PRENATAL DIAGNOSIS
Prenat Diagn 2010; **30**: 000–000.
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.2578

The relationship between first trimester fetal growth, pregnancy-associated plasma protein A levels and birthweight

O. Habayeb[1], A. Daemen[2], D. Timmerman[3], B. De Moor[2], G. A. Hackett[1], T. Bourne[3,4] and C. C. Lees[1]*

[1]*Division of Fetal-Maternal Medicine, Cambridge University Hospitals NHS Trust, Hills Road, Cambridge, UK*
[2]*Department of Electrical Engineering, Katholieke Universiteit Leuven, Leuven, Belgium*
[3]*Department of Obstetrics and Gynecology, University Hospitals Leuven, Campus Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium*
[4]*Imperial College London, Hammersmith Campus, Du Cane Road, London, UK*

Objective We sought to define the relationship between first trimester fetal growth, pregnancy-associated plasma protein A (PAPP-A) levels and birthweight.

Methods Two-hundred and one women with repeat first trimester crown-rump length (CRL) measurements were included. In 194, the first trimester PAPP-A value was known and in 169 there was complete data including birthweight. Fetal growth curves were derived using functional linear discriminant analysis (FLDA) and growth compared between those with >90th, 10th to 90th and <10th percentile PAPP-A multiple of median (MoM) levels and birthweight percentiles.

Results Median maternal age was 35 years, gestation at PAPP-A sampling and of first scan was 11 weeks. Median delivery gestation was 40 weeks and birthweight 3425 g. There was no association between first trimester fetal CRL growth and either PAPP-A MoM percentile or birthweight percentile. There was a significant correlation between PAPP-A MoM and birthweight percentile ($p = 0.0004$).

Conclusions First trimester fetal growth rate is not related to birthweight percentile or first trimester PAPP-A levels. Irrespective of gestation, a low PAPP-A is associated with delivery of a smaller baby, and a high PAPP-A with a larger baby. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: PAPP-A; first trimester fetal growth; CRL; birthweight; FLDA; functional linear discriminant analysis

INTRODUCTION

The association between low pregnancy-associated plasma protein A (PAPP-A) at 11 to 14 weeks and delivery of a small for gestational age (SGA) baby has been known for a number of years (Ong *et al.*, 2000; Smith *et al.*, 2002, 2006; Krantz *et al.*, 2004; Spencer *et al.*, 2008). Early first trimester PAPP-A levels can predict early pregnancy failure (Westergaard *et al.*, 1983) and the risk of aneuploidy, particularly Down syndrome (Bindra *et al.*, 2002). These studies investigated the level of serum PAPP-A measured at the time of first trimester aneuploidy screening, namely at 11 to 14 weeks. There are no published data regarding the relationship between earlier first trimester PAPP-A measurements and either first trimester growth or eventual birthweight.

To date, first trimester fetal growth has been inferred by comparing single 'one off' measurements of fetal size based on either crown-rump length (CRL) or gestational sac (GS) size (Mantoni and Pedersen, 1982; Falco *et al.*, 1996; Reljic, 2001; Choong *et al.*, 2003) to expected measurements based on cross-sectional presumed normative data. The difficulty with this approach is that interpreting fetal size depends on the gestation assigned to that fetus. The result is that if a fetus is already small when it is measured, it may be inappropriately assigned a gestational age that is less than the true value. On the other hand, as fetal growth rate in the first trimester is almost linear in normal pregnancies not destined to miscarry (Bottomley *et al.*, 2009), comparisons of first trimester fetal growth are less sensitive to errors or variation in gestational age assessment. In women undergoing assisted reproduction, first trimester fetal size has been shown to be related to birthweight (Bukowski *et al.*, 2007). The relationship between the rate of fetal growth, as opposed to 'one off' measurements of size in the first trimester with either subsequent birthweight percentiles or with early PAPP-A has not been defined.

