PRENATAL DIAGNOSIS
Prenat Diagn 2000; **20**: 311–317.

Is maternal serum total hCG a marker of trisomy 21 in the first trimester of pregnancy?

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In a study of 130 first trimester cases of trisomy 21 and 959 controls we have shown that the median MoM for alpha-fetoprotein (AFP) is lower (0.82) and that for total human chorionic gonadotrophin (hCG) is higher (1.31) than in the control group. For AFP 15.3% of cases were below the 5th centile and for total hCG 19.8% were above the 95th centile. The median shift observed for AFP and total hCG is poorer than that for pregnancy associated plasma protein-A (PAPP-A) or free β-hCG and together with maternal age, AFP and total hCG could only be expected to detect 40% of cases. In combination with PAPP-A, total hCG would identify 52% of cases, somewhat less than the 67% observed with free β-hCG and PAPP-A. However, we have demonstrated for total hCG a significant temporal change in median MoM with gestational age. Before 70 days the median MoM was less than 0.5, between 70 and 83 days this increased to 1.13, and between 84 and 97 days this increased to 1.52. This median shift has significant implications for interpreting previous studies and even more significant implications for detection rates. When population parameters specific to the gestational age in question are used, detection rates with total hCG and PAPP-A increase from 47% at 70–83 days to 60% at 84–97 days. This observation explains much of the confusion around total hCG in the first trimester and shows the importance of selecting analyte pairs and population parameters appropriate to the time in gestation when screening is performed. Copyright © 2000 John Wiley & Sons, Ltd.

INTRODUCTION

Screening for trisomy 21 in the second trimester by the measurement of maternal serum biochemical markers has become an accepted part of obstetric practice in many countries (Cuckle *et al*., 1995; Palomaki *et al*., 1997). In routine practice a combination of maternal age with serum alpha-fetoprotein (AFP) and either free β-human chorionic gonadotrophin (hCG), with or without unconjugated oestriol can identify approximately 65% of cases for a 5% false positive rate (Crossley *et al*., 1994; Wald *et al*., 1992; Goodburn *et al*., 1994; Macri and Spencer, 1996; Spencer, 1999a). In the first trimester of pregnancy much interest is focused on the use of fetal nuchal translucency (NT) thickness in combination with appropriate biochemical markers. Of the biochemical markers investigated, AFP and total hCG appear to be weak markers (Aitken *et al*., 1993) in the first trimester, while only free β-hCG (Spencer *et al*., 1992; Spencer, 1997) and pregnancy associated plasma protein-A (PAPP-A) have been shown to be of any value (Brambati *et al*., 1991; Spencer *et al*., 1994; Wald *et al*., 1996). Large-scale studies (Spencer *et al*., 1999a) have shown that detection rates of 89–90% can be achieved at a 5% false positive rate when ultrasound, maternal serum PAPP-A and free β-hCG are combined together at 10–14 weeks of gestation. Furthermore, the use of rapid immunodiagnostic technology has allowed the delivery of such screening within a 1 h time frame, leading to the development of a one-stop clinic for assessment of risk of fetal abnormality (OSCAR) (Spencer, 1999b).

Despite a general consensus that total hCG is of little value in screening for trisomy 21 in the first trimester (Wald *et al*., 1997), sporadic reports in small series continue to claim that total hCG is of value at this time (Forest *et al*., 1995; Haddow *et al*., 1998; Casals *et al*., 1999). We have therefore sought to clarify this situation by analysing AFP and total hCG in a large series of trisomy 21 cases, comparing these markers with other first trimester marker data largely from our previous study (Spencer *et al*., 1999a).

