PRENATAL DIAGNOSIS, VOL. **17:5:** 407–413 (1997)

SCREENING FOR DOWN SYNDROME PREGNANCY USING β-CORE FRAGMENT: PROSPECTIVE STUDY

TAICHI ISOZAKI[1], GLENN E. PALOMAKI[2], RAY O. BAHADO-SINGH[1,3] AND LAURENCE A. COLE[1]*

[1]*Department of Obstetrics and Gynaecology, Yale University School of Medicine, New Haven, CT, U.S.A.*
[2]*Foundation for Blood Research, Scarborough, ME, U.S.A.*
[3]*Department of Genetics, Yale University School of Medicine, New Haven, CT, U.S.A.*

Received 3 October 1996
Revised 21 November 1996
Accepted 25 November 1996

SUMMARY

Two recent publications by Cuckle *et al.*, and one each by Canick *et al.* and Kellner *et al.*, describe the use of urine β-core fragment measurements as a screening test for Down syndrome pregnancies. Median levels of over 5·4 MOM were reported for cases of Down syndrome, with an over 72 per cent detection rate for a 5 per cent false-positive rate. Urine β-core fragment was suggested as a superior screening test for Down syndrome pregnancies. These four studies were retrospectives, with samples from affected cases collected at different sites from those from normal cases. In the present study, prospective data were collected for 726 pregnancies over a 9-month period at a single medical centre. Fresh samples were assayed continuously, without knowledge of the karyotype. Urinary β-core fragment levels in 709 unaffected samples continually declined from 12 to 24 weeks of pregnancy. A logarithmic fit was optimal for the median curve. The log standard deviation of unaffected samples was 0·368. All 13 Down syndrome cases had levels exceeding 1·0 MOM, with a median value of 4·1 MOM. Eight of 13 Down syndrome cases (62 per cent) had levels exceeding the 95th centile. Results have not been adjusted for maternal age, which may improve the detection rate. The results reported here, while less impressive than those reported previously, confirm the usefulness of urine β-core fragment as a screening test for Down syndrome. Because of the prospective nature of this study, the 62 per cent sensitivity suggested here might be more representative of the true performance of urinary β-core fragment in clinical practice than the higher rates observed in previous studies. Results for this single urine test are similar to those for triple screen and other serum combination tests. Single analyte urine β-core fragment tests, or β-core fragment combination protocols, may eventually replace serum analytes in screening for Down syndrome pregnancies.

Prenat. Diagn. 17: 407–413, 1997.
No. of Figures: 2. No. of Tables: 1. No. of References: 23.

KEY WORDS: Down syndrome; β-core fragment; human chorionic gonadotropin; screening

INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two dissimilar subunits, α- and β-, linked non-covalently. At least five hCG-related molecules can be detected in pregnancy serum. These are non-nicked hCG (the biologically active hormone), deactivated or nicked hCG (that with a cut in the β-subunit peptide chain between residues 47 and 48), free α-subunit, free β-subunit, and nicked free β-subunit (Cole *et al.*, 1991, 1993). The same five molecules can be detected in urine. In addition, β-core fragment, a degraded molecule for comprising two β-subunit fragments, namely residues 6–40 and 55–92 held together by disulphide bonds, can

*Correspondence to: Laurence A. Cole, PhD, Associate Professor, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, U.S.A.

Contract grant sponsor: NIH; Contract grant number: CA46828.

CCC 0197–3851/97/050407–07 $17.50

also be detected in urine (Birken *et al.*, 1988). The β-core fragment results from the degradation of hCG and free β-subunit, by a complex pathway, which seemingly involves nicking of hCG by macrophage enzymes, subsequent splitting of nicked hCG into subunits, and ultimately the degradation of nicked free β-subunit in the kidney (Kardana and Cole, 1994; Lefort *et al.*, 1986). Levels of β-core fragment may reflect a combination of hormone production and macrophage and other degradative enzyme activities.

