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Human placental lactogen is a first-trimester maternal serum marker of Down syndrome

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Background Human placental lactogen (hPL) is synthesised by the placenta and found in maternal serum. We analysed the potential of hPL as a first-trimester maternal serum-screening marker for fetal Down syndrome (DS).

Materials and Methods hPL was quantified by ELISA in 47 DS pregnancies and 136 controls in gestational weeks 8–13. Distributions of log multiples of the median (MoMs) were established. The quantity of hPL in DS screening was estimated using Monte Carlo simulation methods.

Results The mean log10 MoM hPL was −0.1995 (SD: 0.1993) in affected and 0.0026 (SD: 0.2129) in control pregnancies. This corresponds to a MoM of 0.63 in DS pregnancies. hPL correlated significantly with log10 MoM values of hCGβ ($r = 0.320$) and PAPP-A ($r = 0.590$) in controls, but not with hCGβ ($r = 0.228$) or PAPP-A ($r = 0.090$) in DS pregnancies. The inclusion of hPL in the double test (PAPP-A + hCGβ) increased the detection rate from 67 to 75% for a false-positive rate of 5%.

Conclusion hPL is a DS screening marker that is applicable at weeks 9–13 and could be included in multiple marker first-trimester screening for DS. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: human chorionic somatotropin; hCS; hPL; prenatal screening; first trimester

INTRODUCTION

First-trimester combined serum and ultrasound screening, using the parameters pregnancy-associated plasma protein-A (PAPP-A), the free β-form of human chorionic gonadotrophin (hCGβ) and the size of the nuchal translucency (NT), has become state of the art for prenatal screening for Down syndrome (DS) (Spencer *et al*., 1999). However, the performance of the screening still leaves room for improvement as the detection rate is only in the order of 80% for a 2% false-positive rate (Spencer *et al*., 1999), and the administration of both a serum test and an ultrasound test to all pregnant women may not be cost-effective (Christiansen and Olesen, 2002). Several suggestions have been made to improve the performance and efficiency, for example, integrated screening models where first-trimester screening is combined with second-trimester testing (Wald *et al*., 1999a), contingent and sequential screening models where an initial test defines the need for further testing in the first (Christiansen and Olesen, 2002) and second trimester (Benn *et al*., 2005) and—most recently—repetitive use of the same markers (Wright and Bradbury, 2005). Another option is the inclusion of new first-trimester maternal serum markers for DS. Several such markers have been suggested, for example, ADAM 12 (Laigaard *et al*., 2003), the proform of eosinophil major basic protein (ProMBP) (Christiansen *et al*., 1999), pregnancy-specific glycoprotein 1 (SP1) (Qin *et al*., 1997) and inhibin A (Christiansen and Norgaard-Pedersen, 2005). The introduction of such markers has been hampered either by the lack of readily—and commercially—available robust assays and/or the finding that the discriminatory ability of the markers is the most early in pregnancy (ProMBP (Christiansen *et al*., 1999) and SP1 (Wald *et al*., 1999b)), where it is difficult to get blood samples from pregnant women.

The majority of known maternal serum markers for DS are pregnancy-associated proteins synthesised by the placenta, and all such proteins could potentially be markers for DS.

Human placental lactogen (hPL) is a polypeptide of 191 aminoacids (Barrera-Saldana *et al*., 1983) constitutively synthesised by the placenta (Weinstein *et al*., 1982) and found in increasing concentrations in maternal serum through pregnancy (Grumbach *et al*., 1968). The synthesis of hPL is restricted to the extravillous trophoblast (Sasagawa *et al*., 1987) the syncytiotrophoblast and the trophoblast differentiating into syncytiotrophoblast (Fujimoto *et al*., 1986; Klassen *et al*., 1989; Musicki *et al*., 1997) from where it is released to the maternal circulation (Fujimoto *et al*., 1986). In placentae from DS pregnancies, the differentiation of villous trophoblast into syncytiotrophoblast is impaired and the synthesis—*in vitro*—by cultured DS placentae—of hPL is markedly reduced (Frendo *et al*., 2000). If these *in vitro* findings are representative of the trophoblast differentiation into syncytiotrophoblast *in vivo*, it is likely that the maternal serum concentration of hPL will be reduced in DS pregnancies and hPL may be a maternal serum marker of fetal DS.