METHODS

The study population was derived from three groups of women. The first comprised women with singleton pregnancies who were referred to the Harris Birthright Centre for fetal karyotyping, because screening by a combination of maternal age and fetal NT at 10–14 weeks in their hospital identified these patients as being at high risk for trisomy 21 (Snijders *et al*., 1998). The second group comprised self-referred women for assessment of risk. Blood samples were collected from women at the time of the scan and the serum was aliquoted and stored at -20°C prior to blinded retrospective analysis. Gestational age was determined

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Received: 25 October 1999
Revised: 5 January 2000
Accepted: 21 January 2000

by measurement of fetal crown–rump length (CRL). Pregnancy outcome was ascertained in all women. The 90 trisomy 21 cases formed part of a previous study of the markers free β-hCG and PAPP-A (Spencer *et al.*, 1999a). To supplement these a third group of 40 samples from cases of trisomy 21 were obtained from the Glasgow centre as part of an ongoing study (Berry *et al.*, 1997), which included the Combined Ultrasound and Biochemistry study (CUBS) in Scotland. In this series, total hCG, AFP, PAPP-A and free β-hCG were all measured at the same time.

To establish suitable reference data a control group of samples spreading the gestational ranges 10–14 weeks were obtained from samples collected as part of routine first trimester screening in the OSCAR clinic (Spencer, 1999b) at Harold Wood in patients with a normal pregnancy outcome. All samples were analysed for PAPP-A and free β-hCG within 30 min of sample collection and samples were then subsequently frozen as aliquots at -20°C. Pregnancy outcome was ascertained in all women. To supplement these, a further 90 control samples in the gestational period 6–9 weeks were obtained from the Glasgow centre.

In total, serum samples from 130 pregnancies affected by trisomy 21 were available for biochemical analysis. A control group of 959 cases resulting in the birth of an unaffected baby were used to establish median and reference data. Table 1 shows the characteristics of the trisomy 21 population and also that for the control population.

Maternal serum AFP and total hCG were measured over a period of three days on the Kryptor analyser—a random access immunoassay analyser using time-resolved amplified cryptate emission (TRACE) technology and the CIS automated assays (CIS UK Ltd., High Wycombe, Bucks, UK). The between day precision of these assays (CV%) was 4.7% for AFP at 11 kU/l and 3.2% for total hCG at 105 800 U/l.

Statistical analysis

Weighted regression analysis was carried out to derive the relationship between marker levels and gestational age. To correct for marker variation with gestational age each value was converted to MoMs from the respective median marker levels in unaffected pregnancies of the same gestational age. Statistical analysis of data was performed using Excel and Astute, a statistical software add-in for Microsoft Excel 5 (DDU Software, University of Leeds, U.K.) or Analyse-It (Smart Software, Leeds, U.K.). The performance of various marker combinations as potential screening procedures was examined using standard statistical modelling techniques (Royston and Thompson, 1992). Using the observed population parameters for AFP and total hCG and those for other markers detailed in a previous study (Spencer *et al.*, 1999a), a series of 15 000 random MoM values were selected for each marker from within the gaussian distributions of the log MoM of affected and unaffected pregnancies. These values were then used to calculate likelihood ratios for the combinations. The likelihood ratios were then used together with the age-related risk of trisomy 21 in the first trimester (Snijders *et al.*, 1995) to calculate the expected detection rate of affected pregnancies at a fixed false positive rate, in a population with the maternal age distribution of pregnancies in England and Wales (OPCS, 1994).

Table 1—Median and range for maternal age, gestational age, fetal crown–rump length, maternal weight and sample storage time in the trisomy 21 group and the control group

	Trisomy 21	Controls
No.	130	959
Maternal age (years)	37 (17–45)	28.8 (15–45)
Gestational age (days)	86 (43–97)	83 (42–97)
Crown–rump length (mm)	61 (42–84)	56 (36–84)
Maternal weight (kg)	64 (44–133)	65 (36–120)
Sample storage time (days)	937 (150–2317)	435 (350–502)

