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Early Pregnancy Levels of Pregnancy-Associated Plasma Protein A and the Risk of Intrauterine Growth Restriction, Premature Birth, Preeclampsia, and Stillbirth

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The risk of adverse perinatal outcome among 8839 women recruited to a multicenter, prospective cohort study was related to maternal circulating concentrations of trophoblast-derived proteins at 8–14 wk gestation. Women with a pregnancy-associated plasma protein A (PAPP-A) in the lowest fifth percentile at 8–14 wk gestation had an increased risk of intrauterine growth restriction [adjusted odds ratio, 2.9; 95% confidence interval (CI), 2.0–4.1], extremely premature delivery (adjusted odds ratio, 2.9; 95% CI, 1.6–5.5), moderately premature delivery (adjusted odds ratio, 2.4; 95% CI, 1.7–3.5), preeclampsia (adjusted odds ratio, 2.3; 95% CI, 1.6–3.3), and stillbirth (adjusted odds ratio, 3.6; 95% CI, 1.2–11.0). The strengths of the associations were similar when the test was performed before 13 wk gestation or between 13 and 14 wk gestation. In contrast, levels of free β-human CG, another circulating protein synthesized by the syncytiotrophoblast, were not predictive of later outcome in multivariate analysis. PAPP-A has been identified as a protease specific for IGF binding proteins. We conclude that control of the IGF system in the first and early second trimester trophoblast may have a key role in determining subsequent pregnancy outcome. (*J Clin Endocrinol Metab* 87: 1762–1767, 2002)

INTRAUTERINE GROWTH RESTRICTION and preterm birth are major determinants of perinatal morbidity and mortality. Much of routine prenatal care involves detecting women at increased risk of these adverse events and targeting intensive monitoring and interventions. Standard reviews of fetal physiology suggested that variation in human fetal growth was largely a phenomenon of the second half of pregnancy (1), and it is during this phase of pregnancy when women receive the bulk of prenatal care. However, a previous study showed that embryos and fetuses that were smaller than expected in the first trimester of pregnancy were more likely to have pregnancy complications, including intrauterine growth restriction and preterm birth (2). In the present study, we investigated whether circulating concentrations of two trophoblast-derived proteins in early pregnancy [pregnancy-associated plasma protein A (PAPP-A) and free β-subunit human CG (FβhCG)] might identify women at increased risk of subsequent adverse perinatal outcomes.

Materials and Methods

Blood samples were obtained from women between 8 and 14 wk gestation, attending 15 maternity hospitals in southern Scotland, as part of a prospective, nonintervention multicenter study on combined ultrasound and biochemical screening for Down's syndrome, which is being reported elsewhere (Crossley *et al.*, submitted for publication). Women were sent an information leaflet along with their booking notification and were invited to participate in the study when first attending for prenatal care. Gestational age at the time of recruitment was assessed by crown-rump length (CRL) and/or biparietal diameter, as recommended, using previously described protocols (3). Signed consent was obtained from all patients, and ethical approval was obtained from the institutional committees of all participating centers and from the regional multicenter ethics committee. No results were reported to either the obstetrician or patient, and prenatal care was not modified in any way by participation in the study.

Seven data collection

Relevant patient and pregnancy information were entered on the study data form, which was sent along with a clotted blood sample to the study coordinating center, where the data were entered into the study database. Samples were assayed for PAPP-A and FβhCG using the *Kryptor* immunoassay analyzer (Brahms, Berlin, Germany; formerly supplied by CIS-Bio International, Burgess Hill, UK). Kryptor assays are based on time- resolved amplified cryptate emission technology. PAPP-A exists complexed 2:2 with the precursor of eosinophil major basic protein, and the PAPP-A assay measures this PAPP-A/precursor of eosinophil major basic protein complex. The detection limit of the assay is 0.004 IU/liter (International reference preparation 78/610), and the coefficient of variation was found to be 3.5% at 4.58 IU/liter and 5.7% at 12.90 IU/liter. The FβhCG assay is specific for the unbound β-subunit of hCG and measures both nicked and unnicked forms. The detection limit of the assay is 0.1 IU/liter (International reference preparation 75/551), and the coefficient of variation was found to be 4.3% at 17.6 IU/liter and 3.5% at 48.4 IU/liter. FβhCG and PAPP-A levels were converted to multiples of the median (MOMs) using the CRL or biparietal diameter measurement when the sample was obtained as an estimate of gestation. Because PAPP-A levels are reduced in smokers by around 15%, when compared with nonsmokers (4), separate medians were used for each group. PAPP-A and FβhCG MOM values were corrected for maternal weight using reciprocal-linear regression (5). Outcome data were gathered from each woman's clinical record, after

Abbreviations: BMI, Body mass index; BW, birth weight; CI, confidence interval; CRL, crown-rump length; FβhCG, free β-human CG; IGFBP, IGF binding protein; MOM, multiple of the median.

delivery, using predefined criteria and coded by a team of two research midwives, two obstetricians, and two clinical scientists.

