

PRENATAL SONOGRAPHIC FEATURES OF FETUSES IN TRISOMY 13 PREGNANCIES (IV)

Chih-Ping Chen^{1,2,3,4,5,6*}

Departments of ¹Obstetrics and Gynecology and ²Medical Research, Mackay Memorial Hospital, Taipei, ³Department of Biotechnology, Asia University, ⁴School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, and ⁵Institute of Clinical and Community Health Nursing and ⁶Department of Obstetrics and Gynecology, National Yang-Ming University, Taipei, Taiwan.

SUMMARY

Prenatal ultrasound is a powerful tool to detect structural abnormalities associated with the fetuses in trisomy 13 pregnancies. This article provides a comprehensive review of the prenatal sonographic markers of trisomy 13 in the first trimester, including fetal nuchal translucency thickness, fetal heart rate, fetal nasal bone, fetal tricuspid regurgitation, ductus venous flow, fetal crown-rump length, fetal trunk and head volume, fetal frontomaxillary facial angle, gestational sac volume and umbilical cord diameter, along with biochemical markers such as maternal serum free β -human chorionic gonadotropin, maternal serum pregnancy-associated plasma protein-A, maternal serum placental growth factor, and the fetal and total cell-free DNA concentration in the maternal circulation. [*Taiwan J Obstet Gynecol* 2010;49(1):3-12]

Key Words: biochemical markers, congenital malformations, prenatal diagnosis, trisomy 13, ultrasound

Introduction

Prenatal ultrasound is a powerful tool to detect structural abnormalities associated with the fetus in trisomy 13 pregnancies [1-3]. This article provides a comprehensive review of the prenatal sonographic markers of trisomy 13 in the first trimester, including fetal nuchal translucency thickness, fetal heart rate, fetal nasal bone, fetal tricuspid regurgitation, ductus venous flow, fetal crown-rump length (CRL), fetal trunk and head volume, fetal frontomaxillary facial (FMF) angle, gestational sac volume (GSV) and umbilical cord diameter (UCD), along with biochemical markers such as maternal serum free β -human chorionic gonadotropin (hCG), maternal serum pregnancy-associated plasma protein-A (PAPP-A), maternal serum placental growth factor (PGF), and

the fetal and total cell-free DNA concentration in the maternal circulation.

Maternal age is the most important risk factor related to fetal trisomy 13 [4,5]. In the first trimester, trisomy 13 is associated with increased maternal age, increased fetal nuchal translucency (NT) thickness, increased fetal heart rate (FHR), and decreased maternal serum levels of free β -hCG and PAPP-A [6]. Fetuses with trisomy 13 may present with major structural abnormalities such as omphalocele, holoprosencephaly (HPE), megacystis and congenital heart defects [1,7], smaller CRL and trunk and head volume [8-10], increased FMF angle in the presence of HPE [11], smaller GSV [12], smaller placental volume [13], reduced placental vascularization [14], a decreased level of maternal serum PGF [15], absence of the nasal bone [16-18], tricuspid regurgitation [19], and increased impedance to flow in the ductus venosus [20] in the first trimester. First-trimester maternal serum biochemistry in combination with maternal age, FHR, fetal NT thickness, fetal structural abnormalities on ultrasound and other sonographic markers such as fetal nasal bone, fetal tricuspid regurgitation and fetal ductus blood flow aid early diagnosis of trisomy 13.



ELSEVIER

*Correspondence to: Dr Chih-Ping Chen, Department of Obstetrics and Gynecology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei, Taiwan.
E-mail: cpc_mmh@yahoo.com
Accepted: September 21, 2009

Fetal Nuchal Translucency Thickness

In the first trimester of pregnancy, the term *nuchal translucency* refers to the ultrasound finding of subcutaneous collection of fluid behind the fetal neck irrespective of whether the collection of fluid is septated and whether it is confined to the neck or envelopes the whole fetus [21]. Increased fetal NT thickness refers to the measurement of the vertical thickness in the mid-sagittal section of the fetus that is equal to or above the 95th centile of the reference range [22]. Fetal abnormalities are associated with the thickness of the fetal NT rather than the appearance of fetal NT [21,23]. In 1998, the Fetal Medicine Foundation First Trimester Screening Group suggested that the optional gestational age for the measurement of fetal NT is 11 gestational weeks to 13 gestational weeks and 6 days (13⁺⁶ weeks), with the corresponding minimum fetal CRL of 45 mm and the maximum CRL of 84 mm, and found that the 95th centile of NT increased linearly with fetal CRL from 2.1 mm at a CRL of 45 mm to 2.7 mm at a CRL of 84 mm; by contrast, the 99th centile did not change with CRL and it was approximately 3.5 mm [22].

Increased fetal NT thickness is associated with trisomy 13, trisomy 18, trisomy 21, Turner syndrome, other sex chromosome abnormalities as well as many fetal anomalies and genetic syndromes [24–27]. In a study of 20,804 pregnancies, including 164 cases of chromosomal abnormalities, Pandya et al [28] found that NT thickness increased significantly with CRL and that increased NT thickness occurred in 66 of 86 fetuses with Down syndrome (76.7%) and in 61 of 78 fetuses with other chromosomal abnormalities (78.2%). In a study of 1,015 pregnancies with increased NT thickness, Pandya et al [29] found that the incidence of Turner syndrome was ninefold higher and the incidence of triploidy was eightfold higher than expected, but the incidence of other sex chromosome abnormalities was similar to that expected. Pandya et al [29] also found that the observed numbers of trisomies 21, 18 and 13 in fetuses with NT thicknesses of 3 mm, 4 mm, 5 mm and ≥ 6 mm were approximately three times, 18 times, 28 times and 36 times higher, respectively, than the numbers expected on the basis of maternal age. In a study of 42,619 pregnancies in 20 British centers, Snijders et al [30] found that the incidence of increased NT thickness $> 95^{\text{th}}$ centile for trisomy 21, trisomy 18, trisomy 13, other trisomies, Turner syndrome, other sex aneuploidies and triploidy was 111 of 147 (75.5%), 47 of 60 (78.3%), 14 of 17 (82.4%), 4 of 6 (66.7%), 17 of 18 (94.4%), 4 of 12 (33.3%) and 6 of 9 fetuses (66.7%), respectively. In a study of 91,091 singleton pregnancies, including 106 fetuses with trisomy 18, Sherod et al [31] found that

