Indian J Hematol Blood Transfus (Apr-June 2015) 31(2):286–291
DOI 10.1007/s12288-014-0448-3

ORIGINAL ARTICLE

Free Protein S Reference Ranges in Gravidas Without Hereditary and Acquired Thrombophilia

Ahmet Basaran MD · Özgür Deren MD · Yahya Buyukasik MD · Mustafa Basaran MD

Received: 21 April 2014 / Accepted: 5 August 2014 / Published online: 17 August 2014

Abstract We carried out a retrospective cohort study to construct reference ranges for free protein S (FPS) levels during pregnancy and identify any conditions or factors that may affect FPS levels. Patients that were ordered thrombophilia screening tests during gestational period were identified. Patients demonstrated to have hereditary or acquired thrombophilia were excluded. Reference ranges were constructed using regression analysis. Outcome of the index pregnancy and pregnancy complications was used to identify any confounding factors. A total of 455 pregnant women were included. The quadratic equation for FPS according to gestational age (GA) was [75.497 + (−1.516*GA) + 0.018*GA*GA]. FPS level and GA were negatively correlated (Spearmans rho statistic $[r_s] = -0.436$, $p = 0.001$). FPS level and fetal growth restriction (FGR) were negatively correlated ($[r_s] = -0.093$, $p = 0.049$). FPS level and placental abruption were positively correlated ($[r_s] = 0.098$, $p = 0.039$). Stepwise linear regression model constructed to predict FPS level with gestational age, placental abruption and FGR as the predictor variables. Gestational age was the only variable retaining statistically significant relation with FPS level ($\chi^2 = 0.216$, $df = 3$, $p = 0.001$). FPS levels decrease significantly throughout gestation in gravidas without hereditary and/or acquired thrombophilias. In patients without thrombophilia FPS levels are not associated with pregnancy complications. The obtained reference intervals may be useful for the clinicians ordering FPS during pregnancy.

Keywords Thrombophilia · Free protein S · Protein S · Pregnancy

Abbreviations

APC	Activated protein C
C4BP	C4b-binding protein
D&C	Dilatation & curettage
FGR	Fetal growth restriction
FPS	Free protein S
GA	Gestational age
GH	Gestational hypertension
PPROM	Preterm-premature rupture of membranes
PS	Protein S
PVD	Placental vascular disorders

Introduction

Still birth, severe fetal growth restriction, placental abruption, early onset and severe preeclampsia can be observed in 0.2–3 % of all pregnancies [1]. Although not all, these pregnancy complications were related to placental vascular disorders (PVD). Several systematic reviews and few prospective studies linked PVD with hereditary and/or acquired thrombophilias [2, 3]. In addition to the hereditary and acquired thrombophilias, pregnancy itself is an acquired hypercoagulable state that enhances thrombogenic potential of these disorders. During

A. Basaran MD (✉) · M. Basaran MD
Obstetrics & Gynecology Department, Konya Training and Research Hospital, Kılıçarslan mah, Nurdağ Sk. Sinanoba sitesi, B-blok No:19 Selçuklu, 42080 Konya, Turkey
e-mail: dr_ahmetbasaran@yahoo.com

Ö. Deren MD
Division of Perinatology, Obstetrics & Gynecology Department, Hacettepe University School of Medicine, Ankara, Turkey

Y. Buyukasik MD
Division of Hematology, Internal Medicine Department, Hacettepe University School of Medicine, Ankara, Turkey

pregnancy significant variation occurs in the levels of endogenous anti-coagulant proteins [4]. Similarly, there is significant decrease in the levels of free protein S (FPS) which is thought to be physiologic. Theoretically, this decrease in protein S (PS) and FPS levels may also be offered to cause predisposition to thrombosis and PVD. In order to differentiate between normal and abnormal during pregnancy cut-off values had been previously proposed for clinical use [5, 6]. However, reference curves for the gestational age in weeks for FPS haven't been established. Herein, we construct reference ranges of FPS and analyze factors affecting FPS level in a cohort of patients without hereditary and acquired thrombophilia.

Materials and Methods

We performed a retrospective cohort study at obstetrics and gynecology department of Hacettepe University School of Medicine. Institutional review board approved the study (protocol FON05/33). Medical records of the patients who had admitted to our department between 2001 and 2004 were retrieved from the database system and archives. Consecutive patients whom were ordered thrombophilia screening tests during the gestational period were identified. The battery of ordered screening tests was antithrombin, protein C, FPS, activated protein C (APC) resistance, homocystein, lupus anticoagulant, antiphospholipid antibodies, factor V leiden mutation, prothrombin G20210A mutation. Tests were performed once during pregnancy and each patient was included for one time in the study. Patients demonstrated to have hereditary or acquired thrombophilia were excluded from the study. In the context of acquired thrombophilia, revised Sapporo criteria were used for the classification of antiphospholipid antibody syndrome [7]. Patients using anticoagulant treatment, patients with deep vein thrombosis and pulmonary embolism were also excluded from the study. Complications that developed during the index pregnancy were used to analyze and detect any factors or conditions affecting the levels of FPS. Patients, whose thrombophilia tests that were ordered during or after the diagnosis of adverse pregnancy outcome/complication, were also excluded from the study.

