Maternal Serum Pregnancy-Associated Plasma Protein A and Fetal Nuchal Translucency Thickness for the Prediction of Fetal Trisomies in Early Pregnancy

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Objective: **To determine if the risk for fetal trisomies during the first trimester of pregnancy can be derived by combining data from maternal serum pregnancy-associated plasma protein A (PAPP-A) and fetal nuchal translucency thickness.**

Methods: **Pregnancy-associated plasma protein A was measured in samples from 87 singleton pregnancies with fetal chromosomal abnormalities (45 trisomy 21, 19 trisomy 18, eight trisomy 13, 11 sex chromosome aneuploidies, four triploidies) and 348 chromosomally normal controls at 10–13 weeks' gestation. Likelihood ratios for trisomies 21, 18, and 13 in relation to PAPP-A, in multiples of the normal median (MoM) for crown-rump length, were derived from the overlapping gaussian frequency distribution curves for normal and abnormal pregnancies.**

Results: **In the chromosomally normal group, maternal serum PAPP-A correlated significantly with fetal crown-rump length ($r = 0.421$, $P < .0001$). In the chromosomally abnormal group, the median PAPP-A was significantly lower than in the normal controls. The respective median values expressed in MoM for trisomies 21, 18, and 13 and other aneuploidies were 0.5 MoM (90% confidence interval [CI] 0.09–1.67, $z = 6.0$, $P < .001$), 0.17 MoM (90% CI 0.06–1.45, $z = 6.6$, $P < .001$), 0.25 MoM (90% CI 0.10–0.62, $z = 4.5$, $P < .001$), and 0.72 MoM (90% CI 0.09–2.48, $z = 2.2$, $P < .05$), respectively. There was no significant linear association between PAPP-A and fetal nuchal translucency thickness in either the chromosomally normal ($r = -0.01$, $P = .89$) or abnormal groups ($r = -0.19$, $P = .08$).**

Conclusion: **The risks for fetal trisomies at 10–13 weeks' gestation can be derived by combining data on maternal age, maternal serum PAPP-A, and fetal nuchal translucency thickness.** *(Obstet Gynecol 1994;84:918–22)*

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In pregnancies with fetal trisomies 21, 18, and 13, maternal serum levels of pregnancy-associated plasma protein A (PAPP-A) are decreased during the first trimester.[1–5] In addition, these trisomies are associated with increased fetal nuchal translucency thickness at 10–13 weeks' gestation.[6,7] The aim of the present study was to determine if the risk for fetal trisomies during the first trimester of pregnancy can be derived by combining data from maternal serum PAPP-A and fetal nuchal translucency thickness.

Materials and Methods

Pregnancy-associated plasma protein A was measured in samples from 87 singleton pregnancies with fetal chromosomal abnormalities (45 trisomy 21, 19 trisomy 18, eight trisomy 13, 11 sex chromosome aneuploidies, four triploidies) at 10–13 weeks' gestation. Each of these samples was matched for crown-rump length, maternal age, and storage time with four samples from pregnancies with chromosomally normal fetuses ($n = 348$). To allow examination of the association between nuchal translucency thickness and maternal serum PAPP-A in the chromosomally normal group, cases were preselected for increased nuchal translucency thickness. This study includes previously published findings[5] from 32 pregnancies with chromosomally abnormal fetuses and 134 with chromosomally normal fetuses. The maternal blood samples had been taken immediately before chorionic villus sampling or early amniocentesis for fetal karyotyping because of parental anxiety, advanced maternal age, or increased fetal nuchal translucency thickness (at least 3 mm).

Maternal serum was stored at −20C until assay. Biochemical analysis was performed without knowl-

