First-Trimester Placentation and the Risk of Antepartum Stillbirth

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Stillbirth affects approximately 1 in 200 pregnancies and is therefore approximately 10 times more common than sudden infant death syndrome. In approximately 85% of stillbirths, death of the fetus occurs prior to labor. The main epidemiological factors associated with an increased risk of stillbirth are advanced gestational age, advanced maternal age, nulliparity, high parity, smoking, obesity, and poor obstetric history. However, most of these associations are relatively weak. Effective interventions have been described to reduce perinatal mortality, such as Doppler ultrasonography of umbilical artery blood flow and induction of labor, which is used in prolonged pregnancy. However, application of these interventions requires identification of women at high risk of stillbirth.

We have shown that the risk of a number of pregnancy complications, such as preterm birth and low birth weight, is determined, at least in part, during the first trimester of pregnancy. However, it is not known whether the risk of stillbirth is also determined during the first trimester. In the present large-scale, multicenter, prospective cohort study, we determined the risk of antepartum stillbirth in relation to maternal serum levels of 2 proteins derived from the placenta—placental growth factor (PIGF) and the free β subunit of human chorionic gonadotropin (HCG)—measured during the first 10 weeks after conception.

METHODS
We used data from the Combined Ultrasound and Biochemical Screening (CUBS) study, a prospective, multicenter study of screening for Down syndrome in routine mid-trimester care. The CUBS study evaluated the use of ultrasound measurement of fetal nuchal translucency in combination with analysis of maternal serum levels of PAPP-A and free β subunit of HCG as a first-trimester screening test for Down syndrome in a routine pre-natal care setting.

Context
Preterm birth and low birth weight are determined, at least in part, during the first trimester of pregnancy. However, it is unknown whether the risk of stillbirth is also determined during the first trimester.

Objective
To determine whether the risk of antepartum stillbirth varies in relation to circulating markers of placental function measured during the first trimester of pregnancy.

Design, Setting, and Participants
Multicenter, prospective cohort study (conducted in the United Kingdom from 1998 through 2000) of 7934 women who had singleton births at or after 24 weeks' gestation, who had blood taken during the first 10 weeks after conception, and who were entered into national registries of births and perinatal deaths.

Main Outcome Measures
Antepartum stillbirths and stillbirths due to specific causes.

Results
There were 8 stillbirths among the 400 women with levels of pregnancy-associated plasma protein A (PAPP-A) in the lowest fifth percentile compared with 17 among the remaining 7534 women (incidence rate per 10000 women per week of gestation: 13.4 vs 1.4, respectively; hazard ratio [HR], 9.2 [95% confidence interval (CI), 4.0-21.4]; P < .001). When analyzed by cause of stillbirth, low level of PAPP-A was strongly associated with stillbirth due to placental dysfunction, defined as abruptio or unexplained stillbirth associated with growth restriction (incidence rate: 11.7 vs 0.3, respectively; HR, 46.0 [95% CI, 11.9-178.0]; P < .001), but was not associated with other causes of stillbirth (incidence rate: 1.7 vs 1.1, respectively; HR, 1.4 [95% CI, 0.2-10.6]; P = .75). There was no relationship between having a low level of PAPP-A and maternal age, ethnicity, parity, height, body mass index, race, or marital status. Adjustment for maternal factors did not attenuate the strength of associations observed. There was no association between maternal circulating levels of the free β subunit of human chorionic gonadotropin and stillbirth risk.

Conclusion
The risk of stillbirth in late pregnancy may be determined by placental function in the first 10 weeks after conception.
nental clinic setting. Information leaflets about the study were sent to women with the notification of their first appointment for prenatal care. Those women whose first visit was within 14 weeks’ gestation were invited to participate and those who agreed signed a consent form. Participation in the study involved obtaining a measurement of nuchal translucency at the time of the first ultrasound and an additional blood sample at the time of phlebotomy for routine prenatal investigations. No results were reported to either the obstetrician or patient and prenatal care was not modified in any way due to participation in the study. Ethical approval was obtained from the ethics committee for Scottish Multicenter Research. Fifteen Scottish maternity units participated during a 2-year period between 1997 and 1999 and 98.6% of records came from births in 11 of the hospitals. Ninety-eight percent of the births occurred between May 1998 and July 2000. Births to women recruited to the study constituted 28.6% of all births in the 11 hospitals during that period.