Down syndrome (trisomy 21) is the most common chromosomal cause of mental retardation. Biochemical analyte levels can be altered in Down syndrome pregnancies and can be used to assess risk. In the late 1980s, a triple screen test was developed, comprising hCG α-fetoprotein, and unconjugated oestriol, for detecting Down syndrome and other aneuploidies in second-trimester pregnancies (Bogart *et al.*, 1987; Wald *et al.*, 1988; Canick, 1990). More recently, serum free β-subunit tests and free β-subunit/α-fetoprotein combinations have been introduced as alternative tests for screening second-trimester pregnancies (Macri *et al.*, 1990; Spencer *et al.*, 1991; Spencer *et al.*, 1993). The best free β-subunit combination, or the optimal triple screen test, however, detects only 60–70 per cent of Down syndrome cases, with a 5 per cent false positive rate. At these detection and false-positive rates, a multitude of unnecessary amniocenteses are performed and a significant minority of aneuploidies are overlooked.

Recently, Cuckle *et al.* (1994) measured urine β-core fragment in seven Down syndrome and 67 unaffected pregnancy urines. They found that urine β-core fragment was a better discriminator of Down syndrome in second-trimester pregnancies than the serum tests, and proposed that measurements of this urine analyte might replace existing serum screening tests. A year later, the same group published the results of a larger study with 24 Down syndrome and 294 unaffected pregnancies (Cuckle *et al.*, 1995). They reported an 80 per cent detection rate for Down syndrome pregnancies with a 5 per cent false-positive rate. In the same year, Canick *et al.* (1995) reported similar results in a study of 14 Down syndrome and 91 unaffected cases—88 per cent detection of a 5 per cent false-positive rate. A sensitivity of 80 per cent or more for a single assay is outstanding and a major improvement over existing technology.

Here, we present the first prospective study using 726 routinely collected urine samples assayed twice weekly for β-core fragment without being frozen, collected over 9 months at a single prenatal diagnostic testing centre.

MATERIALS AND METHODS

Urine samples were collected from women with singleton pregnancies at 12–24 weeks of gestation, coming for amniocentesis at the Maternal–Fetal Medicine Unit at Yale-New Haven Hospital. Between August 1995 and May 1996, 726 urine samples were volunteered (whilst waiting or during preparation for amniocentesis). Oral consent was sought, using a protocol approved by Yale University Human Investigation Committee. Urines were collected from 709 women with a normal karyotype, 13 with Down syndrome, two cases of Edward syndrome (trisomy 18), and two with Patau syndrome (trisomy 13) pregnancies. Of 726 patients, 71 per cent came for amniocentesis because of age-related anxiety (over 35 years old); 23 per cent because of a positive triple screen test; 1·1 per cent because of abnormal ultrasound; and 3·7 per cent because of a previous aneuploid pregnancy or other reason. A breakdown of patients, gestational age, maternal age, and karyotype is presented in Table I. We are aware that 13 cases of Down syndrome is high for 726 amniocenteses. We have no obvious explanation for this high incidence.

Urine samples were refrigerated immediately after collection. Twice each week, urine samples were carried to the laboratory and assayed blindly for β-core fragment levels. Results were entered into a Microsoft Excel-7 computer spreadsheet. Gestational age, determined by ultrasound, was obtained from the Maternal–Fetal Medicine Unit computer. Two to three weeks after urine collection, the karyotype was obtained from the records at the Prenatal Diagnosis Service, in the Department of Genetics at Yale University. Maternal age and karyotype were also entered into the computer spreadsheet.

β-core fragment levels were determined by the B210 assay, as described previously (Cole *et al.*, 1994). This is a two-step sandwich assay. Briefly, microtitre plates are coated with monoclonal antibody B210 (gift from O'Connor and Canfield at Columbia University, New York, NY, U.S.A.); urine samples are added; and β-core fragment is extracted. Plates are washed and peroxidase-labelled hCGβ antisera (Bios Specific, Emmerville,