Selection of study cohort

The database included 9002 records of singleton pregnancies where values for PAPP-A, FβhCG, and gestational age at the time of sampling were documented and outcome data were available. We excluded 121 (1.3%) records with missing values for birth weight (BW), 68 (0.8%) with missing values for perinatal outcome (*i.e.* stillbirth or livebirth), 99 (1.1%) records where the gestational age at delivery was outside the range of 24–43 wk, and 26 (0.3%) records where the karyotype was abnormal or missing. This left a study group of 8839 (some records had multiple exclusions or missing values).

Definitions and denominators

Nulliparous women were defined as women either having their first pregnancy or women whose births were preceded only by pregnancies that resulted in abortion before 24 wk gestation. Gestational age was defined as the number of completed weeks of gestation. A small-for-gestational-age baby was defined as a liveborn baby that was less than the fifth percentile of BW for the given week of gestation, using percentiles derived from 409,541 live births in Scotland between 1992–1998 (G. C. S. Smith, unpublished data). The denominator was all live births. Very preterm delivery was defined as birth of a live-born baby between 24 and 32 wk gestation inclusive and the denominator was all live births at or after 24 wk gestation. Moderately preterm delivery was defined as live births between 33 and 36 wk gestation inclusive and the denominator was all live-births at or after 33 wk gestation. Spontaneous preterm birth was defined as vaginal delivery of a liveborn baby between 24–36 wk where labor had not been induced. The denominator was spontaneous preterm births plus term births. Stillbirth was defined as delivery of a dead baby at or after 24 wk gestational age and the denominator was all births at or after 24 wk gestational age. Preeclampsia was defined as pregnancy-induced hypertension with proteinuria. Maternal age was defined as the age of the mother, in completed years, at term. Maternal height was measured in centimeters, maternal weight was measured in kilograms at the time of blood sampling, and body mass index (BMI) was calculated using weight divided by height squared. Nonsmoking was defined as never having smoked, at the time of first attendance for prenatal care; exsmokers were defined as women who stopped smoking either before or during pregnancy; and smokers were defined as women who smoked throughout pregnancy.

Statistical analyses

Separate analyses were undertaken for five dichotomous outcomes: delivery of a small-for-gestational-age baby, moderately preterm delivery, extremely preterm delivery, preeclampsia, and stillbirth. Univariate comparisons of dichotomous data were performed using the chi-square test (more than five observations in all cells) or Fisher's exact test (five or fewer observations in one or more cells). Ordinal data were compared using the chi-square test for trend. The *P* values for all hypotheses tests were two-sided. Crude and adjusted odds ratios were obtained using logistic regression analysis (6). Height and BMI were categorized into strata, age was dichotomized into <35 and ≥ 35, and gestational age at the time of sampling was dichotomized into <13 wk and ≥ 13 wk. The statistical significance of interaction terms was assessed using the likelihood ratio test. The goodness quality of fit of models was assessed using the Hosmer and Lemeshow test based on deciles of probability. All statistical analyses were performed using the Stata software package (Stata Corporation, College Station, TX), version 7.0.

Results

Among the study group, there were missing values for parity in 512 records (5.8%), for height in 739 (8.4%), for BMI in 814 (9.2%), for smoking status in 342 (3.9%), for ethnicity in 557 (6.3%), and for maternal age in 49 (0.6%). The basic demographic and outcome data are given in Table 1. Values of PAPP-A measured in the study ranged from 0.09–27.70 IU/liter, and values of FβhCG ranged from 3.2–265 IU/liter.

When the proportion of adverse events was compared among deciles of PAPP-A, the lowest decile of PAPP-A consistently had the highest proportion of adverse outcomes (Fig. 1). Moreover, there was an association between intrauterine growth restriction and moderately premature delivery across the whole range of PAPP-A (Fig. 1). When outcomes were compared in relation to FβhCG, there was a trend for increased proportions of growth-restricted babies with decreasing FβhCG, but not with any of the other outcomes (Fig. 2). The number of stillbirths was too small to test for trend, but the highest proportion of stillbirths was seen in the lowest deciles of both PAPP-A and FβhCG (Figs. 1 and 2).