the incidence of increased NT thickness $> 95^{\text{th}}$ centile for trisomy 18 was 84 in 106 (79.2%), and that trisomy 18 was associated with early onset intrauterine growth restriction in 21 of 98 fetuses (21.4%), decreased FHR in 6 of 58 fetuses (10.3%) and exomphalos in 22 of 84 fetuses (26.2%). In a study of 100,311 singleton pregnancies, including 46 fetuses with trisomy 13, Snijders et al [32] found that the incidence of increased NT thickness $> 95^{\text{th}}$ centile for trisomy 13 was 33 of 46 fetuses (71.7%) and that trisomy 13 was associated with normal fetal growth in 41 of 46 fetuses (89.1%), increased FHR in 16 of 25 fetuses (64%), HPE in 11 of 46 fetuses (23.9%) and exomphalos in 3 of 46 fetuses (6.5%). In a study of 11,315 pregnancies, including 2,168 fetuses with chromosomal abnormalities, Kagan et al [33] found that the overall incidence of chromosomal abnormalities increased with NT thickness from 7.1% (507/7,109) for 95th centile for CRL -3.4 mm to 20.1% (423/2,101) for 3.5–4.4 mm, 45.8% (321/701) for 4.5–5.4 mm, 50.1% (219/437) for 5.5–6.4 mm, 70.6% (218/309) for 6.5–7.4 mm, 70.8% (148/209) for 7.5–8.4 mm, 75% (126/168) for 8.5–9.4 mm, 84.1% (74/88) for 9.5–10.4 mm, 70.3% (45/64) for 10.5–11.4 mm and 70.7% (87/123) for ≥ 11.5 mm. Kagan et al [33] also found that in the majority of fetuses with trisomy 21, the NT thickness was < 4.5 mm, whereas in the majority of fetuses with trisomies 13 and 18, it was 4.5–8.4 mm, and in Turner syndrome, it was ≥ 8.5 mm.

Fetal Heart Rate

Various studies have shown that FHR increases from 110 beats/min (bpm) at week 5 of gestation to 170 bpm at week 9 of gestation, and then gradually decreases to 150 bpm by week 14 of gestation, which suggests functional maturation of the parasympathetic system [34–36]. In normal pregnancies, there is a decrease in FHR between weeks 10 and 14 of gestation [37,38]. In a study of 6,903 normal singleton pregnancies, Hyett et al [37] found that FHR decreased from a mean value of 171 bpm at the week 10 of gestation to 156 bpm at week 14, and there was no significant association between FHR and fetal NT thickness. Shulman et al [39] reported prenatal diagnosis of trisomy 13 distinguished by persistent fetal tachycardia. The patient was a 34-year-old, gravida 3, para 2, woman who was referred for counseling and chorionic villus sampling because of advanced maternal age and a child with Down syndrome. Ultrasonography showed a singleton at 9.7 weeks of gestation with no fetal or placental structural abnormalities, except for persistent fetal tachycardia with a FHR of 190 bpm.

The patient underwent chorionic villus sampling (CVS) at 10 weeks of gestation when FHR was measured at 190 bpm before and after CVS. CVS revealed trisomy 13, and the pregnancy was terminated. Trisomy 13 was confirmed in cultured mesenchymal core cells and fetal tissue. In a study of 25,000 normal singleton pregnancies, Liao et al [38] found that the FHR decreased from a mean value of 170 bpm at a CRL of 35 mm to 150 bpm at a CRL of 84 mm.

In first-trimester studies, fetal tachycardia was associated with trisomy 13, trisomy 21 and Turner syndrome, and fetal bradycardia was associated with trisomy 18 and triploidy [37,38]. In a study of 85 pregnancies with trisomy 21 fetuses, 34 with trisomy 18, 16 with trisomy 13, 19 with Turner syndrome and eight with triploidy, Hyett et al [37] found that the FHR was significantly higher in trisomy 21, trisomy 13 and Turner syndrome fetuses and significantly lower in trisomy 18 and triploidy fetuses than that in the normal group. In a study of 554 pregnancies with trisomy 21 fetuses, 219 with trisomy 18, 95 with trisomy 13, 50 with triploidy, 115 with Turner syndrome and 28 with sex chromosome abnormalities other than Turner syndrome, Liao et al [38] found that FHR was significantly higher in trisomy 21, trisomy 13 and Turner syndrome fetuses and significantly lower in trisomy 18 and triploidy fetuses than that in the normal group. Furthermore, the FHR in other sex chromosome abnormalities was not significantly different from that in the normal group. The authors also found that the FHR was above the 95th centile of the normal range in 10%, 52% and 67% of the fetuses with trisomy 21, Turner syndrome and trisomy 13, respectively, and that FHR was below the 5th centile of the normal range in 19% and 30% of the fetuses with trisomy 18 and triploidy, respectively.

The pathogenesis of fetal tachycardia in trisomy 13 includes structural cardiac abnormalities and a compensatory mechanism to increase cardiac output in fetuses with left heart obstruction [37,40,41]. In a study of 17 fetuses with trisomy 13 in the first trimester, Hyett et al [41] found that 15 of 16 hearts had cardiac abnormalities, which included ventricular septal defects, valvular defects and agenesis of the pulmonary valve. Furthermore, 15 of 15 great vessels had anomalies, including a decrease in both the aortic valve and aortic isthmus, an increase in the ratio of aortic valve to aortic isthmus and of ductus arteriosus to aortic isthmus, while three of 15 fetuses had truncus arteriosus. In fetuses with trisomy 13, the compensatory mechanism to increase cardiac output in those with narrowing of the outflow tract from the left ventricle is likely mediated by baroreceptor activity in the aortic arch [37, 38,40–42]. The same mechanism may also be involved

in tachycardia in fetuses with trisomy 21 or Turner syndrome [37]. In fetuses with trisomy 18 or triploidy, bradycardia may be related to early onset of intrauterine growth restriction, severe developmental delay, and a preterminal event in intrauterine fetal death [37,38].

Papageorghiou et al [42] demonstrated that at the ultrasound scan performed at weeks 11–13⁺⁶, trisomy 13 was associated with easily detectable major structural anomalies, high NT thickness, and tachycardia. The authors performed a study of 181 fetuses with trisomy 13 to examine the sonographic features of trisomy 13 at weeks 11–13⁺⁶ of gestation. They found HPE, omphalocele and/or megacystis in 92 fetuses (50.8%), FHR above the 95th centile in 129 fetuses (71.3%), FHR above the 99th centile in 93 fetuses (51.4%), and NT thickness above the 95th centile in 141 fetuses (77.9%). They estimated that including FHR in NT screening improved the detection rate of trisomy 13 by approximately 5%. Papageorghiou et al [42] found that in trisomy 13 fetuses, the mean delta FHR was significantly higher than normal (mean, 15 bpm above the normal; 95% confidence interval, CI, 14–16 bpm; $p < 0.0001$), and the likelihood ratio for trisomy 13 increased exponentially with delta FHR. Papageorghiou et al [42] also found that when the FHR was > 20 bpm above the normal mean for CRL (approximately 185 bpm at a CRL of 45 mm and 175 bpm at a CRL of 85 mm), the likelihood ratio for trisomy 13 was 495.4 (95% CI, 285.9–858.4) in 31.5% (57/181) of trisomy 13 fetuses versus only 0.06% of normal controls. Papageorghiou et al [42] concluded that, at the 11–13⁺⁶-week sonographic screening, the measurement of fetal NT and FHR, and the examination of HPE, omphalocele and megacystis, can identify over 90% of fetuses with trisomy 13.