Recently, fetal growth has been studied using the statistical technique of functional linear discriminant analysis (FLDA) in normal pregnancies and those that result in miscarriage (Bottomley *et al.*, 2009). In that study, the fetal growth pattern discrimated between normal and abnormal fetal outcome better than simple comparisons of fetal size for gestation. FLDA uses curves instead of one-dimensional variables as predictor

*Correspondence to: C. C. Lees, Consultant in Obstetrics and Fetal-Maternal Medicine, Rosie Maternity, Addenbrooke's Hospital, Cambridge University Hospitals NHS Trust, Hills Road, Cambridge CB2 2QQ, UK.
E-mail: Christoph.Lees@Addenbrookes.nhs.uk

Received: 6 May 2010
Revised: 20 May 2010
Accepted: 23 May 2010

variables, distinguishing between groups by maximising the ratio of the between-group variation to the within-group variation. FLDA is particularly useful where only fragments of the curves for individual cases are observed.

An understanding of the relationship between early first trimester serum PAPP-A and fetal growth could help appropriate scheduling of clinical follow-up. We therefore investigated the association between early first trimester PAPP-A and birthweight percentile, early PAPP-A and first trimester fetal growth and finally the relationship between first trimester growth and birthweight percentiles.

METHODS

Data were collected from women attending consecutively for phlebotomy to measure early serum PAPP-A as part of a modified trisomy 21 screening programme between September 2007 and May 2008. Patients were not included where the pregnancy ended in first trimester miscarriage or where a chromosomal abnormality was detected prenatally. The data were collected as part of a registered clinical audit as part of routine clinical management and anonymised before analysis.

All women had undergone two or more first trimester ultrasound scans between 6 and 14 weeks of gestation for which CRL measurements were available. Scans were performed on General Electric E8, Voluson 730 Pro (GE Healthcare, Fairfield, CT, USA), Siemens Acuson Sequoia or Antares (Siemens Ultrasound, Munich, Germany). The estimated due date (EDD) was derived from an ultrasound scan performed between 10 and 13 weeks using Robinson CRL charts (Robinson and Fleming,1974) in accordance with National guidance (NICE, 2008). The 10- to 13-week scan was taken as the reference scan even if an earlier ultrasound scan had been performed, and all gestation data normalised to this scan. In one case of *in vitro* fertilisation (IVF) pregnancy, gestation was derived by taking the fertilisation date as day 14.

Birthweight data were retrieved from the hospital's electronic birth register and expressed as percentiles in relation to the gestational age at delivery. PAPP-A was measured using the AutoDELFIA® PAPP-A kit (PerkinElmer LAS (UK) Ltd., Beaconsfield, UK) and expressed as multiples of the expected median for a pregnancy of the same gestation multiple of median (MoM) corrected for maternal weight, ethnicity and smoking status. The rate of growth was calculated as the difference between CRL measurements in millimetres divided by the difference in gestational age in weeks.

Statistical analysis

The relationship between the various variables was tested using the Spearman correlation coefficient. CRL growth curves were obtained with FLDA, an extension of classical linear discriminant analysis (James and Hastie, 2001). In this data set, CRL has been measured multiple times during the first trimester. FLDA was used to analyse the rate of change in CRL as a function of gestational age, for its ability to discriminate growth according to birthweight and PAPP-A MoM. Serial observations from each individual were modelled with a spline function (a curved line formed by two or more vertices), parameterised with a basis function multiplied by a five-dimensional coefficient vector. These vectors were used to estimate the mean coefficient vector of each group with groups defined as <10th percentile, between 10th and 90th percentile and >90th percentile for birthweight or PAPP-A MoM.

RESULTS

Two-hundred and one patients were studied, constituting 437 scans; in 166 cases there were 2 scans and in 35 there were 3 scans. The PAPP-A values were known in 194/201 patients. Of these, 174 were Caucasian, 4 Asian, 4 African or Caribbean and 12 other ethnicity. Six were smokers and the median maternal weight was 65 kg (range 45–102.2). Birthweight data were available in 169/201 patients.

Where PAPP-A was the comparator, the dataset with complete PAPP-A measurements was analysed and where birthweight was the comparator, the dataset with complete birthweight data was analysed.