Here, we examine whether hPL can be used as a maternal serum marker for fetal DS in the first trimester and estimate the effect of including hPL as an extra

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marker in combination with PAPP-A and hCGβ when screening a population of pregnant women for DS.

MATERIALS AND METHODS

Serum samples

Serum samples from 47 pregnant women (median age: 37.7 years, range: 24.0–47.9 years, sampled at median gestational age 75 days, range: 58–95 days) with a DS fetus and 136 control pregnant women (median age: 36.4 years, range: 21.8–43.8 years, sampled at median gestational age 74 days, range: 56–95 days) were retrieved from samples received as part of routine first-trimester serum screening (PAPP-A and hCGβ) performed at the Statens Serum Institut (SSI), Copenhagen, and stored in a biobank as part of the Pregnancy Screening Registry at the SSI. Samples from both DS pregnancies and controls were matched for maternal age ($\pm$5 years) and sampled at the same time period and stored at $-20\,^{\circ}$C prior to analysis. All DS cases were identified by cross-referencing with the Danish Cytogenetic Central Registry, and none of the controls were registered with chromosomal disease. All cases were singleton pregnancies. Information about the gestational age, based on the crown rump length (CRL) and maternal age, was obtained from referral sheets.

Biochemical measurements

The hPL concentrations were determined using a modified version of a commercially available sandwich ELISA (hPL ELISA, enzyme immunoassay (EIA)-1283, DRG Instruments GmBH, Marburg, Germany), where a lower standard at 0.02 mg/L was added to the standards and the sample incubation time was increased to 3 h. The detection limit was 0.02 mg/L. The interassay coefficient of variation was below 15%.

The concentration of PAPP-A and hCGβ was determined in maternal sera as part of the routine screening on the analytical platforms AutoDelfia (PerkinElmer, Turku, Finland) or Kryptor (Brahms, Berlin) using kits and procedures provided by the manufacturers.

Data analysis

A normal median of hPL was established from hPL concentrations measured in control pregnancies using log-regression of hPL on gestational age in days. The normal medians were used to transform hPL concentrations in both controls and affected pregnancies into multiples of the normal median (MoMs) and the distribution of log10 MoM hPL was established in both groups. Correlations were performed a.m. Pearson. Compatibility with the normal distribution was assessed by the Shapiro–Wilk's test. Means were compared using the Mann–Whitney U-test.

Stepwise multiple logistic regression

The relative discriminatory significance of maternal age, gestational age, PAPP-A, hCGβ and hPL was assessed by stepwise multiple logistic regression with the dependent variable defined as zero in DS cases and one in controls. Age was introduced as years, gestational age as days and biochemical parameters as log10 MoM values. As some of the markers correlate significantly in at least one of the groups, we also included the interaction parameters hPL*PAPP-A, hPL*hCGβ and PAPP-A*hCGβ. Initially, the effect of maternal age and each of the biochemical markers was assessed followed by stepwise inclusion of the other markers. A final model was obtained by eliminating non-significant ($p < 0.05$) contributors to the model.

Monte Carlo simulation of screening performance

The performance of various marker combinations as potential screening procedures in a standardised population was examined using standard statistical modelling techniques (Larsen *et al*., 1998). Using the observed population parameters for hPL and published parameters for PAPP-A and hCGβ (Spencer *et al*., 1999), a series of random cut-off MoM values were selected from the distributions in unaffected and affected pregnancies. These values—in combination with the empirically established correlations between the markers—were then used to calculate likelihood ratios for the combinations. Lower truncation limits at -0.5 log10 MoM and -0.8 log10 MoM were used for hCGβ and PAPP-A respectively. Upper truncation limits were 0.8 log10 MoM and 0.5 log10 MoM for hCGβ and PAPP-A respectively. 0.8 log10 MoM and -0.8 log10 MoM were used as upper and lower truncation limits for hPL. The likelihood ratios were then used together with the age-related risk of DS at birth (Cuckle *et al*., 1987) to calculate the expected detection rate of affected pregnancies at various false-positive rates in a population with a standardised maternal age distribution (van der Veen *et al*., 1997).