RESULTS

Table 2 shows the observed and regressed median values for total hCG and AFP. Total hCG MoM and AFP MoM in both the trisomy 21 group and in the control group fitted a gaussian distribution after removal of outliers outside of three standard deviations (number of outliers were seven (0.7%) for total hCG in controls and five (4%) in the trisomy group; for AFP, one (0.1%) in controls and four (3%) in the trisomy group). After $\log_{10}$ transformation, Kolmogorov–Smirnov and Anderson Darling tests showed linearity at the 0.01 probability level for both affected and control populations. Figures 1 and 2 show the probability distributions for both analytes in each study population. Table 3 summarizes the statistical parameters associated with the distributions of the biochemical markers. Correlation of AFP and total hCG in controls and affected populations showed r values of 0.008 and 0.073 respectively. Correlation with free β-hCG and PAPP-A measured previously (Spencer *et al.*, 1999a) and in the additional 40 cases showed a correlation (r) of 0.0847 for free β-hCG and AFP, 0.1859 for PAPP-A and AFP, and 0.4272 for PAPP-A and total hCG in the affected populations. In the unaffected population the correlation between PAPP-A and total hCG was 0.2145.

Figures 3 and 4 show the individual total hCG and AFP MoM's for the trisomy 21 cases. The median MoM for total hCG was 1.31 and this was significantly higher than the control median (MannWhitney U test, $p<0.0001$). Of these cases, 26/130 (20.0%) were above the 95th centile of normal (1.859 MoM). The median MoM for AFP was 0.82 and this was significantly lower than the control median (Mann Whitney U test, $p<0.0001$). Of these cases, 20/130 (15.4%) were below the 5th centile of normal (0.516 MoM). In the 90 cases which were analysed in our previous study of free β-hCG and PAPP-A (Spencer *et al.*, 1999a) the median MoM in this same group was 2.03 and 0.51 respectively, demonstrating the greater

Table 2—Observed and regressed median levels of AFP and total hCG in unaffected pregnancies

Week	Mean gestation (days)	Median observed AFP (kU/l)	Regressed median AFP (kU/l)	Median observed total hCG (U/l)	Regressed median total hCG (U/l)
6	44	3.1	2.9	71036	72200
7	52	5.2	4.0	87820	88100
8	59	5.7	5.1	104800	100210
9	67	6.2	6.9	114800	125147
10	74	8.4	8.9	84670	82461
11	80	11.1	11.0	79230	72318
12	87	14.1	14.3	63370	62051
13	94	17.5	18.4	53760	53241

shift in median MoM for these two markers compared with total hCG and AFP. Adding to this analysis the 40 additional cases from the Glasgow centre gave an overall median free β-hCG MoM of 2.04 and 0.50 for PAPP-A. The log MoM standard deviation for free β-hCG and PAPP-A was 0.281 and 0.292, these were not significantly different from our original series (Spencer *et al.*, 1999a).

When the observed statistical parameters were used in the mathematical model of a population with a maternal age distribution of pregnancies in England and Wales, the estimated detection rates using various marker combinations with maternal age at a fixed false positive rate of 5% varied from 31% for AFP and maternal age or total hCG and maternal age, to 40% when both markers were combined with maternal age. If PAPP-A was used (using population data from Spencer *et al.* (1999a), then in combination with total hCG and maternal age, detection rates would increase to 52% compared with the 67% obtained using free β-hCG, PAPP-A and maternal age (Spencer *et al.*, 1999a). We estimated that adding AFP to either of the two marker combinations of total hCG and PAPP-A or free β-hCG, would increase the detection rate by less than 0.5%.