Pregnancy outcome was ascertained by record linkage to the Scottish Morbidity Record2 and the Scottish Stillbirth and Infant Death Enquiry.4 The Scottish Morbidity Record is a national registry of pregnancy outcome data and the Scottish Stillbirth and Infant Death Enquiry is a national registry that routinely classifies all perinatal deaths in Scotland. Both registries are close to 100% complete and are described in detail elsewhere.1,9 The study cohort for the current analysis was defined by women who participated in the CUBS study, had a PAPP-A level recorded prior to 91 days’ gestation (equivalent to <77 days after conception) assessed by crown-rump length, and were linked to the Scottish Morbidity Record in which singleton birth occurred at or after 24 weeks’ gestation. This cutoff was chosen because ascertainment of stillbirths at less than 24 weeks’ gestation is incomplete and the causes are not defined in the Scottish Stillbirth and Infant Death Enquiry.5 There were no data on spontaneous and therapeutic abortions.

Definitions and Denominators
Maternal height, smoking status, marital status, ethnicity, and body mass index (weight in kilograms divided by the height in meters squared) were ascertained at the time of the first prenatal visit. Maternal age was defined as the age at delivery. Socioeconomic status was estimated based on the postcode of residence, using Carstairs socioeconomic deprivation categories10 (based on 1991 Census data on car ownership, unemployment, overcrowding, and social class within postcode sectors of residence, which contain approximately 1600 residents). Women were categorized into quintiles of socioeconomic deprivation. The gestational age at birth was defined as completed weeks of gestation. Race was self-reported by questionnaire (options were white and other). Many studies have shown disparity in stillbirth risk comparing racial groups, but our population had a small proportion of non-white women.

The cause of perinatal death was classified by a modified version of the Wigglesworth system.8 Explained stillbirths were defined as those in which there was an apparent direct cause, such as fetal abnormality, placental abruption, or maternal diabetes. All other stillbirths were classified as unexplained. Unexplained stillbirths were subdivided into those that were small for gestational age (SGA; in the smallest fifth percentile for sex and week of gestation) and those appropriate for gestational age. Unexplained SGA stillbirths were assumed to reflect chronic placental insufficiency. Therefore, stillbirths due to abruption or SGA unexplained stillbirths were considered collectively as stillbirths due to placental causes.

Statistical Analyses
Levels of PAPP-A and free β subunit of HCG were expressed as multiples of the median for gestational age, which is the convention for biochemical indices in pregnancy that vary with week of gestation. Because PAPP-A levels vary inversely with maternal weight, multiples of the median were corrected for maternal weight using reciprocal-linear regression. This method is widely used in prenatal screening and is described in detail elsewhere.11 Separate multiples of the median for PAPP-A level were estimated for smokers because PAPP-A level is reduced by 15% among smokers.9 Univariate comparison of continuous variables was performed using the Mann-Whitney test and of categorical data using the Fisher exact test. All P values were 2-sided. Statistical significance was assumed at P<.05.

The association between PAPP-A level and stillbirth was assessed by comparing women with levels in the lowest fifth percentile with women in other percentiles. We previously showed that a low PAPP-A level was associated with a range of adverse outcomes. However, we also studied PAPP-A level as a continuous variable and categorized it by quintiles. The risk of stillbirth was compared between groups using time to event analyses in which week of gestation from 24 weeks onward was used as the time scale. The gestational age at delivery was taken as the time of the event in the case of antepartum stillbirth or the time of censoring in the case of all other births. This method uses ongoing pregnancies as the denominator, as previously suggested,12 but accounts for censoring due to birth and allows multivariate analysis2 and can be used in situations in which not all individuals would ultimately experience the event.13 This analytic approach allows assessment of the relative risk accounting for variation in the duration of pregnancy. Survival data were plotted as a cumulative percentage of the event, which is recommended for rare outcomes,14 and univariate statistical comparisons were made using the log-rank test. Crude and adjusted hazard ratios were estimated using a Cox proportional hazards model.15 The proportional hazards assumption was tested using the global test of Grambsch.
and Therneau. Goodness of fit was assessed by the global test described by May and Hosmer. Missing values were imputed using multiple imputation. All statistical analyses were performed using STATA statistical software (version 8.2, STATA Corp, College Station, Tex).