Kagan et al [6] showed a significant change in FHR in trisomy 13 fetuses but a very small change in FHR in trisomy 21 and trisomy 18 fetuses compared with normal controls. In their study, in 395 fetuses with trisomy 21, the mean FHR was above the appropriate mean for gestation in unaffected pregnancies by about 1 bpm and there was no evidence of a change with gestation. In 122 fetuses with trisomy 18, the mean FHR was below the appropriate mean for gestation in unaffected pregnancies by about 3 bpm and there was no evidence of a change with gestation. In 61 fetuses with trisomy 13, the mean FHR was above the appropriate mean for gestation in unaffected pregnancies by about 20 bpm at 11 weeks of gestation, 17 bpm at 12 weeks of gestation and 14 bpm at 13 weeks of gestation. Kagan et al [6] found that in fetuses with trisomy 13, the FHR was above the 95th and 99th centiles of unaffected pregnancies in 85.2% and 62.3% of fetuses, respectively. By contrast, in fetuses with trisomy 21, the FHR was above the 95th and 99th

centiles of unaffected pregnancies in 13.7% and 6.3% of cases, respectively, and, in fetuses with trisomy 18, the FHR was below the 5th and 1st centiles of unaffected pregnancies in 17.2% and 9.2% of fetuses, respectively.

Kagan et al [6] also showed that the inclusion of FHR with first-trimester maternal serum biochemistry in combination with maternal age and fetal NT thickness improved the detection rate of trisomy 13 but did not improve the detection rate of trisomy 18 or trisomy 21. According to their study, at a 0.2% false-positive rate, the estimated detection rates of trisomy 13 using the risk algorithm for trisomy 13 based on maternal age+fetal NT, maternal age+fetal NT+serum biochemistry, and maternal age+fetal NT+serum biochemistry+FHR were 45%, 77% and 87%, respectively. At a 0.2% false-positive rate, the estimated detection rates of trisomy 18 using the risk algorithm for trisomy 18 based on maternal age+fetal NT, maternal age+fetal NT+serum biochemistry and maternal age+fetal NT+serum biochemistry+FHR were 61%, 93% and 91%, respectively. At a 3% false-positive rate, the estimated detection rates of trisomy 21 using the risk algorithm for trisomy 21 based on maternal age+fetal NT, maternal age+fetal NT+serum biochemistry, and maternal age+fetal NT+serum biochemistry+FHR were 75%, 89% and 90%, respectively.

Fetal Nasal Bone

The fetal nasal bone should be examined at weeks 11⁺⁰–13⁺⁶ of gestation on a midsagittal section of the fetal profile, with software adjusting for potential confounding factors [16–18]. The incidence of fetuses with an absent nasal bone decreases with fetal CRL, increases with NT thickness, and is substantially higher in fetuses of Afro-Caribbean origin than in Caucasians [17,18]. Absence of the nasal bone is associated with aneuploidy. In a study that evaluated the incidence of nasal hypoplasia among fetuses of southern Chinese women, Wong et al [43] found that the incidence of an absent nasal bone in fetuses with a normal karyotype was 1 in 114 fetuses (0.88%), which is comparable with Caucasian fetuses. This suggests that assessing the ossification of the fetal nasal bone can be used in southern Chinese women as a supplementary screening test for Down syndrome. Prefumo et al [44] also showed that first-trimester ultrasound failed to demonstrate the fetal nasal bone twice as often in mothers of African origin compared with Caucasians, but the failure to visualize the fetal nasal bone was not significantly higher in women of Asian origin than in women of Caucasian origin. In a meta-analysis of 15,822 fetuses included in the

studies by Cicero et al [16–18], Otano et al [45], Orlandi et al [46], Senat et al [47], Viora et al [48], Wong et al [43], Zoppi et al [49] and Nicolaides [27], with successful examination in 15,413 of 15,822 (97.4%) of all pregnancies, absence of the nasal bone was found in 176 of 12,652 (1.4%) chromosomally normal fetuses and in 274 of 397 (69%) fetuses with trisomy 21. Cicero et al [18] showed that the incidence of an absent nasal bone at weeks 11⁺⁰–13⁺⁶ of gestation in fetuses with trisomy 21, trisomy 18, trisomy 13, triploidy, Turner syndrome, other sex chromosome abnormalities (XXY, XXX and XYY) and other aneuploidies was 229 of 333 (68.8%), 68 of 124 (54.8%), 13 of 38 (34.2%), 0 of 19 (0%), 5 of 46 (10.9%), 1 of 20 (5.0%) and 8 of 48 fetuses (16.7%), respectively.

Fetal Tricuspid Regurgitation

Fetal tricuspid regurgitation is measured by pulsed-wave Doppler on an apical four-chamber view at weeks 11⁺⁰–13⁺⁶ of gestation. Fetal tricuspid regurgitation is related to an increased risk of aneuploidy [19,50–52]. Falcon et al [52] reported tricuspid regurgitation in 77 of 114 fetuses with trisomy 21 (67.5%), 14 of 42 fetuses with trisomy 18 (33.3%), and 58 of 1,323 euploid fetuses (4.4%). Kagan et al [19] reported tricuspid regurgitation in 68 of 122 fetuses with trisomy 21 (55.5%), 12 of 36 fetuses with trisomy 18 (33.3%), six of 20 fetuses with trisomy 13 (30%), three of eight fetuses with Turner syndrome (37.5%), and 181 of 19,614 euploid fetuses (0.9%).

Ductus Venosus Flow

Abnormal ductus venosus flow with the presence of a reversed *a* wave is caused by increased impedance to flow in the ductus venosus and is related to an increased risk of aneuploidy [20,53–59]. In a meta-analysis of the incidence of abnormal ductus venosus flow in the first trimester in euploid fetuses (*n*=6,790), and in those with trisomy 21 (*n*=185), trisomy 18 (*n*=28), trisomy 13 (*n*=11), Turner syndrome (*n*=13) and other chromosomal abnormalities (*n*=18), Maiz et al [20] reported that abnormal ductus venosus flow was observed in 5.2% of euploid fetuses and in 70.8%, 89.3%, 81.8%, 76.9% and 55.6% of the fetuses with trisomy 21, trisomy 18, trisomy 13, Turner syndrome and other chromosomal abnormalities, respectively. Maiz et al [20], in their own study at weeks 11⁺⁰–13⁺⁶ weeks of gestation, showed that the incidence of abnormal ductus venosus flow was observed in 3.2% (622/19,614) of euploid fetuses and

in 66.4% (81/122), 58.3% (21/36), 55.0% (11/20) and 75.0% (6/8) of the fetuses with trisomy 21, trisomy 18, trisomy 13 and Turner syndrome, respectively.