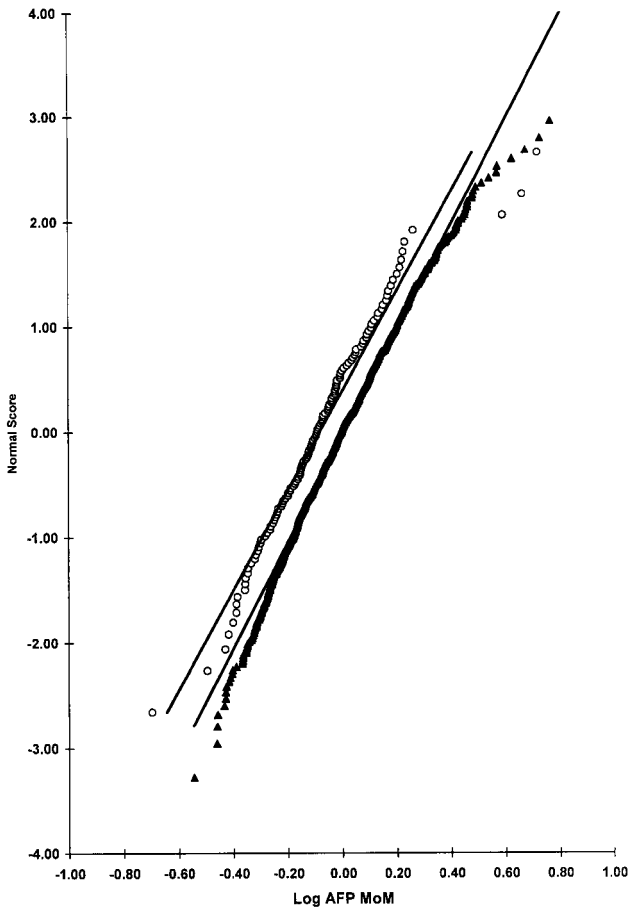

Figure 1—Probability plot of $\log_{10}$ AFP MoM in normal pregnancies (▲) and Down syndrome pregnancies (○)

Examination of Figure 4 indicates that the degree of elevation for total hCG MoM in cases of trisomy 21 may be related to the gestational age. When the study group was analysed by gestational age bands a significant difference in median MoM was noted for total hCG but not for AFP. With total hCG the median MoM in trisomy 21 pregnancies less than 70 days was 0.48, rising to 1.13 at 70–83 days and to 1.52 at 84–97 days. The difference between the 70–83 day median and the 84–97 day median was highly significant ($p = 0.004$, Mann Whitney U test). The correlation coefficient between $\log_{10}$ total hCG MoM and gestational age in days was 0.444.

When we concentrated our modelling analysis on those cases between 10 and 13 weeks and split them into two groups based on those between 70–83 days and those 84–97 days, the population parameters for total hCG shown in Table 4 were found. Using these population parameters and those for PAPP-A across the 70–97 day gestational period from our previous study (Spencer *et al.*, 1999a), modelled detection rates were 47% at 70–83 days and 60% at 84–97 days, each at a 5% false positive rate.

DISCUSSION

In large-scale studies of first trimester maternal serum (Spencer *et al.*, 1999a), the markers free β-hCG and PAPP-A alone have been shown to identify 33% and 38% of cases of Down syndrome at a 5% false positive rate. When each marker is combined with maternal

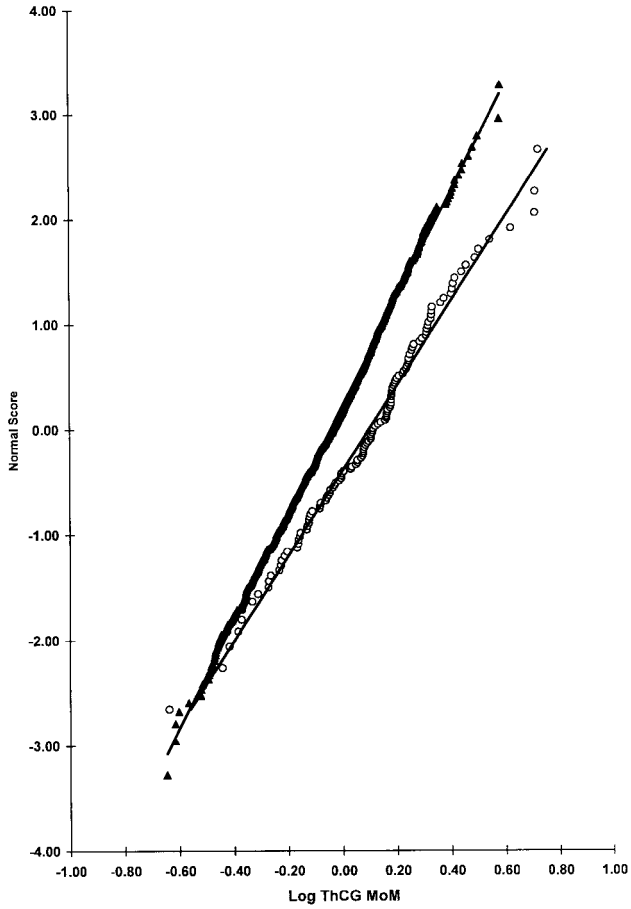

Figure 2—Probability plot of $\log_{10}$ total hCG MoM in normal pregnancies (▲) and Down syndrome pregnancies (○)

