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Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free β-hCG and fetal nuchal translucency thickness

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ABSTRACT

The aim of this prospective study was to measure the contribution of maternal serum free β-human chorionic gonadotropin (β-hCG) in a screening program for fetal trisomy 21 based on fetal nuchal translucency in the first trimester of pregnancy. The maternal serum was collected at the time of the ultrasound scan and assayed without knowledge of the nuchal translucency measurement or karyotype. A total of 2529 pregnancies were examined (normal group, n = 2427; trisomy 21 group, n = 102). Maternal serum free β-hCG was significantly associated with gestational age and maternal weight. In the trisomy 21 group the free β-hCG was significantly higher than in the normals, being above the 95th centile in 29% of the cases. There was no significant association between the deviation from the mean for free β-hCG and nuchal translucency thickness in either the normal or the trisomy 21 groups. When maternal serum free β-hCG was added to a model based on maternal age and fetal nuchal translucency thickness, the detection rate for trisomy 21 was increased from 80% to 85%.

INTRODUCTION

At 10–13 weeks of gestation the majority of fetuses with trisomy 21 have an abnormal accumulation of fluid behind the neck, which can be visualized by ultrasound examination as increased nuchal translucency thickness[1]. A multicenter study of more than 20 000 pregnancies has demonstrated that first-trimester screening, by a combination of fetal nuchal translucency thickness and maternal age, could identify 80% of fetuses with trisomy 21 for a 5% false-positive rate[2]. The aim of this study was to determine the contribution of maternal serum free β-human chorionic gonadotropin (β-hCG) in developing a model to calculate the risk for trisomy 21 by combining data from maternal age, fetal nuchal translucency thickness and maternal serum free β-hCG. Previous studies have reported that in trisomy 21 pregnancies maternal serum free β-hCG is increased during both the second and the first trimesters and the increase is unrelated to fetal nuchal translucency thickness[3–6].

PATIENTS AND METHODS

During a 6-month period (October 1994 to April 1995), blood was obtained from 2561 women with singleton pregnancies who participated in a screening study involving ultrasound examination at 10–14 weeks of gestation. The women were counselled that their blood was to be used for research purposes and not for the assessment of risks in their pregnancy. The study was approved by the Hospital Ethics Committee.

Maternal blood samples were taken immediately before ultrasound examination. All pregnancies were dated by fetal crown–rump length and after measurement of nuchal translucency thickness the parents were counselled as to their estimated risk for fetal trisomies. Karyotyping was performed in 692 cases because of increased nuchal translucency thickness (n = 366), advanced maternal age (n = 264), previous chromosomally abnormal child (n = 12) or parental anxiety (n = 50). Demographic details and clinical details, including maternal weight, were entered in a database at the time of examination, and pregnancy outcome, karyotype and biochemical results were added as soon as these became available. Pregnancy outcome was obtained from the maternity units or the patients themselves.

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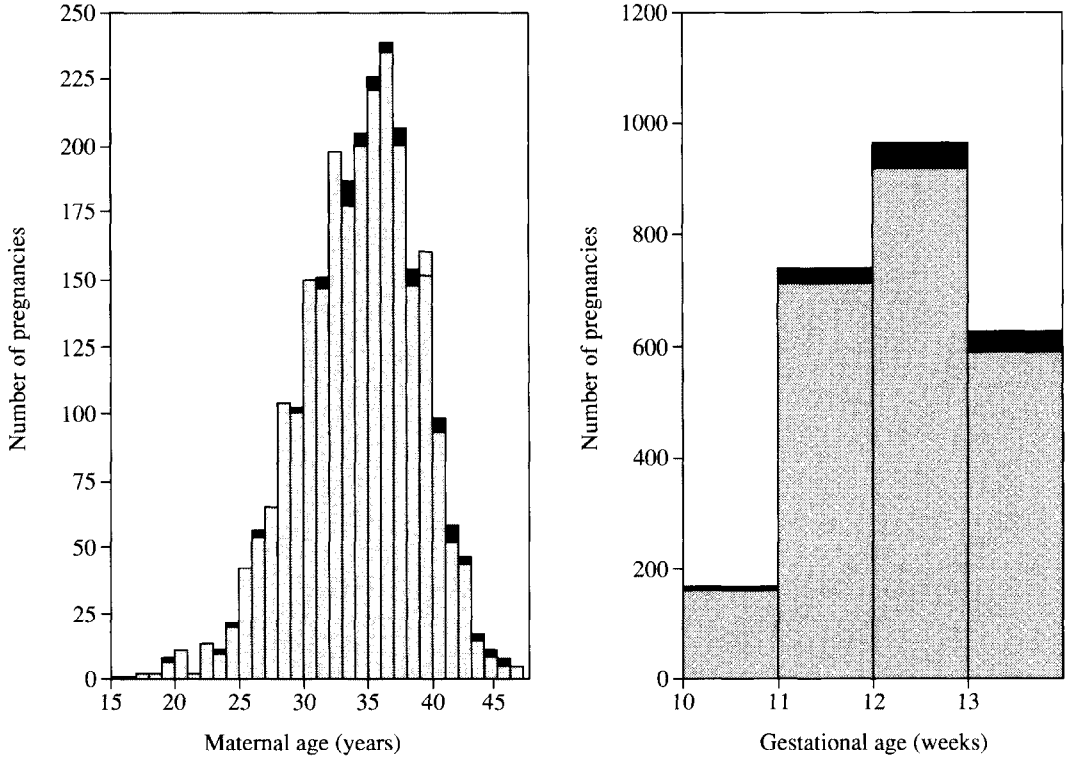