Screening for Aneuploidy by Maternal Age, Fetal NT, Maternal Serum Free β -hCG, Maternal Serum PAPP-A and FHR, with Fetal Nasal Bone, Tricuspid Regurgitation or Ductus Venosus Flow

Trisomy 21 is characterized by increased maternal age, increased NT thickness, increased maternal serum free β -hCG, and decreased maternal serum PAPP-A. Meanwhile, trisomy 18 is characterized by increased maternal age, increased NT thickness, decreased FHR, decreased maternal serum free β -hCG, and decreased maternal serum PAPP-A. Trisomy 13 is characterized by increased maternal age, increased NT thickness, increased FHR, decreased maternal serum free β -hCG, and decreased maternal serum PAPP-A [4,5,22,32,37,38,42,60–68].

Spencer et al [61] reported a median of 0.506 multiples of the median (MoM) for maternal serum free β -hCG and a median of 0.248 MoM for maternal serum PAPP-A in 42 cases of trisomy 13 pregnancies at weeks 10–14 of gestation. Screening for fetal trisomies in a one-step clinic for assessment of risk for fetal abnormalities (OSCAR) program with fetal NT and maternal serum free β -hCG and PAPP-A at 10–14 weeks of gestation can identify about 90% of trisomy 21 pregnancies at a false-positive rate of 5% [69], about 86% of trisomy 18 pregnancies at a false-positive rate of 0.5% [60], and about 84% of trisomy 13 pregnancies at a false-positive rate of 0.1% [61]. Spencer and Nicolaides [63] also used a first-trimester trisomy 13/trisomy 18 risk algorithm that combined fetal NT thickness, maternal serum free β -hCG and PAPP-A to examine 45 cases of trisomy 13 and 59 cases of trisomy 18 at 11–14 weeks of gestation. That study showed a median of 2.819 MoM for NT, a median of 0.375 MoM for maternal serum free β -hCG, and a median of 0.201 MoM for maternal serum PAPP-A in the trisomy 13/trisomy 18 group. Spencer and Nicolaides [63] predicted that the combined trisomy 13/trisomy 18 algorithm can identify 95% of fetuses with trisomy 13 and trisomy 18 with a risk cut-off of 1 in 150 and a false-positive rate of 0.3%. In a study of 12,339 women with singleton pregnancies presenting at 10–14 weeks of gestation who were offered a combination of maternal serum free β -hCG and PAPP-A, and fetal NT thickness, with the option of an invasive diagnostic test at an estimated risk of ≥ 1 in 300 of carrying a fetus with trisomy 21, trisomy 18 or trisomy 13, Spencer et al [62] found that the uptake of

first-trimester screening and invasive testing in the higher-risk group was 97.5% and 77%, respectively, and that the detection rates of trisomy 21, trisomy 18, trisomy 13, Turner syndrome, other sex chromosome abnormalities, triploidy and all aneuploidies were 23 of 25 (92%), 11 of 11 (100%), 4 of 4 (100%), 4 of 4 (100%), 2 of 2 (100%), 5 of 5 (100%) and 49 of 51 fetuses (96.1%), respectively.

Kagan et al [65] performed a prospective screening program for aneuploidy in singleton pregnancies at weeks 11⁺⁰–13⁺⁶ of gestation that included 56,376 normal cases, 395 with trisomy 21, 122 with trisomy 18, and 61 with trisomy 13. They found that at a 3.1% false-positive rate using algorithms developed to calculate patient-specific risks for each of the three trisomies based on maternal age, fetal NT thickness, FHR, and maternal serum free β -hCG and PAPP-A, the estimated detection rates for trisomies 21, 18 and 13 were 91%, 97% and 94%, respectively. In a prospective screening program for aneuploidy in singleton pregnancies at weeks 11⁺⁰–13⁺⁶ of gestation, including 19,614 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (euploid group), 122 with trisomy 21, 36 with trisomy 18, 20 with trisomy 13 and eight with Turner syndrome, Kagan et al [70] found that with a fixed risk cut-off of 1 in 100 for the total risk for trisomies 21, 18 and 13 and Turner syndrome, a standardized false-positive rate of 3.0%, the detection rates for trisomies 21, 18 and 13 and Turner syndrome 91%, 100%, 100% and 100%, respectively, with a screening test that included maternal age, fetal NT thickness, FHR, and maternal serum free β -hCG and PAPP-A. Kagan et al [70] also found that including nasal bone assessment in all pregnancies before the combined test reduced the false-positive rate to 2.5% without changing the detection rate. Kagan et al [19] also found that with the inclusion of the assessment of tricuspid flow to detect tricuspid regurgitation in all pregnancies before the combined test, the detection rates of trisomy 21, trisomy 18, trisomy 13 and Turner syndrome were 96%, 92%, 100% and 100%, respectively.

In a prospective screening program for aneuploidy in singleton pregnancies at weeks 11⁺⁰–13⁺⁶ of gestation, including 19,614 pregnancies with euploid fetuses, 122 with trisomy 21, 36 with trisomy 18, 20 with trisomy 13 and eight with Turner syndrome, Maiz et al [20] found that including ductus venosus flow with maternal age, fetal NT thickness, FHR, and maternal serum free β -hCG and PAPP-A would detect 96%, 92%, 100% and 100% of trisomies 21, 18 and 13 and Turner syndrome, respectively, at a false-positive rate of 3.0%. The same detection rates were achieved with the two-stage strategy, in which the first-stage screening using the combined test

in all patients was followed by second-stage assessment of the a wave in those with an intermediate risk of 1 in 51 to 1 in 1,000 after the first stage, at a false-positive rate of 2.6%; assessment of ductus venosus flow was required in only 15% of the total population.

Fetal Crown-rump Length, and Fetal Trunk and Head Volume

Compared with euploid babies, the birth weight deficit in aneuploid babies is about 10–15% for trisomy 21 and Turner syndrome, about 20% for trisomy 13, and about 30% for trisomy 18 [71,72]. In the first trimester, gestational age and fetal growth are essentially assessed by two-dimensional ultrasound measurement of CRL. Drugan et al [73] found a lag of at least 7 days in CRL-based estimates of gestational age in 4.3% of fetuses with chromosomal abnormalities, compared with 1.7% of normal fetuses in the first trimester, and suggested that the smaller than expected first-trimester fetus is at risk for chromosomal abnormalities. Kuhn et al [74] measured CRL in 135 chromosomally abnormal fetuses and 700 chromosomally normal fetuses at 10–13 weeks of gestation. They found that the median CRL in fetuses with trisomy 18 ($n=32$) was significantly reduced and that the median CRLs in fetuses with trisomy 21 ($n=72$), trisomy 13 ($n=11$), 45,X ($n=5$), 47,XXX ($n=6$), 47,XXY ($n=6$) and triploidy ($n=3$) were not shorter than normal. Schemmer et al [75] measured CRLs in 196 chromosomally abnormal fetuses and in 1,929 chromosomally normal fetuses in the first trimester and found that the mean CRLs and growth rates were significantly reduced in fetuses with trisomy 18 ($n=49$), trisomy 13 ($n=19$) and triploidy ($n=8$). However, these growth parameters were not significantly reduced in fetuses with trisomy 21 ($n=92$), Turner syndrome ($n=8$) or other sex chromosomal abnormalities ($n=20$). Bahado-Singh et al [76] found that there was significant CRL shortening in trisomy 18 fetuses with observed/expected values ≤ 0.80 (odds ratio, 13.78; 95% CI, 5.64–33.88; $p < 0.000001$), there was evidence of CRL shortening in trisomy 13 fetuses with observed/expected values ≤ 0.90 (odds ratio, 3.64; 95% CI, 1.08–12.96; $p < 0.03$), and there was no significant CRL shortening in trisomy 21 fetuses with observed/expected values ≤ 0.92 (odds ratio, 0.86; 95% CI, 0.5–1.47; $p=0.6$).