RESULTS
The linked database contained 11729 records of women who had a PAPP-A level recorded and had an entry in the Scottish Morbidity Record. In 3 records <0.1%, the gestational age at delivery was less than 24 weeks, leaving a cohort of 11726 singleton births at or after 24 weeks' gestation. Among these, 7934 (67.7%) were assayed prior to 13 weeks' gestation (equivalent to 77 days after conception). The median gestational age at sampling was 11.9 weeks (69 days after conception; interquartile range [IQR], 65-73 days). There was no relationship between low PAPP-A level and maternal characteristics (Table 1). Circulating serum levels of PAPP-A (expressed in multiples of the median for gestational age) were lower in male fetuses compared with female median [IQR], 0.97 [0.67-1.41] vs 1.03 [0.71-1.46], respectively; P<.001).

There were 25 (0.3%) antepartum stillbirths in the study group. An autopsy was performed on 19 (76%) of the stillborns. Ten stillbirths were attributed to placental causes—4 due to...

### Table 1. Maternal Characteristics in Relation to Levels of PAPP-A in the First 10 Weeks After Conception*

<table>
<thead>
<tr>
<th>Percentile of PAPP-A Level</th>
<th>≤Fifth (n = 400)†</th>
<th>&gt;Fifth (n = 7534)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
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<tr>
<td>Married</td>
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<td>Other</td>
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<tr>
<td>Deprivation quintile</td>
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<tr>
<td>1 (Least deprived)</td>
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<td>2</td>
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<tr>
<td>5 (Most deprived)</td>
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<td>Ethnicity</td>
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<td>White</td>
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<td>No. of previous births</td>
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<td>No. of previous abortions</td>
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<td>Height, median (IQR), cm</td>
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<tr>
<td>Body mass index, median (IQR)‡</td>
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<td>Missing data</td>
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</tbody>
</table>

**Table 2. Low PAPP-A in the First 10 Weeks After Conception and the Risk of Stillbirth**

<table>
<thead>
<tr>
<th>Stillbirth Category</th>
<th>No. (%) in ≤Fifth Percentile of PAPP-A Level (n = 400)</th>
<th>Incidence Rate†</th>
<th>No. (%) in &gt;Fifth Percentile of PAPP-A Level (n = 7534)</th>
<th>Incidence Rate‡</th>
<th>Unadjusted HR (95% CI)</th>
<th>P Value</th>
<th>Adjusted HR (95% CI)‡</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cause</td>
<td>8 (2.0)§</td>
<td>13.4</td>
<td>17 (0.2)</td>
<td>1.4</td>
<td>9.2 (4.0-21.4)</td>
<td>&lt;.001</td>
<td>9.4 (4.1-21.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Due to abruption</td>
<td>3 (0.8)</td>
<td>5.0</td>
<td>1 (&lt;0.1)</td>
<td>0.1</td>
<td>58.0 (50.5-567.3)</td>
<td>&lt;.001</td>
<td>60.5 (50.1-597.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>All unexplained</td>
<td>4 (1.0)</td>
<td>6.7</td>
<td>12 (0.2)</td>
<td>0.9</td>
<td>6.6 (2.1-20.4)</td>
<td>.001</td>
<td>7.2 (2.3-22.4)</td>
<td>.001</td>
</tr>
<tr>
<td>Unexplained SGA</td>
<td>4 (1.0)</td>
<td>6.7</td>
<td>2 (&lt;0.1)</td>
<td>0.2</td>
<td>0.4 (6.3-288.3)</td>
<td>&lt;.001</td>
<td>0.46 (6.8-236.2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>All placenta-related</td>
<td>11 (1.2)§</td>
<td>11.7</td>
<td>3 (&lt;0.1)</td>
<td>0.3</td>
<td>46.0 (11.9-178.0)</td>
<td>&lt;.001</td>
<td>12.6 (13.3-207.8)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

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**Abbreviations:** IQR, interquartile range; PAPP-A, pregnancy-associated plasma protein A; SGA, small for gestational age.

*The earliest stillbirth occurred at 24 weeks and the latest occurred at 41 weeks.