RESULTS

The maternal serum log10 concentration of hPL in 136 control pregnancies are shown as a function of gestational age in Figure 1. The hPL concentration increased with gestational age ($r = 0.81$, $p < 10^{-36}$). A log-regression of hPL on gestational age gave the regression line log10 hPL = 0.0376*days -3.4506, Figure 1. The residuals were normally distributed and the regression line—the median formula—was used to calculate normal medians for each day. These medians were used to transform concentrations of hPL in maternal sera into MoMs for the appropriate gestational age. The MoMs were log-normally distributed (Shapiro–Wilk's

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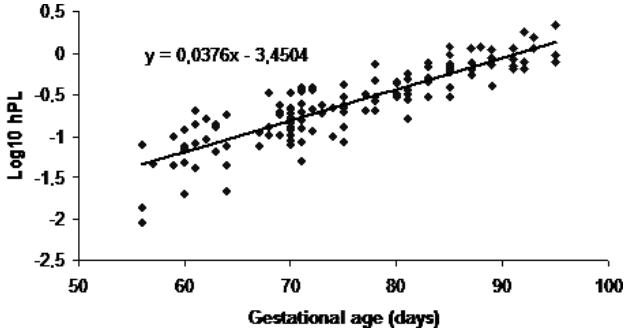

Figure 1—Scatterplot and regression line of maternal serum hPL concentration values as a function of gestational age in 136 control pregnancies. The regression line is shown

Table 1—The distribution of log10 MoM hPL in DS and control pregnancies

	N	Mean	SD	*W*	*p*
DS	47	−0.1995	0.1963	0.978	0.659
Controls	136	0.0026	0.2129	0.979	0.106

$W = 0.98$, $p = 0.11$). The distribution of log10 hPL MoMs in controls is given in Table 1.

The median formula obtained from controls was also used to calculate MoM values of the maternal serum concentrations of hPL concentrations in DS pregnancies. The hPL MoM values were log-normally distributed ($W = 0.98$, $P = 0.66$). The median hPL MoM in DS pregnancies was 0.63. The distribution of log10 MoM hPL values in DS pregnancies is given in Table 1. The cumulative distribution of MoM values in DS and control pregnancies is given in Table 2. The individual log10 MoM hPL values are depicted as a function of the gestational age in Figure 2. There was no change in log10 hPL MoMs with gestational age throughout the week 8–13 gestational age window ($r = -0.1143$, $p = 0.44$). Likewise, when the log10 MoM hPL in DS cases from weeks 8–10 were compared with the DS cases from weeks 11–13, no significant difference ($p = 0.44$) was found. The log10 hPL MoM values in DS pregnancies were significantly lower when compared to the log10 hPL MoM values in control pregnancies ($p < 10^{-7}$). The log10 MoM hPL values in control pregnancies did not correlate with maternal age ($r = 0.004$, $p = 0.96$).

The correlation between log10 MoM hPL and the log10 MoM PAPP-A and log10 MoM hCGβ was examined in both controls and DS pregnancies. In controls, the correlations between hPL and hCGβ ($r = 0.320$, $p = 0.0001$), and PAPP-A ($r = 0.590$, $p < 10^{-13}$) were significant. In DS pregnancies, neither the correlation between hPL and hCGβ ($r = 0.228$, $p = 0.12$) nor that between hPL and PAPP-A ($r = 0.09$, $p = 0.55$) was significant. The absence of a significant correlation in DS pregnancies between PAPP-A and hPL was also apparent when the gestational age windows of weeks 8–10 and 11–13 were analysed separately (Data not shown). In Figures 3 and 4, the relation between PAPP-A and hPL and between hCGβ and hPL log10 MoM values is shown respectively in controls and DS pregnancies.

Table 2—Cumulative distribution of hPL MoM values in 136 control pregnancies and 47 DS pregnancies

hPL MoM	Controls (% of total)	DS (% of total)
≥1 MoM	71 (52%)	5 (11%)
<1 MoM	65 (48%)	42 (89%)
<0.9 MoM	50 (37%)	39 (83%)
<0.8 MoM	41 (30%)	35 (74%)
<0.7 MoM	26 (19%)	31 (66%)
<0.6 MoM	17 (13%)	19 (40%)
<0.5 MoM	9 (7%)	12 (26%)
<0.4 MoM	6 (4%)	7 (15%)
<0.3 MoM	2 (1%)	1 (2%)

