

# First-trimester ultrasound screening for chromosomal defects

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## INTRODUCTION

This article examines a new method of screening for chromosomal abnormalities that is essentially based on two observations made more than 100 years ago. The first observation was by Dr Langdon Down who in 1866 reported that the skin of affected individuals appears to be too large for their bodies<sup>1</sup>. In 1876, Fraser and Mitchell published a detailed description of 62 patients and noted an association between the condition and advanced maternal age<sup>2</sup>. It is now known that the excess skin of individuals with Down syndrome can be visualized by ultrasonography as increased nuchal translucency in the first 3 months of intrauterine life. Furthermore, increased translucency at 10–14 weeks of gestation is a common phenotypic expression of many trisomies, Turner syndrome and triploidy.

## METHODS OF SCREENING FOR CHROMOSOMAL DEFECTS

Screening for fetal trisomy 21 was introduced in the early 1970s, when laboratory techniques were developed for determining the fetal karyotype. Initially, screening was based on maternal age and the 'high-risk' women who were offered amniocentesis were aged 37 years or more. This group constituted about 5% of the pregnant population and contributed about 30% of trisomy 21 babies.

In the late 1980s, a new method of screening was introduced that takes into account not only maternal age but also the concentration of various fetoplacental products in the maternal circulation. At 16 weeks of gestation, the median maternal serum concentrations of  $\alpha$ -fetoprotein, estriol and chorionic gonadotropin (total, free  $\alpha$ - and free  $\beta$ -hCG) in trisomy 21 pregnancies are sufficiently different from the median in normal pregnancies to allow the use of combinations of some or all of these substances to select a 'high-risk' group. This method of screening is proving to be more effective than maternal age alone and, for the same

rate of invasive testing (about 5%), it can identify about 60% of the fetuses with trisomy 21.

In the 1990s, screening by a combination of maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation was introduced<sup>3,4</sup> and is now proven to identify more than 80% of affected fetuses for a false-positive rate of about 5%<sup>5</sup>. When fetal heart rate and maternal serum free  $\beta$ -hCG are also taken into account, the detection rate of chromosomal defects is about 90%<sup>6,7</sup>.

## CHROMOSOMAL DEFECTS IN RELATION TO MATERNAL AGE AND GESTATION

Estimates of risks for trisomy 21 for each maternal age first became available in the late 1980s; these were derived from the data of surveys in live births<sup>8,9</sup>. While such data are useful for counselling parents as to the risk of giving birth to a baby with trisomy 21, with the introduction of antenatal screening it has become necessary to establish prevalences for all chromosomal defects at different stages of pregnancy. For example, exomphalos is associated with trisomy 18 but the risk for this chromosomal defect increases with maternal age and decreases with gestation; the frequency of trisomy 18 in the presence of exomphalos is therefore lower if the maternal age is 20 years rather than 40 years and it is higher if the gestation is 12 weeks rather than 20 weeks<sup>10</sup>. Similarly, to determine the significance of a given ultrasonographic marker for any chromosomal abnormality, it is essential to know the prevalence of the abnormality at the gestation under study, based on the maternal age distribution of the population that is examined.

Snijders and colleagues (1995)<sup>11</sup> combined data from studies on mid-trimester amniocentesis and first-trimester chorion villus sampling to estimate the prevalence of a

**Table 1** Estimated risk for trisomies 21, 18 and 13 (1/number given in the table) in relation to maternal age and gestation<sup>11</sup>

Maternal age (years)	Trisomy 21			Trisomy 18			Trisomy 13		
	12 weeks	20 weeks	40 weeks	12 weeks	20 weeks	40 weeks	12 weeks	20 weeks	40 weeks
20	898	1175	1527	2484	4897	18 013	7826	14 656	42 423
25	795	1040	1352	2200	4336	15 951	6930	12 978	37 567
30	526	688	895	1456	2869	10 554	4585	8 587	24 856
35	210	274	356	580	1142	4 202	1826	3 419	9 896
40	57	74	97	157	310	1 139	495	927	2 683

wide range of chromosomal defects at different gestations in relation to trisomy 21 in live births. Maternal age and gestational age-specific risks were then calculated by multiplying the maternal age-specific prevalences of trisomy 21 in live births with the relative prevalence at a given gestation (Table 1).

For each chromosomal defect, it is possible to calculate the risk of intrauterine lethality (Table 2) from the differences in prevalence at 40 weeks and prevalence at a given gestation. For example, in trisomy 21, if the prevalence at 40 weeks is 1.00, the prevalences at 16 and 12 weeks of gestation are 1.46 and 1.69, respectively; the rate of intrauterine lethality between 16 weeks and 40 weeks is 32%  $[(1.46 - 1.00)/1.46]$ , and between 12 and 40 weeks is 41%  $[(1.69 - 1.00)/1.69]$ <sup>11</sup>.

## ASSESSMENT OF NUCHAL TRANSLUCENCY THICKNESS

During the second and third trimesters of pregnancy, abnormal accumulation of fluid behind the fetal neck can be classified as nuchal cystic hygromas, which are associated with Turner syndrome, and nuchal edema, which has a diverse etiology including trisomies, cardiovascular and pulmonary defects, skeletal dysplasias, congenital infection and metabolic and hematological disorders<sup>12</sup>. Furthermore, the chromosomally normal fetuses had a very poor prognosis because, in many cases, there was an underlying skeletal dysplasia, genetic syndrome or cardiac defect<sup>12</sup>. In the first trimester, the term translucency is used, because this is the ultrasonographic feature that is observed; during the second trimester, the translucency usually resolves and, in a few cases, it evolves into either nuchal edema or cystic hygromas with or without generalized hydrops.

### Measurement

Transabdominal ultrasound examination is performed to obtain a sagittal section of the fetus for measurement of fetal crown-rump length. The maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine is measured<sup>3</sup>. Care is taken to distinguish between fetal skin and amnion because, at this gestation, both structures appear as thin membrane. This is achieved by waiting for spontaneous fetal movement away from the amniotic membrane; alternatively, the fetus is bounced off the amnion by asking the mother to cough and/or by tapping the maternal abdomen (Figure 1).

**Table 2** Estimates for the rate of spontaneous loss in fetuses with various chromosomal defects<sup>11</sup>

Chromosomal defect	Estimated loss rate (%)	
	From 12 to 40 weeks	From 16 to 40 weeks
Trisomy 21	41	32
Trisomy 18	86	74
Trisomy 13	82	71
Turner syndrome	75	52
47,XXX	~ 5	~ 3
47,XXX	~ 5	~ 3
47,XYY	~ 5	~ 3
Triploidy	> 99	> 99



**Figure 1** Ultrasound picture demonstrating measurement of fetal crown-rump length and nuchal translucency thickness of 2.2 mm. The amnion can be seen separate from the fetal skin

All sonographers performing fetal scans should be capable of measuring reliably the crown-rump length and obtaining a proper sagittal view of the fetal spine. For such sonographers, it is easy to acquire, within a few hours, the skill to measure accurately nuchal translucency thickness.

### Repeatability

A potential criticism of screening by ultrasound is that scanning requires not only highly skilled operators but it is also prone to operator variability. This issue was addressed by a prospective study at 10–14 weeks of gestation in which the translucency was measured by two of four operators in 200 pregnant women<sup>13</sup>. This study demonstrated that, after an initial measurement, the second one made

by the same (intra-) observer or another (inter-) observer varies from the first by less than 0.54 mm and 0.62 mm, respectively in 95% of the cases. Additionally, the study demonstrated that the caliper placement repeatability was similar to the intra-observer and inter-observer repeatability, suggesting that a large part of the variation in measurements can be accounted for by the placement of the calipers rather than the generation of the image. Digital image processing and automation of caliper placement should reduce the differences in measurement. In the meantime, it is best to take the mean of two good measurements rather than one.

### Increase with gestational age

In a multicenter study involving more than 20 000 pregnancies, the fetal nuchal translucency thickness increased with crown-rump length<sup>5</sup>. Therefore, in determining whether a given nuchal translucency thickness is increased, it is essential to take gestation into account.