†Incidence expressed per 10000 women per week of gestation from 24 weeks.

‡Attributable fraction = 28.4%.

§Attributable fraction = 68.4%.
abruption and 6 were SGA unexplained stillbirths. An autopsy was performed on all of the SGA unexplained stillbirths. Among the 400 women with PAPP-A levels in the lowest fifth percentile (<0.4 multiples of the median), 8 (2%) had a stillbirth due to any cause, 3 (0.8%) had a stillbirth due to abruption, 4 (1.0%) had a stillbirth due to SGA unexplained stillbirth, and 7 (1.8%) had a stillbirth due to placental causes. When compared with the rest of the population, a low PAPP-A level was associated with a 9.2-fold risk of all-cause stillbirth (Figure 1A), a 58.0-fold risk of stillbirth due to abruption, a 40.0-fold risk of SGA unexplained stillbirth, and a 46.0-fold risk of stillbirth due to placental dysfunction (Figure 1B). There were 2 stillbirths due to chromosomal abnormality in the study group and one of these was to a mother with a low PAPP-A level. This was the sole stillbirth among this group not due to a placental cause.

The proportion of stillbirths due to placental dysfunction was 7 of 8 among women with a low PAPP-A level compared with 3 of 17 in the other women (P = .002). Figure 2 illustrates the relationship between quintiles of PAPP-A level and the risk of stillbirth due to placental dysfunction. There were no stillbirths due to placental dysfunction among women with PAPP-A levels in the upper 3 quintiles. There was no relationship between free β subunit of HCG quintile and the risk of all-cause stillbirth (P = .59) or stillbirth due to placental dysfunction (Figure 2).

The numbers of all-cause stillbirths were sufficient to test the goodness of fit of the multivariate model, to test the proportional hazards assumption, and to determine whether there were any interactions between PAPP-A and the other factors. There was no evidence of poor fit (P = .93), there was no evidence of nonproportionality (P = .25), and there were no statistically significant interactions between PAPP-A level and the other maternal characteristics (all P > .01). When treated as a continuous variable, the hazard ratio associated with an increase in multiples of the median for PAPP-A level was 0.13 (95% confidence interval [CI], 0.04-0.40) and for free β subunit of HCG was 1.06 (95% CI, 0.67-1.69). The multiples of the median of PAPP-A level among stillbirths caused by placental dysfunction was 0.34 (IQR, 0.27-0.45) and for other births was 1.00 (IQR, 0.69-1.43) (P < .001).

**COMMENT**

The main finding of this study is that women in the first 10 weeks after conception with circulating levels of PAPP-A in the lowest fifth percentile had a greater than 40-fold risk of having an intravascular fetal death due to placental dysfunction, which was independent of maternal characteristics. There was no relationship between maternal circulating levels of free β subunit of HCG and stillbirth due to any cause. The strengths of the present study are that all data were collected prospectively and all outcomes were defined independently of the study. Because level of PAPP-A was not used in the clinical estimation of Down syndrome risk, there is no potential for confounding due to bias in the use of invasive procedures. The study design excluded women who had a therapeutic
abortion due to a chromosomal abnormality. It is likely that some of these women would have had low PAPP-A levels. However, because chromosomal abnormalities are relatively rare, excluding these women would have virtually no effect on the strength of the association between stillbirth and placental dysfunction. Our data suggest that the placental dysfunction causing stillbirth may be an end point of impaired placental function in the first 10 weeks after conception.

We had previously described a 3.6-fold risk of all-cause stillbirth among women with low levels of PAPP-A.8 This was based on partial follow-up of the CUBS cohort.8 However, 40% of the 8839 women in that analysis were sampled in the second trimester and partial follow-up included insufficient numbers of women sampled in the first trimester for subanalysis. Moreover, it was not a prior hypothesis that levels of PAPP-A would be associated with stillbirth and detailed information on the cause of stillbirth was not retrieved in our previous study. We addressed these weaknesses in the present study by linking the entire CUBS cohort to a national database of perinatal deaths. Consequently, in the present study, we had data for almost 8000 women sampled before 13 weeks' gestation and stillbirths could be classified according to the cause. This is the first study, to our knowledge, which demonstrates an association between a biochemical measurement in the first trimester and the risk of stillbirth. Moreover, the strength of the association between PAPP-A level and stillbirth caused by placental dysfunction is one of strongest described for this outcome.