The median age at the time of the PAPP-A measurement was 35 years (range 22–49); median gestation at PAPP-A sampling was 11 weeks (range 8–13). The median (range) for the first scan ($n = 201$) was 75 days (43–92), for the second scan ($n = 201$) was 88 days (66–98) and for the third ($n = 35$) was 89 days (82–95). The median difference between scans 1 and 2 was 12 days; between scans 2 and 3 was 7 days.

Distributions for birthweight and PAPP-A

The median gestation at delivery was 40 weeks (range 30–42) and median birthweight 3425 g (range 1350–5040). Birthweight was normally distributed with mean birthweight 3383 g ([10th–90th percentile], [2684–3933]). PAPP-A MoM showed a positively skewed distribution with median MoM 1.048 ([10th–90th percentile], [0.48–2.053]). Birthweight percentiles were derived from these data on which the subsequent analyses were based.

CRL growth and birthweight using FLDA

Curves were constructed with FLDA, taking all available CRL measurements per subject. The cases were split into three groups for comparison: Those where birthweight was <10th percentile, birthweight 10th to 90th percentile and birthweight >90th percentile. For each group, the mean FLDA line was obtained as the average across all curves of cases belonging to that group.

These relationships are shown in Figure 1a and b. There was no difference between these CRL growth

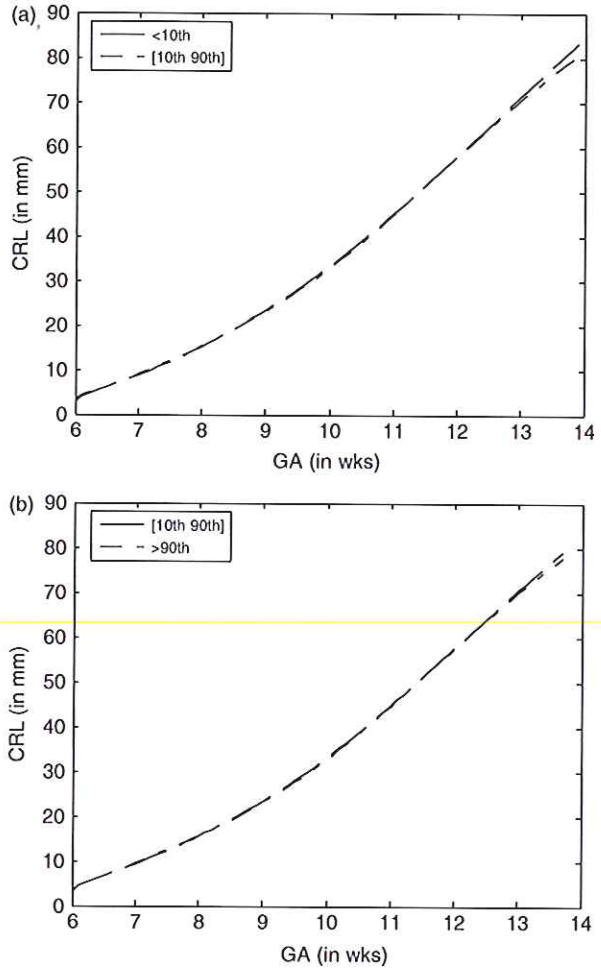


Figure 1—The relationship between CRL growth and birthweight. Curves were constructed with FLDA, taking all available CRL measurements per subject. Birthweights were divided into three groups: <10th percentile, 10th to 90th percentile and >90th percentile. For each group, the mean FLDA line was obtained as the average across all curves of cases belonging to that group. There was no difference between these CRL growth curves as can be seen from the almost complete superimposition regardless of the birthweight percentile <10th percentile compared to 10th to 90th percentiles (a) and >90th percentile compared to 10th to 90th percentiles (b)

curves as can be seen from the almost complete superimposition regardless of the birthweight percentile.

CRL growth and PAPP-A using FLDA

Analogous to the birthweight comparisons, curves for CRL growth were constructed with FLDA with the cases split into three groups for comparison: Those where PAPP-A was <10th percentile, PAPP-A 10th to 90th percentile and PAPP-A >90th percentile.