## NUCHAL TRANSLUCENCY AND CHROMOSOMAL DEFECTS

In the early 1990s, several reports of small series in high-risk pregnancies demonstrated a possible association between increased nuchal translucency and chromosomal defects in the first trimester of pregnancy (Table 3)<sup>3,14-31</sup>.

Although the mean prevalence of chromosomal defects in 20 series involving a total of 1698 patients was 29%, there were large differences between the studies, with the prevalence ranging from 19 to 88%. This variation in

results presumably reflects differences in the maternal age distributions of the populations examined and differences in the definition of minimum thickness of the abnormal translucency, ranging from 2 to 10 mm.

Subsequently, a series of screening studies in high-risk pregnancies was carried out; these involved measurement of nuchal translucency thickness immediately before fetal karyotyping, mainly for advanced maternal age. These studies, involving a total of 1273 pregnancies, reported that the nuchal translucency thickness was above the 95th centile of the normal range in about 80% of trisomy 21 fetuses<sup>3,4</sup>. Similar findings were obtained in an additional four studies of pregnancies undergoing first-trimester fetal karyotyping<sup>24,25,27,28</sup>. However, in another study involving 1819 pregnancies, nuchal translucency thickness of > 3 mm identified only 30% of the chromosomally abnormal fetuses (no data were provided specifically for trisomy 21) and the false-positive rate was 3.2%<sup>23</sup>.

An additional finding of the screening studies in high-risk pregnancies was that the prevalence of chromosomal defects is dependent on both the fetal nuchal translucency thickness and maternal age<sup>4,17,18</sup>. For example, in a study of 1015 pregnancies with increased fetal nuchal translucency thickness at 10-14 weeks of gestation, the observed numbers of trisomies 21, 18 and 13 in fetuses with translucencies of 3 mm, 4 mm, 5 mm, and > 6 mm were approximately 3 times, 18 times, 28 times and 36 times higher than the respective number expected on the basis of maternal age (Figure 2); the incidences of Turner syndrome and triploidy were 9 times and 8 times higher but the incidence of other sex chromosome aneuploidies was similar to that expected<sup>18</sup>.

**Table 3** Summary of reported series on first-trimester fetal nuchal translucency providing data on gestational age in weeks, criteria for diagnosis of increased nuchal translucency thickness and the presence of associated chromosomal defects

Author	Gestational age (weeks)	Nuchal translucency thickness (mm)	n	Abnormal karyotype						
				Total		Tr21	Tr18	Tr13	45,X	Other
				n	%					
Johnson <i>et al.</i> , 1993 <sup>14</sup>	10-14	≥ 2.0	68	41	60	16	9	2	9	5
Hewitt <i>et al.</i> , 1993 <sup>15</sup>	10-14	≥ 2.0	29	12	41	5	3	1	2	1
Shulman <i>et al.</i> , 1992 <sup>16</sup>	10-13	≥ 2.5	32	15	47	4	4	3	4	—
Nicolaides <i>et al.</i> , 1992 <sup>3,4</sup>	10-13	≥ 3.0	88	33	38	21	8	2	—	2
Pandya <i>et al.</i> , 1994 <sup>17,18</sup>	10-13	≥ 3.0	1015	193	19	101	51	13	14	15
Szabo and Gellen, 1990 <sup>19</sup>	11-12	≥ 3.0	8	7	88	7	—	—	—	—
Wilson <i>et al.</i> , 1992 <sup>20</sup>	8-11	≥ 3.0	14	3	21	—	—	—	1	2
Ville <i>et al.</i> , 1992 <sup>21</sup>	9-14	≥ 3.0	29	8	28	4	3	1	—	—
Trauffer <i>et al.</i> , 1994 <sup>22</sup>	10-14	≥ 3.0	43	21	49	9	4	1	4	3
Brambati <i>et al.</i> , 1995 <sup>23</sup>	8-15	≥ 3.0	70	13	19	?	?	?	?	?
Comas <i>et al.</i> , 1995 <sup>24</sup>	9-13	≥ 3.0	51	9	18	4	4	—	—	1
Szabo <i>et al.</i> , 1995 <sup>25</sup>	9-12	≥ 3.0	96	43	45	28	10	—	2	3
Nadel <i>et al.</i> , 1993 <sup>26</sup>	10-15	≥ 4.0	63	43	68	15	15	1	10	2
Savoldelli <i>et al.</i> , 1993 <sup>27</sup>	9-12	≥ 4.0	24	19	79	15	2	1	1	—
Schulte-Valentin <i>et al.</i> , 1992 <sup>28</sup>	10-14	≥ 4.0	8	7	88	7	—	—	—	—
van Zalen-Sprock <i>et al.</i> , 1992 <sup>29</sup>	10-14	≥ 4.0	18	5	28	3	1	—	1	1
Cullen <i>et al.</i> , 1990 <sup>30</sup>	11-13	≥ 6.0	29	15	52	6	2	—	4	3
Suchet <i>et al.</i> , 1992 <sup>31</sup>	8-14	≥ 10.0	13	8	62	—	—	—	7	1
<b>Total</b>	<b>8-15</b>		<b>1698</b>	<b>495</b>	<b>29</b>	<b>245</b>	<b>116</b>	<b>25</b>	<b>59</b>	<b>39</b>

Tr21, trisomy 21; Tr18, trisomy 18; Tr13, trisomy 13; 45,X, Turner syndrome

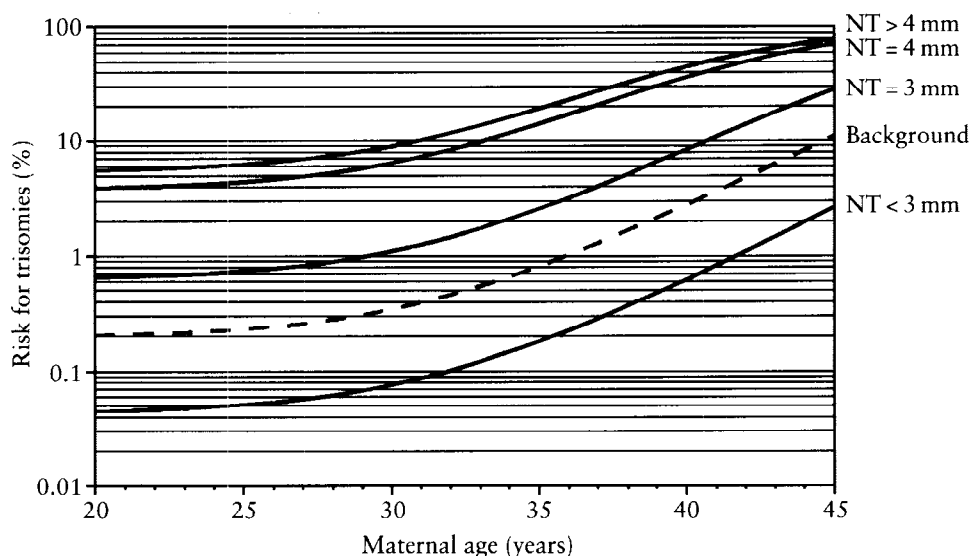


Figure 2 Adjusted risk for trisomy 21 with maternal age according to nuchal translucency (NT) thickness measurements of < 3 mm, 3 mm, 4 mm and more than 4 mm

### Screening in unselected populations

#### *The Frimley Park and St. Peter's study*

Frimley Park Hospital and St. Peter's Hospital, Chertsey, are general hospitals within the National Health Service offering routine antenatal care and their combined annual number of deliveries is approximately 6000. Prior to the introduction of nuchal translucency scanning, the policy of these hospitals was to offer amniocentesis to women aged 35 years or more. During 1993, there were 11 fetuses with Down syndrome and only two of these were detected prenatally<sup>32</sup>. Subsequently, nuchal translucency screening at 10–14 weeks of gestation was introduced and the implementation of this policy was achieved without the need for increasing the number of staff or the equipment. Women with fetal translucency of 2.5 mm or more were offered fetal karyotyping. In addition, women aged 35 years or more were offered amniocentesis at 16 weeks' gestation. The data of the first 5 months after the introduction of the new policy were analyzed following completion of the pregnancies<sup>32</sup>. During this period, 74% of women delivering in the two hospitals attended for first-trimester scanning and the nuchal translucency was successfully measured in all pregnancies. The translucency was raised in 3.6% of cases and the total percentage of invasive procedures was 5.1%. All four cases of Down syndrome that occurred in this period were diagnosed prenatally.