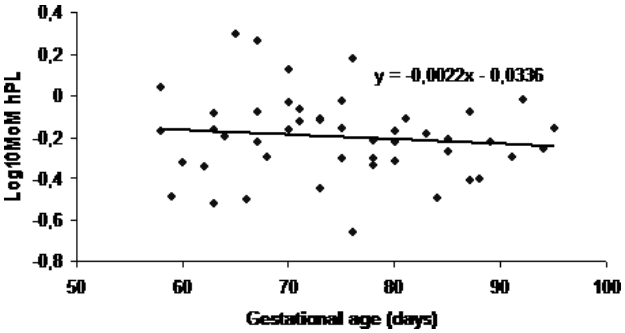

Figure 2—Scatterplot and regression line of log10 MoM hPL values in 47 DS pregnancies as a function of gestational age. The regression line is shown

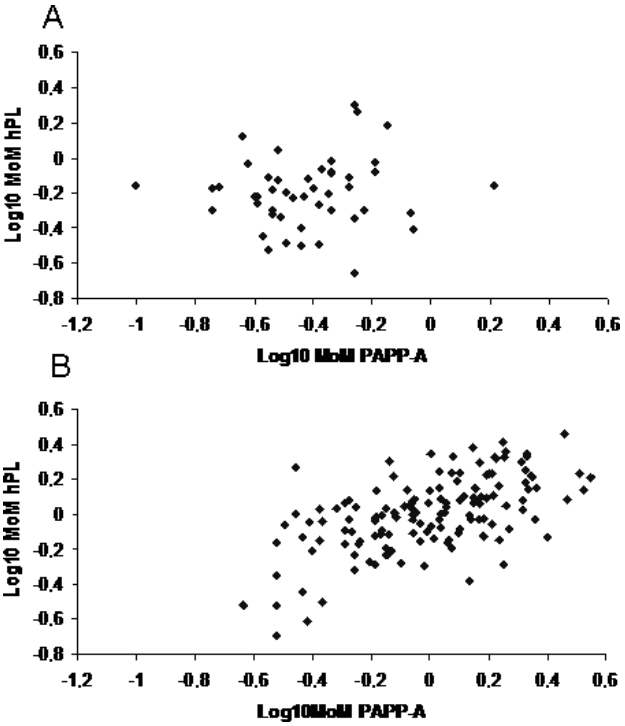

Figure 3—The relation between log10 MoM hPL and log10 MoM PAPP-A in (A) DS pregnancies and (B) control pregnancies

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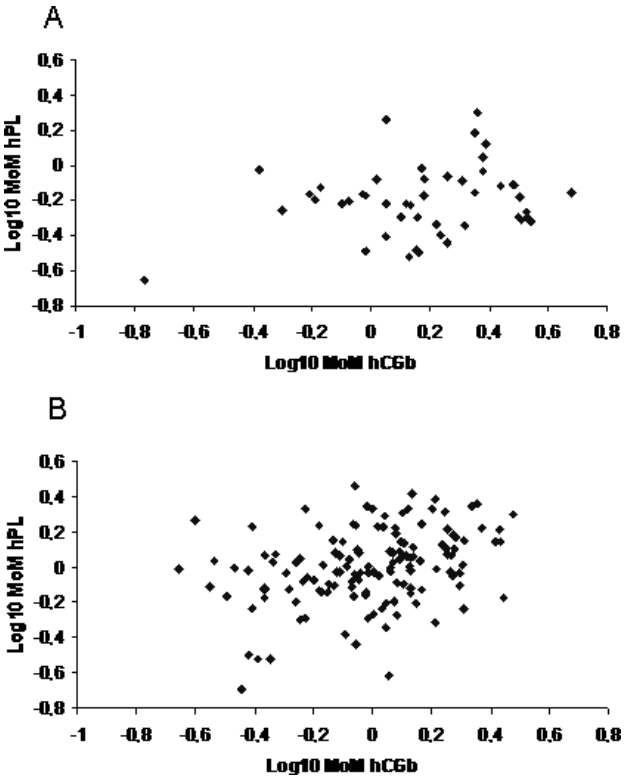

Figure 4—The relation between log10 MoM hPL and log10 MoM hCGβ in (A) DS pregnancies and (B) control pregnancies