These relationships are shown in Figure 2a and b. There was no difference between these CRL growth curves as can be seen from the almost complete superimposition regardless of the PAPP-A percentile.

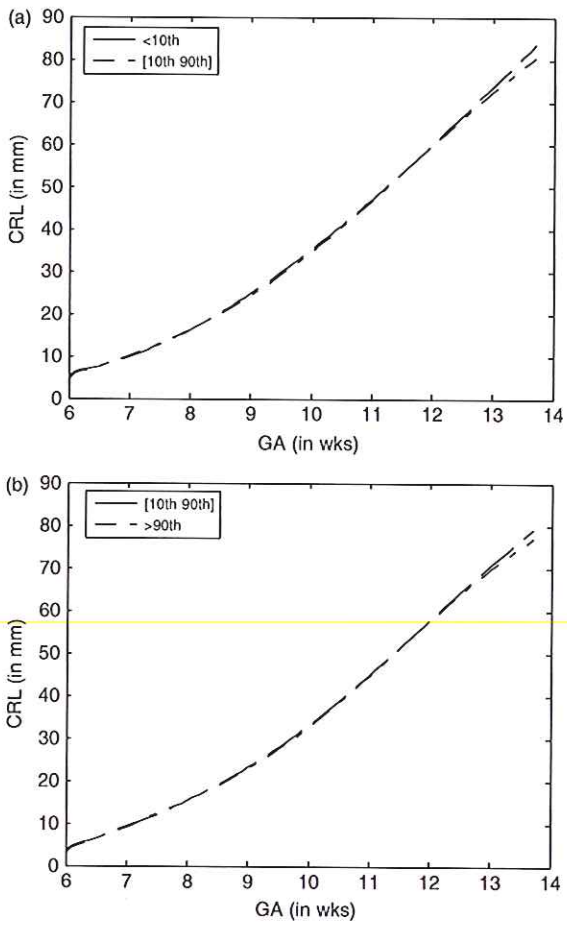


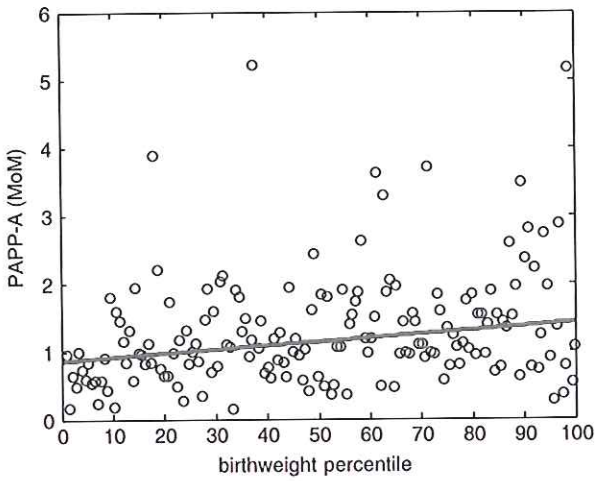
Figure 2—The relationship between CRL growth and PAPP-A MoM. Curves were constructed with FLDA, taking all available CRL measurements per subject. PAPP-A MoM values were divided into three groups: <10th percentile, 10th to 90th percentile and birthweight >90th percentile. For each group, the mean FLDA line was obtained as the average across all curves of cases belonging to that group. There was no difference between these CRL growth curves as can be seen from the almost complete superimposition regardless of the PAPP-A MoM <10th percentile compared to 10th to 90th percentiles (a) and >90th percentile compared to 10th to 90th percentiles (b)

PAPP-A (MoM) in relation to birthweight percentile

In 163 cases, there were data for both PAPP-A and birthweight. There was a highly significant correlation between PAPP-A MoM and birthweight percentile (Spearman correlation coefficient = 0.2755; $p = 0.0004$; Figure 3).