#### *The University College study*

In a screening study of 1704 women with singleton pregnancies attending University College Hospital, London, for routine antenatal care at 8–14 weeks of gestation, transabdominal ultrasound examination was performed<sup>33</sup>. In 20% of cases, the sonographers forgot to measure the fetal nuchal translucency thickness. In a further 18% of those women where a measurement was attempted, this was

unsuccessful. In 28% of the 1127 cases where measurements were made, the scans were carried out before 10 weeks of gestation. The translucency thickness was  $\geq 3$  mm in 6% of the cases. The population contained three fetuses with trisomy 21, all in women aged  $\geq 39$  years, and increased translucency was found in one.

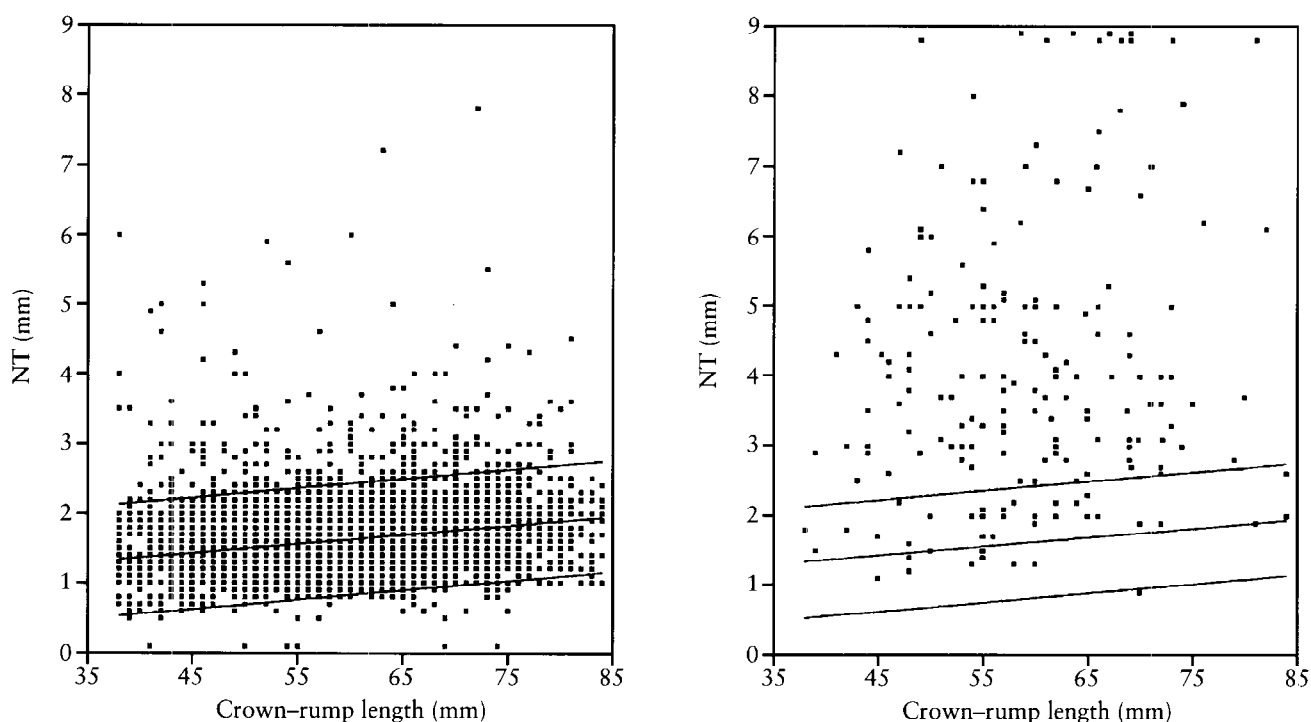
#### *The Austrian study*

In a screening study of 1972 women with singleton pregnancies attending a National Health Service hospital in Vienna for routine antenatal care at 10–13 weeks of gestation, transabdominal ultrasound examination was performed and the fetal nuchal translucency thickness was successfully measured in all cases<sup>34</sup>. The nuchal translucency thickness was  $\geq 2.5$  mm in 1.3% of the cases and this group included 73% of those with chromosomal abnormalities.

#### *The multicenter screening study*

In a multicenter study, at the Harris Birthright Centre and four District General Hospitals, nuchal translucency screening at 10–14 weeks of gestation has been carried out in 20 804 pregnancies, including 164 cases of chromosomal abnormalities<sup>5</sup>. This study has demonstrated that:

- (1) In normal pregnancies, nuchal translucency thickness increases with gestation (Figure 3);
- (2) In chromosomally abnormal pregnancies, nuchal translucency thickness is increased (Figure 3);
- (3) The risk for trisomies can be derived by multiplying the background maternal age and gestation-related risk by a likelihood ratio, which depends on the degree of deviation in nuchal translucency from the normal median for crown–rump length;



**Figure 3** Fetal nuchal translucency (NT) thickness with crown-rump length in chromosomally normal fetuses (left), and in fetuses with trisomy 21 (right)

- (4) In about 5% of pregnancies, the estimated risk for trisomy 21 was at least 1 in 100 and this group included 80% of fetuses with trisomy 21 and 77% of those with other chromosomal abnormalities.

Because the maternal age of the screened population was higher than in Britain as a whole, it was estimated that the cut-off risk to include 5% of the British population is 1/300; using this cut-off the sensitivity of the test for trisomy 21 was estimated to be about 80%. This study has proven that combining maternal age with fetal nuchal translucency thickness is currently the most sensitive method of screening for chromosomal abnormalities.

### The Fetal Medicine Foundation

The findings of the RADIUS study<sup>35</sup> have demonstrated that inappropriate introduction of new services by inadequately trained personnel and without the provision of audit can lead to the erroneous conclusion that the new service/technology is not beneficial. In addition, such bad medical practices are inevitably detrimental to the health of patients.

It is for this reason that the Fetal Medicine Foundation, a registered charity, was set up. One of the aims is to provide comprehensive training, support and audit for the proper implementation of the 10–14-week scan. The Foundation is offering, free of charge, training courses on many aspects of first-trimester scanning, and has introduced a certificate of competence that can be obtained after passing the appropriate theoretical and practical examinations. The Foundation provides the necessary computer program for calculation of risks only to those sonographers that

receive the appropriate certificate of competence and participate in continuing audit of results.

In Britain, there are now 20 approved centers for carrying out nuchal translucency screening and, by the 1st January 1996, a total of 66 600 singleton pregnancies with live fetuses at 10–14 weeks of gestation had been examined. The first 42 619 completed pregnancies included 147 with trisomy 21 and 130 with other chromosomal defects; using a cut-off risk of 1/300, estimated from the maternal age and nuchal translucency thickness, the sensitivity for trisomy 21 was 86% (Tables 4 and 5).

The 42 619 pregnancies included 20 543 that were examined at the Harris Birthright Research Centre for Fetal Medicine and 22 076 that were screened in 19 other British centers (Table 4). The Harris Birthright Centre recruited self-referred patients and 24.2% were aged 37 years or more and therefore the prevalence of trisomy 21 was high. Consequently, the estimated risk based on maternal age and nuchal translucency thickness was more than 1/300 in 16.1% of the population, including 87% of the trisomy 21 pregnancies. The other 19 centers offered screening as part of routine antenatal care and the screened patients were more representative of the whole British population. In this group, only 6.3% were aged 37 years or more and 6% had an estimated risk of 1/300; the detection rate for trisomy 21 was 84%.