age this increases to 46% and 48% respectively, and when combined together in a three parameter algorithm, detection rates for a 5% false positive rate are increased to 67%. Our study of total hCG and AFP shows these marker performances to be inferior to free β-hCG and PAPP-A, with total hCG detecting only 20% of cases and AFP 15% of cases at a 5% false positive rate. When combined with maternal age these rise to 31% for either marker and as a three parameter algorithm with maternal age a detection rate of 40% can be achieved. These detection rates appear at odds with those published by Haddow *et al.* (1998), who claimed detection rates of 63% and 60% when maternal age, PAPP-A, and total hCG or free β-hCG were used at a 5% false positive rate. However, as pointed out by Hallahan *et al.* (1998), the observation that total hCG performed better than free β-hCG (29% versus 25% detection rate at a 5% false positive rate) was inconsistent with the distribution parameters provided in the paper, and that on re-analysis the data would show that free β-hCG should have detected 29% compared with 25% for total hCG.

Our current data clearly show that total hCG and AFP are poor markers of trisomy 21 in the first trimester. The median AFP MoM of 0.82 in the Down group is consistent with the summary value of 0.78 in the 16 series (335 cases) summarized in Wald *et al.* (1997). The median total hCG MoM of 1.31 is close to the summary value of 1.29 in the 12 series (352 cases) summarized by Wald *et al.* (1997). However, our data clearly show a gestational age-related increase in median total hCG MoM in cases of trisomy 21. If all of the published series for total hCG are analysed in two groups according to whether the median study gestational age was 11 weeks or older or less than 11 weeks (Table 5), then it is also clear that in general those studies that have claimed total hCG to be useful have higher median MoMs because the study consists predominately of late first trimester samples. The median MoMs in these two similar sized groups (1.199 versus 1.412) show a similar level of difference to our analysis of mid and late first trimester samples.

Temporal changes in median analyte levels across the first and second trimester of affected pregnancies are only now being recognized as large series of data are compiled. It has been known for some time that PAPP-A levels change significantly between the first and second trimester (Berry *et al.*, 1997) but clear demonstration of a gradual change across both periods has only recently been demonstrated (Spencer *et al.*, 1999a). Similarly, changes have recently been demonstrated for trisomy 18 when the reduced PAPP-A levels get progressively lower with advancing gestation (Tul *et al.*, 1999; Spencer *et al.*, 1999b). Free β-hCG demonstrates different temporal changes with the median MoM being highest at 14–16 weeks and gradually falling during the 16–20 (Spencer *et al.*, 1993) and the 10–14 week periods (Spencer *et al.*,

Table 3—Statistical parameters for the various marker distributions (as MoM) in trisomy 21 and control pregnancies

	AFP	Total hCG
$\log_{10}$ mean controls	0.008	−0.0044
$\log_{10}$ SD controls	0.1966	0.1959
$\log_{10}$ mean affected	−0.082	0.097
$\log_{10}$ SD affected	0.2111	0.2479
10th centile controls	0.587	0.489
50th centile controls	1.00	1.00
90th centile controls	1.795	1.554
10th centile affected	0.463	0.593
50th centile affected	0.820	1.310
90th centile affected	1.545	2.522

Table 4—Statistical parameters for total hCG distribution in trisomy 21 and control pregnancies split by gestational age

	70 to 83 days	84 to 97 days
$\log_{10}$ mean controls	−0.09909	−0.00509
$\log_{10}$ SD controls	0.1967	0.1995
$\log_{10}$ mean affected	0.019137	0.16983
$\log_{10}$ SD affected	0.1600	0.2253
Number of affected cases	31	89

