Ultrasound Obstet Gynecol 2016; **48**: 631–635
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.17229

Prediction of stillbirth from placental growth factor at 19–24 weeks

J. E. AUPONT*, R. AKOLEKAR*†, A. ILLIAN*, S. NEONAKIS* and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK; †Department of Fetal Medicine, Medway Maritime Hospital, Gillingham, UK*

KEYWORDS: placental growth factor; pyramid of care; stillbirth; uterine artery Doppler

ABSTRACT

Objectives *To investigate whether the addition of maternal serum placental growth factor (PlGF) measured at 19–24 weeks' gestation improves the performance of screening for stillbirth that is achieved by a combination of maternal factors, fetal biometry and uterine artery pulsatility index (UtA-PI) and to evaluate the performance of screening with this model for all stillbirths and those due to impaired placentation and unexplained or other causes.*

Methods *This was a prospective screening study of 70 003 singleton pregnancies including 268 stillbirths, carried out in two phases. The first phase included prospective measurement of UtA-PI and fetal biometry, which were available in all cases. The second phase included prospective measurement of maternal serum PlGF, which was available for 9870 live births and 86 antepartum stillbirths. The values of PlGF obtained from this screening study were simulated in the remaining cases based on bivariate Gaussian distributions, defined by the mean and standard deviations. Multivariable logistic regression analysis was used to determine whether the addition of maternal serum PlGF improved the performance of screening that was achieved by a combination of maternal factors, fetal biometry and UtA-PI.*

Results *Significant contribution to the prediction of stillbirth was provided by maternal factor-derived* a-priori *risk, multiples of the median values of PlGF, UtA-PI and fetal biometry Z-scores. A model combining these variables predicted 58% of all stillbirths and 84% of those due to impaired placentation, at a false-positive rate of 10%. Within the impaired-placentation group, the detection rate of stillbirth < 32 weeks' gestation was higher than that of stillbirth ≥ 37 weeks (97%* vs *61%; P < 0.01).*

Conclusions *A high proportion of stillbirths due to impaired placentation can be identified effectively in the second trimester of pregnancy using a combination of maternal factors, fetal biometry, uterine artery Doppler and maternal serum PlGF. Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

A screening study of 70 003 singleton pregnancies at 19–24 weeks' gestation, including 268 stillbirths, reported that 59% of antepartum stillbirths were associated with pre-eclampsia (PE), birth of a small-for-gestational-age (SGA) neonate or placental abruption, and these were attributed to impaired placentation; 41% were due to other or unexplained causes[1]. Screening for stillbirth by a combination of factors from maternal characteristics and medical history predicted 34% of stillbirths due to impaired placentation and 23% of those that were due to other or unexplained causes, at a false-positive rate (FPR) of 10%[1,2]. Prediction of stillbirth due to impaired placentation was improved substantially by a model combining maternal factors, fetal biometry and uterine artery pulsatility index (UtA-PI), with a detection rate (DR) of 75% at a FPR of 10%[1].

Placental growth factor (PlGF) is an angiogenic protein produced by the placenta and is implicated in trophoblastic invasion of maternal spiral arteries[3,4]. Maternal serum levels in the first, second and third trimesters are decreased in pregnancies with impaired placentation that develop PE and those that deliver SGA neonates[5–15]. There is also evidence that measurement of serum PlGF at 11–13 weeks' gestation is useful in predicting stillbirth[16].

The objective of this study was to investigate whether measurement of maternal serum placental growth factor (PlGF) at 19–24 weeks' gestation improves the

Correspondence to: Prof. R. Akolekar, Fetal Medicine Research Institute, King's College Hospital, 16–20 Windsor Walk, Denmark Hill, London SE5 8BB, UK (e-mail: ranjit.akolekar@nhs.net)