We then determined the ability of the lowest 5% of MOMs

TABLE 1. Characteristics and outcomes of study group (n = 8839)

Study group characteristics		
Age (yr)	Median (IQR)	30.7 (26.9–34.0)
	Age > 35 yr	1693 (19.2%)
Parity	Median (IQR)	1 (0–1)
	Nulliparous	3924 (44.4%)
Ethnicity	Non-Caucasian	221 (2.5%)
Smoking status	Nonsmokers	5976 (67.6%)
	Exsmokers	703 (8.0%)
	Current smokers	1818 (20.6%)
Height (cm)	Median (IQR)	163 (159–168)
Weight (kg)	Median (IQR)	63.9 (57.5–72.1)
BMI	Median (IQR)	23.8 (21.7–26.6)
Gestational age at sampling	Median (IQR) (in days)	87 (81–93)
	<13 wk	5388 (60.4%)
Outcome data		
BW (g)*	Median (IQR)	3450 (3110–3780)
Less than 5th percentile[a]		370 (4.2%)
Gestational age at delivery[a]	24–32 wk	86 (1.0%)
	33–36 wk	326 (3.7%)
	37–43 wk	8405 (95.3%)
Stillbirths		22 (0.3%)

Data are number (%) unless stated otherwise. IQR, Interquartile range.
[a] Excludes stillbirths.

