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Maternal Serum Screening for Down Syndrome in the First Trimester: Experience from Belarus

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We have carried out a large retrospective study of α-fetoprotein (AFP), free-β human chorionic gonadotrophin (hCG) and pregnancy-associated plasma protein (PAPP-A) in the first trimester of pregnancy. Unlike other studies all women had routine ultrasound dating, carried out during a nuchal translucency measurement project. A total of 13 477 serum samples were tested for AFP and 11 659 for free β-hCG. A subset of 1564 samples from unaffected pregnancies were also tested for PAPP-A on a case–control basis. All three markers were also determined in 31 samples from pregnancies with Down syndrome. Equations were derived to express results in multiples of the median using both gestational age and crown–rump length and to adjust for maternal weight. Statistical modelling with Gaussian distribution parameters obtained in the study were used to predict the detection rate for a 5 per cent false-positive rate. The predicted rates were: 73.7 per cent for all three markers; 69.1 per cent for PAPP-A and free β-hCG; 47.4 per cent for PAPP-A and AFP; 57.6 per cent for free β-hCG and AFP. As these rates are similar to those in the second trimester, health planners may now want to consider a change in policy from second-trimester to first-trimester screening with biochemical markers. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; maternal serum; first trimester; screening; parameters

INTRODUCTION

There are several possible maternal serum markers for Down syndrome that could be used to screen for the disorder in the first trimester of pregnancy (see Wald *et al.* (1997) for a recent review). Of these, α-fetoprotein (AFP) and free-β human chorionic gonadotrophin (hCG) are already well-established second-trimester markers. A further marker, PAPP-A, would appear to have a high discriminatory power in the first trimester but this diminishes as pregnancy progresses. In addition there is now a powerful ultrasound marker, nuchal translucency (NT), which could be used between 10 and 14 weeks' gestation (Snijders *et al.*, 1998). The optimal policy would be to combine both biochemical and ultrasound screening. Not only would this make the best use of all marker information, but the availability of ultrasound gestational dating would maximize the yield from the biochemical test. We have carried out a large population study of AFP, free β-hCG and PAPP-A in the first trimester of pregnancy among women having routine ultrasound dating as part of NT measurement.

METHODS

In January 1996 we began a pilot intervention study of ultrasound NT screening for Down syndrome prior to 14 weeks' gestation among women having antenatal care in Minsk. As a related project we collected a serum sample from every pregnant woman after she had been examined by ultrasound. In the initial phase of the project we stored the serum samples, at $-20°C$, for later biochemical analysis whereas later they were tested straight away. Each sample was tested for AFP and free β-hCG using a dual-labelled time-resolved fluorescent assay (DELFIA™, EG&G Wallac Oy, Turku, Finland). A total of 11 659 samples were tested with this assay and a further 1818 were tested for AFP only using an enzyme immuno-assay (Roche Inc., Moscow, Russia) which we found to give identical results when a series of 200 samples were tested by both methods. So a total of 13 477 samples were tested for AFP. The series included 129 twins and one triplet pregnancy.

A subset of samples was also tested retrospectively for pregnancy-associated plasma protein (PAPP-A) using a time-resolved fluorescent assay (DELFIA™, EG&G Wallac Oy, Turku, Finland) as a case–control study. All prenatal diagnoses of fetal abnormalities made in Belarus are reported by the five regional genetics centres to the Centre for Medical Genetic Services in Minsk. The National Monitoring System for Birth Defects, situated in our Institute, collects information on all infants born in Belarus with serious abnormalities. We used both sources to identify which pregnancies in the study had either prenatal diagnosis of Down syndrome or subsequently delivered an affected infant. For each case we chose 50 matched controls for PAPP-A analysis. The controls were matched according to gestational age (same crown–rump length) and duration of storage (within same week). The cases were placed at random within the series of samples being tested.