Accepted: 15 August 2016

performance of screening for stillbirths that is achieved by a combination of maternal factors, fetal biometry and UtA-PI and to evaluate the performance of screening with this model for all stillbirths and those due to impaired placentation and unexplained or other causes.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for routine pregnancy care at 19 + 0 to 24 + 6 weeks' gestation at King's College Hospital and Medway Maritime Hospital, UK, between March 2006 and October 2015[1]. Gestational age was determined from the measurement of fetal head circumference (HC) in the second trimester or crown–rump length in the first trimester[17,18]. The study was carried out in two phases: in the first phase, we recorded maternal characteristics and medical history, performed ultrasound examination for measurement of fetal HC, abdominal circumference (AC) and femur length (FL) and measured UtA-PI by transvaginal color Doppler ultrasound[17,19]. In the second phase, we also measured maternal serum concentration of PlGF using automated analyzers which provide reproducible results within 40 min of blood sampling (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA or Cobas e411 system, Roche Diagnostics, Penzberg, Germany).

Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the ethics committee of both participating hospitals. The inclusion criteria were women with a singleton pregnancy who delivered a phenotypically normal live birth or stillbirth ≥ 24 weeks' gestation. Pregnancies with aneuploidy, major fetal abnormality, and those ending in a miscarriage, termination of pregnancy or stillbirth due to intrapartum causes were excluded.

Data on pregnancy outcome were obtained from the maternity hospital records or the general practitioners of the women. The hospital maternity records of all women with antepartum stillbirth were reviewed to determine whether the death was associated with PE, placental abruption or a birth weight $<10^{th}$ percentile for gestational age[20] or it was due to other causes or was unexplained.

Statistical analysis

The measured values of PlGF and UtA-PI were $\log_{10}$ transformed to ensure homogeneity of variance and make the distributions Gaussian, and each measured value was expressed as a multiple of the normal median (MoM) after adjustment for characteristics found to provide a substantial contribution to the $\log_{10}$ transformed value[21,22]. The observed measurements of fetal HC, AC and FL were expressed as the respective *Z*-score corrected for gestational age[17]. The measured values of UtA-PI and fetal biometry were available in all cases in the study population of 70 003 singleton pregnancies. Maternal serum PlGF measurements were available in 9956 pregnancies, including 86 with a stillbirth. In all stillbirths and subgroups of stillbirths, the mean and SD of $\log_{10}$MoM PlGF values were estimated; the values for PlGF were then simulated in the remaining cases in the study population, based on the bivariate Gaussian distributions of the marker in stillbirths and live births, defined by the mean and SD ($\log_{10}$MoM). The *a-priori* risk for stillbirths was estimated from the algorithm derived from multivariable logistic regression analysis of maternal characteristics and history, as described previously[2]. Univariable and multivariable logistic regression analyses was used to determine the significance of the contribution of these biomarkers in the prediction of stillbirth and whether the addition of serum PlGF ($\log_{10}$ MoM) improved the performance of screening that was achieved by a combination of maternal factors, *Z*-scores of fetal biometry and MoM values of UtA-PI[1]. The variables which provided a significant contribution in the multivariable analysis were used to determine the patient-specific risk of stillbirth using the equation odds/(1 + odds), where odds = e^Y and Y was estimated from the coefficients of variables in the logistic regression analysis. The distribution of patient-specific risks was used to determine the performance of screening by receiver–operating characteristics (ROC) curve analysis and the DR and FPR were estimated.

The statistical software package SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for the data analyses.

RESULTS

Study population

The maternal and pregnancy characteristics of the total study population of 70 003 singleton pregnancies are as previously described (Table S1)[1]. Maternal serum PlGF values were available in 9956 singleton pregnancies, including 9870 live births and 86 antepartum stillbirths; maternal and pregnancy characteristics of this group are shown in Table S2.