CRL growth rate in relation to birthweight and PAPP-A

The rate of CRL growth was derived by ($CRL_2 - CRL_1$) (mm)/($GA_2 - GA_1$) (weeks). This was plotted against birthweight percentile and PAPP-A MoM. The median rate of growth in this gestation range was 12.4 mm/week. There was no relationship between the rate of first



Color Figure - Online only

Figure 3—PAPP-A (MoM) in relation to birthweight percentile. There was a highly significant correlation between PAPP-A MoM and birthweight percentile (Spearman correlation coefficient = 0.2755; $p = 0.0004$) ($n = 163$)

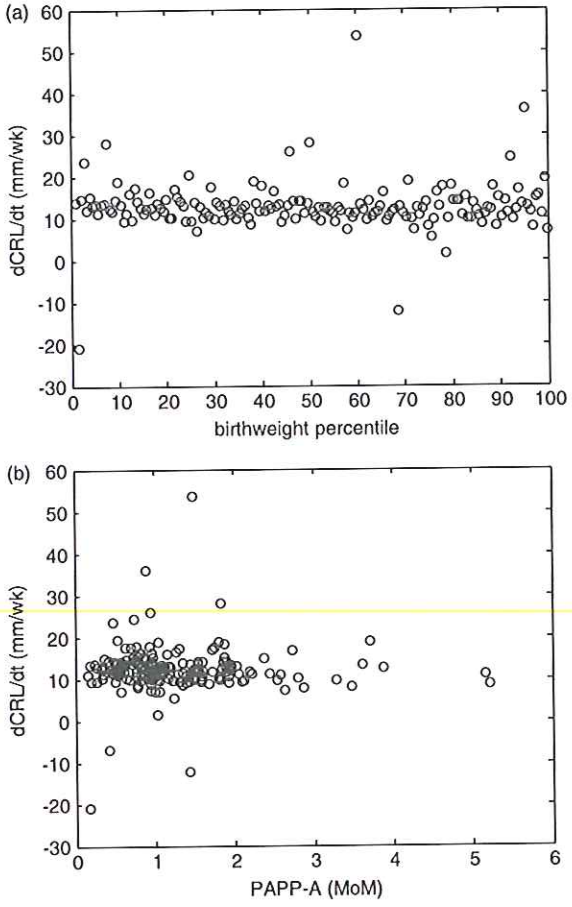
trimester growth and either PAPP-A MoM or birthweight percentile (both Spearman correlation coefficients −0.11; p values 0.12 and 0.15, respectively) (Figure 4a and b).

Rate of CRL growth with gestation

The CRL growth rate (dCRL/dt in mm/week) for each case is plotted against the mid-point gestation between which the ultrasound scans were carried out (Figure 5).

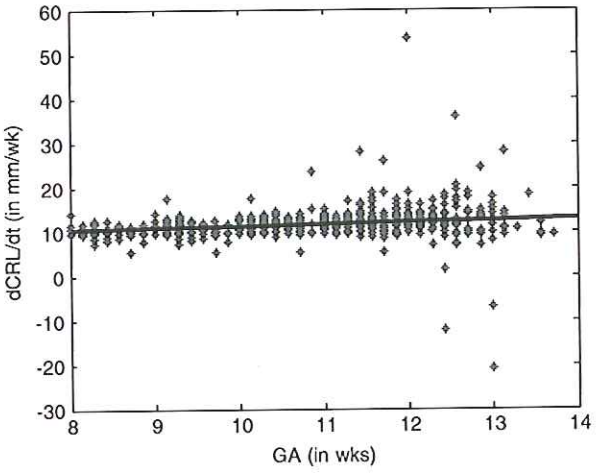
DISCUSSION

We have used FLDA, a statistical technique only recently applied to fetal biometry (Bottomley *et al.*, 2009), to describe embryonic and fetal growth in the first trimester of pregnancy. Using FLDA, a single curve is derived from multiple longitudinal observations from each patient. In this study, two or three data-points per patient have been used. In this way, each patient curve is treated independently thus eliminating potential bias from non-independent data. Although there are no other published reports of the relationship between first trimester fetal growth rate (as assessed by change in CRL with time) and birthweight, the use of FLDA derived growth rate calculations are likely to be robust in that repeat data-points from the same patient are not assumed to be independent. Previous studies have tended to pool data from several pregnancies to generate 'growth curves' introducing the potential for incorrect inferences to be drawn by analysis of data-points that are treated as if they were independent. Our data suggest that fetal growth, as evidenced both by the graphs showing the change in CRL with gestation and by the incremental growth rate in CRL in the first trimester (mm/week), is not related either to birthweight at delivery or first trimester PAPP-A.