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Table 1—Median level of AFP, free β-hCG and PAPP-A according to gestational age in weeks

Gestation (weeks)	AFP		Free β-hCG		PAPP-A	
	Number	Median (iu/ml)	Number	Median (ng/ml)	Number	Median (miu/l)
8	105	4.73	60	73.3	17	644
9	357	5.51	245	71.9	82	865
10	1968	7.21	1632	61.8	229	1900
11	4632	9.95	4039	52.6	500	2398
12	4345	13.95	3770	43.9	503	3800
13	1944	17.60	1797	34.0	233	5724

None of the first-trimester biochemical information was used in the clinical management of the pregnancy. The results of ultrasound NT screening were used clinically but we adopted what is now known to be a suboptimal approach of using a fixed 3 mm cut-off. All women in Minsk have biochemical screening for Down syndrome from 15 weeks of gestation. This uses two markers, AFP and hCG (DIAplus-Roche, Moscow, Russia), with a cut-off risk of 1 in 360 at the expected date of delivery.

27 cases of Down syndrome were screened in the first trimester including 4 where the pregnancy ended in birth. NT measurement was possible in 23 cases resulting in 8 with positive screening results having invasive prenatal diagnosis (5 in the first trimester and 3 in the second), and a further 15 cases were detected following second-trimester biochemical screening. In order to increase the power of our study we supplemented the series with 4 additional cases identified in women who participated in a previous pilot study of first-trimester ultrasound screening for fetal malformations. Samples from all 31 cases were tested for PAPP-A together with 1564 unaffected controls which met the matching criteria. Hence, in total, 31 serum samples from pregnancies with Down syndrome were tested for all three markers.

All results were expressed as multiples of the gestation-specific median (MOMs) in unaffected pregnancies. For AFP and free β-hCG the expected normal median values used to calculate MOMs were derived both from the crown–rump length (CRL) directly and from the gestational age based on the CRL (Robinson and Fleming, 1975). The median level of all three markers was calculated for each completed week of gestation. The expected values were obtained by regression of median level on median days weighted for the number of women tested. A similar process was carried out with CRL. For AFP and free β-hCG there were sufficient data to calculate medians for each mm of CRL; this was not possible for PAPP-A so that the weekly medians were used but the regression was for median level on median CRL in each week. Maternal weight was available in most pregnancies and to adjust the MOM values for weight each was divided by the expected weight-specific MOM. This was obtained by regression analysis using an inverse model (Neveaux *et al.*, 1996).

The Gaussian distribution parameters of $\log_{10}$ MOM were estimated for Down syndrome and unaffected singleton pregnancies. The mean was estimated from the log median value. To avoid the undue influence of occasional outliers the standard deviation was calculated from the 10th–90th centile range divided by 2.563. Correlation coefficients were obtained directly after excluding outlying values exceeding three standard deviations from the mean. The predicted detection rate for a 5 per cent false-positive rate was calculated from these parameters by standard statistically modelling techniques (Royston and Thompson, 1992). The maternal age distribution used for this purpose was that of the 104 000 women screened in Minsk during the period 1991–1997.

RESULTS

Table 1 shows the median levels of AFP, free β-hCG and PAPP-A in singleton unaffected pregnancies for each completed week of gestation. The best fitting regression curves were:

$$\text{AFP} = 32.2272 - 1.03532\text{day} + 0.00946354\text{day}^2;$$
$$\text{free } \beta\text{-hCG} = 127.923 - 0.491708\text{day} - 0.0055259\text{day}^2; \text{ and}$$
$$\text{PAPP-A} = 18691.5 - 576.876\text{day} + 4.68407\text{day}^2.$$

Figure 1 shows the comparable results for AFP and free β-hCG for each CRL. The two regression curves and that for PAPP-A using median for each week instead of median days were:

$$\text{AFP} = -3.15173 + 0.282966\text{crl} + 0.000076297\text{crl}^2;$$
$$\text{free } \beta\text{-hCG} = 98.5006 - 0.12534\text{crl} + 0.00326422\text{crl}^2; \text{ and}$$
$$\text{PAPP-A} = -410.446 + 32.0223\text{crl} + 0.690769\text{crl}^2.$$

For MOM calculation in the rest of this paper these CRL-based equations are used.