The results from the Harris Birthright Centre demonstrate that, in a population in which on the basis of maternal age at least 24.2% would have been classified as high risk, nuchal translucency screening reduced the need for invasive testing to 16.1% and yet the sensitivity of the test for trisomy 21 was 87%. The results from the other centers prove that, provided there is adequate training and audit,

**Table 4** Results of the Fetal Medicine Foundation multicenter screening study for trisomy 21 based on the combination of maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation in 42 619 completed singleton pregnancies. Figures in parentheses are percentages

	HBC*		19 DGH <sup>†</sup>		Total	
	All cases	Tr21	All cases	Tr21	All cases	Tr21
Total number of pregnancies	20 543	104	22 076	43	42 619	147
Maternal age > 37 years	4 978 (24)	68 (65)	1 393 (6)	17 (39)	6 370 (15)	85 (58)
Estimated risk > 1/300 all cases	3 302 (16)	91 (87)	1 316 (6)	36 (84)	4 618 (11)	127 (86)
Estimated risk > 1/100 all cases	1 194 (6)	82 (79)	518 (2)	28 (68)	1 712 (4)	110 (75)

\*, HBC, Harris Birthright Centre; <sup>†</sup>, DGH, District General Hospitals; Tr 21, trisomy 21

**Table 5** Results of the screening study for trisomy 21 based on the combination of maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation from 42 619 completed singleton pregnancies examined in 20 British centers under the auspices of the Fetal Medicine Foundation. Figures in parentheses are percentages

Karyotype	Total	Nuchal translucency > 95 <sup>th</sup> centile	Risk > 1/300
Trisomy 21	147	111 (76)	127 (86)
Trisomy 18	60	47 (78)	52 (87)
Trisomy 13	17	14 (82)	16 (94)
Other trisomy	6	4 (67)	6 (100)
Turner syndrome	18	17 (94)	18 (100)
Sex aneuploidies	12	4 (33)	9 (75)
Triploidy	9	6 (67)	6 (67)
Other	8	4 (50)	6 (75)

nuchal translucency screening, as part of routine antenatal care, can identify 84% of trisomy 21 fetuses.

## NUCHAL TRANSLUCENCY AND FETAL HEART RATE

In an ultrasound screening study of fetal nuchal translucency thickness at 10–14 weeks of gestation, the fetal heart rate was also measured<sup>7</sup>. In 6903 normal singleton pregnancies, fetal heart rate decreased from a mean of 171 beats/min at 10 weeks of gestation to 156 beats/min at 14 weeks. In 85 trisomy 21 pregnancies, the mean fetal heart rate was significantly higher than in the normal group (Figure 4). Similarly, the heart rate was increased in 16 fetuses with trisomy 13 and in 19 with Turner syndrome. In contrast, the heart rate was decreased in 34 fetuses with trisomy 18 and in eight with triploidy.

In both the trisomy 21 pregnancies and the normal pregnancies, there was no significant association between nuchal translucency thickness and fetal heart rate. Therefore, fetal nuchal translucency and heart rate can be combined in calculating the risk for trisomies. In a study of 6961 pregnancies at 10–14 weeks of gestation, it was estimated that inclusion of fetal heart rate with maternal age and fetal nuchal translucency thickness can improve the sensitivity of screening for trisomy 21 by about 5%<sup>7</sup>.

## NUCHAL TRANSLUCENCY AND MATERNAL SERUM BIOCHEMISTRY

In trisomy 21 during the first trimester of pregnancy, the maternal serum concentration of free beta human chorionic gonadotropin (free  $\beta$ -hCG) is higher and pregnancy-associated plasma protein A (PAPP-A) is lower than in chromosomally normal pregnancies<sup>36–46</sup> (Figures 5 and 6). Pregnancy-specific  $\beta$ -1 glycoprotein (SP1) and  $\alpha$ -fetoprotein do not provide useful distinction between affected and normal pregnancies<sup>40,47</sup>.

Studies examining the relationship between maternal serum PAPP-A or free  $\beta$ -hCG concentrations and fetal nuchal translucency thickness have demonstrated no significant association between biochemistry and ultrasound findings in either the chromosomally normal or the trisomy 21 pregnancies<sup>7,41,46</sup>. Therefore, maternal serum PAPP-A and free  $\beta$ -hCG and fetal nuchal translucency can be combined in calculating risks for fetal trisomies. In a study of 2529 pregnancies at 10–14 weeks of gestation, it was estimated that inclusion of maternal serum free  $\beta$ -hCG with maternal age and fetal nuchal translucency thickness can improve the sensitivity of screening for trisomy 21 by about 5%<sup>6</sup>.

## PATHOLOGICAL FINDINGS IN TRISOMIC FETUSES WITH INCREASED TRANSLUCENCY

Hyett and colleagues<sup>48–50</sup> reported on the pathological examination of the fetal heart and great arteries in chromosomally abnormal fetuses with increased nuchal translucency at 10–14 weeks of gestation. After suction termination of pregnancy, the heart and great arteries were identified, fixed with paraformaldehyde and examined using a step-wise microdissection method and scanning electron microscopy.

The most common cardiac lesions seen in fetuses affected by trisomy 21 were atrioventricular or ventricular septal defect<sup>48</sup>, whereas trisomy 18 was associated with ventricular septal defects and/or polyvalvular abnormalities<sup>49</sup>. Additionally, the aortic isthmus was significantly narrower than in normal fetuses and the degree of narrowing of the isthmus was significantly greater in fetuses with high nuchal translucency thickness<sup>50</sup>. It could, therefore, be

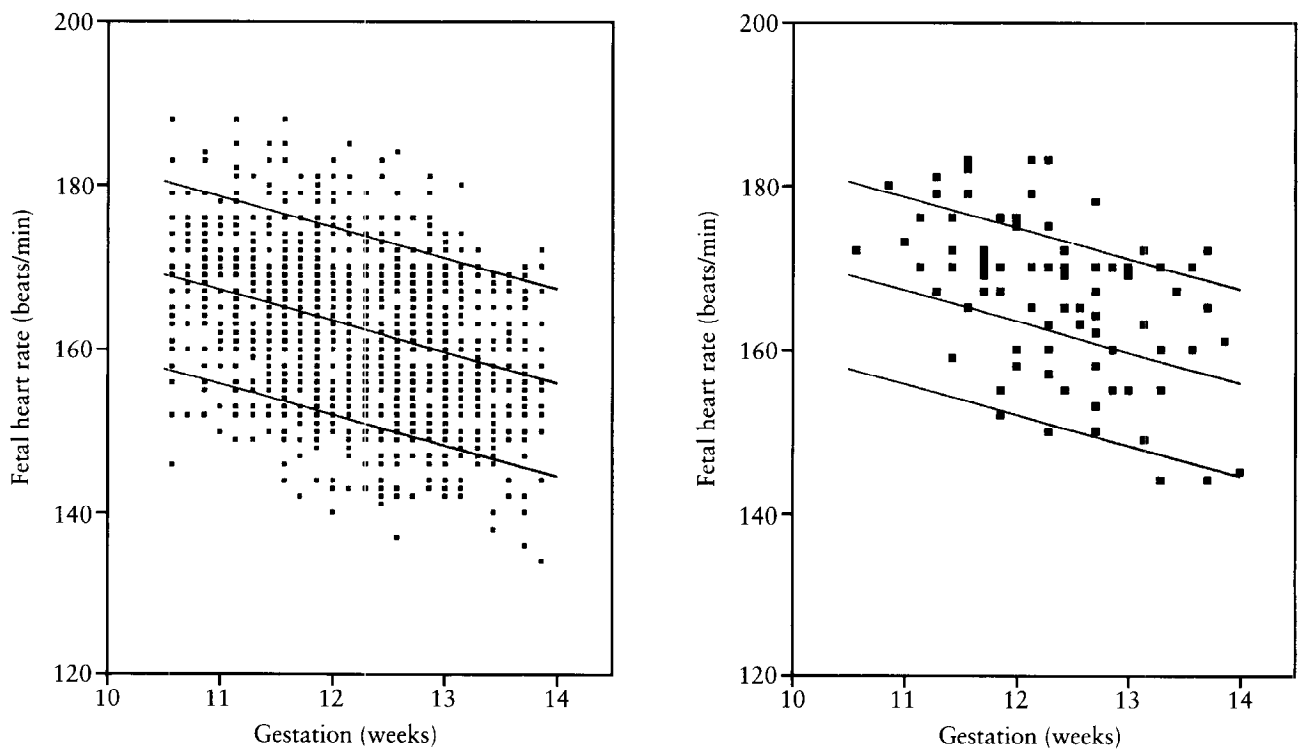


Figure 4 Fetal heart rate with gestation in chromosomally normal fetuses (left) and in fetuses with trisomy 21 (right)<sup>7</sup>