With the introduction of three-dimensional ultrasound, the measurement of fetal trunk and head volume offers a new approach for the early diagnosis of fetal growth restriction [8–10]. Falcon et al [9] performed a study of fetal trunk and head volume in 500 euploid fetuses, 72 trisomy 21 fetuses, 29 trisomy 18 fetuses,

14 trisomy 13 fetuses, 14 Turner syndrome fetuses and 11 triploid fetuses at weeks 11⁺⁰–13⁺⁶ of gestation. They found that compared with the normal group, fetuses with trisomy 21 and Turner syndrome had similar CRL for gestational age ($p=0.335$ and $p=0.317$, respectively), and the fetal trunk and head volumes were about 10–15% lower ($p < 0.001$ and $p=0.004$, respectively). However, in trisomy 18, trisomy 13 and triploidy fetuses, the deficit in CRL was less than 15% ($p < 0.001$), while the deficit in fetal trunk and head volume was about 45% ($p < 0.001$). Falcon et al [10] also calculated the fetal head-to-trunk volume ratio in chromosomally abnormal fetuses at weeks 11⁺⁰–13⁺⁶ of gestation and found that in trisomy 13, trisomy 18 and triploidy fetuses, the growth restriction was asymmetrical with the trunk being more severely compromised than the head. By contrast, in trisomy 21 and Turner syndrome, the growth restriction was symmetrical with the trunk and head being equally affected.

Fetal Frontomaxillary Facial Angle

Measurement of the fetal FMF angle is a sonographic method used to define the relative portion of the maxilla to the forehead at weeks 11⁺⁰–13⁺⁶ of gestation [11, 77–79]. Fetuses with trisomy 21 and trisomy 18 are associated with midfacial hypoplasia and a significantly larger FMF angle than that in euploid fetuses at weeks 11⁺⁰–13⁺⁶ of gestation [11,77–79]. However, in fetuses with trisomy 13, the FMF angle at weeks 11⁺⁰–13⁺⁶ of gestation is only increased in cases with HPE [11]. In a study of 23 fetuses with trisomy 13, Borenstein et al [11] found that in 12 fetuses with HPE, the FMF angle was above the 95th centile of the normal range in 10 fetuses and was not significantly different from the normal range in 11 fetuses without HPE.

Gestational Sac Volume

In a study of the association between chromosomal defects and GSV measured by three-dimensional ultrasound at weeks 11–13⁺⁶ of gestation, Falcon et al [12] found that the mean GSV for gestational age in fetuses with trisomy 21 ($n=45$), trisomy 18 ($n=17$) and Turner syndrome ($n=10$) was not significantly different from that of chromosomally normal fetuses ($n=417$). By contrast, GSV was smaller in those with trisomy 13 ($n=6$) and triploidy ($n=5$) than in chromosomally normal fetuses. Falcon et al [12] also found that the mean GSV for CRL was significantly larger in trisomy 18, smaller in trisomy 13 and triploidy fetuses, and was similar to

normal values in trisomy 21 and Turner syndrome. Meanwhile, the mean CRL for gestational age was significantly smaller than normal in trisomy 18, trisomy 13 and triploidy fetuses. Falcon et al [12] concluded that in fetuses with trisomy 13 and triploidy, the small GSV may be due to early-onset growth restriction and reduced amniotic fluid, and that in trisomy 18, the large GSV may be due to interference with fetal swelling by the associated congenital anomalies.

Umbilical Cord Diameter

Ghezzi et al [80] first suggested UCD at 10–14 weeks of gestation as an ultrasound marker for fetal aneuploidy based on the findings that the proportion of fetuses with an UCD above the 95th centile was higher in fetuses with chromosomal abnormalities than in normal fetuses (5/17 vs. 39/767; $p < 0.01$). Ghezzi et al [80] hypothesized that the underlying pathophysiologic mechanisms leading to an increase in UCD might be associated with increased NT thickness, such as changes in the extracellular matrix components or fetal venous congestion. Axt-Flidner et al [81] found that at 11–14 weeks of gestation, fetuses with chromosomal abnormalities were more likely to have an UCD above the 95th centile. In their study, the proportion of fetuses with UCD above the 95th centile for CRL was higher in aneuploid fetuses compared with euploid fetuses (5/14 vs. 13/285; $p < 0.005$). However, Rembouskos et al [82] found that the UCD in trisomy 21 fetuses ($n = 97$) was significantly lower than normal ($n = 1,150$), but in fetuses with trisomy 18 ($n = 42$), Turner syndrome ($n = 18$), trisomy 13 ($n = 6$), triploidy ($n = 6$) or 47,XXY ($n = 4$), there was no significant difference from the normal value.

Maternal Serum Placental Growth Factor

The first-trimester maternal serum level of PGF was found to be lower in pregnancies with trisomy 13 than in pregnancies with euploid fetuses [15]. In a study of 45 pregnancies with trisomy 21, 45 with trisomy 18 and 493 with normal results at weeks 10–13 of gestation, Spencer et al [83] found that the median maternal serum PGF concentration in the trisomy 21 group was 1.26 MoM, which was significantly higher than that in the control group. Furthermore, the median maternal serum PGF concentration in the trisomy 18 group was 0.889 MoM, which was lower than that in the control group, but did not reach significance. Zaragoza et al [15] performed a study of 90 pregnancies with trisomy 21, 28 with trisomy 18, 19 with trisomy 13, 28 with

Turner syndrome, 10 with triploidy, and 609 with euploid results at weeks 11⁺⁰–13⁺⁶ of gestation. They found that the median values in pregnancies with trisomy 21, trisomy 18, trisomy 13, Turner syndrome and triploidy were 0.707 MoM, 0.483 MoM, 0.404 MoM, 0.534 MoM and 0.531 MoM, respectively, which were significantly lower than the value of 0.991 MoM in the euploid group. Zaragoza et al [15] suggested that incorporating PGF in the first-trimester combined screening for trisomy 21 might improve the detection rates of other major aneuploidies because of the significantly lower serum levels of PGF in trisomy 18, trisomy 13, Turner syndrome and triploid pregnancies.