An empirical assessment of the discriminatory significance of hPL, PAPP-A, hCGβ and maternal age in the examined group of pregnant women was obtained by stepwise multiple logistic regression. Despite the fact that DS and control pregnancies in this study were matched for maternal age ($\pm$5 years), maternal age in combination with any of the three biochemical markers resulted in regression models where both maternal age and the marker were significant ($p < 0.05$) predictors of DS (data not shown). The combination of maternal age, PAPP-A and hCGβ resulted in a model with maternal age not being significant (a result of the fact that samples were matched for maternal age), whereas PAPP-A and hCGβ were significant predictors. The model was good with a McFadden's ρ^2 of 0.514. The final regression model, depicted in Table 3, shows that PAPP-A, hCGβ and hPL and the interaction between PAPP-A and hPL are significant predictors, and the McFadden's ρ^2 is 0.542. Thus, hPL and the interaction of hPL with PAPP-A contribute significantly to the discrimination of DS and control pregnancies—in the particular sample studied.

The estimated population performance of hPL as a maternal serum-screening marker for DS is shown in Table 4. It is seen that adding hPL to the double test leads to an increase of 8% points in the detection rate (DR), from 44 to 52%, for a fixed false-positive rate (FPR) of 1%. This represents an 18% increase in the efficiency of screening. Furthermore, the combination of hPL and hCGβ, that is, a double test where PAPP-A is substituted by hPL, is nearly as efficient as the double test with a DR of 43% compared to a DR of 44% for an FPR of 1%.

DISCUSSION

We have found that hPL is reduced to 0.63 MoM in DS pregnancies and that this is the case uniformly through the gestational age window at weeks 8–13. This is in accordance with the reduced hPL synthesis in cultured DS placentae (Frendo *et al*., 2000). Furthermore, multiple logistic regression, Table 3, showed that hPL in the examined sample is a significant predictor of the fetus suffering from DS. Using hPL parameters derived from the present study, and published distributional parameters for PAPP-A, hCGβ and the maternal age distribution, we also show that the use of hPL as a marker in conjunction with PAPP-A and hCGβ is associated with an increase in the DR from 44 to 52% using a fixed FPR of 1% and an increase from 67 to 75% for a fixed FPR of 5%, compared with the sole use of PAPP-A and hCGβ. It is thus an appreciable improvement in performance that can be obtained by including hPL as a new

Table 3—Significant predictors in multiple logistic regression model of the discrimination between control and DS pregnancies. All continuous parameters were entered as log10 MoM values

Predictor	β	SE	t	P
Constant	−3.828	0.716	−5.347	<0.001
PAPP-A	−10.016	2.199	−4.554	<0.001
hCGβ	5.061	1.176	4.304	<0.001
hPL	−7.092	3.028	−2.342	0.019
PAPP-A[a] hPL	−17.077	8.148	−2.096	0.036

Table 4—Estimated screening performance for DS of hPL and hPL in combination with other first-trimester markers

	Risk cut-off						FPR	
	1 : 100		1 : 250		1 : 400		1%	5%
	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	DR (%)	DR (%)
hPL + age	1.7	24	6.7	44	13.7	59	19	40
hPL + age + PAPP − A	1.6	33	6.0	55	11.8	69	27	51
hPL + age + hCGβ	2.1	54	5.9	69	9.5	76	43	66
PAPP − A + hCGβ + age	2.3	56	6.1	70	9.3	77	44	67
hPL + PAPP − A + hCGβ + age	2.1	63	5.1	76	7.9	82	52	75

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first-trimester marker. Furthermore, the data in Table 2 suggest that PAPP-A may be substituted by hPL in centres offering the double test as this will only result in a decrease in DR by 1% for fixed FPRs of both 1 and 5%.

The strong correlation between hPL and PAPP-A found in control pregnancies is interesting as it could represent a regulatory mechanism of PAPP-A or hPL synthesis; a mechanism that is apparently perturbed in DS pregnancies. This difference in correlation between controls and DS pregnancies may explain the significant contribution to discrimination of the PAPP-A and hPL in controls.

As assays for hPL are widely available and easily modified into assays suitable for large-scale population screening, the inclusion of hPL in practical screening should be easy to implement. However, the relation between hPL and NT has not been established and a final assessment of the potential of hPL as a part of first-trimester screening can only be reached in large prospective studies.