The relationships between the three marker levels and maternal weight are shown in Table 2. Inverse weight regression curves fitted the data well with equations:

$$\text{AFP} = 0.403284 + 35.9545/\text{weight};$$

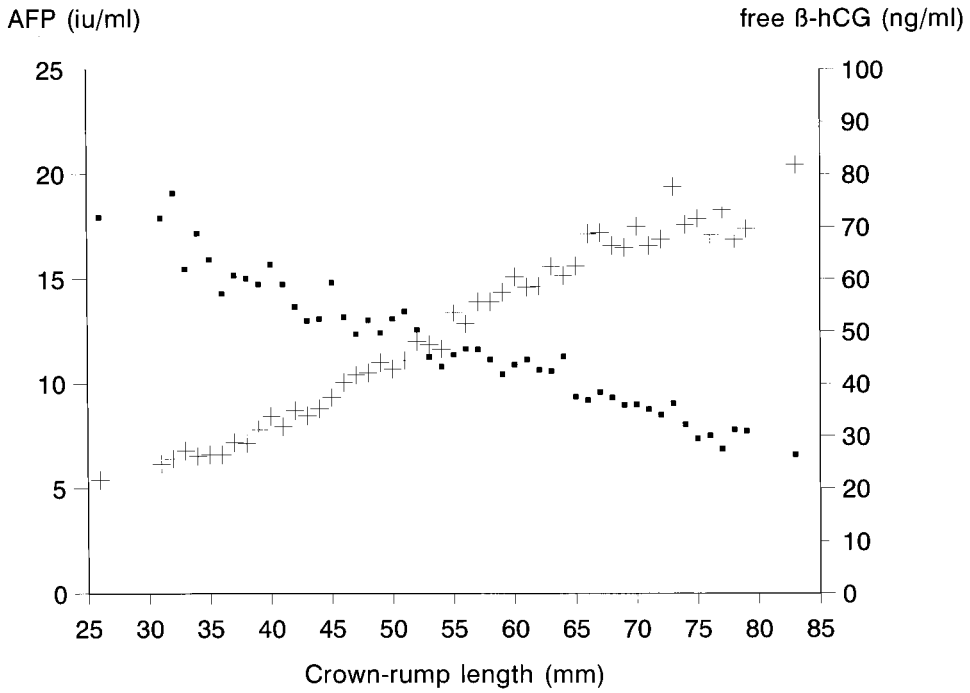

Fig. 1—Median level of AFP (+) and free β-hCG (■) according to CRL in mm; the lowest point is for all values $\leqslant$30 mm (median 26 mm, 9 weeks 3 days) and the highest is for $\geqslant$80 mm (median 83 mm, 14 weeks 0 days)

Table 2—Median level of AFP, free β-hCG and PAPP-A according to maternal weight

Maternal weight (kg)	AFP		Free β-hCG		PAPP-A	
	Number	Median (MOM)	Number	Median (MOM)	Number	Median (MOM)
<45	160	1.31	140	1.40	20	1.17
45–	973	1.17	864	1.16	129	1.36
50–	2503	1.08	2181	1.10	265	1.04
55–	2986	1.04	2611	1.03	345	1.01
60–	2595	0.97	2237	1.01	318	1.02
65–	1604	0.94	1401	0.92	215	0.83
70–	976	0.89	841	0.86	121	0.80
75–	544	0.90	455	0.86	57	0.79
80–	306	0.87	261	0.77	29	0.77
$\geqslant$85+	464	0.83	389	0.69	47	0.79

free β-hCG = 0.235410 + 45.8731/weight; and PAPP-A = 0.133128 + 51.1869/weight.

Table 3 shows the Gaussian parameters in our population. The median in Down syndrome was significantly reduced for AFP (0.71 MOM; 95 per cent confidence interval (CI) 0.55–0.92), and PAPP-A (0.54 MOM; 95 per cent CI 0.40–0.73), and increased for free β-hCG (2.58 MOM; 95 per cent CI 2.02–3.30). Figure 2 shows the maternal age distribution. The median age was 24 years and 7 per cent of women were aged 35 or older. Using these parameters and age distribution the expected detection rate for a 5 per cent false-positive rate for different marker combinations were: 73.7 per cent for all three; 69.1 per cent for PAPP-A and free β-hCG; 47.4 per cent for PAPP-A and AFP; 57.6 per cent for free β-hCG and AFP.