Color Figure - Online only

Figure 4—Rate of CRL growth in relation to birthweight and PAPP-A. Rate of CRL growth was derived by (CRL_2-CRL_1) (mm)/(GA_2-GA_1) (weeks). This was plotted against birthweight percentile (a) and PAPP-A MoM (b). Correlation was checked using Spearman correlation coefficient. There was no relationship between the rate of first trimester growth and either PAPP-A MoM or birthweight percentile (both Spearman correlation coefficients −0.11; p values 0.12 and 0.15, respectively)



Color Figure - Online only

Figure 5—Rate of CRL growth with gestation

A CRL 2 to 6 days smaller than would be expected from the gestational age derived from the date of the last menstrual period is associated with a higher risk of an SGA baby (Smith *et al.*, 1998) and, in women undergoing assisted reproduction an observed first trimester fetal CRL size that is greater than expected has been shown to be associated with a larger birthweight (Bukowski *et al.*, 2007). A reduction in fetal growth rate later in pregnancy assessed by the change in biparietal diameter (BPD) expressed as millimetres of growth per week between the first (11–13 weeks) and second trimesters (17–21 weeks) is associated with adverse perinatal outcome. According to the latter study, the odds ratios (OR) for perinatal death is 4.79 (95% CI, 1.43–15.99) when the growth rate is below the 2.5th percentile, and growth rate is associated with birthweight at delivery (Pedersen *et al.*, 2008).

In a further study where CRL was measured between 11 weeks and 13 weeks 6 days and expressed as an *Z*-score, a correlation with birthweight was found as *Z*-CRL and $\log_{10}$ PAPP-A MoM were positively correlated with *Z*-birthweight (Leung *et al.*, 2008). However, it is not clear how the *Z*-scores were derived as the reference CRL in that study was also used to date the pregnancy (Leung *et al.*, 2008). This illustrates a significant problem when analysing early pregnancy data. In many studies CRLs taken in the first trimester are used to date pregnancies, when it is always possible that pregnancies dated in this way are already small. For example, an 8-week pregnancy by dates may be found to have a CRL measurement appropriate for an embryo of only 7 weeks. The assumption would be that the pregnancy was of an earlier gestation, when in fact it may already be small due to restricted growth or delayed implantation, with the attendant risk of later growth problems (Smith *et al.*, 2002).

In the analyses we present, another potential problem, namely that of inferring first trimester growth from the use of 'one off' size measurements, has been largely overcome as growth rate rather than fetal size is being investigated. The FLDA growth curves that we present are based on dating by first trimester CRL measurement. Although a difference of a few days between ultrasound and last menstrual period dating may occur, the effect of this is likely to be insignificant in this study as fetal growth rate is almost constant in the gestation range studied.

Although we found no relationship between first trimester fetal growth rate and birthweight corrected for gestation, this does not necessarily contradict studies where growth has been inferred from a single CRL measurement and a relationship found between growth and birthweight (Smith *et al.*, 1998; Bukowski *et al.*, 2007). These authors expressed first trimester growth as the difference between observed and expected first trimester CRL related to length of pregnancy from a certain conception date. This would more accurately be described as 'size' rather than growth. No study has corrected for the timing of implantation, even in the case of women undergoing assisted reproduction (Bukowski *et al.*, 2007). It was concluded that first trimester fetal growth, not late embryonic implantation, accounted for the differences shown in birthweight and gestation at delivery. While the conception date in that study was known, the date of implantation was not.