Fetal and Total Cell-free DNA Concentrations in Maternal Circulation

Lo et al [84] first reported significantly higher fetal cell-free DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21 ($n = 13$) compared with those carrying normal fetuses ($n = 37$) at 17–18 weeks of gestation. Zhong et al [85] reported significantly elevated fetal cell-free DNA in maternal plasma in pregnancies with trisomy 21 ($n = 15$) and trisomy 13 ($n = 3$) fetuses and a nonsignificant elevation in trisomy 18 ($n = 6$) compared with the normal ($n = 29$) at 14⁺⁴ weeks of gestation. Ohashi et al [86] reported that the levels of maternal serum fetal cell-free DNA in pregnancies with trisomy 21 ($n = 5$) and trisomy 18 ($n = 3$) fetuses were not significantly different from those in pregnancies with normal fetuses ($n = 55$) at 15–17 weeks of gestation. Hromadnikova et al [87] reported no significant differences in maternal plasma fetal cell-free DNA or total cell-free DNA levels in pregnancies with normal ($n = 13$) and trisomy 21 ($n = 11$) fetuses at 20 weeks of gestation. Lee et al [88] reported significantly elevated fetal cell-free DNA in maternal serum in pregnancies with trisomy 21 ($n = 11$) fetuses compared with normal fetuses ($n = 55$) at 15–19 weeks of gestation. Spencer et al [89] reported that the maternal serum concentration of fetal cell-free DNA was not significantly different, whereas the maternal serum concentration of total cell-free DNA was significantly elevated in pregnancies with trisomy 21 ($n = 10$) fetuses compared with normal fetuses ($n = 10$) at 15 weeks of gestation. Wataganara et al [90] reported that maternal serum fetal cell-free DNA levels were increased in fetuses with trisomy 13 ($n = 5$) but not trisomy 18 ($n = 5$) compared with normal fetuses ($n = 24$) at 15–20 weeks of gestation. Gerovassili et al [91] found that the first-trimester maternal plasma levels of fetal cell-free DNA were not significantly different between pregnancies with normal

fetuses ($n=238$) and pregnancies with trisomy 21 ($n=38$), trisomy 18 ($n=9$), trisomy 13 ($n=4$) and triploidy ($n=5$) fetuses. Similarly, they reported no differences between the first-trimester maternal plasma levels of total cell-free DNA in pregnancies with normal fetuses ($n=264$) and pregnancies with trisomy 21 ($n=72$), trisomy 18 ($n=24$), trisomy 13 ($n=12$), Turner syndrome ($n=16$) and triploidy ($n=8$) fetuses. Gero-vassili et al [91] suggested that the fetal cell-free DNA is not an ideal prognostic marker for chromosomal abnormalities in first-trimester pregnancies.

References

- Chen CP. Prenatal sonographic features of fetuses in trisomy 13 pregnancies (I). *Taiwan J Obstet Gynecol* 2009;48:210–7.
- Chen CP. Prenatal sonographic features of fetuses in trisomy 13 pregnancies (II). *Taiwan J Obstet Gynecol* 2009;48:218–24.
- Chen CP. Prenatal sonographic features of fetuses in trisomy 13 pregnancies (III). *Taiwan J Obstet Gynecol* 2009;48:342–9.
- Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risks for trisomies at 9–14 weeks' gestation. *Prenat Diagn* 1994;14:543–52.
- Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. Maternal age and gestational age-specific risks for chromosomal defects. *Fetal Diagn Ther* 1995;10:356–67.
- Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* 2008;23:1968–75.
- Chen CP, Chien SC. Prenatal sonographic features of trisomy 13. *J Med Ultrasound* 2007;15:58–66.
- Falcon O, Peralta CF, Cavoretto P, Faiola S, Nicolaides KH. Fetal trunk and head volume measured by three-dimensional ultrasound at 11⁺⁰ to 13⁺⁶ weeks of gestation in chromosomally normal pregnancies. *Ultrasound Obstet Gynecol* 2005;26:263–6.
- Falcon O, Peralta CF, Cavoretto P, Auer M, Nicolaides KH. Fetal trunk and head volume in chromosomally abnormal fetuses at 11⁺⁰ to 13⁺⁶ weeks of gestation. *Ultrasound Obstet Gynecol* 2005;26:517–20.
- Falcon O, Cavoretto P, Peralta CF, Csapo B, Nicolaides KH. Fetal head-to-trunk volume ratio in chromosomally abnormal fetuses at 11⁺⁰ to 13⁺⁶ weeks of gestation. *Ultrasound Obstet Gynecol* 2005;26:755–60.
- Borenstein M, Persico N, Dagklis T, Faros E, Nicolaides KH. Frontomaxillary facial angle in fetuses with trisomy 13 at 11⁺⁰ to 13⁺⁶ weeks. *Ultrasound Obstet Gynecol* 2007;30:819–23.
- Falcon O, Wegrzyn P, Faro C, Peralta CF, Nicolaides KH. Gestational sac volume measured by three-dimensional ultrasound at 11 to 13⁺⁶ weeks of gestation: relation to chromosomal defects. *Ultrasound Obstet Gynecol* 2005;25:546–50.
- Wegrzyn P, Faro C, Falcon O, Peralta CF, Nicolaides KH. Placental volume measured by three-dimensional ultrasound at 11 to 13⁺⁶ weeks of gestation: relation to chromosomal defects. *Ultrasound Obstet Gynecol* 2005;26:28–32.
- Rizzo G, Capponi A, Cavicchioni O, Vendola M, Arduini D. Placental vascularization measured by three-dimensional power Doppler ultrasound at 11 to 13⁺⁶ weeks' gestation in normal and aneuploid fetuses. *Ultrasound Obstet Gynecol* 2007;30:259–62.
- Zaragoza E, Akolekar R, Poon LC, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11–13 weeks in chromosomally abnormal pregnancies. *Ultrasound Obstet Gynecol* 2009;33:382–6.
- Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides KH. Absence of nasal bone in fetuses with trisomy 21 at 11–14 weeks of gestation: an observational study. *Lancet* 2001;358:1665–7.
- Cicero S, Longo D, Rembouskos G, Sacchini C, Nicolaides KH. Absent nasal bone at 11–14 weeks of gestation and chromosomal defects. *Ultrasound Obstet Gynecol* 2003;22:31–5.
- Cicero S, Rembouskos G, Vandecruys H, Hogg M, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with absent nasal bone at the 11–14 weeks scan. *Ultrasound Obstet Gynecol* 2004;23:218–23.
- Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11⁺⁰ to 13⁺⁶ weeks of gestation. *Ultrasound Obstet Gynecol* 2009;33:18–22.
- Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. *Ultrasound Obstet Gynecol* 2009;33:512–7.
- Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* 1992;304:867–9.
- Snijders RJM, Noble P, Sebire NJ, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998;352:343–6.
- Cullen MT, Gabrielli S, Green JJ, et al. Diagnosis and significance of cystic hygroma in the first trimester. *Prenat Diagn* 1990;10:643–51.
- Souka AP, Snijders RJM, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10–14 weeks of gestation. *Ultrasound Obstet Gynecol* 1998;11:391–400.
- Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol* 2005;192:1005–21.
- Nicolaides KH, Heath V, Cicero S. Increased fetal nuchal translucency at 11–14 weeks. *Prenat Diagn* 2002;22:308–15.
- Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004;191:45–67.
- Pandya PP, Snijders RJM, Johnson SP, De Lourdes Brizot M, Nicolaides KH. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. *Br J Obstet Gynaecol* 1995;102:957–62.
- Pandya PP, Kondylis A, Hilbert F, Snijders RJM, Nicolaides KH. Chromosomal defects and outcome in 1,015 fetuses