Figure 1 Maternal age and gestational age distribution in 2427 normal (gray bars) and 61 trisomy 21 pregnancies (black bars)

Table 1 Maternal age and gestational age distribution in 2427 controls and 61 fetuses with trisomy 21

	Controls		*Trisomy 21*	
	n	%	*n*	%
Maternal age (years)				
< 20	12	0.5	1	1.6
20–24	54	2.2	2	3.3
25–29	362	14.9	2	3.3
30–34	868	35.8	14	23.0
35–39	924	38.1	24	39.3
≥ 40	207	8.5	18	29.5
Gestation (weeks)				
10	165	6.8	2	3.3
11	714	29.4	20	32.8
12	943	38.9	21	34.4
13	605	24.9	18	29.5

The serum samples were stored overnight at 4°C and assayed in one batch the following day, except for samples that were taken on a Friday, when the serum was stored at –20°C and assayed on the following Monday. Biochemical analysis was performed without knowledge of fetal karyotype or nuchal translucency thickness. The concentration of maternal serum free β-hCG was determined using an immunoradiometric assay (CIS, Paris). The detection limit was 0.15 ng/ml, and intra- and inter-assay coefficients of variance were 3.1% and 5.7%, respectively.

The total data set of 2562 cases was searched to identify singleton pregnancies where the fetal karyotype was normal ($n = 563$) and/or where no obvious fetal abnormality was visualized at birth ($n = 229$), or at ultrasound examination at 20 weeks of gestation ($n = 1635$) in those with continuing pregnancies. In addition, the data set was searched to identify all singleton pregnancies where trisomy 21 was diagnosed ($n = 61$). Excluded were pregnancies with a chromosomal abnormality other than trisomy 21 ($n = 50$), those that resulted in spontaneous loss ($n = 10$) and those where parents opted for elective termination after the diagnosis of fetal malformations ($n = 14$).

Regression analysis was applied to determine the inter-relation of maternal serum free β-hCG, gestation by crown–rump length and maternal weight. The equation that described the relationship between maternal serum free β-hCG, maternal weight and fetal crown–rump length was used to calculate for each pregnancy the number of standard deviations by which the maternal serum free β-hCG concentration differed from the appropriate normal mean for maternal weight and fetal crown–rump length (delta value).

To obtain a sufficiently large sample for evaluation of the distribution of delta free β-hCG in trisomy 21 pregnancies, findings in the 61 fetuses from the present data set were compared and combined with those in 41 cases that were the subject of a previous study[3]. The mean and standard deviations for delta free β-hCG were similar (see Results), and therefore the combined group was used to describe the distribution of delta values in trisomy 21. Distributions of delta values in the trisomic and normal pregnancies were examined for normality. Likelihood ratios for trisomy 21 were determined by dividing the height of the Gaussian curve for trisomic