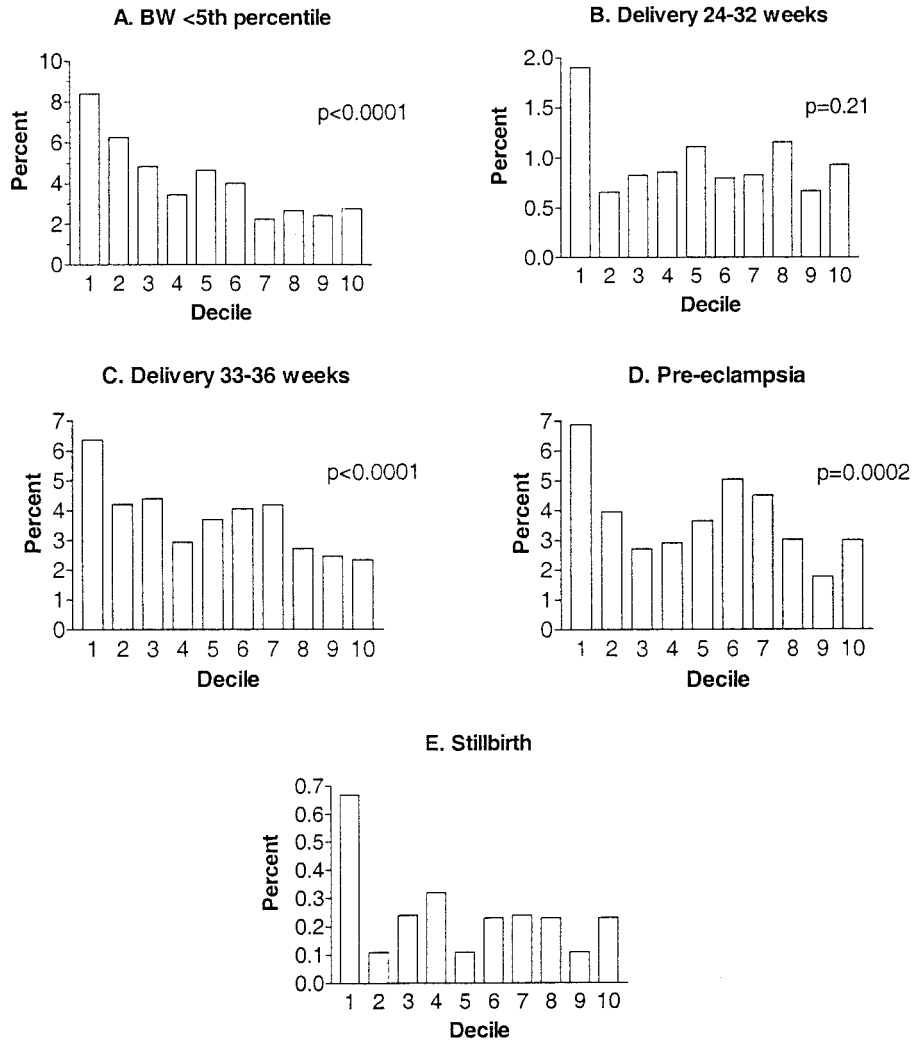

FIG. 1. Proportion of adverse perinatal outcomes related to PAPP-A decile. The *P* value is the chi-square test for trend. When data from the smallest decile were excluded, the test for trend remained statistically significant for BW less than fifth percentile ($P < 0.0001$) and delivery between 33–36 wk ($P = 0.006$) but was no longer statistically significant for preeclampsia ($P = 0.22$). The chi-square test was not performed on stillbirth data, because of the small number of adverse events.

for each serum marker to identify women at increased risk of adverse outcomes in later pregnancy (Table 2). Women with the lowest 5% of PAPP-A MOMs were at increased risk of intrauterine growth restriction, moderately and extremely premature birth, preeclampsia, and stillbirth. Women with the lowest 5% of FβhCG MOMs were at increased risk of intrauterine growth restriction but none of the other outcomes.

In multivariate analysis (Table 3), PAPP-A remained highly significantly predictive of all adverse outcomes when adjusted for FβhCG, BMI, height, ethnicity, parity, smoking status, maternal age, and gestational age at the time of sampling. There were no statistically significant interactions between PAPP-A and the other covariates. FβhCG was no longer significantly positively predictive of any adverse outcome when adjusted for PAPP-A, BMI, height, ethnicity, parity, smoking status, maternal age, and gestational age at the time of sampling. There were no statistically significant interactions between FβhCG and any of these other covariates. When the analysis was confined to spontaneous preterm births, there was a positive association with the lowest 5% of PAPP-A MOMs [adjusted odds ratio, 2.0; 95% confidence interval (CI), 1.2–3.3; $P = 0.005$] but no association with the lowest 5% of FβhCG MOMs (adjusted odds ratio, 0.7; 95% CI, 0.4–1.4; $P = 0.32$).

Discussion

In this study, we demonstrate that maternal circulating concentrations of PAPP-A at 8–14 wk gestation are significantly predictive of adverse perinatal outcome in later pregnancy. The strength of the association between PAPP-A and outcome before 13 wk gestation was similar to that at ≥13 wk gestation. These data indicate that, in a proportion of women, adverse pregnancy outcome is determined in the first trimester of pregnancy.

We had previously studied ultrasonic measurement of the CRL of the fetus and found that embryos and fetuses that were smaller than expected in the first trimester were more likely to be low BW, low BW at term, in the smallest fifth percentile of weight for gestational age, and born extremely prematurely (2). That study had certain weaknesses. First, the fetus might be smaller than expected because of variation in the assumed day of ovulation. Second, we were able to obtain an ideal menstrual history and early ultrasound in only approximately 10% of women, which meant that the