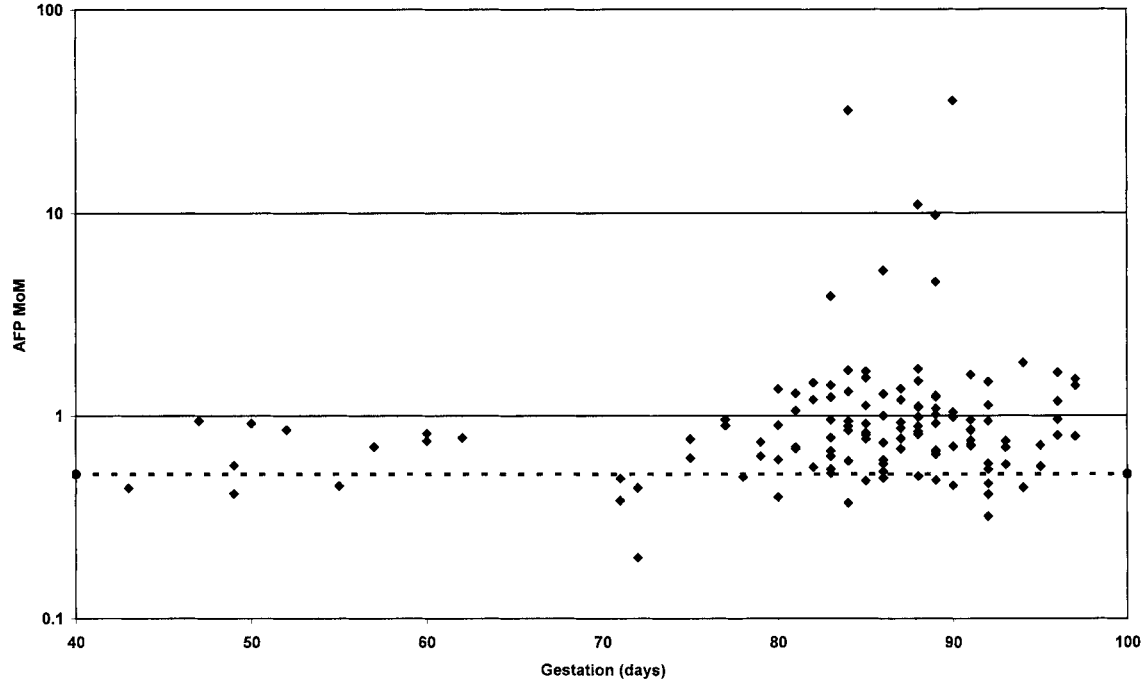

Figure 3—Distribution of AFP MoM with gestational age in Down syndrome cases. Dotted line is the 5th centile of normality

1999a), and falling precipitously prior to 10 weeks (Spencer *et al.* (this article)).

Studies that have suggested that total hCG and PAPP-A can be a useful first trimester screening combination have shown detection rates of 63% (Haddow *et al.*, 1998), very similar to our data for samples collected during the 85–97 day period. Such studies have been biased by the inclusion of samples at the very end of the first trimester. In screening practice samples will be collected over a much wider gestational range and be matched to the time period that nuchal translucency is valid (73–97 days). We have shown therefore that temporal changes in analyte median MoM in affected cases will have considerable importance and impact on detection rates and will require us to focus screening on a defined optimum period using specifically optimized algorithms (Reynolds *et al.*, 1998).