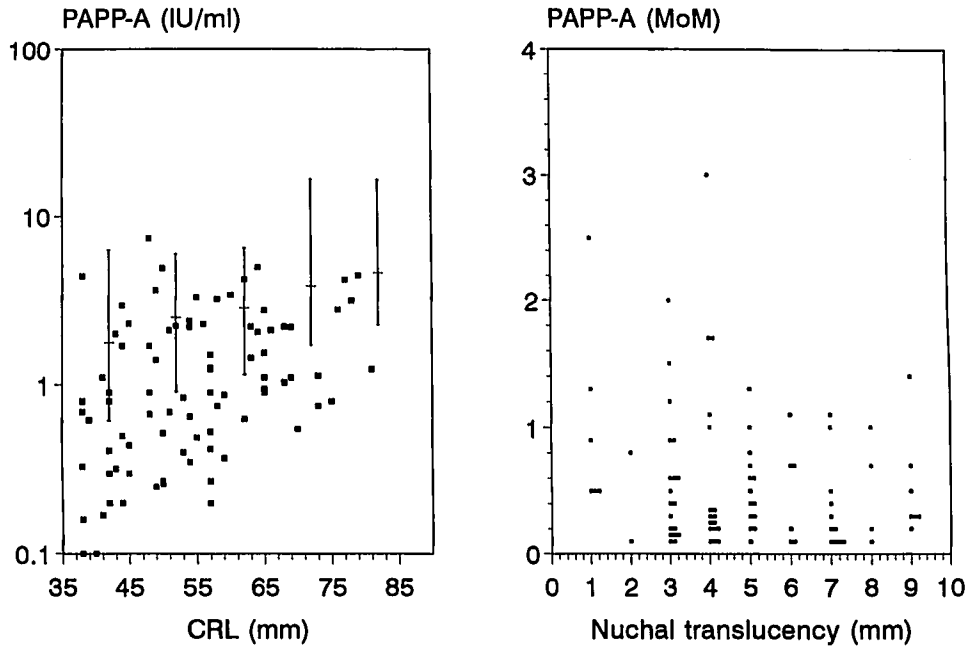

Figure 1. Individual values for maternal serum pregnancy-associated plasma protein A (PAPP-A) in 72 chromosomally normal fetuses in relation to fetal crown-rump length (the bars indicate the median, fifth, and 95th percentiles in chromosomally normal fetuses) and values expressed as multiples of the normal median (MoM) for crown-rump length (CRL) in relation to fetal nuchal translucency thickness.

edge of fetal karyotype or nuchal translucency thickness. Pregnancy-associated plasma protein A was measured by a double-sandwich, time-resolved immunofluorometric assay with chelated europium as a label. The antibody to PAPP-A binding immunoglobulin (Ig) (Dakopatts-Dako, Glostrup, Denmark) was a polyclonal rabbit IgG in a stabilized solution at 14.3 mg/mL (batch 11). The purity of the coating antibody was enhanced by negative affinity chromatography over an immobilized normal pooled pregnancy serum fraction obtained by pressure filtration through a 300,000-dalton cutoff membrane (Sartorius-Filtration, Goettingen, Germany). Details of the method were published previously.[5] The limit of detection of the assay was 0.02 mIU/mL and the intra- and inter-assay variations were 8.2 and 11.6%, respectively.

The normal medians of PAPP-A for fetal crown-rump length were calculated from the chromosomally normal group. Values for PAPP-A in the chromosomally normal and abnormal groups were expressed as multiples of the normal median (MoM) for crown-rump length. The significance of differences between groups was examined using rank analysis of variance. Regression analysis was used to examine the association between nuchal translucency thickness and PAPP-A levels. Likelihood ratios for trisomies 21, 18, and 13 in relation to PAPP-A in MoM were derived from the overlapping gaussian frequency distribution curves for normal and abnormal pregnancies as described by Macintosh et al.[8]

Results

The median maternal age was 38 years (range 22–45), the median gestational age was 82 days (range 70–97), and the median crown-rump length was 53 mm (range 38–87). In the chromosomally normal group, the fetal nuchal translucency thickness was less than 3 mm in 200 cases and at least 3 mm in 148. Maternal serum PAPP-A correlated significantly with fetal crown-rump length ($\log_{10}$ [PAPP-A] = −0.173 + 0.011 × crown-rump length; standard deviation [SD] = 0.25, $r = 0.421$, $n = 348$, $P < .0001$), and there was no significant association between PAPP-A and nuchal translucency thickness ($r = -0.01$, $P = .89$).

In the chromosomally abnormal group, the median PAPP-A was significantly lower than in the chromosomally normal group ($z = 9.02$, $P < .0001$) (Figure 1). The fetal nuchal translucency thickness was less than 3 mm in eight cases and at least 3 mm in 77. There was no significant association between PAPP-A and nuchal translucency thickness ($r = -0.19$, $n = 86$, $P = .08$).