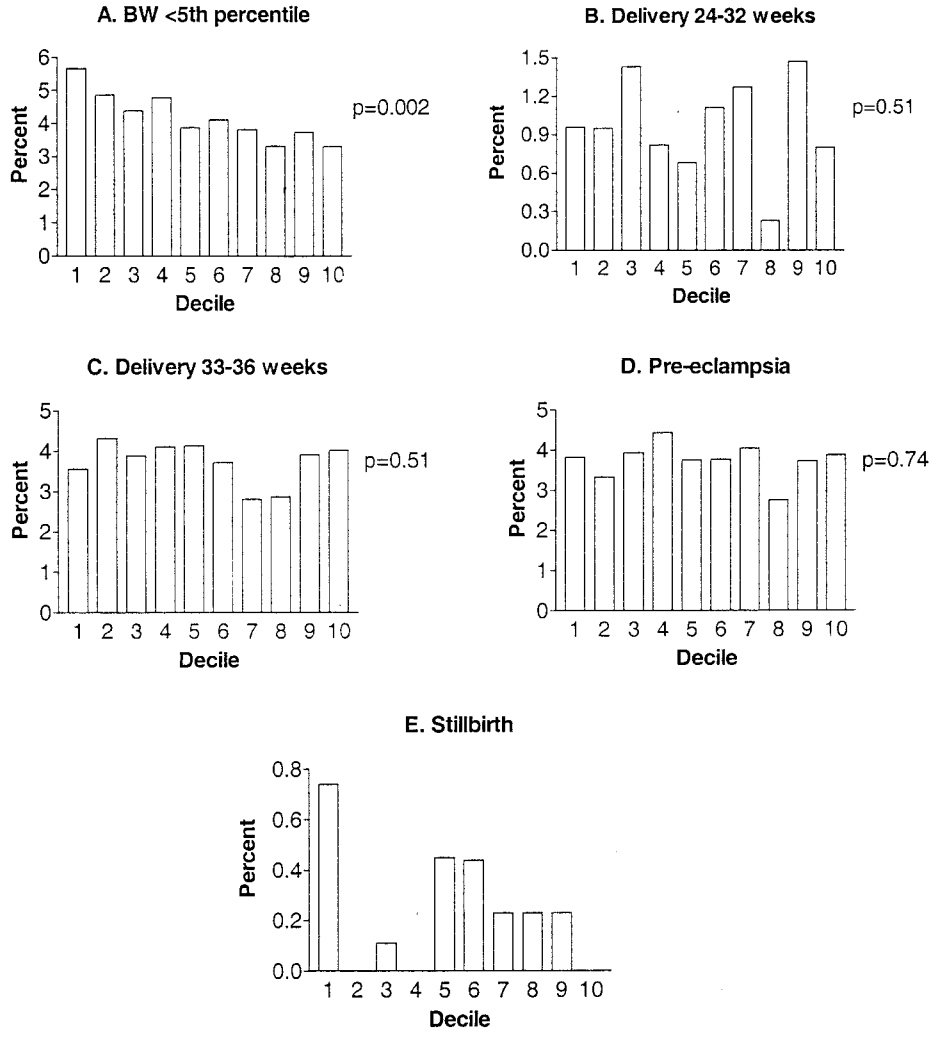

FIG. 2. Proportion of adverse perinatal outcomes related to FβhCG decile. The *P* value is the chi-square test for trend. When data from the smallest decile were excluded, the test for trend remained statistically significant for BW less than fifth percentile ($P = 0.03$). The chi-square test was not performed on stillbirth data, because of the small number of adverse events.

TABLE 2. Univariate analysis of first-trimester biochemistry and perinatal outcomes

	PAPP-A				FβhCG			
	Smallest 5th percentile (n = 461)	>5th Percentile (n = 8378)	Odds ratio (95% CI)	*P*	Smallest 5th percentile (n = 456)	>5th Percentile (n = 8383)	Odds ratio (95% CI)	*P*
BW < 5th percentile for gestational age	46 (10.0%)	324 (3.9%)	2.8 (2.0–3.8)	<0.0001	30 (6.6%)	340 (4.1%)	1.7 (1.1–2.5)	0.008
Delivery, 24–32 wk	12 (2.6%)	74 (0.9%)	3.0 (1.6–5.5)	0.0002	6 (1.3%)	80 (1.0%)	1.4 (0.6–3.1)	0.44
Delivery, 33–36 wk	35 (7.9%)	291 (3.5%)	2.2 (1.6–3.4)	<0.0001	10 (2.2%)	316 (3.8%)	0.6 (0.3–1.1)	0.09
Preeclampsia	35 (7.6%)	296 (3.5%)	2.1 (1.6–3.2)	<0.0001	19 (4.1%)	312 (3.7%)	1.1 (0.7–1.8)	0.63
Stillbirth	4 (0.9%)	18 (0.2%)	4.0 (1.4–11.5)	0.03	3 (0.7%)	19 (0.2%)	2.9 (0.9–9.8)	0.10

Statistical comparison by χ^2 test or Fisher's exact test, as appropriate.

technique could not be used as a screening test. Our current findings of an association between low PAPP-A and adverse outcome provide additional weight to our hypothesis that adverse perinatal outcome may be determined in early pregnancy. These observations suggest that measurement of specific circulating trophoblast-derived proteins in the first trimester of pregnancy may provide a potential screening tool to identify women at increased risk of subsequent adverse pregnancy outcome.