Biomarkers in outcome groups

In pregnancies with stillbirth, compared to live births, PlGF MoM was lower (0.65 *vs* 1.00, $P < 0.0001$), UtA-PI MoM was higher (1.37 *vs* 1.00, $P < 0.0001$) and *Z*-scores of HC, AC and FL were lower (−0.20 *vs* −0.01, $P < 0.0001$; −0.29 *vs* 0.00, $P < 0.0001$; −0.12 *vs* −0.01, $P = 0.012$, respectively). In pregnancies with stillbirth due to impaired placentation, compared to live births, the PlGF MoM was lower (0.42 *vs* 1.00, $P < 0.0001$), UtA-PI MoM was higher (1.68 *vs* 1.00, $P < 0.0001$) and *Z*-scores of HC, AC and FL were lower (−0.38

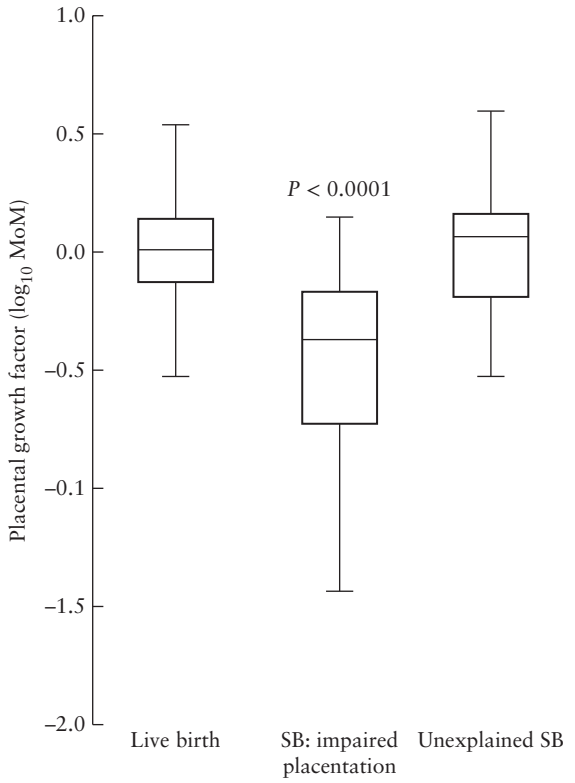

Figure 1 Box-and-whisker plot of placental growth factor in live births, unexplained stillbirths (SB) and SB due to impaired placentation. Boxes with internal lines represent median and interquartile range and whiskers are range. MoM, multiples of the median.

vs −0.01, $P < 0.0001$; −0.66 *vs* 0.00, $P < 0.0001$; −0.46 *vs* −0.01, $P < 0.0001$, respectively); in stillbirths due to unexplained causes there were no significant differences from live births for any of the biomarkers (Table S3 and Figure 1).

Prediction of stillbirth and performance of combined screening

The results of univariable and multivariable regression analyses are shown in Table S4. In the multivariable regression analysis, there was a significant contribution to the prediction of stillbirth due to impaired placentation from maternal factor-derived *a-priori* risk, PlGF MoM, UtA-PI MoM and *Z*-scores of HC and AC, but not FL, ($R^2 = 0.482$; $P < 0.0001$). The performance of screening for stillbirth with these variables is shown in Table 1 and Figure 2.

The DR for all stillbirths, at a 10% FPR, increased from 55% when screening by a combination of maternal factors, fetal biometry and UtA-PI to 58% with the addition of serum PlGF; the respective detection rates for stillbirth due to impaired placentation were 75% and 84%. Within the impaired placentation group, the DR of screening with the combined model was higher for stillbirths < 32 weeks' gestation than that of stillbirth ≥ 37 weeks (97% *vs* 61%; $P < 0.01$).

DISCUSSION

Main findings of the study

The findings of the study demonstrate that a high proportion of stillbirths due to impaired placentation can be identified effectively by second-trimester screening using a combination of maternal factors, serum PlGF, fetal biometry and UtA-PI. Such combined screening at 19–24 weeks' gestation can potentially predict 84% all stillbirths due to impaired placentation, at a 10% FPR; the performance of screening is better for stillbirths < 32 weeks' gestation (97%) than those that occur at term (61%).