Table I—Breakdown of patients according to karyotype

Karyotype	Gestational age		Maternal age	MOM
Normal karyotype	**mean 16·4 ± 4·0 weeks**	***n*=709**	**mean 35·4 ± 4·0 years**	**1·0***
46,XX	mean 16·5 ± 3·9 weeks	*n*=369	mean 35·3 ± 3·9 years	1·0*
46,XY	mean 16·4 ± 4·1 weeks	*n*=340	mean 35·5 ± 4·1 years	1·0*
Tested due to age	mean 15·9 ± 1·2 weeks	*n*=512	mean 37·1 ± 2·2 years	0·97*
Tested due to triple screen†	mean 17·8 ± 1·3 weeks	*n*=163	mean 30·6 ± 4·1 years	1·26*
Aneuploid	**mean 17·5 ± 2·8 weeks**	***n*=17**	**mean 37 ± 4·3 years**	4·1*
47,XX,+21	13 weeks 6 days		41	4·7
47,XX,+21	15 weeks 0 days		38	5·2
47,XY,+21	15 weeks 3 days		‡	29·1
47,XX,+21	15 weeks 6 days		36	2·9
47,XX,+21	16 weeks 3 days		42	1·4
47,XX,+21	16 weeks 5 days		37	1·0
47,XX,+21	17 weeks 6 days		44	5·6†
47,XX,+21	18 weeks 5 days		37	4·4
47,XY,+21	18 weeks 5 days		‡	4·1
47,XY,+21	18 weeks 5 days		‡	1·2
47,XY,+21	20 weeks 4 days		31	4·0
47,XY,+21	21 weeks 0 days		‡	1·7
47,XY,+21	24 weeks 0 days		29	17·7
47,XX,+18	13 weeks 0 days		39	0·32
47,XY,+18	17 weeks 2 days		‡	0·02
47,XY,+13	15 weeks 4 days		38	23·6
47,XY,+13	18 weeks 5 days		35	0·35

*Median MOM.
†Tested due to positive triple screen.
‡Samples were coded to protect patient privacy; age not retrievable after coding.

CA, U.S.A.) are added to quantitate bound β-core fragment. After a further wash, substrate is added and peroxidase enzyme activity is measured spectrometrically. Urine samples were diluted in the range 1–50 to 1–10 000 with buffer for this assay. Initially, to ensure the integrity of the assay, all samples were tested at two dilutions (1 to 100 and 1 to 1000). Further dilutions were made as needed. The B210 assay was standardized with β-core fragment batch SB455 (Birken, Columbia University, New York, NY, U.S.A.), calibrated by amino acid analysis. The B210 assay detects only β-core fragment, with less than 0·3 per cent cross-reactivity with free β-subunit and hCG.

Urines were collected at random times with no uniformity in the time of voiding. β-core fragment levels were normalized to adjust for variations in urine concentration by dividing by creatinine concentration. Creatinine concentration was determined using the Sigma Chemical Co. spectrometric creatinine kit (St Louis, MO, U.S.A.) and the dictated procedures. Immunoassay results (ng/ml) were normalized to urine creatinine concentration (ng/mg creatinine).

Results were analysed at the Foundation for Blood Research in Maine, using their published β-core fragment MOM methods (Canick *et al.*, 1995). A simple logarithmic fit was found to be optimal for smoothing. The observed medians are reported along with the standard deviation, estimated by the 10th–90th centile difference divided by 2·56.

RESULTS

Figure 1 shows the relationship between urine β-core fragment concentration and gestational age in 709 unaffected and 17 karyotypically abnormal pregnancies. The unaffected pregnancy data best fit a log Gaussian distribution (between the fifth and 95th centiles) and the equation $\log_{10}(x)=4{\cdot}244-0{\cdot}0754(y)$, where x is the concentration of β-core fragment (ng/mg creatinine) and y