Figure 2 shows individual values, expressed as MoM for crown-rump length, for chromosomally normal and abnormal fetuses. In fetuses with trisomy 21, the median PAPP-A was 0.5 MoM (90% confidence interval [CI] 0.09–1.67, $z = 6.0$, $P < .001$); in those with trisomy 18, it was 0.17 MoM (90% CI 0.06–1.45, $z = 6.6$, $P < .001$); in those with trisomy 13, it was 0.25 MoM (90% CI 0.10–0.62, $z = 4.5$, $P < .001$); and in those with other chromosomal abnormalities, it was 0.72 MoM (90% CI

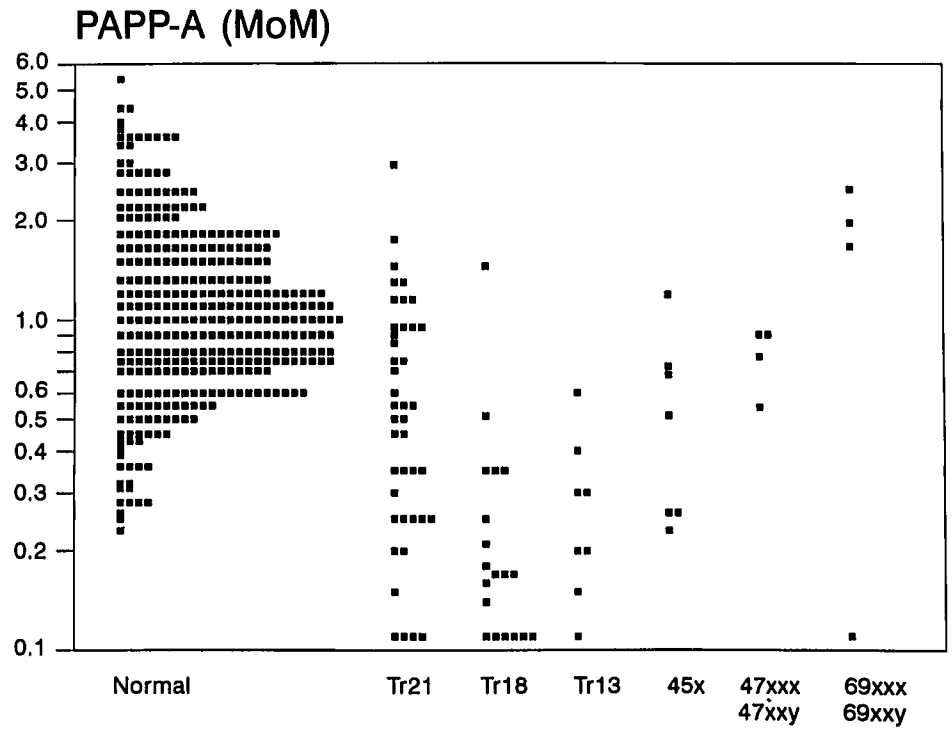

Figure 2. Individual values for maternal serum pregnancy-associated plasma protein A (PAPP-A) (in multiples of the normal median [MoM] for crown-rump length) in 348 chromosomally normal and 72 abnormal fetuses. Chromosomal abnormalities included trisomy 21 (Tr21; $n = 45$), trisomy 18 (Tr18; $n = 19$), trisomy 13 (Tr13; $n = 8$), Turner syndrome (45X; $n = 7$), 47,XXX or 47,XXY ($n = 4$), and triploidy (69XXX or 69XXY; $n = 4$).

0.09–2.48, $z = 2.2$, $P < .05$). Table 1 shows the percentage of pregnancies with chromosomally normal and trisomic fetuses for different cutoff levels of PAPP-A.

Frequency distributions for PAPP-A in the chromosomally normal group and in the groups with trisomy 21 and trisomy 18 or 13 are shown in Figure 3. Table 2 shows the likelihood ratios for trisomy 21, trisomies 18 and 13, and all three trisomies in relation to PAPP-A. Since there is no significant association between maternal serum PAPP-A levels and fetal nuchal translucency thickness, an estimate of an individual woman's risk for fetal trisomies can be obtained by multiplying the left side of the odds ratio of the risk based on age and nuchal translucency thickness[7,9] with the appropriate likelihood ratio for PAPP-A. Figure 4 illustrates the levels of PAPP-A for different maternal ages and fetal nuchal translucency thickness for a 1:110 risk for fetal trisomy, which is the maternal age-related risk for a 37-year-old woman at 9–14 weeks' gestation.[10] In the group with fetal nuchal translucency greater than 3 mm, the risk for fetal trisomy is more than 1:110, irrespective of maternal serum PAPP-A.