Table 1 Performance of screening for all stillbirths and those due to impaired placentation, based on maternal factors and a combination of maternal factors with fetal biometry, uterine artery pulsatility index (UtA-PI) and placental growth factor (PlGF) at 19–24 weeks' gestation

			Detection rate (% (95% CI))	
Outcome	n	*AUC (95% CI)*	*5% FPR*	*10% FPR*
All stillbirths	268			
Maternal factors + biometry + UtA-PI		0.748 (0.711–0.785)	45.1 (39.1–51.0)	54.7 (48.7–60.6)
Maternal factors + biometry + UtA-PI + PlGF		0.781 (0.746–0.817)	50.7 (44.7–56.7)	57.6 (51.7–63.5)
Stillbirth from impaired placentation	159			
Maternal factors + biometry + UtA-PI		0.904 (0.875–0.933)	69.8 (62.7–76.9)	74.8 (68.1–81.6)
Maternal factors + biometry + UtA-PI + PlGF		0.950 (0.932–0.967)	76.1 (69.5–82.7)	83.6 (77.8–89.4)
Stillbirth < 32 weeks	90			
Maternal factors + biometry + UtA-PI		0.952 (0.921–0.982)	85.6 (78.4–92.9)	87.8 (81.0–94.6)
Maternal factors + biometry + UtA-PI + PlGF		0.990 (0.983–0.998)	94.4 (89.7–99.2)	96.7 (93.1–100.0)
Stillbirth < 37 weeks	126			
Maternal factors + biometry + UtA-PI		0.929 (0.899–0.959)	79.4 (72.3–86.5)	82.5 (75.9–89.1)
Maternal factors + biometry + UtA-PI + PlGF		0.973 (0.960–0.985)	84.1 (77.7–90.5)	89.7 (84.5–95.0)
Stillbirth ≥ 37 weeks	33			
Maternal factors + biometry + UtA-PI		0.810 (0.743–0.877)	33.3 (17.2–49.4)	45.5 (28.4–62.4)
Maternal factors + biometry + UtA-PI + PlGF		0.863 (0.802–0.923)	45.5 (28.5–62.5)	60.6 (43.9–77.3)

AUC, area under the receiver–operating characteristics curve; FPR, false-positive rate.

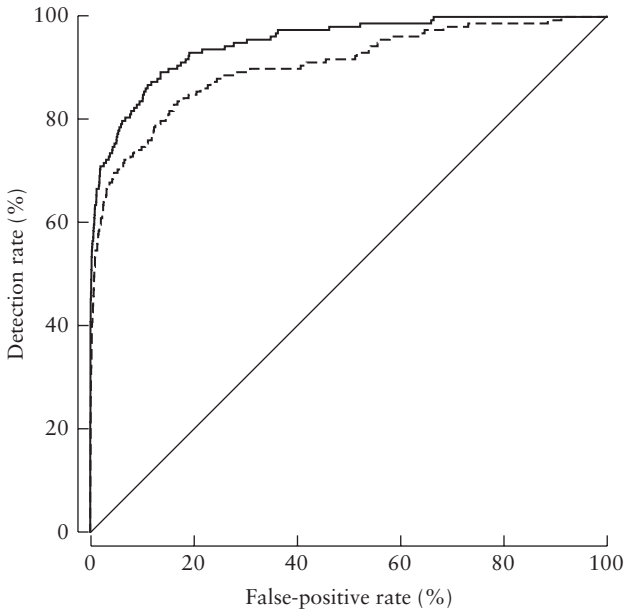

Figure 2 Receiver–operating characteristics curves for prediction of stillbirth due to impaired placentation from screening with a combination of maternal factors, fetal biometry and uterine artery pulsatility index (UtA-PI) (– – –) and with the addition of maternal serum placental growth factor (——).

Strengths and limitations

The strengths of this screening study are first, examination of a large population of pregnant women attending routine assessment at 19–24 weeks' gestation, second, systematic recording of data on maternal characteristics and medical history to identify known risk factors associated with stillbirth, third, use of a specific methodology and appropriately trained doctors to measure UtA-PI, fourth, use of automated machines to provide accurate measurement of maternal serum PlGF within 40 min of sampling, fifth, expression of the values of biomarkers as MoMs after adjusting for factors that affect the measurements, and sixth, use of multivariable regression analysis to take into account possible interrelations between the different variables to define the relative predictive value of each factor.