Among the twins tested the median values were: 2.20 for AFP (126 pregnancies), 2.15 MOM for free β-hCG (116) and 1.55 MOM for PAPP-A (22). The triplet pregnancy had values of 3.62 MOM for AFP and 1.43 MOM for free β-hCG; PAPP-A was not tested.

DISCUSSION

In a large-scale evaluation of the three most likely first-trimester Down syndrome screening markers we have derived the equations needed to express results in MOMs and to adjust for maternal weight. Statistical

Table 3—Parameters of Gaussian distributions on $\log_{10}$ scale

	Down syndrome	Unaffected pregnancies
Mean (untransformed MOM)		
AFP	− 0.146 (0.71)	0.000 (1.00)
Free β-hCG	0.412 (2.58)	0.000 (1.00)
PAPP-A	− 0.267 (0.54)	− 0.006 (0.99)
Standard deviation		
AFP	0.253	0.226
Free β-hCG	0.240	0.295
PAPP-A	0.289	0.237
*Correlation coefficient (*p-*value)*		
AFP and free β-hCG	− 0.105 (0.58)	0.100 (0.0001)
AFP and PAPP-A	− 0.018 (0.92)	0.048 (0.12)
Free β-hCG and PAPP-A	0.338 (0.07)	0.140 (0.0001)

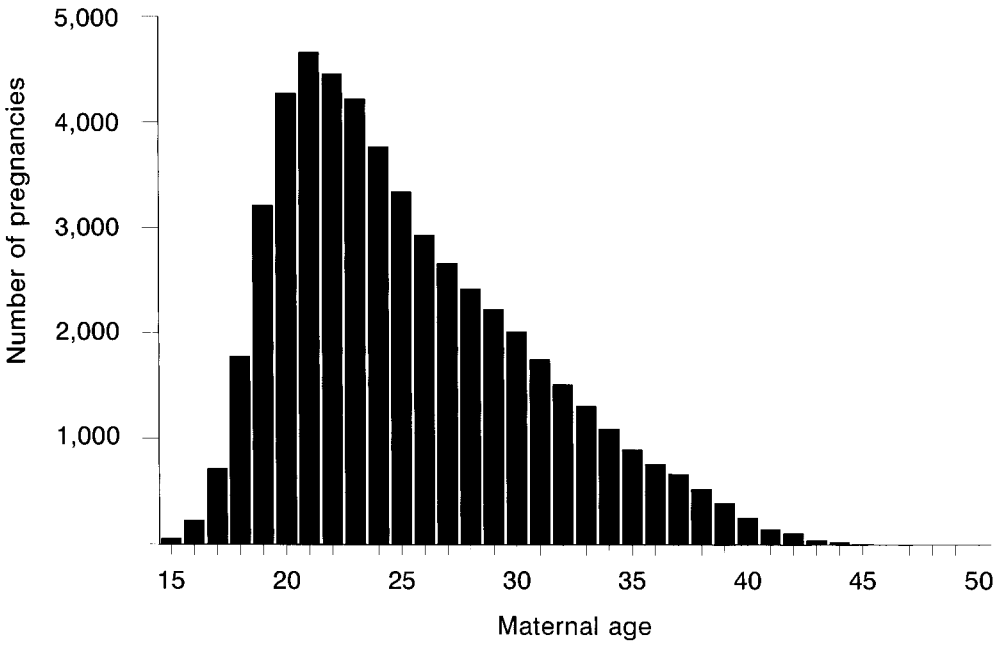

Fig. 2—Histogram of the maternal age distribution among screened women in Minsk

modelling with the Gaussian distribution parameters obtained in the study predict that mutiple-marker screening will yield a high detection rate.

All three markers changed with gestational age in a manner readily described by a simple quadratic equation. In contrast, when measuring hCG or free β-hCG in the second trimester a complex additive–exponential regression is usually needed. Similar shaped equations could be derived using either gestational age in days based on CRL or CRL directly. There was a strong negative correlation with maternal weight for each marker used which was well characterized by an inverse regression equation.