We advance a possible alternative hypothesis that first trimester growth in chromosomally normal embryos is in fact constant and birthweight may be different because a later implanting embryo is smaller than expected leading to a difference between observed and expected CRL (although not growth), and this effect continues into the second and third trimesters. These embryos may lead to pregnancies that have a higher chance of adverse outcome; the likelihood of early pregnancy failure is known to be greater with delayed implantation in pregnancies where both embryo transfer and implantation dates were known (Wilcox *et al.*, 1999). As we have dated all pregnancies from a 10- to 13-week scan, we cannot infer from this study a relationship between fetal size (a 'one off' CRL) and birthweight, but do report on the relationship between growth rate and birthweight.

Further, our results also suggest that first trimester growth rate is not related to early PAPP-A levels. These findings are consistent with previous reports in relation to fetal size that suggest independence between CRL measurements and PAPP-A levels (Smith *et al.*, 1998); however, no studies have addressed the relationship between PAPP-A and first trimester growth rate as measured by CRL. In our study all babies were chromosomally normal, although in the case of some chromosomal abnormalities both the first trimester growth rate and PAPP-A levels tend to be low (Spencer and Nicolaides, 2002). It has been known for several years that low PAPP-A levels are associated with a higher likelihood of a baby being born SGA with birthweight <10th percentile (Pihl *et al.*, 2008) and adverse pregnancy outcome (Ong *et al.*, 2000; Smith *et al.*, 2002, 2006; Krantz *et al.*, 2004; Spencer *et al.*, 2008). Thus, the association between low PAPP-A and birthweight is not explained by an association with first trimester fetal growth or indeed size.

A recent study demonstrated a significant relationship between first trimester PAPP-A and birthweight (Peterson and Simhan, 2008). The difficulty in interpreting this relationship is that birthweight was not normalised for gestation and therefore gestation at delivery might have been a confounding variable, particularly in the context of preterm delivery. We have overcome this by relating earlier first trimester PAPP-A MoM to birthweight percentile, the latter being independent of gestation at delivery.

A potential weakness of this and other studies on this subject relates to normalising PAPP-A levels to gestation-related percentiles or MoM. It is customary in spontaneous conceptions to date a pregnancy from a first trimester CRL measurement, and then to relate the PAPP-A level to this derived gestation. In this way, a smaller than expected embryo may in fact have a PAPP-A level related more to its size rather than its true gestation from conception. The only way to overcome this bias would be to date a pregnancy from implantation date, for which there is as yet no clinically robust methodology.

Our data suggest that where early first trimester PAPP-A levels are low, they are not predictive of first trimester growth, nor is first trimester growth (as opposed to 'size') predictive of final birthweight percentile. However, PAPP-A is significantly correlated with birthweight across the entire range of PAPP-A MoMs (Figure 4). By inference, where PAPP-A MoM is low, fetal growth must slow at some point between the end of the first trimester and delivery. However, it is not clear at which point in pregnancy the level of PAPP-A starts to reflect variations in fetal growth, nor can this information be derived from our data. One could speculate that the consequence of a combination of late implantation, low PAPP-A and a smaller than expected fetal size might be associated with abnormal placental implantation, manifesting itself in the late first trimester when the 'switch' from histiotrophic to haematotrophic placentation occurs and is evidenced by maternal and fetal Doppler changes (Jauniaux *et al.*, 1992). There is in fact an association between low PAPP-A in the first trimester and reduced fetal growth rate between 18 and 24 weeks with the reduction persisting into the third trimester (Fox *et al.*, 2009). The clinical importance of this finding is that if ultrasound surveillance is instituted in the context of low PAPP-A levels where the fetus is thought to be chromosomally normal, it would be most likely to be usefully performed after the first trimester, probably in the late second and/or third trimesters.

REFERENCES

Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. 2002. One-stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15030 pregnancies. *Ultrasound Obstet Gynecol* **20**: 219–225.

Bottomley C, Daemen A, Mukri F, *et al.* 2009. Functional linear discriminant analysis: a new longitudinal approach to the assessment of embryonic growth. *Hum Reprod* **24**: 278–283.

Bukowski R, Bukowski R, Smith GC, *et al.* 2007. Fetal growth in early pregnancy and risk of delivering low birthweight infant: prospective cohort study. *BMJ* **334**: • 336.