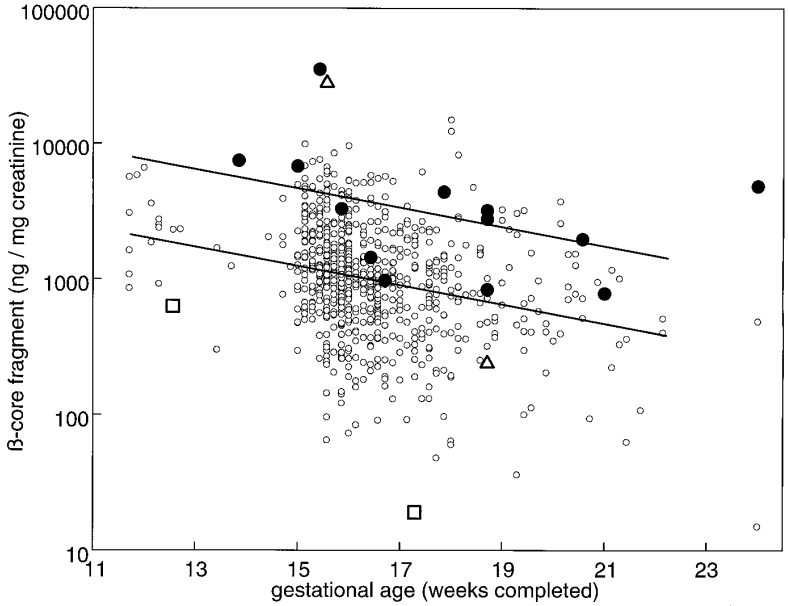

Fig. 1—Levels of β-core fragment in urine samples from 726 pregnancies, 13 affected with Down syndrome (●) two with Edward syndrome (□), and two with Patau syndrome (△), and 709 controls (○). The lower line is the 50th centile for unaffected pregnancies; the upper line is the 95th centile

is gestational age (weeks). The log median for unaffected samples was 0·000 and the log standard deviation was 0·368.

Table I shows the karyotype, gestational and maternal ages, and MOM values for the 17 aneuploidies. All 13 Down syndrome cases had levels exceeding 1·0 MOM, with a median value of 4·1 MOM. This represents a significant elevation of β-core fragment levels ($P=0{\cdot}008$; two-sided probability Wilcoxon rank test). As shown in Fig. 1, eight of 13 Doiwn syndrome cases (62 per cent) had levels exceeding the 95th centile of unaffected pregnancies.

The levels were very low for both Edward syndrome cases, at 0·32 and 0·02 MOM, respectively. By comparison, the lowest value for unaffected samples was 0·06 MOM. Sporadic results were obtained with the two Patau syndrome cases, 23·6 and 0·35 MOM, respectively.

We tested the ability of spot creatinine determinations to adjust urine β-core fragment levels over the urine concentration range. When β-core fragment MOM values were plotted against creatinine concentration, an almost straight line response was observed with nearly zero slope (Fig. 2). The median MOM values were 0·91, 1·03, 1·17, 0·96, and 0·96 for creatinine concentrations 0–0·5, 0·5–1·0, 1·0–1·5, 1·5–2·0, and 2·0–2·5, respectively (intercept 1·0 MOM, slope 0·006 MOM/mg creatinine, $r^2=0{\cdot}002$).

DISCUSSION

To date, six papers have been published describing β-core fragment measurements in unaffected and Down syndrome pregnancies. Four of these papers (Cuckle *et al.*, 1994, 1995; Canick *et al.*, 1995; Kellner *et al.*, 1996) report Down syndrome cases with median values exceeding 5·4 MOM and detection rates over 72 per cent (at 5 per cent false-positive rate). These findings for a single Down syndrome test are without equal and suggest β-core fragment as a complete replacement for serum triple screen or free β-subunit screening protocols. Two other papers, however, report much less impressive results (Hayashi and Kozu, 1995; Spencer *et al.*, 1996).

Hayashi and Kozu (1995) used the Wako Inc. Wakotest β-core kit (Osaka, Japan) to evaluate β-core fragment levels in unaffected and Down syndrome pregnancies. They found median values

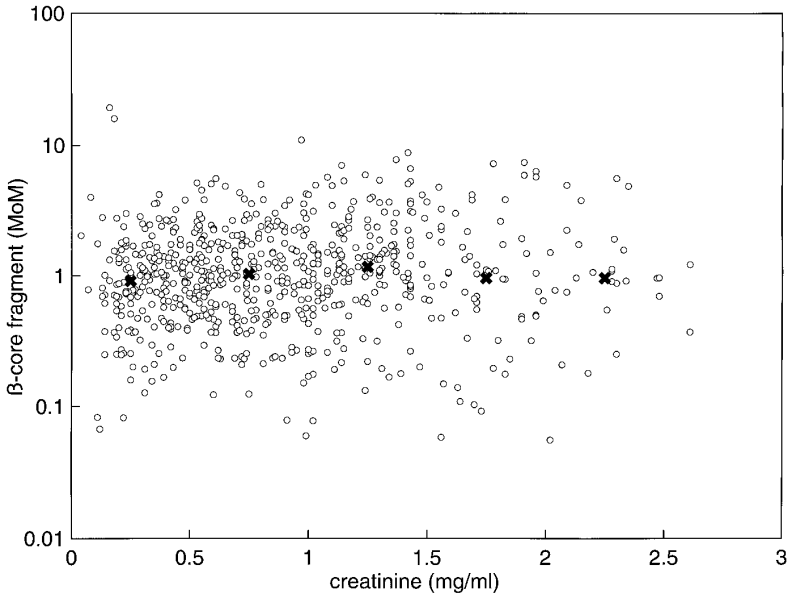

Fig. 2—Relationship between MOM values for unaffected urine samples ($n=709$, ○) and creatinine concentration. Symbols (**X**) mark median MOM values for 0–0·5, 0·5–1·0, 1·0–1·5, 1·5–2·0, and 2·0–2·5 mg/ml creatinine