In Down syndrome the median marker levels observed were consistent with other publications. In a meta-analysis of 16 studies including a total of 326 affected pregnancies tested before 15 weeks' gestation the overall median AFP level was 0.79 MOM with 95 per cent CI 0.74–0.86 MOM (van Lith, 1997). In similar meta-analyses the overall medians were 1.91 MOM (95 per cent CI 1.75–2.07) for free β-hCG (9 studies, 336 affected pregnancies; van Lith (1996) and 0.39 MOM (95 per cent CI 0.34–0.44) for PAPP-A (12 studies, 297 pregnancies: Wald *et al.* (1997)). Thus, the confidence intervals on our medians overlap with those published.

PAPP-A and free β-hCG are more discriminatory first-trimester markers than AFP or unconjugated oestriol and several estimates of the detection rate for a 5 per cent false-positive rate using the two-marker combination have been published. Our own predicted detection rate of 69.1 per cent is higher than others based on single series-derived parameters: 49 per cent (Berry *et al.*, 1997), 51 per cent (Spencer *et al.*, 1994), 56 per cent (Forest *et al.*, 1997), 60 per cent (Haddow *et al.*, 1998), 62 per cent (Wald *et al.*, 1996) and 63 per cent (Krantz *et al.*, 1996). One possible explanation for the variation in detection rates between studies is

differences in antibody used in the PAPP-A assay; those which cross-react with eosinophil major basic protein are less specific (Christiansen and Norgaard-Pedersen, 1996; Qin *et al*., 1998). The best estimate of the detection rate for PAPP-A and free β-hCG is probably 60 per cent, a prediction obtained using parameters derived from meta-analysis (van Lith, 1996). This is similar to the rate we currently achieve by two-marker screening in the second trimester. For example, a modelling exercise for the 104 000 pregnant women screened in Minsk with AFP and hCG, including 195 with Down syndrome predicts a 61 per cent detection rate for a 5 per cent false-positive rate.

An advantage of using AFP as well as free β-hCG is that they allow screening over a wide gestational range, from 8 to 22 weeks. Thus, a screening laboratory already performing second-trimester screening can readily extend their range to the first trimester. They could then improve the detection rate by adopting a trimester-specific third marker: PAPP-A for the first trimester and, say, unconjugated oestriol or inhibin A for the second. The predicted first-trimester detection rate for a 5 per cent false-positive rate using the three marker combination with parameters derived from our data was 73.7 per cent, a 5 per cent increase in detection over PAPP-A and free β-hCG alone. The predicted increase from other studies was: 2 per cent (Spencer *et al*., 1994), 5 per cent (Berry *et al*., 1997) and 5 per cent (Forest *et al*., 1997).

There are a number of factors which will determine exactly what gestational period within the first trimester Down syndrome screening should be offered. One limitation is the gestational period when it is safe to carry out CVS. Taking into consideration methodological limitations and contradictory results of various studies on the safety of CVS (Kuliev *et al*., 1996; Firth *et al*., 1994; Evans and Hamerton, 1996) this procedure should not be done before 10 weeks. Secondly, if, as is likely, a combined ultrasound and biochemical approach is introduced, the lower limit of satisfactory NT measurement of 10 weeks' gestation (R. Snijders, personal communication) needs to be taken into account. Lastly, the median level of PAPP-A in Down syndrome approaches the level for unaffected pregnancies towards the end of the first trimester and the beginning of the second. Thus, the period of 10–12 weeks' gestation would appear to be an optimal 'window' for first-trimester screening. Health planners will need to take this into consideration when introducing combined prenatal screening in the first trimester.

A Royal College of Obstetricians and Gynaecologists Study Group (1997) has recommended that there is now sufficient evidence to consider that specific serum markers for Down syndrome at 9–13 weeks' gestation (notably PAPP-A and free-β hCG) may be as effective as those serum markers in established use at 15–22 weeks' gestation. On the basis of our experience in this pilot study we strongly concur with this recommendation. Moreover, a recent survey of women's opinions found that most of those having second-trimester serum screening for Down syndrome would have preferred earlier testing (Kornman *et al*., 1997). The main reasons given were an easier termination, if required, and earlier reassurance. In the face of the scientific evidence and the preferences of women many health planners may now want to consider a change in policy.

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REFERENCES

Berry E, Aitken DA, Crossley JA, Macri JN, Connor JM. 1997. Screening for Down's syndrome: changes in marker levels and detection rates between first and second trimesters. *Br J Obstet Gynaecol* **104:** 811–817.