Choong S, Rombauts L, Ugoni A, Meagher S. 2003. Ultrasound prediction of risk of spontaneous miscarriage in live embryos from assisted conceptions. *Ultrasound Obstet Gynecol* **22**: 571–577.

Falco P, Milano V, Pilu G, *et al.* 1996. Sonography of pregnancies with first-trimester bleeding and a viable embryo: a study of prognostic indicators by logistic regression analysis. *Ultrasound Obstet Gynecol* **7**: 165–169.

Fox NS, Shalom D, Chasen ST. 2009. Second-trimester fetal growth as a predictor of poor obstetric and neonatal outcome in patients with low first-trimester serum pregnancy-associated plasma protein-A and a euploid fetus. *Ultrasound Obstet Gynecol* **33**: 34–38.

James G,• Hastie T. 2001. Functional linear discriminant analysis for irregularly sampled curves. *J R Stat Soc Ser B (Stat Methodol)* **63**: 533–550.

Jauniaux E, Jurkovic D, Campbell S, Hustin J. 1992. Doppler ultrasonographic features of the developing placental circulation: correlation with anatomic findings. *Am J Obstet Gynecol* **166**: 585–587.

Krantz D, Goetzl L, Simpson JL, *et al.* 2004. Association of extreme first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. *Am J Obstet Gynecol* **191**: 1452–1458.

Leung TY, Sahota DS, Chan LW, *et al.* 2008. Prediction of birthweight by fetal crown-rump length and maternal serum levels of pregnancy-associated plasma protein-A in the first trimester. *Ultrasound Obstet Gynecol* **31**: 10–14.

Mantoni M, Pedersen JF. 1982. Fetal growth delay in threatened abortion: an ultrasound study. *Br J Obstet Gynaecol* **89**: 525–527.

National• Institute of Health and Clinical Excellence (NICE). 2008. Antenatal care-routine care for the healthy pregnant woman. 73–76.

Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. 2000. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* **107**: 1265–1270.

Pedersen NG, Wøjdemann KR, Scheike T, Tabor A. 2008. Fetal growth between the first and second trimesters and the risk of adverse pregnancy outcome. *Ultrasound Obstet Gynecol* **32**: 147–154.

Peterson SE, Simhan HN. 2008. First-trimester pregnancy-associated plasma protein A and subsequent abnormalities of fetal growth. *Am J Obstet Gynecol* **198**: e43–e45.

Pihl K, Larsen T, Krebs L, Christiansen M. 2008. First trimester maternal serum PAPP-A, beta-hCG and ADAM12 in prediction of small-for-gestational-age fetuses. *Prenat Diagn* **28**: 1131–1135.

Reljic M. 2001. The significance of crown-rump length measurement for predicting adverse pregnancy outcome of threatened abortion. *Ultrasound Obstet Gynecol* **17**: 510–512.

Robinson HP, Fleming JEE. 1974. A critical evaluation of sonar 'crown-rump length' measurements. *BJOG* **82**: 702–710.

Smith GC, Smith MF, McNay MB, Fleming JE. 1998. First-trimester growth and the risk of low birthweight. *N Engl J Med* **339**: 1817–1822.

Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. 2002. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab* **7**: 1762–1767.

Smith GC, Shah I, Crossley JA, *et al.* 2006. Pregnancy-associated plasma protein A and alpha-fetoprotein and prediction of adverse perinatal outcome. *Obstet Gynecol* **107**: 161–166.

Spencer K, Nicolaides KH. 2002. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal translucency thickness, maternal serum free beta-hCG and PAPP-A. *Prenat Diagn* **22**: 877–879.

Spencer K, Cowans NJ, Avgidou K, Molina F, Nicolaides KH. 2008. First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses. *Ultrasound Obstet Gynecol* **31**: 15–19.

Westergaard JG, Sinosich MJ, Bugge M, Madsen LT, Teisner B, Grudzinskas JG. 1983. Pregnancy-associated plasma protein A in the prediction of early pregnancy failure. *Am J Obstet Gynecol* **145**: 67–69.

Wilcox AJ, Baird DD, Weinberg CR. 1999. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med* **340**: 1796–1799.