of only 1·3 MOM in Down syndrome cases. They inferred that β-core fragment may be no better than free β-subunit or hCG. This study, however, was flawed, since the Wakotest β-core kit is not a conventional β-core fragment assay. The assay was designed for tumour marker applications. It measures hCG free β-subunit using a free β-subunit standards curve. The assay cross-reacts with β-core fragment (Cole, 1995; Cole *et al.*, 1997; Tanaka and Cole, 1994).

Recently, Spencer *et al.* (1996) evaluated β-core fragment as a screening test for Down syndrome pregnancies. They reported median values for affected cases of 2·35 MOM. This study may also be flawed. The unaffected samples described in this paper were collected at different sites to those from Down syndrome cases. An irregular median curve is described for 400 unaffected pregnancy samples, rising from 10 weeks to a peak at 13 weeks' gestation (instead of peaking at 10 weeks), then declining. This contrasts with the continuous declining curve, 11 weeks onwards, observed in the present study, the results in the four other β-core fragment and Down syndrome pregnancy publications, and the findings of DeMedeiros *et al.* (1992), the keynote study for β-core fragment levels during pregnancy (819 urine samples). The irregular curve was accompanied by an unduly high log standard deviation (0·42 versus 0·31–0·37 in the present study and in all the other four publications).

The first four papers have other less obvious limitations. Collection and testing of samples is restrospective with a disproportionate number of affected samples. In all four papers, cases were collected either at multiple sites or at different locations to unaffected samples.

Here, we present the results of a continuous prospective study, blindly testing β-core fragment levels at a single amniocentesis testing centre. After 9 months, accumulated β-core fragment data were analysed. A continuous decline was observed in the concentration of β-core fragment in 709 unaffected samples from 12 to 24 weeks of gestation. Using a logarithmic median curve, a log standard deviation of 0·368 was recorded. Over the study period, 13 Down syndrome samples were collected. All 13 had levels exceeding 1·0 MOM, and a median value of 4·1 MOM. Eight of 13 cases exceeded the 95th centile, indicating 62 per cent sensitivity at a 5 per cent false-positive rate. The results reported here, while less impressive than those reported in the four papers (Cuckle *et al.*, 1994, 1995; Canick *et al.*, 1995; Kellner *et al.*,

1996), confirm the usefulness of urine β-core fragment as a superior screening test for Down syndrome screening. Because of the prospective nature of this study, the 62 per cent sensitivity suggested here might be more representative of the performance of β-core fragment in clinical practice than the 72 per cent or greater sensitivity suggested in the four earlier publications. Data (4·1 MOM, 62 per cent sensitivity) were determined using a simple algorithm combining gestational age and β-core fragment concentration. More complex algorithms incorporating maternal age and other variables, as used in other β-core fragment studies and in the triple screen test, may to a small extent improve the detection rate of this test.

Twenty-three per cent of samples were from patients who were offered amniocentesis because of a positive triple screen test. The one Down syndrome case originally referred for amniocentesis because of a positive triple screen was also positive in the β-core fragment test. The median β-core fragment level in the 163 unaffected cases referred to the amniocentesis centre because of a positive triple screen was 1·26 MOM, versus 0·97 MOM for the 512 unaffected cases coming because of age-related concerns. The higher median β-core fragment level in the triple screen-positive group may be due to the selection of samples with raised hCG, or raised related-molecule levels (detected by the triple screen test). To a small extent, this group biases or unduly raises the median β-core fragment level in the control category. It may also, to a small degree, cause an underestimation of the median MOM and predictive values for Down syndrome pregnancies.