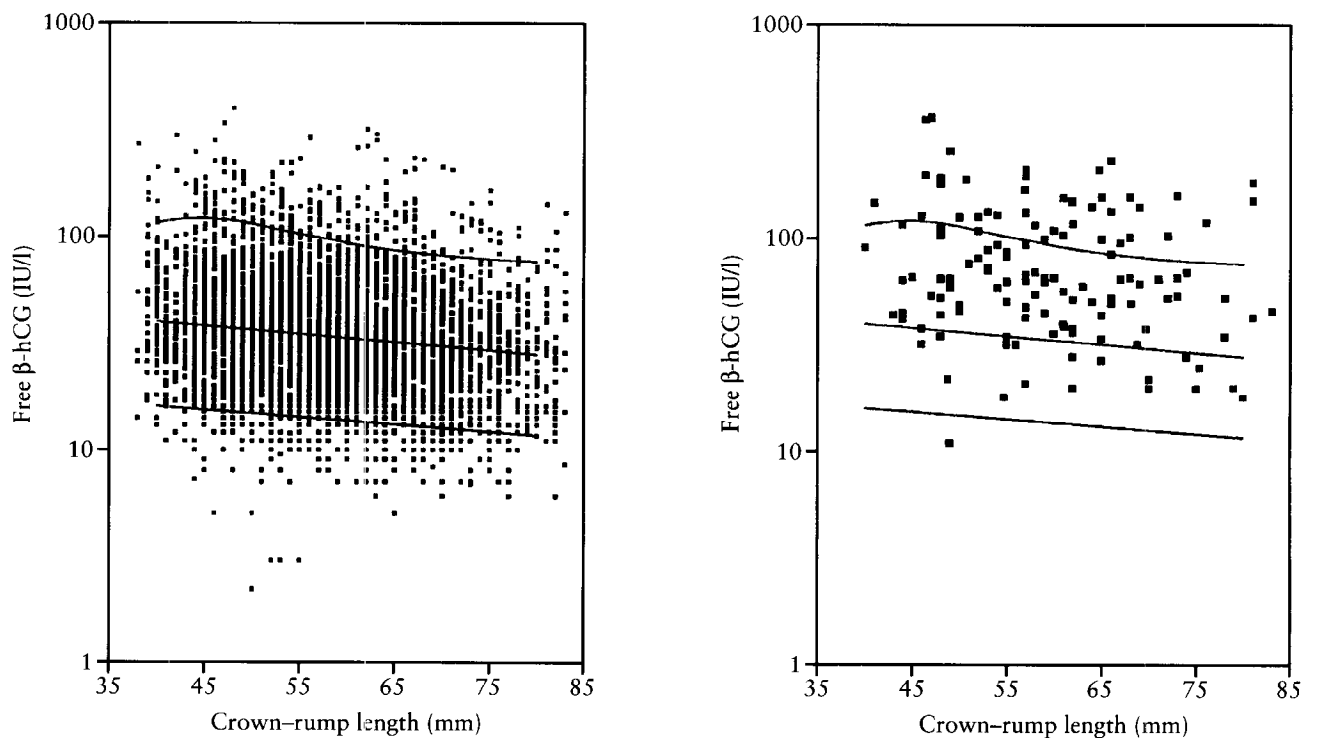


Figure 5 Maternal serum free  $\beta$ -hCG with crown-rump length in chromosomally normal pregnancies (left) and in those with trisomy 21 (right)<sup>6</sup>

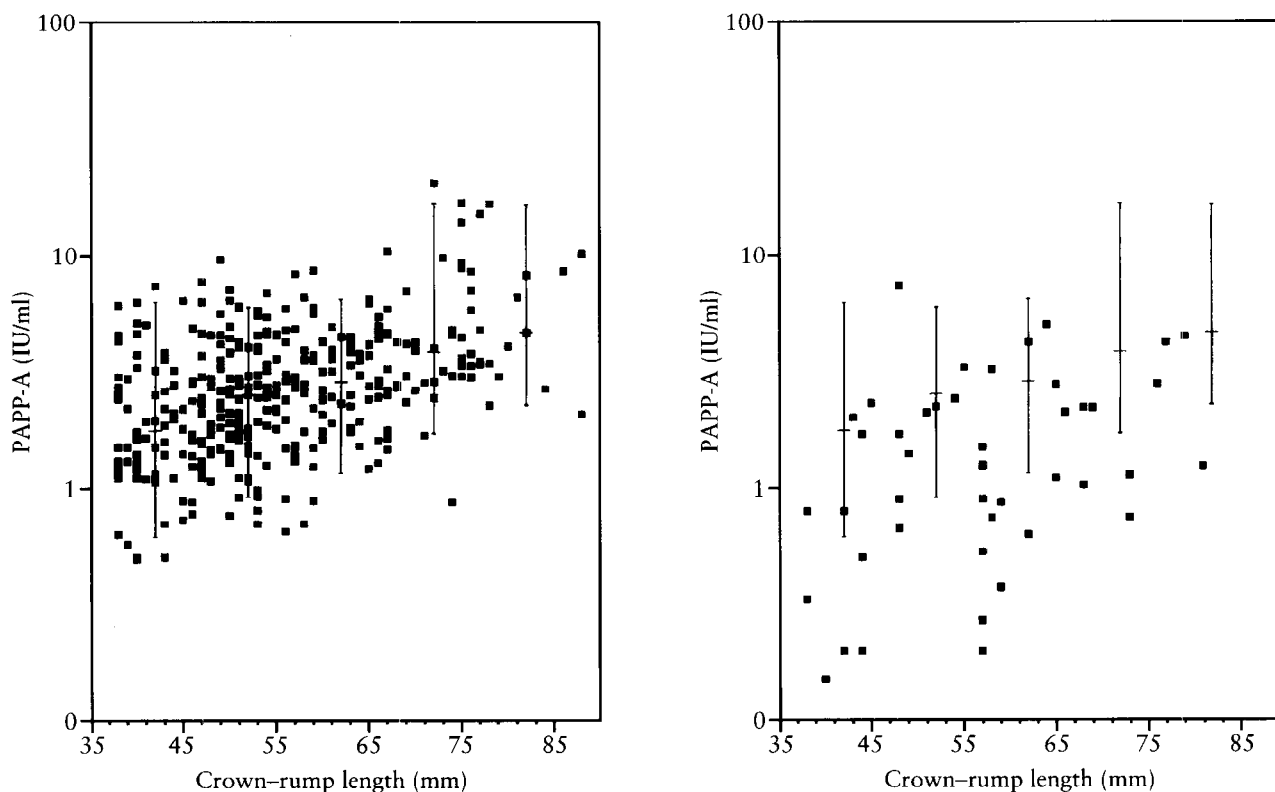


Figure 6 Maternal serum PAPP-A with crown-rump length in chromosomally normal pregnancies (left) and in those with trisomy 21 (right)<sup>41</sup>

postulated that narrowing of the aortic isthmus may be one of the underlying mechanisms for increased nuchal translucency thickness.