Table 1. False-Positive and Detection Rates for Fetal Trisomies with Different Cutoff Points of Maternal Serum PAPP-A

PAPP-A MoM	Controls ($n = 348$)	Trisomy 21 ($n = 45$)	Trisomy 18 or 13 ($n = 27$)	Trisomy 21, 18, or 13 ($n = 72$)
≤1.0	175 (50%)	37 (82%)	26 (96%)	63 (88%)
≤0.8	121 (35%)	31 (69%)	26 (96%)	57 (79%)
≤0.6	54 (16%)	28 (62%)	25 (93%)	53 (74%)
≤0.4	17 (5%)	20 (44%)	24 (89%)	44 (61%)
≤0.2	0	8 (18%)	15 (56%)	23 (32%)

PAPP-A = pregnancy-associated plasma protein A; MoM = multiples of the normal median.

Discussion

The data of this study confirm that maternal serum PAPP-A is reduced in pregnancies with fetal chromosomal abnormalities.[1–5] On the basis of our results, a policy of screening for fetal trisomies by maternal serum PAPP-A and offering invasive testing to those with levels of 0.4 MoM or less could potentially identify 44% of fetuses with trisomy 21 and 89% of those with trisomies 18 or 13, for a false-positive rate of 5%. This would compare favorably with traditional screening by maternal age and offering invasive testing to women with a minimum age of 37 years, which for a false-positive rate of 5% identifies 25–30% of trisomic fetuses.

In this study, PAPP-A was examined in relation to fetal crown-rump length rather than gestation calculated from the last menstrual period (LMP). This is essential for screening studies because in 10–45% of pregnancies, women are uncertain of their LMP or they have irregular menstrual cycles[11,12]; even in those with

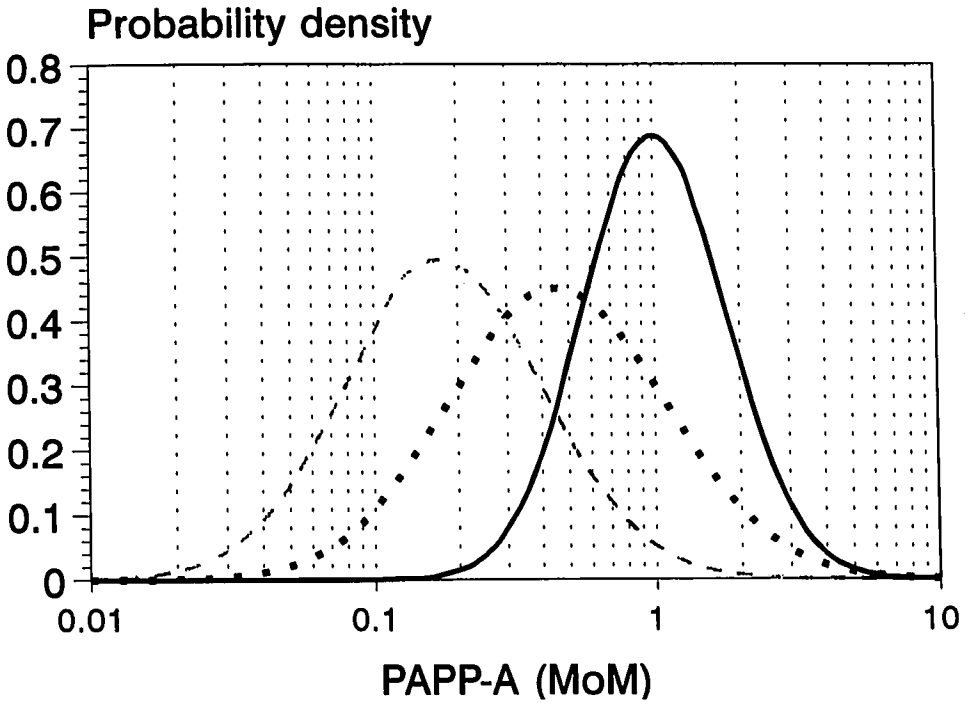

Figure 3. Probability density in relation to pregnancy-associated plasma protein A (PAPP-A) (expressed as multiples of the normal median [MoM] for crown-rump length) for pregnancies with chromosomally normal fetuses (*solid line*), fetuses with trisomy 21 (*dotted line*), and fetuses with trisomy 18 or 13 (*dashed line*).