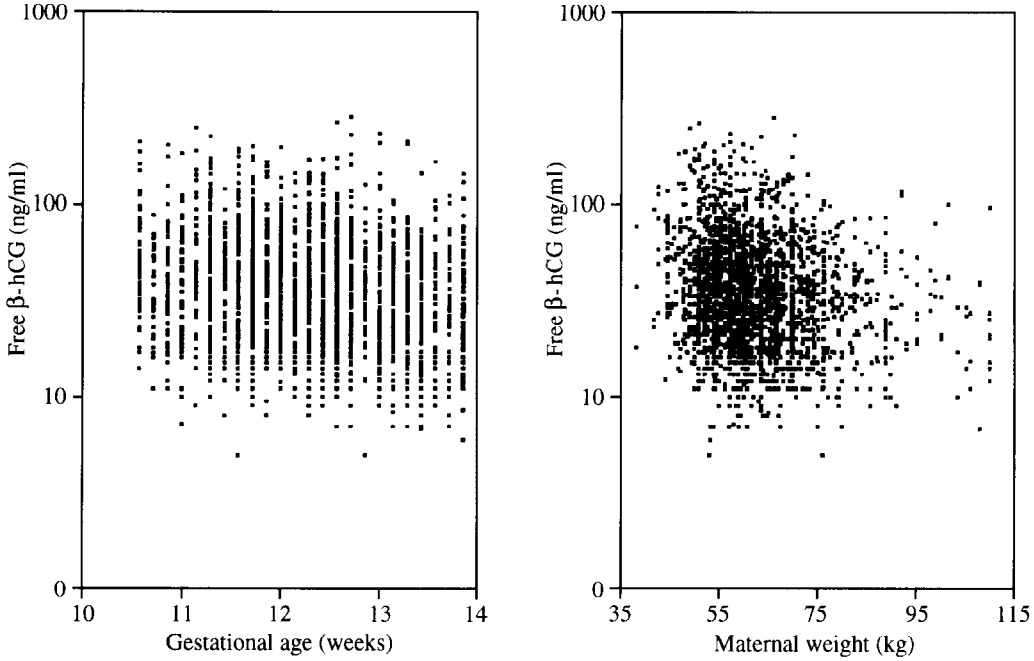

Figure 2 Relationship between maternal serum free β-hCG and gestational age (left) and maternal weight (right) in 2427 normal pregnancies

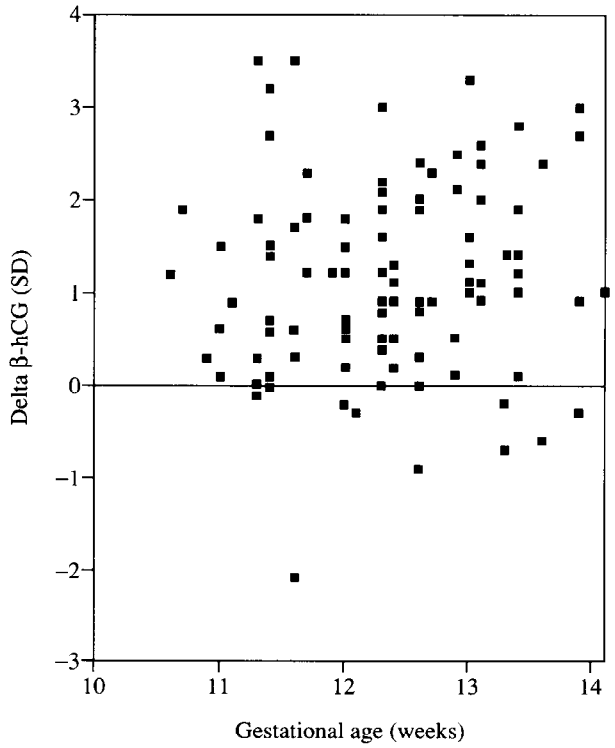

Figure 3 Deviation of maternal serum free β-hCG from the appropriate normal mean for gestation and maternal weight in 102 trisomy 21 pregnancies. There was a no significant change in the mean deviation with gestation

pregnancies by the height of the curve in the normal group.

Regression analysis was applied to examine the relationship between deviation of nuchal translucency thickness[2] and delta free β-hCG, both for the normal and for the trisomic group. Since both parameters were found to be independent (see Results), a combined likelihood ratio was derived by multiplying the likelihood ratio for fetal nuchal translucency[2] with that for maternal serum free β-hCG. To calculate adjusted risks for trisomy 21, the background risk for gestational age and maternal age[7] was expressed as an odds ratio and multiplied by the appropriate likelihood ratio.

