrops	CVS indicated T18. TOP
12	Lumbosacral spina bifida with Arnold-Chiari malformation, omphalocele, bladder exstrophy and fixed flexion deformities of the lower limbs with polydactyly. Possible amniotic rupture syndrome, body stalk anomaly or OEIS complex	38.8	N/A	TOP before first trimester anatomy ultrasound
13	Pentalogy of Cantrell	30.6	N/A	TOP before first trimester anatomy ultrasound
14	Possible horseshoe kidney, bladder could not be identified	39.7	Renal agenesis	Normal microarray. TOP
15	TRAP sequence	Twin 1: 40.7 Twin 2: 29.5	Twin 1: Normal Twin 2 (TRAP fetus): hydrops fetalis, acrania, univentricular heart and bilateral syndactyly	Cord ligation of TRAP fetus at 14 weeks gestation, subsequent miscarriage of healthy twin
16	Dilated fourth ventricle (minor abnormality)	42.2	No abnormalities detected	Normal mid-trimester morphology ultrasound

CRL: crown-rump length, cfDNA: cell-free DNA, N/A: not available, TOP: termination of pregnancy, TRAP sequence: twin reverse arterial perfusion sequence, T13: trisomy 13, T18: trisomy 18, T21: trisomy 21, CVS: chorionic villous sampling, OEIS: omphalocele, exstrophy of the cloaca, imperforate anus, and spinal defects.

FIGURES

Figure 1: Flowchart of study participants