The main weakness of this study is the relatively small number of events, which leads to wide 95% CIs. However, the lower limit of the 95% CI of the hazard ratio for placental causes of stillbirth was 12 and this study is, therefore, powered to demonstrate a strong association. Many studies of stillbirth are limited by incomplete ascertainment of cases. However, in the present study ascertainment of events is likely to be close to 100% because it is a legal requirement to register a stillbirth in Scotland and the perinatal death database used is virtually 100% complete when compared against death registries.3 The present study could be criticized because the women received prenatal care relatively early so the CUBS cohort may not be representative of the general population. However, the study was prospective and ascertainment of stillbirth and definition of its cause for the present analysis was completely independent of the CUBS study. It is unlikely, therefore, that there are biases among women with low levels of PAPP-A that might lead to a spurious association with stillbirth. Moreover, adjustment for a range of maternal characteristics, including all the previously described maternal characteristics associated with stillbirth,3 did not affect the strength of the association with PAPP-A level. Given the above, it is unlikely that the association between PAPP-A level and stillbirth is due to confounding. The primary purpose of the CUBS study was to assess methods for Down syndrome screening and the association described with stillbirth is the result of a secondary analysis. However, the strength and statistical significance of the associations observed make it unlikely that these are chance findings.

The current study focused on stillbirths at or after 24 weeks' gestation because the data available on these events are close to 100% complete. The data sources used in the present study are less robust for fetal losses between 20 and 23 weeks' gestation.1 Further studies will be required to determine the association between PAPP-A level and losses at earlier gestational ages. However, the present study is clinically relevant because the stillbirths occurred at gestational ages in which the fetus is viable. Interventions have been described that have been shown to reduce perinatal mortality among high-risk women, specifically, Doppler ultrasonography of umbilical artery blood flow4 and induction of labor in prolonged pregnancy.5 This raises the possibility that PAPP-A level might be clinically useful when combined with an intervention. The positive predictive value for placental causes of stillbirth was 1.8% among women with a PAPP-A level in the lowest fifth percentile. Although low, given the rarity of this event, this may justify closer prenatal surveillance and elective delivery prior to 40 weeks' gestation. However, further studies are required to confirm this association before clinical practice is changed. Furthermore, it may be that other proteins derived from the placenta in the first trimester have a higher positive predictive value. This is an area that we are currently studying.

A specific association between PAPP-A level and stillbirth is biologically plausible. PAPP-A has been identified as a protease for insulinlike growth factor binding proteins 4 and 5.20 Messenger RNA for PAPP-A has been identified in placental X cells and syncytiotrophoblast and the protein has been localized to placental septae, anchoring villi, and chorionic villi.21 Low levels of PAPP-A would be expected to lead to lower levels of free insulinlike growth factor. The insulinlike growth factor 2 is thought to have a key role in trophoblast function22 and, therefore, it is plausible that low levels of PAPP-A reflect poor placental function in early pregnancy. Consistent with this, it has recently been shown that mice homozygous for targeted disruption of the PAPP-A gene exhibit severe early onset intrauterine growth restriction.23 Level of PAPP-A is unlikely to be acting simply as a marker of placental volume. There was no association between stillbirth risk and levels of free β subunit of HCG, which is a protein derived from the placenta that is not involved in the control of the insulinlike growth factor system. The current findings indicate that catastrophic complications of late pregnancy may be determined by impaired placental function in the first 10 weeks after conception, which precedes prenatal care.

Author Contributions: Dr Smith had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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FIRST-TRIMESTER PAPP-A AND STILLBIRTH

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Drafting of the manuscript: Smith.
Critical revision of the manuscript for important intellectual content: Smith, Crossley, Aitken, Pell, Cameron, Connor, Dobbie.
Statistical analysis: Smith, Crossley.
Obtained funding: Smith, Aitken, Pell, Cameron, Connor.
Administrative, technical, or material support: Smith, Connor, Dobbie.
Analysis and interpretation of data: Dobbie.
Acquisition of data: Crossley, Aitken, Connor, Dobbie.
Study supervision: Smith, Aitken.
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