### LETHALITY OF TRISOMY 21 FETUSES WITH INCREASED TRANSLUCENCY

Screening for chromosomal defects in the first rather than the second trimester has the advantage of earlier prenatal diagnosis and consequently less traumatic termination of pregnancy for those couples that choose this option. A potential disadvantage is that earlier screening preferentially identifies those chromosomally abnormal pregnancies that are destined to miscarry. Approximately 40% of affected fetuses die between 12 weeks of gestation and term<sup>51</sup>. This issue of preferential intrauterine lethality of chromosomal defects is, of course, a potential criticism of all methods of antenatal screening, including second-trimester maternal serum biochemistry; the estimated rate of intrauterine lethality between 16 weeks and term is about 30%<sup>51</sup>. This section examines the interrelation between increased nuchal translucency in trisomy 21 and fetal lethality.

#### Decision to continue with the pregnancy after the diagnosis of trisomy 21

In a study of 108 fetuses with trisomy 21 diagnosed in the first trimester because of increased nuchal translucency thickness, in five cases the parents chose to continue with

the pregnancy whereas in 103 they had termination; trisomy 21 was also diagnosed in one of the fetuses in a twin pregnancy where the parents elected to avoid invasive prenatal diagnosis or selective fetocide<sup>51</sup>. In five of the six fetuses, the nuchal translucency resolved and at the second-trimester scan the nuchal fold thickness was normal (less than 7 mm)<sup>51</sup>. All six trisomy 21 babies were born alive. One had a major atrioventricular septal defect and died at the age of 6 months. Another two of the babies had small ventricular septal defects and these are being managed conservatively awaiting spontaneous closure. These data suggest that increased nuchal translucency does not necessarily identify those trisomic fetuses that are destined to die *in utero*.

#### Decision to terminate the pregnancy after the diagnosis of trisomy 21

In a study of 70 pregnancies where trisomy 21 was diagnosed at 12 (range 11–14) weeks of gestation and where the parents opted for elective termination which was carried out at 14 (12–20) weeks, ultrasound examination to establish viability was carried out at the time of chorion villus sampling and just before termination<sup>52</sup>. Eight fetuses died in the interval between chorion villus sampling and termination and the rate of lethality increased with translucency thickness from 5.3% for those with translucency of 0–3 mm to 23.5% for translucency of > 7 mm.

Even if one assumes that the relative rate of intrauterine lethality of trisomy 21 fetuses according to translucency

thickness stays the same throughout pregnancy, it was estimated that a policy of screening by maternal age and fetal nuchal translucency followed by selective termination of affected fetuses would be associated with at least a 70% reduction in the live birth incidence of trisomy 21<sup>52</sup>.

### Data from the Fetal Medicine Foundation multicenter study

In the 42 619 completed pregnancies that were screened in the multicenter study, it was estimated that 108 babies with trisomy 21 would have been born had there not been any antenatal screening. The estimate was made from the maternal age distribution of the population and the maternal age-related prevalence of trisomy 21 in live births. In reality, there were only 17 live births with trisomy 21 and seven of these were in the screen-positive group (risk more than 1/300), but the parents chose not to have prenatal diagnosis or decided to continue with the pregnancy despite the prenatal diagnosis of trisomy 21. Similarly, of the 130 trisomy 21 fetuses that were diagnosed prenatally, 120 were in the screen-positive group and ten in the screen-negative group.

On the extreme assumption that all ten screen-negative pregnancies with trisomy 21 that were diagnosed antenatally would have resulted in live births had the pregnancies not been terminated, then the number of trisomy 21 live births in the screen-negative group would have been 20. Therefore, screening by a combination of maternal age and fetal nuchal translucency and selective termination of affected fetuses has now proven to reduce the potential live birth prevalence of trisomy 21 by at least 81% (88 of 108).

### NUCHAL TRANSLUCENCY IN MULTIPLE PREGNANCIES

During the last 20 years, both the average maternal age and the use of assisted reproduction techniques have increased with a consequent increase in the number of multiple pregnancies at high risk of chromosomal defects. In multiple pregnancies compared to singletons, prenatal diagnosis is complicated because, first, effective methods of screening, such as maternal serum biochemistry, are not applicable; second, the techniques of invasive testing may provide uncertain results or may be associated with higher risks of miscarriage; and, third, the fetuses may be discordant for an abnormality in which case one of the options for the subsequent management of the pregnancy is selective fetocide.

In singleton pregnancies, the method of choice for fetal karyotyping may be chorion villus sampling because of the advantages of early diagnosis, whilst, in twin pregnancies, the method of choice for fetal karyotyping is amniocentesis. However, cytogenetic results from amniocentesis are not usually available until around 18–20 weeks of gestation; if one fetus is chromosomally abnormal and the parents choose selective fetocide, the risk of miscarriage is three times higher than with fetocide before 16 weeks<sup>53</sup>. Therefore, there is a need for selection of the appropriate

diagnostic technique depending on the likelihood for selective fetocide; if the risk is high (more than 1 in 50), then chorion villus sampling should be the technique of choice, otherwise amniocentesis is preferable<sup>54</sup>.

Pandya and colleagues<sup>55</sup> examined nuchal translucency thickness of each fetus in eight twin pregnancies where karyotyping at 10–14 weeks of gestation demonstrated that at least one of the fetuses was chromosomally abnormal. Eight fetuses had trisomy 21 and two had trisomy 18. The nuchal translucency thickness was more than 2.5 mm in nine (90%) of the trisomic fetuses and in one of the chromosomally normal ones.

In the Harris Birthright screening study at 10–14 weeks there were 20 543 singleton pregnancies and 392 twin pregnancies. This study demonstrated that, in twin pregnancies, screening for trisomy 21 by measurement of fetal nuchal translucency thickness and maternal age had a similar sensitivity to that found in singletons. However, the false-positive rate of the test is higher in twin compared to singleton pregnancies, due to a higher prevalence of increased translucency in chromosomally normal fetuses from monochorionic pregnancies (about 9%, compared to 6% in singletons). The most likely explanation for this high false-positive rate is that increased nuchal translucency in one of the fetuses in monochorionic twins is an early manifestation of heart failure due to twin-to-twin transfusion syndrome.

### INCREASED TRANSLUCENCY IN CHROMOSOMALLY NORMAL FETUSES

In two studies examining a total of 32 chromosomally normal fetuses with increased nuchal translucency ( $\geq 2$  mm), there were four terminations of pregnancy (three because of progressive hydrops and one because of amnion disruption sequence), one intrauterine death in a fetus with obstructive uropathy, one spontaneous abortion and 26 live births; 23 were healthy, two had non-specific dysmorphic features and one had Noonan syndrome<sup>14,22</sup>.

Shulman and colleagues<sup>56</sup> reported on 32 chromosomally normal fetuses with increased nuchal translucency ( $\geq 2.5$  mm). In one case, there were persistent hygromas that were successfully repaired at birth and in the other 31 cases the translucency resolved by 20 weeks and all babies were healthy at birth; follow-up examination at 12 months demonstrated normal growth and development in all infants.

Pandya and colleagues<sup>17,18</sup> reported on the outcome of 565 chromosomally normal fetuses with nuchal translucency 3–9 mm. The prevalence of structural defects, mainly cardiac, diaphragmatic, renal and abdominal wall, was approximately 4%, which is higher than would be expected in an unselected population. Additionally, fetuses with increased translucency, as with nuchal edema in later pregnancy, may be at increased risk of rare genetic syndromes such as Stickler syndrome, Smith–Lemli–Opitz syndrome, Jarco Lavine syndrome or arthrogyposis<sup>57,58</sup>. The overall survival, taking into account perinatal deaths and

terminations of pregnancy for fetal defects, decreased with nuchal translucency thickness from 97% for 3 mm to 53% for  $\geq 5$  mm<sup>18</sup>.