RESULTS

The median maternal age was 34 (range 15–47) years and the median gestation by crown–rump length was 12 (range 10–14) weeks (Figure 1, Table 1). In the normal group (563 cases where karyotyping was performed and 1864 where a normal karyotype was assumed), maternal serum free β-hCG was significantly associated with both gestational age and maternal weight (Figure 2; $\log_{10}$ (β-hCG) = 2.6440 – 0.0675 × gestation in weeks – 0.0044 × maternal weight in kilograms; standard deviation = 0.27059; $F = 89.9$; d.f. = 2424; $p < 0.0001$).

The two groups of fetuses with trisomy 21 (61 from the current study group and 41 from a previous study) were not significantly different with respect to the mean or variance of delta free β-hCG (95% CI for the difference −0.52 to 0.31; $t = 0.50$, $p = 0.62$). Therefore, findings were combined. In the total group of 102 trisomic pregnancies, the mean delta free β-hCG was 1.13, which is significantly higher than the mean in the normal group ($F = 126.9$; d.f. = 2.527; $p < 0.0001$) and there was no significant association with gestational age (Figure 3; $r = 0.09$; $p = 0.35$). The values were above the 95th centile in 29% ($n = 30$) of the cases. Distributions of delta free β-hCG and likelihood ratios for trisomy 21 based on actual data, and on Gaussian curves, are shown in Figures 4 and 5.

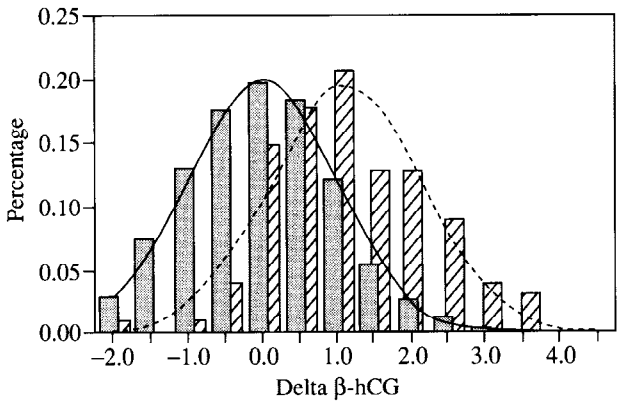

Figure 4 Distribution of delta values for maternal serum free β-hCG in 2427 normal (stippled bars) and 102 trisomy 21 pregnancies (hatched bars). For the distribution of delta values in trisomy 21, data from the 61 cases in the present study were combined with those from 41 cases examined previously[3]. Curves assume a Gaussian distribution

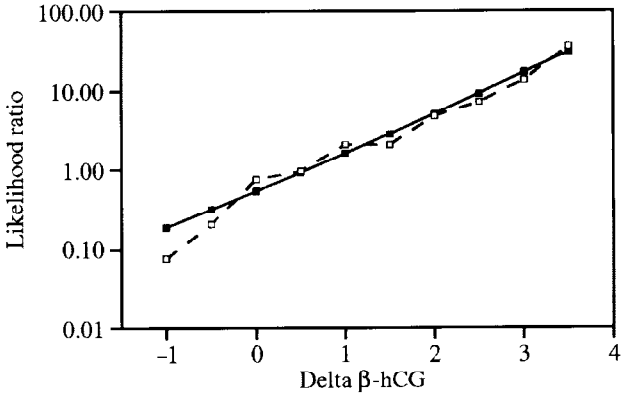

Figure 5 Comparison of likelihood ratios based on actual data (- - -) to those based on the Gaussian distribution curves (—)