The performance of β-core fragment for Down syndrome screening is higher than that of any single serum test and similar to that of the triple screen or free β-subunit double screen combination test. Combining β-core fragment with other urine or serum analytes may further enhance the screening efficiency. Recently, Kellner *et al.* (1996) tested the combination of urinary β-core fragment and total oestriol in 32 cases of Down syndrome and 206 unaffected pregnancies. They detected 80 per cent of Down syndrome cases at a 5 per cent false-positive rate, or 60 per cent of cases at a 1·3 per cent false-positive rate. Tests using a combination of β-core fragment and total oestriol, or possibly combinations with other urine analytes, provide superior results. Such tests are likely to eventually replace the current serum analyte assay protocols. Maternal serum α-fetoprotein is an important test for neural tube defects. If both serum α-fetoprotein and urine β-core fragment are determined, an algorithm connecting both assays may be considered. The cost of collecting, transporting, and testing both urine and serum samples might be a concern. A urine replacement test for serum α-fetoprotein should be identified.

Variable results were observed with non-Down syndrome aneuploidies. The levels were very low for 2 of 2 Edward syndrome cases. One case had levels below the lowest of 709 unaffected samples. Similar, extraordinarily low β-core fragment levels were noted in Edward syndrome cases by Canick *et al.* (1995). These findings are consistent with the very low hCG levels, which are also associated with this aneuploidy (Barkai *et al.*, 1991). A relationship may exist between extremely low β-core fragment levels (below the first centile) and this aneuploidy. Disparate results were observed with the two Patau syndrome cases: one sample had a high MOM value, while the other had a low value. Larger studies are needed with a more significant number of Edward and Patau syndrome cases to investigate a screening application.

In this study and all the preceding β-core fragment Down syndrome screening publications, spot urine β-core fragment levels were normalized to creatinine concentration. We investigated the ability of the creatinine levels to correctly normalize β-core fragment values over the urine concentration range. In a plot of β-core fragment MOM values against creatinine concentration, a virtually straight line response was observed with almost zero slope. This means that β-core fragment in all concentrations of urine, whether very low, as may be observed in the afternnon or evening after a large liquid intake, or high, as observed in first morning urines or after dehydration, may be correctly adjusted by normalization to creatinine concentration. We infer that spot creatinine measurements are suitable for normalizing β-core fragment values to urine concentration for Down syndrome screening applications.

ACKNOWLEDGEMENTS

We wish to acknowledge particularly Dr Maurice J. Mahoney of the Department of Human Genetics at Yale University and the genetics counsellors, Jodi Rucquoi, Elizabeth Merrill, Miriam S. Di Maio, Joann Stanis, and Sarah Turk, who asked patients to volunteer and collected urine samples for this project. We also wish to

thank Dr Howard Cuckle, University of Leeds, U.K., for his notes and advice on this paper. Research was supported by NIH grant CA46828 to L. A. Cole.

REFERENCES

Barkai, G., Chaki, R., Sochat, M., Goldman, B. (1991). Human chorionic gonadotrophin and trisomy 18, *Am. J. Med. Genet.*, **41,** 52–53.

Birken, S., Armstrong, E.G., Kolks, M.A.G., Cole, L.A., Agosto, G.M., Krichevsky, A., Canfield, R.E. (1988). The structure of the human chorionic gonadotropin β-subunit core fragment from pregnancy urine, *Endocrinology*, **123,** 572–583.

Bogart, M.H., Pandian, M.R., Jones, O.W. (1987). Abnormal maternal serum hCG levels in pregnancies with fetal chromosome abnormalities, *Prenat. Diagn.*, **7,** 623–630.

Canick, J.A. (1990). Screening for Down syndrome using maternal serum alpha-fetoprotein, unconjugated estriol and hCG, *J. Clin. Immunoassay*, **13,** 30–33.

Canick, J.A., Kellner, L.H., Saller, D.N., Jr, Palomaki, G.E., Walker, R.P., Osathanondh, R. (1995). Second trimester levels of maternal urinary gonadotropin peptide in Down syndrome pregnancy, *Prenat. Diagn.*, **15,** 752–759.

Cole, L.A. (1995). Down's syndrome screening using urine β-core fragment test; choice of immunoassay, *Prenat. Diagn.*, **15,** 679–682.