Hyett and colleagues<sup>57</sup> performed pathological studies in fetuses with increased translucency and reported a high prevalence of cardiac abnormalities and genetic syndromes. In this respect, measurement of nuchal translucency thickness may prove to be a useful method of screening for cardiac and other abnormalities in addition to its role in screening for chromosomal defects. It is, therefore, recommended that, in all cases with increased nuchal translucency at 10–14 weeks, detailed ultrasound scans are subsequently performed to diagnose fetal abnormalities and markers of possible genetic syndromes.

## CONCLUSION

The combination of maternal age and fetal nuchal translucency thickness at 10–14 weeks is now proven to be the most effective method of screening for chromosomal abnormalities with a sensitivity for trisomy 21 of more than 80%. This method of screening is also effective in multiple pregnancies. Additionally, increased nuchal translucency is likely to be a useful marker for a wide variety of fetal abnormalities and genetic syndromes. However, as with the introduction of any new technology into routine clinical practice, it is essential that those undertaking the 10–14-week scan are adequately trained and their results are subjected to rigorous audit.

## REFERENCES

- Down, J. L. H. (1866). Observations on an ethnic classification of idiots. *Clinical Lecture Reports, London Hospital*, 3, 259
- Fraser, J. and Mitchell, A. (1876). Kalmuk idiocy. Report of a case with autopsy. *J. Ment. Sci.*, 98, 169–79
- Nicolaides, K. H., Azar, G., Byrne, D., Mansur, C. and Marks, K. (1992). Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *Br. Med. J.*, 304, 867–9
- Nicolaides, K. H., Brizot, M. L. and Snijders, R. J. M. (1994). Fetal nuchal translucency thickness: ultrasound screening for fetal trisomy in the first trimester of pregnancy. *Br. J. Obstet. Gynaecol.*, 101, 782–6
- Pandya, P. P., Snijders, R. J. M., Johnson, S. J., Brizot, M. and Nicolaides, K. H. (1995). Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. *Br. J. Obstet. Gynaecol.*, 102, 957–62
- Noble, P. L., Abraham, H. D., Snijders, R. J. M., Sherwood, R. and Nicolaides, K. H. (1995). Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free  $\beta$ -hCG and fetal nuchal translucency thickness. *Ultrasound Obstet. Gynecol.*, 6, 390–5
- Hyett, J. A., Noble, P. L., Snijders, R. J. M., Montenegro, N. and Nicolaides, K. H. (1996). Fetal heart rate in trisomy 21 and other chromosomal abnormalities at 10–14 weeks of gestation. *Ultrasound Obstet. Gynecol.*, 7, in press
- Cuckle, H. S., Wald, N. J. and Thompson, S. G. (1987). Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br. J. Obstet. Gynaecol.*, 94, 387–402
- Hecht, C. A. and Hook, E. B. (1994). The imprecision in rates of Down's syndrome by 1-year maternal age intervals: a critical analysis of rates used in biochemical screening. *Prenat. Diagn.*, 14, 729–38
- Snijders, R. J. M., Sebire, N. J., Souka, A., Santiago, C. and Nicolaides, K. H. (1995). Fetal exomphalos and chromosomal defects: relationship to maternal age and gestation. *Ultrasound Obstet. Gynecol.*, 6, 250–5
- Snijders, R. J. M., Sebire, N. J. and Nicolaides, K. H. (1995). Maternal age and gestational age specific risk for chromosomal defects. *Fetal Diagn. Ther.*, 10, 356–67
- Nicolaides, K. H., Azar, G., Snijders, R. J. M. and Gosden, C. M. (1992). Fetal nuchal oedema: associated malformations and chromosomal defects. *Fetal Diagn. Ther.*, 7, 123–31
- Pandya, P. P., Altman, D., Brizot, M. L., Pettersen, H. and Nicolaides, K. H. (1995). Repeatability of measurement of fetal nuchal translucency thickness. *Ultrasound Obstet. Gynecol.*, 5, 334–7
- Johnson, M. P., Johnson, A., Holzgreve, W., Isada, N. B., Wapner, R. J., Treadwell, M. C., Heeger, S. and Evans, M. (1993). First-trimester simple hygroma: cause and outcome. *Am. J. Obstet. Gynecol.*, 168, 156–61
- Hewitt, B. (1993). Nuchal translucency in the first trimester. *Aust. NZ J. Obstet. Gynaecol.*, 33, 389–91
- Shulman, L. P., Emerson, D., Felker, R., Phillips, O., Simpson, J. and Elias, S. (1992). High frequency of cytogenetic abnormalities with cystic hygroma diagnosed in the first trimester. *Obstet. Gynecol.*, 80, 80–2
- Pandya, P. P., Brizot, M. L., Kuhn, P., Snijders, R. J. M. and Nicolaides, K. H. (1994). First trimester fetal nuchal translucency thickness and risk for trisomies. *Obstet. Gynecol.*, 84, 420–3
- Pandya, P. P., Kondylios, A., Hilbert, L., Snijders, R. J. M. and Nicolaides, K. H. (1995). Chromosomal defects and outcome in 1015 fetuses with increased nuchal translucency. *Ultrasound Obstet. Gynecol.*, 5, 15–19
- Szabo, J. and Gellen, J. (1990). Nuchal fluid accumulation in trisomy-21 detected by vaginal sonography in first trimester. *Lancet*, 336, 1133
- Wilson, R. D., Venir, N. and Faquharson, D. F. (1992). Fetal nuchal fluid – physiological or pathological? – in pregnancies less than 17 menstrual weeks. *Prenat. Diagn.*, 12, 755–63
- Ville, Y., Lalondrelle, C., Doumerc, S., Daffos, F., Frydman, R., Oury, J. F. and Dumez, Y. (1992). First-trimester diagnosis of nuchal anomalies: significance and fetal outcome. *Ultrasound Obstet. Gynecol.*, 2, 314–16
- Trauffer, M. L., Anderson, C. E., Johnson, A., Heeger, S., Morgan, P. and Wapner, R. J. (1994). The natural history of euploid pregnancies with first-trimester cystic hygromas. *Am. J. Obstet. Gynecol.*, 170, 1279–84
- Brambati, B., Cislighi, C., Tului, L., Alberti, E., Amidani, M., Colombo, U. and Zuliani, G. (1995). First-trimester Down's syndrome screening using nuchal translucency: a prospective study. *Ultrasound Obstet. Gynecol.*, 5, 9–14
- Comas, C., Martinez, J. M., Ojuel, J., Casals, E., Puerto, B., Borrell, A. and Fortuny, A. (1995). First-trimester nuchal edema as a marker of aneuploidy. *Ultrasound Obstet. Gynecol.*, 5, 26–9
- Szabo, J., Gellen, J. and Szemere, G. (1995). First-trimester ultrasound screening for fetal aneuploidies in women over 35 and under 35 years of age. *Ultrasound Obstet. Gynecol.*, 5, 161–3
- Nadel, A., Bromley, B. and Benacerraf, B. R. (1993). Nuchal thickening or cystic hygromas in first- and early second-trimester fetuses: prognosis and outcome. *Obstet. Gynecol.*, 82, 43–8
- Savoldelli, G., Binkert, G., Achermann, J. and Schmid, W. (1993). Ultrasound screening for chromosomal anomalies in the first trimester of pregnancy. *Prenat. Diagn.*, 13, 513–18
- Schulte-Vallentin, M. and Schindler, H. (1992). Non-echogenic nuchal oedema as a marker in trisomy 21 screening. *Lancet*, 339, 1053