REFERENCES

Barrera-Saldana HA, Seeburg PH, Saunders GF. 1983. Two structurally different genes produce the same secreted human placental lactogen hormone. *J Biol Chem* **258**: 3787–3793.

Benn P, Wright D, Cuckle H. 2005. Practical strategies in contingent sequential screening for Down syndrome. *Prenat Diagn* **25**: 645–652.

Christiansen M, Norgaard-Pedersen B. 2005. Inhibin A is a maternal serum marker for Down's syndrome early in the first trimester. *Clin Genet* **68**: 35–39.

Christiansen M, Olesen LS. 2002. An increase in cost-effectiveness of first trimester maternal screening programmes for fetal chromosome anomalies is obtained by contingent testing. *Prenat Diagn* **22**: 482–486.

Christiansen M, Oxvig C, Wagner JM, *et al*. 1999. The proform of eosinophil major basic protein: a new maternal serum marker for Down syndrome. *Prenat Diagn* **19**: 905–910.

Cuckle HS, Wald NJ, Thompson SG. 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.

Frendo JL, Vidaud M, Guibourdenche J, *et al*. 2000. Defect of villous cytotrophoblast differentiation into syncytiotrophoblast in Down's syndrome. *J Clin Endocrinol Metab* **85**: 3700–3707.

Fujimoto S, Hamasaki K, Ueda H, Kagawa H. 1986. Immunoelectron microscope observations on secretion of human placental lactogen (hPL) in the human chorionic villi. *Anat Rec* **216**: 68–72.

Grumbach MM, Kaplan SL, Sciarra JJ, Burr IM. 1968. Chorionic growth hormone-prolactin (CGP): secretion, disposition, biologic activity in man, and postulated function as the "growth hormone" of the 2d half of pregnancy. *Ann N Y Acad Sci* **148**: 501–531.

Klassen ME, Nachtigal MW, Cattini PA. 1989. Human chorionic somatomammotropin gene expression in primary placental cell cultures. *Placenta* **10**: 321–329.

Laigaard J, Sorensen T, Frohlich C, *et al*. 2003. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. *Prenat Diagn* **23**: 1086–1091.

Larsen SO, Christiansen M, Norgaard-Pedersen B. 1998. Calculation of roc curves in multidimensional likelihood ratio based screening with Down's syndrome as a special case. *J Med Screen* **5**: 57–62.

Musicki B, Pepe GJ, Albrecht ED. 1997. Functional differentiation of placental syncytiotrophoblasts during baboon pregnancy: developmental expression of chorionic somatomammotropin messenger ribonucleic acid and protein levels. *J Clin Endocrinol Metab* **82**: 4105–4110.

Qin QP, Christiansen M, Nguyen TH, Sorensen S, Larsen SO, Norgaard-Pedersen B. 1997. Schwangerschaftsprotein 1 (SP1) as a maternal serum marker for Down syndrome in the first and second trimesters. *Prenat Diagn* **17**: 101–108.

Sasagawa M, Yamazaki T, Endo M, Kanazawa K, Takeuchi S. 1987. Immunohistochemical localization of HLA antigens and placental proteins (alpha hCG, beta hCG CTP, hPL and SP1 in villous and extravillous trophoblast in normal human pregnancy: a distinctive pathway of differentiation of extravillous trophoblast. *Placenta* **8**: 515–528.

Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.

van der Veen V, Beekhuis JR, Cornel MC, Mantingh A, de Walle HE, de Wolf BT. 1997. A demographic approach to the assessment of Down syndrome screening performance. *Prenat Diagn* **17**: 717–724.

Wald NJ, Watt HC, Hackshaw AK. 1999a. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. *N Engl J Med* **341**: 461–467.

Wald NJ, Watt HC, Norgaard-Pedersen B, Christiansen M, International Prenatal Screening Research Group. 1999b. SP1 in pregnancies with Down syndrome in the first trimester of pregnancy. *Prenat Diagn* **19**: 517–520.

Weinstein D, Galski H, Schenker JG, *et al*. 1982. The synthesis and secretion of human placental lactogen (hPL) in cultured term placenta. *Mol Cell Endocrinol* **26**: 189–199.

Wright DE, Bradbury I. 2005. Repeated measures screening for Down's Syndrome. *BJOG* **112**: 80–83.

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