Cole, L.A., Kardana, A., Andrade-Gordon, P., Gawinowicz, M.A., Morris, J.C., Bergert, E.R., O'Connor, J., Birken, S. (1991). The heterogeneity of hCG: III. The occurrence, biological and immunological activities of nicked hCG, *Endocrinology*, **129,** 1559–1567.

Cole, L.A., Kardana, A., Park, S.-Y., Braunstein, G.D. (1993). The deactivation of hCG by nicking and dissociation, *J. Clin. Endocrinol. Metab.*, **76,** 704–710.

Cole, L.A., Kardana, A., Seifer, D.B., Bohler, H.C. (1994). Urine hCG β-subunit core fragment, a sensitive test for ectopic pregnancy, *J. Clin. Endocrinol. Metab.*, **78,** 497–499.

Cole, L.A., Isozaki, T., Palomaki, G., Canick, J., Iles, R., Kellner, L., Saller, D.N., Jr, Cuckle, H. (1997). Detection of β-core fragment in second trimester Down's syndrome pregnancies, *Rec. Adv. Prenat. Diagn. Aneuploidy*, in press.

Cuckle, H.S., Iles, R.K., Chard, T. (1994). Urinary β-core human chorionic gonadotropin: a new approach to Down's syndrome screening, *Prenat. Diagn.*, **14,** 953–958.

Cuckle, H.S., Iles, R.K., Sehmi, I.H., Chard, T., Oakey, R.E., Davies, S., Ind, T. (1995). Urinary multiple marker screening for Down's syndrome, *Prenat. Diagn.*, **15,** 745–751.

DeMedeiros, S.F., Amato, F., Mathews, C.D., Norman, R.J. (1992). Urinary concentration of β-core fragment of hCG throughout pregnancy, *Obstet. Gynecol.*, **80,** 223–228.

Hayashi, M., Kozu, H. (1995). Maternal urinary β-core fragment of hCG/creatinine ratios and fetal chromosomal abnormalities in the second trimester of pregnancy, *Prenat. Diagn.*, **15,** 11–16.

Kardana, A., Cole, L.A. (1994). hCG β-subunit nicking enzymes in pregnancy and cancer patient serum, *J. Clin. Endocrinol. Metab.*, **79,** 761–767.

Kellner, L.H., Canick, J.A., Palomaki, G.E., Saller, D.N., Lambert-Meserlian, G.M., Neuer, M., Osathanondh, R., Bombard, A.T. (1996). Urinary markers: a new approach to screening for Down syndrome in the second trimester, *Am. J. Hum. Genet.*, **59,** A41 (207).

Lefort, G.P., Stolk, J.M., Nisula, B.C. (1986). Renal metabolism of the beta-subunit of human choriogonadotropin in the rat, *Endocrinology*, **119,** 924–931.

Macri, J.N., Kasturi, R.V., Krantz, D.A., Cook, E.J., Moore, N.D., Young, J.A., Romero, K., Larsen, J.W., Jr. (1990). Maternal serum Down syndrome screening: free beta-protein is a more effective marker than human chorionic gonadotropin, *Am. J. Obstet. Gynecol.*, **163,** 1248–1253.

Spencer, K. (1991). Evaluation of an assay of the free beta-subunit of choriogonadotropin and its potential value in screening for Down's syndrome, *Clin. Chem.*, **37,** 809–814.

Spencer, K., Macri, J.N., Anderson, R.W., Aitken, D.A., Berry, E., Crossley, J.A., Wood, P.J., Coombes, E.J., Stroud, M., Worthington, D.J. (1993). Dual analyte immunoassay in neural tube defect and Down's syndrome screening: results of a multicentre clinical trial, *Ann. Clin. Biochem.*, **30,** 394–401.

Spencer, K., Aitken, D.A., Macri, J.N., Buchanan, P.D. (1996). Urine free beta hCG and beta core in pregnancies affected by Down's syndrome, *Prenat. Diagn.*, **16,** 605–613.

Tanaka, A., Cole, L.A. (1994). Improved methods for detecting beta-core in normal and cancer patient urines, *Clin. Chem.*, **40,** 2317–2318.

Wald, N.J., Cuckle, H.S., Densem, J.W. (1988). Maternal serum unconjugated estriol as an antenatal screening test for Down syndrome, *Br. J. Obstet. Gynaecol.*, **95,** 334–335.