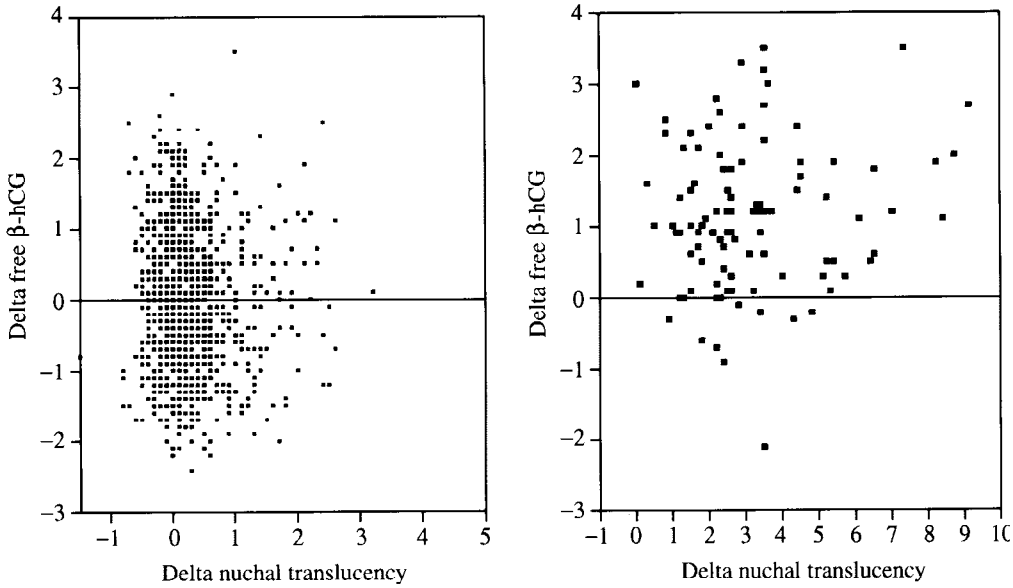

Figure 6 Delta values for maternal serum free β-hCG in relation to delta values for fetal nuchal translucency thickness in 2427 normal (left) and 102 trisomy 21 pregnancies (right)

For the screened population (2427 normal and 61 trisomic pregnancies) background risks were calculated, with maternal age and gestation taken into account[7]. To calculate adjusted risks, the background risk was expressed as an odds ratio and multiplied by the appropriate likelihood ratio. There was no significant association between delta free β-hCG and delta nuchal translucency in either the normal ($r = -0.04$) or the trisomy 21 ($r = 0.13$) groups (Figure 6, Table 2). Therefore, the likelihood ratio for fetal nuchal translucency thickness can be multiplied by the likelihood ratio for maternal serum free β-hCG to obtain a likelihood ratio based on both parameters. False-positive rates and detection rates for different screening strategies are compared in Figure 7. In the present population, for a false-positive rate of 5%, screening by maternal age alone would detect 29% of trisomy 21 pregnancies. Combining information from maternal age and maternal serum free β-hCG would improve the detection rate by 7% to 36%. When maternal serum free β-hCG is added to the model based on maternal age and fetal nuchal translucency, the detection rate would be increased by 5% to 85% (Figure 7).

DISCUSSION

The data of this study are compatible with findings in previous reports that in pregnancies with fetal trisomy 21 maternal serum concentrations of free β-hCG at 10–14 weeks of gestation are increased[3,5,8]. Immaturity of the fetoplacental function or a change in the balance of placental vs. fetal protein secretion are the two main theories proposed to explain the endocrinological changes in the maternal serum of trisomy 21

Table 2 Median, 10th and 90th centiles for delta maternal serum free β-hCG in relation to delta fetal nuchal translucency thickness (NT)

	Controls			*Trisomy 21*		
Delta NT	*Median*	*10th*	*90th*	*Median*	*10th*	*90th*
> median	0.0	−1.3	1.3	1.0	0.0	2.4
> 90th centile	−0.1	−1.3	1.3	1.0	−0.1	2.4
>95th centile	−0.1	−1.3	1.2	1.0	−0.1	2.4
> 99th centile	0.0	−1.2	1.2	1.1	−0.1	2.4
Total	0.0	−1.3	1.3	1.0	0.0	2.4

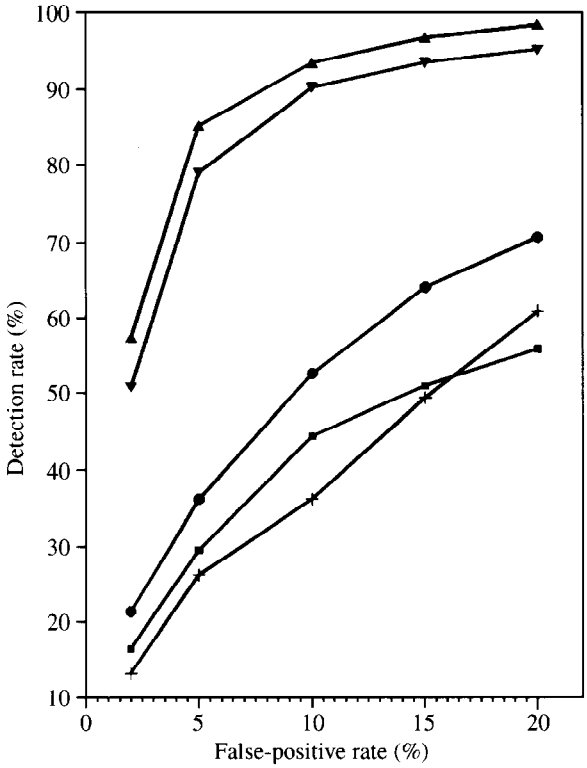

Figure 7 Operator receiver plot if screening was based on maternal age alone (+), maternal serum free β-hCG (■) maternal age and maternal serum free β-hCG (●), maternal age and fetal nuchal translucency thickness (▼) or maternal age, maternal serum free β-hCG and fetal nuchal translucency thickness (▲)