29. Van Zalen-Sprock, M. M., Van Vugt, J. M. G. and Van Geijn, H. P. (1992). First-trimester diagnosis of cystic hygroma – course and outcome. *Am. J. Obstet. Gynecol.*, **167**, 94–8
30. Cullen, M. T., Gabrielli, S., Green, J. J., Rizzo, N., Mahoney, M. J., Salafia, C., Bovicelli, L. and Hobbins, J. C. (1990). Diagnosis and significance of cystic hygroma in the first trimester. *Prenat. Diagn.*, **10**, 643–51
31. Suchet, I. B., Van der Westhuizen, N. G. and Labatte, M. F. (1992). Fetal cystic hygromas: further insights into their natural history. *Can. Assoc. Radiol. J.*, **6**, 420–4
32. Pandya, P. P., Goldberg, H., Walton, B., Riddle, A., Shelley, S., Snijders, R. J. M. and Nicolaides, K. H. (1995). The implementation of first-trimester scanning at 10–13 weeks' gestation and the measurement of fetal nuchal translucency thickness in two maternity units. *Ultrasound Obstet. Gynecol.*, **5**, 20–5
33. Bewley, S., Roberts, L. J., Mackinson, M. and Rodeck, C. (1995). First trimester fetal nuchal translucency: problems with screening the general population. II. *Br. J. Obstet. Gynaecol.*, **102**, 386–8
34. Ewigman, B. G., Crane, J. P., Frigoletto, F. D., Lefevre, M. L., Bain, R. P., McNellis, D. and the RADIUS Study Group (1993). The effect of prenatal ultrasound screening on perinatal outcome. *N. Engl. J. Med.*, **329**, 821–7
35. Hafner, E., Schuchter, K. and Philipp, K. (1995). Screening for chromosomal abnormalities in an unselected population by fetal nuchal translucency. *Ultrasound Obstet. Gynecol.*, **6**, 330–3
36. Wald, N., Stone, R., Cuckle, H. S., Grudzinskas, J. G., Barkai, G., Brambati, B., Teisner, B. and Fuhrmann, W. (1992). First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's syndrome. *Br. Med. J.*, **305**, 28
37. Brambati, B., Macintosh, M. C. M., Teisner, B., Maguiness, S., Shrimanker, K., Lanzani, A., Bonacchi, I., Tului, L., Chard, T. and Grudzinskas, T. J. (1993). Low maternal serum level of pregnancy associated plasma protein (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *Br. J. Obstet. Gynaecol.*, **100**, 324–6
38. Hurlley, P. A., Ward, R. H. T., Teisner, B., Iles, R. K., Lucas, M. and Grudzinskas, J. G. (1993). Serum PAPP-A measurements in first-trimester screening for Down syndrome. *Prenat. Diagn.*, **13**, 903–8
39. Muller, F., Cuckle, H., Teisner, B. and Grudzinskas, J. G. (1993). Serum PAPP-A levels are depressed in women with fetal Down syndrome in early pregnancy. *Prenat. Diagn.*, **13**, 633–6
40. Bersinger, N. A., Brizot, M. L., Johnson, A., Snijders, R. J. M., Abbott, J., Schneider, H. and Nicolaides, K. H. (1994). First trimester maternal serum pregnancy-associated plasma protein A and pregnancy-specific  $\beta$ 1-glycoprotein in fetal trisomies. *Br. J. Obstet. Gynaecol.*, **101**, 970–4
41. Brizot, M. L., Snijders, R. J. M., Bersinger, N. A., Kuhn, P. and Nicolaides, K. H. (1994). Maternal serum pregnancy associated placental protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. *Obstet. Gynecol.*, **84**, 918–22
42. Ozturk, M., Milunsky, A., Brambati, B., Sachs, E. S., Miller, S. and Wands, J. R. (1990). Abnormal maternal serum levels of human chorionic gonadotropin free subunits in trisomy 18. *Am. J. Med. Genet.*, **36**, 480–3
43. Aitken, D. A., McCaw, G., Crossley, J. A., Berry, C., Connor, J. M., Spencer, K. and Macri, J. N. (1993). First-trimester biochemical screening for fetal chromosome abnormalities and neural tube defects. *Prenat. Diagn.*, **13**, 681–9
44. Macri, J. N., Kasuuri, R. V., Krantz, D. A., Cook, E. J., Moore, N. D., Young, J. A., Romero, K. and Larsen, J. W. (1990). Maternal serum Down syndrome screening: free beta protein is a more effective marker than human chorionic gonadotropin. *Am. J. Obstet. Gynecol.*, **163**, 1248–53
45. Macintosh, M. C., Iles, R., Teisner, B., Sharma, V., Chard, T. and Grudzinskas, J. G. (1994). Maternal serum human chorionic gonadotrophin and pregnancy associated plasma protein A, markers for fetal Down syndrome at 8–14 weeks. *Prenat. Diagn.*, **14**, 203–8
46. Brizot, M. L., Snijders, R. J. M., Butler, J., Bersinger, N. A. and Nicolaides, K. H. (1995). Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. *Br. J. Obstet. Gynaecol.*, **102**, 127–32
47. Brizot, M. L., Kuhn, P., Bersinger, N. A., Snijders, R. J. M. and Nicolaides, K. H. (1995). First trimester maternal serum alpha-fetoprotein in fetal trisomies. *Br. J. Obstet. Gynaecol.*, **102**, 31–4
48. Hyett, J. A., Moscoso, G. and Nicolaides, K. H. (1995). First trimester nuchal translucency and cardiac septal defects in fetuses with trisomy 21. *Am. J. Obstet. Gynecol.*, **172**, 1411–13
49. Hyett, J. A., Moscoso, G. and Nicolaides, K. H. (1995). Cardiac defects in trisomy 18 fetuses affected by increased first trimester nuchal translucency. *Fetal Diagn. Ther.*, **10**, 381–6
50. Hyett, J. A., Moscoso, G. and Nicolaides, K. H. (1995). Increased nuchal translucency in trisomy 21 fetuses: relation to narrowing of the aortic isthmus. *Hum. Reprod.*, **10**, 3049–51
51. Pandya, P. P., Snijders, R. J. M., Johnson, S. and Nicolaides, K. H. (1995). Natural history of trisomy 21 fetuses with fetal nuchal translucency. *Ultrasound Obstet. Gynecol.*, **5**, 381–3
52. Hyett, J. A., Sebire, N. J., Snijders, R. J. M. and Nicolaides, K. H. (1996). Intrauterine lethality of trisomy 21 fetuses with increased nuchal translucency thickness. *Ultrasound Obstet. Gynecol.*, in press
53. Evans, M. I., Goldberg, J. D., Dommergues, M., Wapner, R. J., Lynch, L., Dock, B. S., Horenstein, J., Golbus, M. S., Rodeck, C. H., Dumez, Y., Holzgreve, W., Timor-Tritsch, I., Johnson, M. P., Isada, N. B., Monteagudo, A. and Berkowitz, R. L. (1994). Efficacy of second-trimester selective termination for fetal abnormalities: international collaborative experience among the world's largest centers. *Am. J. Obstet. Gynecol.*, **171**, 90–4
54. Sebire, N. J., Noble, P. L., Psarra, A., Papapanagiotou, G. and Nicolaides, K. H. (1996). Fetal karyotyping in twin pregnancies: selection of technique by measurement of fetal nuchal translucency thickness. *Br. J. Obstet. Gynaecol.*, in press
55. Pandya, P. P., Hilbert, F., Snijders, R. J. M. and Nicolaides, K. H. (1995). Nuchal translucency thickness and crown-rump length in twin pregnancies with chromosomally abnormal fetuses. *J. Ultrasound Med.*, **14**, 565–8
56. Shulman, L. P., Emerson, D. S., Grevengood, C., Felker, R. E., Gross, S. J., Phillips, O. P. and Elias, S. (1994). Clinical course and outcome of fetuses with isolated cystic nuchal lesions and normal karyotypes detected in the first trimester. *Am. J. Obstet. Gynecol.*, **171**, 1278–81
57. Hyett, J. A., Moscoso, G., Papapanagiotou, G., Perdu, M. and Nicolaides, K. H. (1996). Abnormalities of the heart and great vessels in chromosomally normal fetuses with increased nuchal translucency thickness at 10–13 weeks of gestation. *Ultrasound Obstet. Gynecol.*, **7**, in press
58. Hyett, J. A., Clayton, P. T., Moscoso, G. and Nicolaides, K. H. (1995). Increased first trimester nuchal translucency as a prenatal manifestation of Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.*, **58**, 374–6