Christiansen MM, Norgaard-Pedersen B. 1996. Maternal serum screening for Down syndrome in first trimester using schwangerschaftsprotein 1, PAPP-A/proMBP-complex and the proform of eosinophil major basic protein as markers. In: Grudzinskas JG, Ward RHT (eds) *Screening for Down's Syndrome in the First Trimester*. London: Royal College of Obstetricians and Gynaecologists; 148–162.

Evans JA, Hamerton JL. 1996. Limb defects and chorionic villus sampling. *Lancet* **347:** 484–485.

Firth HV, Boyd PA, Chamberlain PF, Mackenzie IZ, Morris-Kay GM, Huson SM. 1994. Analysis of limb reduction defects in babies exposed to chorionic villus sampling. *Lancet* **343:** 1069–1071.

Forest J-C, Masse J, Moutquin J-M. 1997. Screening for Down syndrome during first trimester: a prospective study using free β-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Clin Biochem* **30:** 333–338.

Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A. 1998. Screening of maternal serum for fetal Down's syndrome in the first trimester. *N Engl J Med* **338:** 955–961.

Kornman LH, Wortelboer MJM, Beekhuis JR, Morssink LP, Mantingh A. 1997. Women's opinions and the implications of first- versus second-trimester screening for fetal Down's syndrome. *Prenat Diagn* **17:** 1011–1018.

Krantz DA, Larsen JW, Buchanan PD, Macri JN. 1996. First-trimester Down syndrome screening: free β-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Am J Obstet Gynecol* **174:** 612–616.

Kuliev A, Jackson L, Froster U, Brambati B, Simpson JL, Verlinsky Y, Ginsberg N, Smidt-Jensen S, Zakut H. 1996. Chorionic villus sampling safety. *Am J Obstet Gynecol* **174:** 807–811.

Neveux N, Palomaki GE, Larrivee DA, Knight GJ, Haddow JE. 1996. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenat Diagn* **16:** 1115–1119.

Qin Q-P, Christiansen M, Oxvig C, Pettersson K, Sotrup-Jensen L, Koch C, Norgaard-Pedersen B. 1998. Double-monoclonal immunofluorometric assays for pregnancy-associated plasma protein A/proeosinophil major basic protein (PAPP-A/proMBP) complex in first-trimester maternal serum screening for Down syndrome. *Clin Chem* **43:** 2323–2332.

Robinson HP, Fleming JEE. 1975. A critical evaluation of sonar crown–rump length measurements. *Br J Obstet Gynaecol* **82:** 702–710.

Royal College of Obstetricians and Gynaecologists. 1997. Recommendations arising from the 32nd Study Group: screening for Down's syndrome in the first trimester. In: Grudzinskas JG, Ward RHT (eds) *Screening for Down's Syndrome in the First Trimester*. London: Royal College of Obstetricians and Gynaecologists; 353–356.

Royston P, Thompson SG. 1992. Model-based screening by risk with application to Down's syndrome. *Stat Med* **11:** 257–268.

Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. *Lancet* **352:** 343–346.

Spencer K, Aitken DA, Crossley JA, McCaw G, Berry E, Anderson R, Connor JM, Macri JN. 1994. First trimester biochemical screening for trisomy 21: the role of free beta hCG, alpha fetoprotein and pregnancy associated plasma protein A. *Ann Clin Biochem* **31:** 447–454.

van Lith JMM. 1996. Markers for Down's syndrome in early pregnancy. *Early Hum Develop* **47** (Suppl.): 27–29.

van Lith JMM. 1997. Maternal serum markers for aneuploidy in early pregnancy. In: Grudzinskas JG, Ward RHT (eds) *Screening for Down's Syndrome in the First Trimester*. London: Royal College of Obstetricians and Gynaecologists; 163–169.

Wald NJ, George L, Smith D, Densem JW, Petterson K. 1996. Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. *Br J Obstet Gynaecol* **103:** 407–412.

Wald NJ, Kennard A, Hackshaw A, McGuire A. 1997. Antenatal screening for Down's syndrome. *J Med Screen* **4:** 181–246.