pregnancies[9]. However, recent studies examining placental mRNA expression of β-hCG have suggested that the increase in maternal serum β-hCG found in trisomy 21 is likely to be the consequence of a change in the post-translation phase of biosynthesis of the hCG molecule, rather than a change during transcription or translation[10].

The present study confirms our previous finding that both in normal and trisomy 21 pregnancies the deviation in maternal serum concentrations of free β-hCG is independent of the deviation in fetal nuchal translucency thickness[3]. Consequently, the estimate of the risk for trisomy 21 based on nuchal translucency thickness and maternal age can easily be adjusted to include information on maternal serum free β-hCG.

The effectiveness of screening by maternal age and nuchal translucency is much higher (80%) than screening by maternal age and serum free β-hCG (36%). Therefore, the first parameter to be used in any model for screening in the first trimester would be fetal nuchal translucency thickness. Since the detection rate with such a model is high, the additional contribution of any subsequent marker is inevitably small. Adding maternal serum free β-hCG improved the detection rate of our model for first-trimester screening based on maternal age and fetal nuchal translucency by approximately 5%. It is estimated that with the expanded model, for a 5% false-positive rate, 85% of fetuses with trisomy 21 could be identified.

First-trimester ultrasound scanning is becoming an integral part of routine antenatal care, because it establishes whether the fetus is alive, the gestational age, the number of fetuses and chorionicity, the chances of a chromosomal abnormality and presence or absence of major structural defects. In terms of screening for chromosomal abnormalities, an advantage of ultrasound, in contrast to biochemical screening, is that it provides an immediate assessment of risk so that parents can be counselled directly. If this advantage is to be retained in future methods of screening that combine data from ultrasound and biochemistry it is necessary for the maternal blood to be analyzed a few days before scanning; the final calculation of risk and counselling could then be carried out at the time of scanning. The feasibility of such an approach and the cost-effectiveness of adding biochemical testing to nuchal translucency screening in order to improve detection from about 80% to 85% remain to be determined.

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REFERENCES

1. 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
2. Pandya, P. P., Johnson, S., Brizot, M. L., Snijders, R. J. M. and Nicolaides, K. H. (1995). Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10–14 weeks gestation. *Br. J. Obstet. Gynaecol.*, **102**, 957–62
3. 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
4. Macri, J. N., Kasturi, R. V., Krantz, D. A., Cook, E. J., Moore, N. D., Young, J. A., Romero, K. and Larson, Jr, 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
5. Spencer, K., Aitken, D. A., Crossley, J. A., McGraw, G., Berry, E., Anderson, R., Connor, J. M. and Macri, J. N. (1994). Free beta human choriogonadotropin in Down's

syndrome screening: a multicentre study of its role compared with other biochemical markers. *Ann. Clin. Biochem.*, **31**, 447–54
6. Wald, N. J., Densem, J., Stone, R. and Cheng, R. (1992). The use of free β-hCG in antenatal screening for Down's syndrome. *Br. J. Obstet. Gynecol.*, **100**, 550–7
7. Snijders, R. J. M., Holzgreve, W., Cuckle, H. and Nicolaides, K. H. (1994). Maternal age-specific risk for trisomies at 9–14 weeks gestation. *Prenat. Diagn.*, **14**, 543–52
8. Macri, J. N., Spencer, K., Aitken, D. A., Garver, K., Buchanan, P. D. *et al.* (1993). First-trimester free beta (hCG) screening for Down syndrome. *Prenat. Diagn.*, **13**, 557–62
9. Chard, T. (1991). Biochemistry and endocrinology of the Down's syndrome pregnancy. *Ann. NY Acad. Sci.*, **626**, 580–96
10. Brizot, M. L., Jauniaux, E., McKie, M., Farzaneh, F. and Nicolaides, K. H. (1995). Placental expression of alpha and beta subunits of human chorionic gonadotrophin in early pregnancies with Down's syndrome. *Hum. Reprod.*, **10**, 2506–9