certain dates and regular 28-day cycles, there are considerable variations in the day of ovulation.[13] Since the crown-rump length in fetuses with trisomy 21 or 13 is normal,[14] a policy of routine pregnancy dating by measurement of crown-rump length will not affect the interpretation of results from PAPP-A. However, fetuses with trisomy 18 have shorter crown-rump length[14]; consequently, the MoM for PAPP-A in trisomy 18 would be even lower if pregnancy dating was by LMP rather than by crown-rump length.

Table 2. Likelihood Ratio for Fetal Trisomies in Relation to Maternal Serum PAPP-A

	Likelihood ratio		
PAPP-A MoM	Trisomy 21 (n = 45)	Trisomies 18 or 13 (n = 27)	Trisomies 21, 18, or 13 (n = 72)
0.2	17.7	29.9	23.4
0.25	7.8	10.1	9.3
0.3	4.3	4.4	4.8
0.4	2.0	1.4	2.0
0.5	1.2	0.6	1.1
0.6	0.9	0.3	0.7
0.7	0.7	0.2	0.5
0.8	0.6	0.1	0.4
0.9	0.5	0.1	0.4
1.0	0.5	0.1	0.3
1.2	0.4	0.1	0.3
1.4	0.4	0.0	0.2
1.6	0.4	0.0	0.2
1.8	0.4	0.0	0.2
2.0	0.4	0.0	0.2

Abbreviations as in Table 1.

Our findings demonstrate the feasibility of deriving risks for fetal trisomies at 10–13 weeks' gestation by combining data on maternal age, maternal serum PAPP-A, and fetal nuchal translucency thickness. This has become possible because in pregnancies with chromosomally normal fetuses, the incidence of nuchal translucency thickness of at least 3 mm is independent of maternal age.[7] In addition, as shown in the present study, maternal serum PAPP-A is not significantly associated with nuchal translucency thickness in either the chromosomally normal or abnormal groups.

We have reported previously[7] that fetal nuchal translucency less than 3 mm is associated with a fourfold reduction in maternal age-related risk for trisomy 21, whereas translucencies of 3, 4, and 5 mm or greater are associated with fourfold, 23-fold, and 25-fold increases in maternal age-related risks, respectively.[9] The prevalence of nuchal translucency thickness of 3 mm is approximately 4% and that of more than 3 mm is 1%.[7]

Snijders et al[10] have established that for a 37-year-old woman (the traditional cutoff age for offering invasive testing), the maternal age-related risk for trisomy 21 at 9–14 weeks' gestation is approximately 1:110. This study offers an alternative approach for the selection of women with a minimum risk of 1:110; in addition to maternal age, it includes data on maternal serum PAPP-A and fetal nuchal translucency thickness. Fetal karyotyping could be