There is an extensive literature on the use of trophoblast-derived steroids and proteins, including PAPP-A, as biochemical tests of fetal well-being in the third trimester of pregnancy (7), although these are not currently widely used in antepartum monitoring. Previous studies of first-trimester measurement of PAPP-A and perinatal outcome have reported inconsistent results. One study compared first-trimester PAPP-A levels in 73 babies ultimately born less than the fifth percentile for gestational age and 87 babies ultimately born preterm with matched controls. There was no statistically significant difference between the groups (8). However, another study found a positive correlation between PAPP-A at 8–14 wk and eventual BW (9), and an

TABLE 3. Adjusted odds ratios for adverse perinatal outcomes associated with first-trimester PAPP-A and FβhCG in the lowest 5th percentile

Outcome	PAPP-A <5th percentile		FβhCG <5th percentile	
	Adjusted odds ratio (95% CI)	*P*	Adjusted odds ratio (95% CI)	*P*
BW < 5th percentile	2.9 (2.0–4.1)	<0.001	1.3 (0.9–2.0)	0.15
Delivery, 24–32 weeks	2.9 (1.6–5.5)	0.001	1.1 (0.5–2.7)	0.77
Delivery, 33–36 weeks	2.4 (1.7–3.5)	<0.001	0.5 (0.3–1.0)	0.04
Preeclampsia	2.3 (1.6–3.3)	<0.001	1.1 (0.7–1.8)	0.64
Stillbirth	3.6 (1.2–11.0)	0.02	2.3 (0.7–8.2)	0.18

Odds ratios are adjusted for BMI, height, smoking status, ethnicity, parity, maternal age, gestational age at the time of sampling, PAPP-A, and FβhCG. Odds ratios for delivery wk 33–36 are also adjusted for interaction between age ≥35 and height.

analysis of 60 *in vitro* fertilization pregnancies described lower concentrations of PAPP-A in the first trimester among 8 women who eventually delivered preterm (10). Another study of 5297 women demonstrated lower PAPP-A levels at 10–14 wk gestation among women who miscarried, delivered babies small for gestational age, and developed preeclampsia (11). However, PAPP-A was used in these women to estimate the risk of the fetus having Down's syndrome, and the study failed to take into account the effect of invasive procedures, which may well have explained the association with loss before 24 wk. Moreover, none of these studies took into account smoking status. Because smoking is associated with many of these outcomes (12) and is also associated with low PAPP-A (4), these studies are difficult to interpret. The advantages of the present study are: that it was a prospective cohort study, that the levels of serum markers did not influence clinical management, that key maternal factors such as weight and smoking were taken into account, and that it included much larger numbers of women who ultimately experienced adverse events.

The pattern of the association varied for different outcomes (Fig. 1). In the case of preeclampsia, stillbirth, and extremely premature birth, the association was only with the smallest decile of PAPP-A. In the case of growth restriction and moderately premature delivery, the association was observed across the whole range of PAPP-A. Moreover, low FβhCG was also associated with growth restriction, although this was lost after adjusting for PAPP-A, whereas low FβhCG was not significantly associated with the other outcomes, even in univariate analysis (Tables 2 and 3). Both PAPP-A and FβhCG are produced by the syncytiotrophoblast (13, 14). It seems likely that these different patterns of association may reflect different pathophysiological mechanisms relating first-trimester trophoblast function and later adverse perinatal outcome. The fact that the strength and pattern of the association differed for the two trophoblast-derived proteins suggests that PAPP-A is not acting as a simple marker of the volume or health of the trophoblast but that the association reflects a specific property of PAPP-A in the physiological regulation of trophoblast function.

The precise mechanisms linking first-trimester levels of trophoblast-derived proteins and adverse outcomes will require further study. PAPP-A has been identified as a protease for IGF binding protein (IGFBP)-4 (15). IGFBPs bind IGF-I and IGF-II, inhibiting their interaction with cell surface receptors and have, therefore, a key role in modulating IGF activity (16). Because PAPP-A breaks down IGFBP (15), low levels of PAPP-A would be expected to be associated with high levels of IGFBP and, therefore, low levels of free IGF. The IGFs have a key role in regulating fetal growth (17). The IGFs have also been shown to control uptake of glucose and amino acids in cultured trophoblast (18) and are thought to have an important role in the autocrine and paracrine control of trophoblast invasion of the decidua (19). The current observation that PAPP-A was predictive of a range of adverse obstetric outcomes implies a fundamental role of this system in development of the placenta in early pregnancy, and the observed association between low PAPP-A and poor perinatal outcome is clearly biologically plausible.

In summary, first-trimester serum concentrations of PAPP-A, a trophoblast-specific protein regulating IGF function, is highly predictive of a range of subsequent adverse pregnancy outcomes. These observations imply that adverse outcome in late pregnancy may be determined in the first trimester of pregnancy, that control of the IGF system in early pregnancy may be critical in normal placental development, and that women at high risk of adverse pregnancy outcome may be identified in very early pregnancy.

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