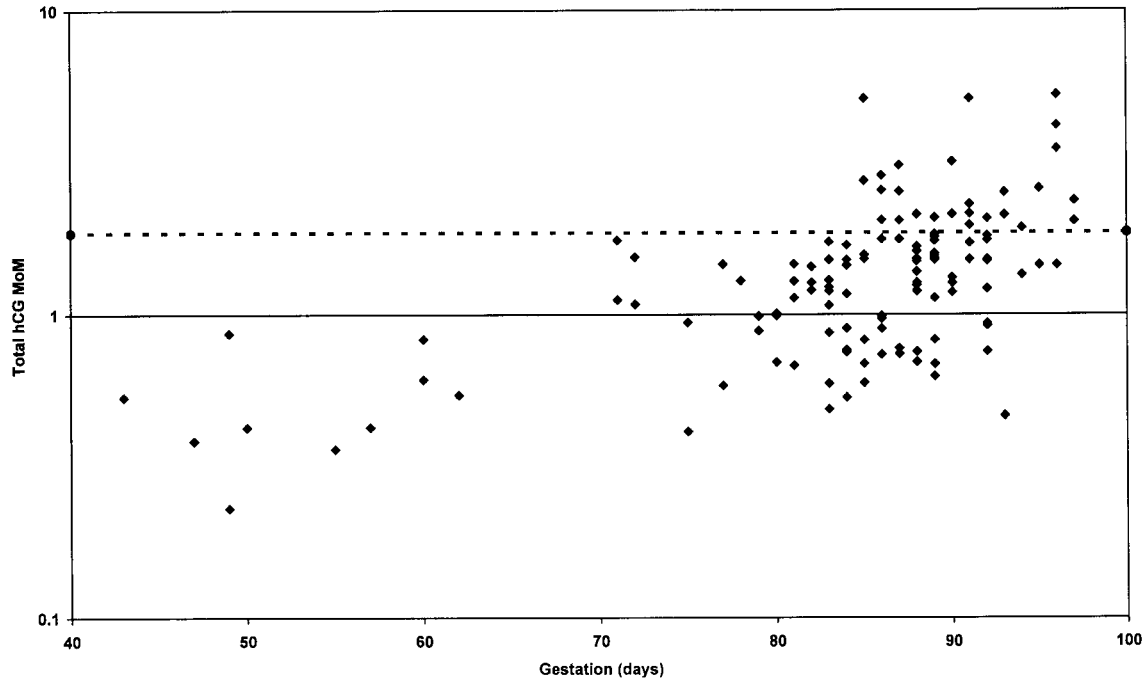

Figure 4—Distribution of total hCG MoM with gestational age in Down syndrome cases. Dotted line is the 95th centile of normality

Table 5—Published studies of total hCG in the first trimester grouped by median gestational age

Study	No. of cases	Median MoM	Weeks' gestation (median and range)
Studies with median gestation <11 weeks			
Brock *et al.* (1990)	21	1.43	10 (7–14)
Johnson *et al.* (1991)	11	0.91	10 (9–12)
Kratzer *et al.* (1991)	17	1.23	10 (9–12)
Hogdall *et al.* (1992)	14	1.10	10 (9–12)
Van Lith *et al.* (1992)	24	1.19	10 (9–12)
Aitken *et al.* (1993)	16	0.97	10 (6–14)
Macintosh *et al.* (1994)	20	1.40	10 (8–14)
Biagiotti *et al.* (1995)	41	1.12	10 (8–12)
Wald *et al.* (1996)	77	1.23	10 (8–14)
Total	**241**	**1.119**	
Studies with median gestation ⩾11 weeks			
Cuckle *et al.* (1988)	22	1.10	11 (7–12)
Crandall *et al.* (1993)	11	1.73	12 (11–14)
Brizot *et al.* (1995)	41	1.50	11 (10–13)
Haddow *et al.* (1998)	48	1.54	11 (9–13)
Casals *et al.* (1999)	32	1.63	12 (10–14)
Spencer *et al.* (this article)	130	1.31	12 (10–14)
Total	**284**	**1.412**	
Other studies with median gestation indeterminate			
Bogart *et al.* (1989)	6	1.17	(9–11)
Isles *et al.* (1993)	25	1.35	(8–14)
Kellner *et al.* (1994)	5	0.90	(8–14)
Forest *et al.* (1995)	12	1.83	(9–13)
Hallahan *et al.* (1998)	63	1.3	(10–13)
All studies	**636**	**1.319**	

ACKNOWLEDGEMENTS

We acknowledge the support of CIS in providing the reagents and instrumentation to carry out this study.

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