- with increased nuchal translucency. *Ultrasound Obstet Gynecol* 1995;5:15-9.
30. Snijders RJM, Johnson S, Sebire NJ, Noble PL, Nicolaides KH. First-trimester ultrasound screening for chromosomal defects. *Ultrasound Obstet Gynecol* 1996;7:216-26.
 31. Sherod C, Sebire NJ, Soares W, Snijders RJM, Nicolaides KH. Prenatal diagnosis of trisomy 18 at the 10-14-week ultrasound scan. *Ultrasound Obstet Gynecol* 1997;10:387-90.
 32. Snijders RJM, Sebire NJ, Nayar R, Souka A, Nicolaides KH. Increased nuchal translucency in trisomy 13 fetuses at 10-14 weeks of gestation. *Am J Med Genet* 1999;86:205-7.
 33. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness and chromosomal defects. *Obstet Gynecol* 2006;107:6-10.
 34. Robinson HP, Shaw-Dunn J. Fetal heart rates as determined by sonar in early pregnancy. *J Obstet Gynaecol Br Commonw* 1973;90:805-9.
 35. Rempen A. Diagnosis of viability in early pregnancy with vaginal sonography. *J Ultrasound Med* 1990;9:711-6.
 36. Wisser J, Dirschedl P. Embryonic heart rate in dated human embryos. *Early Hum Dev* 1994;37:107-15.
 37. Hyett JA, Noble PL, Snijders RJM, Montenegro N, Nicolaides KH. Fetal heart rate in trisomy 21 and other chromosomal abnormalities at 10-14 weeks of gestation. *Ultrasound Obstet Gynecol* 1996;7:239-44.
 38. Liao AW, Snijders RJM, Geerts L, Spencer K, Nicolaides KH. Fetal heart rate in chromosomally abnormal fetuses. *Ultrasound Obstet Gynecol* 2000;16:610-3.
 39. Shulman LP, Emerson DS, Phillips OP. Fetal trisomy 13 distinguished by persistent fetal tachycardia. *Int J Gynaecol Obstet* 1997;57:317-8.
 40. Rudolph AM, Heymann MA. Cardiac output in the fetal lamb: the effects of spontaneous and induced changes of heart rate on right and left ventricular output. *Am J Obstet Gynecol* 1976;124:183-92.
 41. Hyett JA, Moscoso G, Nicolaides KH. Abnormalities of the heart and great arteries in first trimester chromosomally abnormal fetuses. *Am J Med Genet* 1997;69:207-16.
 42. Papageorgiou AT, Avgidou K, Spencer K, Nix B, Nicolaides KH. Sonographic screening for trisomy 13 at 11 to 13⁺6 weeks of gestation. *Am J Obstet Gynecol* 2006;194:397-401.
 43. Wong SF, Choi H, Ho LC. Nasal bone hypoplasia: is it a common finding amongst chromosomally normal fetuses of southern Chinese women? *Gynecol Obstet Invest* 2003;56:99-101.
 44. Prefumo F, Sairam S, Bhide A, Penna L, Hollis B, Thilaganathan B. Maternal ethnic origin and fetal nasal bones at 11-14 weeks of gestation. *BJOG* 2004;111:109-12.
 45. Otano L, Aiello H, Igarzabal L, Matayoshi T, Gadow EC. Association between first trimester absence of fetal nasal bone on ultrasound and Down's syndrome. *Prenat Diagn* 2002;22:930-2.
 46. Orlandi F, Bilardo CM, Campogrande M, et al. Measurement of nasal bone length at 11-14 weeks of pregnancy and its potential role in Down's syndrome risk assessment. *Ultrasound Obstet Gynecol* 2003;22:36-9.
 47. Senat MV, Bernard JP, Boulvain M, Ville Y. Intra- and inter-operator variability in fetal nasal bone assessment at 11-14 weeks of gestation. *Ultrasound Obstet Gynecol* 2003;22:138-41.
 48. Viora E, Masturzo B, Errante G, Sciarrone A, Bastonero S, Campogrande M. Ultrasound evaluation of fetal nasal bone at 11 to 14 weeks in a consecutive series of 1906 fetuses. *Prenat Diagn* 2003;23:784-7.
 49. Zoppi MA, Ibba RM, Axinan C, Floris M, Manca F, Monni G. Absence of fetal nasal bone and aneuploidies at first-trimester nuchal translucency screening in unselected pregnancies. *Prenat Diagn* 2003;23:496-500.
 50. Huggon IC, DeFigueiredo DB, Allan LD. Tricuspid regurgitation in the diagnosis of chromosomal anomalies in the fetus at 11-14 weeks of gestation. *Heart* 2003;89:1071-3.
 51. Faiola S, Tsoi E, Huggon IC, Allan LD, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13⁺6-week scan. *Ultrasound Obstet Gynecol* 2005;26:22-7.
 52. Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11⁺0 to 13⁺6-week scan: association with chromosomal defects and reproducibility of the method. *Ultrasound Obstet Gynecol* 2006;27:609-12.
 53. Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. Screening for chromosomal abnormalities at 11-14 weeks: the role of ductus venosus blood flow. *Ultrasound Obstet Gynecol* 1998;12:380-4.
 54. Antolin E, Comas C, Torrents M, et al. The role of ductus venosus blood flow assessment in screening for chromosomal abnormalities at 10-16 weeks of gestation. *Ultrasound Obstet Gynecol* 2001;17:295-300.
 55. Murta CG, Moron AF, Avila MA, Weiner CP. Application of ductus venosus Doppler velocimetry for the detection of fetal aneuploidy in the first trimester of pregnancy. *Fetal Diagn Ther* 2002;17:308-14.
 56. Zoppi MA, Putzolu M, Ibba RM, Floris M, Monni G. First-trimester ductus venosus velocimetry in relation to nuchal translucency thickness and fetal karyotype. *Fetal Diagn Ther* 2002;17:52-7.
 57. Borrell A, Martinez JM, Seres A, Borobio V, Cararach V, Fortuny A. Ductus venosus assessment at the time of nuchal translucency measurement in the detection of fetal aneuploidy. *Prenat Diagn* 2003;23:921-6.
 58. Toyama JM, Brizot ML, Liao AW, Lopes LM, Nomura RM, Saldanha FA, Zugaib M. Ductus venosus blood flow assessment at 11 to 14 weeks of gestation and fetal outcome. *Ultrasound Obstet Gynecol* 2004;23:341-5.
 59. Prefumo F, Sethna F, Sairam S, Bhide A, Thilaganathan B. First-trimester ductus venosus, nasal bones, and Down syndrome in a high-risk population. *Obstet Gynecol* 2005;105:1348-54.
 60. Tul N, Spencer K, Noble P, Chan C, Nicolaides KH. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free β -hCG and PAPP-A at 10-14 weeks of gestation. *Prenat Diagn* 1999;19:1035-42.
 61. Spencer K, Ong C, Skentou H, Liao AW, Nicolaides KH. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free β -hCG and PAPP-A at 10-14 weeks of gestation. *Prenat Diagn* 2000;20:411-6.
 62. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one stop clinic: a review of three years prospective experience. *BJOG* 2003;110:281-6.