Potential limitations of the study include estimation of performance based on simulation of PlGF values; however, there was no significant difference between the bivariate Gaussian distributions of the measured and simulated values. Another potential limitation is that the performance of screening by a model derived and tested using the same dataset may be overestimated; consequently, the model needs validation in prospective studies.

Comparison with other studies

Previous studies in the second trimester have reported the benefit of incorporating measurement of serum PlGF in models of screening for PE and SGA[7,10,13]. Previous second-trimester studies have highlighted the value of UtA Doppler in screening for stillbirth[1,23–25]. Our study has demonstrated the value of combining maternal factors, fetal biometry, UtA-PI and PlGF in screening for stillbirth.

Clinical implications of the study

Prevention of stillbirth due to impaired placentation could potentially be achieved by a two-stage screening and intervention strategy. The first stage, at 11–13 weeks, would aim at improving placentation through pharmacological interventions such as low-dose aspirin and pravastatin in the high-risk group[26,27]; first-trimester screening by a combination of maternal factors, UtA-PI, fetal ductus venosus PI for veins and maternal serum PlGF could detect about 60% of stillbirths due to impaired placentation, at a 10% FPR[16]. The second stage, at 19–24 weeks, would identify a high-risk group that could benefit from close monitoring for early diagnosis of PE and SGA and appropriate management to prevent stillbirth in such pregnancies; as demonstrated in this study, about 85% of stillbirths could be predicted from combined screening at 20 weeks' gestation.

ACKNOWLEDGMENTS

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116). The reagents and equipment for the measurement of serum placental growth factor were provided by PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland and Roche Diagnostics Limited, Penzberg, Germany.

REFERENCES

1. Akolekar R, Tokunaka M, Ortega N, Syngelaki A, Nicolaides KH. Prediction of stillbirth from maternal factors, fetal biometry and uterine artery Doppler at 19–24 weeks. *Ultrasound Obstet Gynecol* 2016; **48**: 624–630.
2. Yerlikaya G, Akolekar R, McPherson K, Syngelaki A, Nicolaides KH. Prediction of stillbirth from maternal demographic and pregnancy characteristics. *Ultrasound Obstet Gynecol* 2016; **48**: 607–612.
3. Shore VH, Wang TH, Wang CL, Torry RJ, Caudle MR, Torry DS. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast. *Placenta* 1997; **18**: 657–665.
4. Vuorela P, Hatva E, Lymboussaki A, Kaipainen A, Joukov V, Persico MG, Alitalo K, Halmesmaki E. Expression of vascular endothelial growth factor and placenta growth factor in human placenta. *Biol Reprod* 1997; **56**: 489–494.
5. Akolekar R, Zaragoza E, Poon LC, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11 + 0 to 13 + 6 weeks of gestation in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2008; **32**: 732–739.
6. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11–13 weeks gestation. *Am J Obstet Gynecol* 2016; **214**: 103.e1–12
7. Gallo DM, Wright D, Casanova C, Campanero M, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 19–24 weeks' gestation. *Am J Obstet Gynecol* 2016; **214:** 619e1.
8. Tsiakkas A, Saiid Y, Wright A, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 30–34 weeks' gestation. *Am J Obstet Gynecol* 2016; **215**: 87.e1–17.
9. Andrietti S, Silva M, Wright A, Wright D, Nicolaides KH. Competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 35–37 weeks' gestation. *Ultrasound Obstet Gynecol* 2016; **48**: 72–79.
10. Tsiakkas A, Cazacu R, Wright A, Wright D, Nicolaides KH. Serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for preeclampsia. *Ultrasound Obstet Gynecol* 2016; **47**: 472–477.
11. Poon LC, Zaragoza E, Akolekar R, Anagnostopoulos E, Nicolaides KH. Maternal serum placental growth factor (PlGF) in small for gestational age pregnancy at 11(+0) to 13(+6) weeks of gestation. *Prenat Diagn* 2008; **28**: 1110–1115.
12. Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11–13 weeks. *Fetal Diagn Ther* 2011; **29**: 148–154.