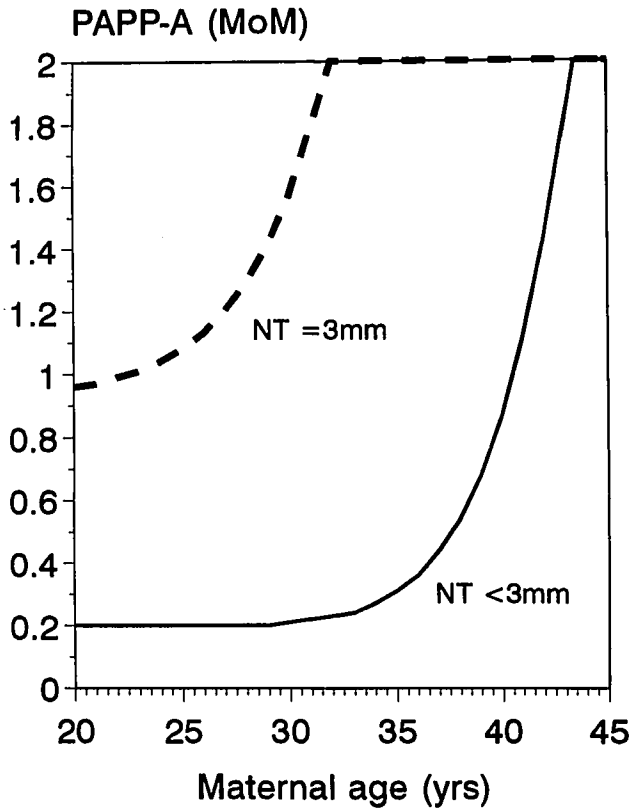

Figure 4. Maternal serum pregnancy-associated plasma protein A (PAPP-A) level (expressed as multiples of the normal median [MoM] for crown-rump length) at which the risk for trisomy 21 is approximately 1:110 in pregnancies with fetal nuchal translucency (NT) less than 3 mm (*solid line*) and pregnancies with translucency of 3 mm (*dashed line*).

offered to all women with fetal nuchal translucency thickness greater than 3 mm. Even for a 20-year-old (maternal age-related risk of 1:700), the risk for trisomies would be at least 1:30; if the maternal serum PAPP-A was as high as 2 MoM (likelihood ratio of 0.4), the risk would still be more than 1:110. For pregnancies with fetal nuchal translucency less than 4 mm, selection of those with a minimum risk of 1:110 would depend on maternal age, translucency thickness, and PAPP-A (Figure 4). The sensitivity and specificity, as well as difficulties in the practical implementation of the proposed alternative method of screening, must be determined.

References

1. Wald N, Stone R, Cuckle HS, et al. First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's syndrome. BMJ 1992;305:28.
2. Brambati B, Macintosh MCM, Teisner B, et al. 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 1993;100:324–6.
3. Hurley PA, Ward RHT, Teisner B, Iles RK, Lucas M, Grudzinkas JG. Serum PAPP-A measurements in first-trimester screening for Down syndrome. Prenat Diagn 1993;13:903–8.
4. Muller F, Cuckle H, Teisner B, Grudzinskas JG. Serum PAPP-A levels are depressed in women with fetal Down syndrome in early pregnancy. Prenat Diagn 1993;13:633–6.
5. Bersinger NA, Brizot ML, Johnson A, et al. First trimester maternal serum pregnancy-associated plasma protein A (PAPP-A) and pregnancy-specific β1-glycoprotein (SP1) in fetal trisomies. Br J Obstet Gynaecol (in press).
6. Nicolaides KH, Azar G, Byrne D, Mansur CA, Marks K. Fetal nuchal translucency: Ultrasound screening for chromosomal defects in the first trimester of pregnancy. BMJ 1992;304:867–9.
7. Nicolaides KH, Brizot ML, Snijders RJM. Fetal nuchal translucency thickness: Ultrasound screening for fetal trisomy in the first trimester of pregnancy. Br J Obstet Gynaecol 1994;101:782–6.
8. Macintosh MCM, Brambati B, Chard T, Grudzinskas JG. Predicting fetal chromosome anomalies in the first trimester using pregnancy associated plasma protein-A: A comparison of statistical methods. Methods Inf Med 1993;32:175–9.
9. Pandya PP, Brizot ML, Kuhn P, Snijders RJM, Nicolaides KH. First-trimester fetal nuchal translucency thickness and risk for trisomies. Obstet Gynecol 1994;84:420–3.
10. Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risk for trisomies at 9–14 weeks' gestation. Prenat Diagn 1994;14:543–52.
11. Campbell S, Warsof SL, Little D, Cooper DJ. Routine ultrasound screening for the prediction of gestational age. Obstet Gynecol 1985;65:613–20.
12. Bergsiø P, Denman III DW, Hoffman J, Meirik O. Duration of human singleton pregnancy. Acta Obstet Gynecol Scand 1994;69: 197–207.
13. Geirsson RT. Ultrasound instead of last menstrual period as the basis of gestational age assignment. Ultrasound Obstet Gynecol 1991;1:212–9.
14. Kuhn P, Brizot ML, Pandya PP, Snijders RJM, Nicolaides KH. Crown-rump length in chromosomally abnormal fetuses at 10–13 weeks' gestation. Am J Obstet Gynecol (in press).

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