63. Spencer K, Nicolaides KH. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal translucency thickness, maternal serum free β -hCG and PAPP-A. *Prenat Diagn* 2002;22:877-9.
64. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005;25:221-6.
65. Kagan KO, Wright D, Maiz N, Pandeva I, Nicolaides KH. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008;32:488-92.
66. Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008;31:618-24.
67. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2008;31:493-502.
68. Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* 2008;31:376-83.
69. Spencer K, Souter V, Tul N, Snijders RJM, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999;13:231-7.
70. Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. *Ultrasound Obstet Gynecol* 2009;33:259-64.
71. Chen ATL, Chan YK, Falek A. The effects of chromosomal abnormalities on birth weight in man, I: sex chromosome disorders. *Hum Hered* 1971;21:543-56.
72. Chen ATL, Chan YK, Falek A. The effects of chromosomal abnormalities on birth weight in man, II: autosomal defects. *Hum Hered* 1972;22:209-24.
73. Drugan A, Johnson MP, Isada NB, et al. The smaller than expected first-trimester fetus is at increased risk for chromosome anomalies. *Am J Obstet Gynecol* 1992;167:1525-8.
74. Kuhn P, Brizot ML, Pandya PP, Snijders RJ, Nicolaides KH. Crown-rump length in chromosomally abnormal fetuses at 10 to 13 weeks' gestation. *Am J Obstet Gynecol* 1995;172:32-5.
75. Schemmer G, Wapner RJ, Johnson A, Schemmer M, Norton HJ, Anderson WE. First-trimester growth patterns of aneuploid fetuses. *Prenat Diagn* 1997;17:155-9.
76. Bahado-Singh RO, Lynch L, Deren O, Morrotti R, Copel JA, Mahoney MJ, Williams J 3rd. First-trimester growth restriction and fetal aneuploidy: the effect of type of aneuploidy and gestational age. *Am J Obstet Gynecol* 1997;176:976-80.
77. Sonek J, Borenstein M, Dagklis T, Persico N, Nicolaides KH. Frontomaxillary facial angle in fetuses with trisomy 21 at 11-13⁺⁶ weeks. *Am J Obstet Gynecol* 2007;196:271.e1-4.
78. Borenstein M, Persico N, Strobl I, Sonek J, Nicolaides KH. Frontomaxillary and mandibulomaxillary facial angles at 11⁺⁰ to 13⁺⁶ weeks in fetuses with trisomy 18. *Ultrasound Obstet Gynecol* 2007;30:928-33.
79. Borenstein M, Persico N, Kagan KO, Gazzoni A, Nicolaides KH. Frontomaxillary facial angle in screening for trisomy 21 at 11⁺⁰ to 13⁺⁶ weeks. *Ultrasound Obstet Gynecol* 2008;32:5-11.
80. Ghezzi F, Raio L, Di Naro E, Franchi M, Buttarelli M, Schneider H. First-trimester umbilical cord diameter: a novel marker of fetal aneuploidy. *Ultrasound Obstet Gynecol* 2002;19:235-9.
81. Axt-Flidner R, Schwarze A, Kreiselmaier P, Krapp M, Smrcek J, Diedrich K. Umbilical cord diameter at 11-14 weeks of gestation: relationship to nuchal translucency, ductus venous blood flow and chromosomal defects. *Fetal Diagn Ther* 2006;21:390-5.
82. Rembouskos G, Cicero S, Papadopoulos V, Tripsanas C, Nicolaides KH. Umbilical cord diameter at 11-14 weeks of gestation: relation to chromosomal defects. *Ultrasound Obstet Gynecol* 2004;23:237-9.
83. Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. First trimester maternal serum placenta growth factor (PlGF) concentrations in pregnancies with fetal trisomy 21 or trisomy 18. *Prenat Diagn* 2001;21:718-22.
84. Lo YMD, Lau TK, Zhang J, et al. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. *Clin Chem* 1999;45:1747-51.
85. Zhong XY, Burk MR, Troeger C, Jackson LR, Holzgreve W, Hahn S. Fetal DNA in maternal plasma is elevated in pregnancies with aneuploid fetuses. *Prenat Diagn* 2000;20:795-8.
86. Ohashi Y, Miharu N, Honda H, Samura O, Ohama K. Quantitation of fetal DNA in maternal serum in normal and aneuploid pregnancies. *Hum Genet* 2001;108:123-7.
87. Hromadnikova I, Houbova B, Hridelova D, et al. Quantitative analysis of DNA levels in maternal plasma in normal and Down syndrome pregnancies. *BMC Pregnancy Childbirth* 2002;2:4.
88. Lee T, LeShane ES, Messerlian GM, et al. Down syndrome and cell-free fetal DNA in archived maternal serum. *Am J Obstet Gynecol* 2002;187:1217-21.
89. Spencer K, de Kok JB, Swinkels DW. Increased total cell-free DNA in the serum of pregnant women carrying a fetus affected by trisomy 21. *Prenat Diagn* 2003;23:580-3.
90. Wataganara T, LeShane ES, Farina A, Messerlian GM, Lee T, Canick JA, Bianchi DW. Maternal serum cell-free fetal DNA levels are increased in cases of trisomy 13 but not trisomy 18. *Hum Genet* 2003;112:204-8.
91. Gerovassili A, Garner C, Nicolaides KH, Thein SL, Rees DC. Free fetal DNA in maternal circulation: a potential prognostic marker for chromosomal abnormalities? *Prenat Diagn* 2007;27:104-10.