13. Poon LC, Lesmes C, Gallo DM, Akolekar R, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by biophysical and biochemical markers at 19–24 weeks. *Ultrasound Obstet Gynecol* 2015; **46**: 437–445.
14. Bakalis S, Peeva G, Gonzalez R, Poon LC, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by biophysical and biochemical markers at 30–34 weeks. *Ultrasound Obstet Gynecol* 2015; **46**: 446–451.
15. Fadigas C, Peeva G, Mendez O, Poon LC, Nicolaides KH. Prediction of small-for-gestational-age neonates: screening by placental growth factor and soluble fms-like tyrosine kinase-1 at 35–37 weeks. *Ultrasound Obstet Gynecol* 2015; **46**: 191–197.
16. Akolekar R, Machuca M, Mendes M, Paschos V, Nicolaides KH. Prediction of stillbirth from placental growth factor at 11–13 weeks. *Ultrasound Obstet Gynecol* 2016; **48**: 618–623.
17. Snijders RJ, Nicolaides KH. Fetal biometry at 14–40 weeks' gestation. *Ultrasound Obstet Gynecol* 1994; **4**: 34–48.
18. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurements. *Br J Obstet Gynaecol* 1975; **182**: 702–710.
19. Albaiges G, Missfelder-Lobos H, Lees C, Parra M, Nicolaides KH. One-stage screening for pregnancy complications by color doppler assessment of the uterine arteries at 23 weeks' gestation. *Obstet Gynecol* 2000; **96**: 559–564.
20. Poon LCY, Tan MY, Yerlikaya G, Syngelaki A, Nicolaides KH. Birth weight in live births and stillbirths. *Ultrasound Obstet Gynecol* 2016; **48**: 602–606.
21. Tayyar A, Guerra L, Wright A, Wright D, Nicolaides KH. Uterine artery pulsatility index in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 689–697.
22. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015; **45**: 591–598.
23. Smith GC, Yu CK, Papageorghiou AT, Cacho AM, Nicolaides KH, Fetal Medicine Foundation Second Trimester Screening Group. Maternal uterine artery Doppler flow velocimetry and the risk of stillbirth. *Obstet Gynecol* 2007; **109**: 144–151
24. Singh T, Leslie K, Bhide A, D'Antonio F, Thilaganathan B. Role of second-trimester uterine artery Doppler in assessing stillbirth risk. *Obstet Gynecol* 2012; **119**: 256–261.
25. Poon LC, Volpe N, Muto B, Yu CK, Syngelaki A, Nicolaides KH. Second-trimester uterine artery Doppler in the prediction of stillbirths. *Fetal Diagn Ther* 2013; **33**: 28–35.
26. Roberge S, Nicolaides K, Demers S, Villa P, Bujold E. Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. *Ultrasound Obstet Gynecol* 2013; **41**: 491–499.
27. Costantine MM, Cleary K, Hebert MF, Ahmed MS, Brown LM, Ren Z, Easterling TR, Haas DM, Haneline LS, Caritis SN, Venkataramanan R, West H, D'Alton M, Hankins G. Safety and pharmacokinetics of pravastatin used for the prevention of preeclampsia in high-risk pregnant women: a pilot randomized controlled trial. *Am J Obstet Gynecol* 2016; **214**: 720.e1–17.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Maternal and pregnancy characteristics in pregnancies that resulted in stillbirth, stratified according to whether this was unexplained or due to impaired placentation, compared with pregnancies that resulted in live birth

Table S2 Maternal and pregnancy characteristics in pregnancies that resulted in stillbirth and had placental growth factor measurements, stratified according to whether this was unexplained or was due to impaired placentation, compared with pregnancies that resulted in live birth

Table S3 Placental growth factor, uterine artery pulsatility index and fetal biometry at 19–24 weeks' gestation in pregnancies with live birth compared to those with a stillbirth

Table S4 Univariable and multivariable logistic regression analyses for prediction of stillbirth due to impaired placentation by maternal factors and combination of placental growth factor, uterine artery pulsatility index and fetal biometry at 19–24 weeks' gestation