Pregnancy complications were defined according to the criteria below:

1. Fetal growth restriction (FGR):
   a. Small for gestational age: Defined as estimated fetal weight <10th percentile for gestational age [8, 9].
   b. FGR due to placental disease: Small for gestational age patients with identified Doppler abnormalities without any congenital anomaly/disorder [10, 11].
2. Gestational hypertension (GH)/Preeclampsia: Defined as normotension prior to 20 weeks gestation, hypertension (single diastolic blood pressure of 110 mm Hg or greater, or consecutive reading of 90 mm Hg or greater on more than one occasion at least 4 h apart, and proteinuria (more than 300 mg per 24 h) developing after 20 weeks [12, 13] [14].
3. Preterm delivery: Defined as delivery occurring prior to 37 completed weeks of gestation, associated with intact membranes [15].
4. Preterm-premature rupture of membranes (PPROM): Rupture of the amniotic membranes with release of the amniotic fluid prior to 37 weeks of gestation [16]. PPROM patients were not added to the preterm delivery group.
5. Oligohydramnios: Four quadrant amniotic fluid index $\leq$5 cm [17].
6. Gestational diabetes: Diagnosed with 100 g oral glucose tolerance test [18] or 75 g oral glucose tolerance test [19].
7. Placental abruption; one or more of the following criteria: [20].
   a. The freshly delivered placenta showing evidence of retroplacental bleeding or clot(s).
   b. Placental abruption diagnosed on prenatal ultrasound.
   c. Histopathologic diagnosis.

Correlation analysis with construction of the correlation matrix between patient demographics, gestational age, pregnancy complications and FPS levels were performed and evaluated in order to search for any factor that may be affecting the FPS levels. Significant correlations with large size effects according to Cohen's guidelines were used to indicate the presence of factors that may affect FPS levels. We did not have any priori assumption about the possible associations between pregnancy complications and FPS levels. Therefore, current study is an exploratory study. The effects of multiple factors that were found to be related with each other were evaluated with linear regression.

To evaluate the mean FPS levels according to trimesters ANNOVA with post hoc tests was performed. To create reference ranges of FPS curve-fitting was used and quadratic equation was obtained. To construct percentiles, standard deviations from four weekly periods were obtained and curve fitting was used to create a cubic equation for standard deviation. Then the formula [mean $\pm$ z-score*standard deviation] was used to construct 10th and 90th percentiles. For the statistical analysis SPSS ® (Statistical Package for Social Sciences) program version 21 for Windows ® was used and $p < 0.05$ was considered significant.

Biochemical Assay

We measured FPS antigen levels by Liatest FPS kit on STA Compact from Diagnostica Stago (Asnieres Sur Seine, France). This is an immunoturbidimetric, antigenic assay for FPS. The intra-and inter-assay variation coefficients at normal levels of FPS are 2.3 and 3.9 %, respectively. The intra-and inter-assay variation coefficients at low levels of FPS are 5.7 and 8.1 %, respectively [21].

Results

In the indicated study period 455 patients were identified according to predefined selection criteria. Demographic characteristics of the patients were summarized in the Table 1. Complications observed in the current pregnancy were summarized in Fig. 1 (interestingly still birth was not observed in this selected group of patients). Fetal congenital anomaly rate was 2.4 %.

Table 1 Demographics

	Mean ± SD/ median	Range
Age	29.2 ± 5.44	17–44
Gravidity	2[a]	1–8
Parity	1[a]	0–6
Abortion	1[a]	0–6
D and C	1[a]	0–4
Living children	1[a]	0–6
Birth weight (g)	3,132 ± 670	253–4,650
Gestational age at delivery (weeks)	37.8 ± 2.9	18–41
Patients with placental abruption	33.3 ± 4.3	28–38

[a] Median values for respective variables

Mean gestational age (GA) at which FPS levels ordered was 20.34 ± 10.3 (range 5–40) weeks. FPS levels were ordered at first trimester, second trimester and third trimester in 36.2, 29.9 and 33.9 % of the patients, respectively. The scatter plot of FPS and superimposed quadratic equation curves were given in Fig. 2. The reference lines represent the mean, 10th and 90th percentiles. For the indicated equations residual plots were obtained and controlled for symmetry and linearity. The quadratic equation of FPS was [75.497 + (−1.516*GA) + 0.018*GA*GA] (R-squared = 0.22). The quadratic equation for standard deviation was [30.074 + (−1.22*GA) + (0.019*GA*GA)] (R-squared = 0.821). Means and 95 % CIs of the mean according to the trimesters were provided in Table 2.

Correlation matrix was constructed for FPS, gestational age, patient demographics, birth weight and pregnancy complications to detect any confounding factors and associations. Statistically significant correlations were observed between FPS level and gestational age (Spearmans rho statistic [r_s] = −0.436, p = 0.001), FPS level and placental abruption ([r_s] = 0.098, p = 0.039), and between FPS level and FGR ([r_s] = −0.093, p = 0.049). Using Cohen's guidelines with the exception of association between FPS and gestational age the effect sizes were small. Moreover, in the correlation matrix placental abruption was also found to be positively correlated with GH/preeclampsia (r_s = 0.111, p = 0.018) and acute fetal distress (r_s = 0.322, p = 0.001).

Linear regression model was constructed to predict FPS level with gestational age, placental abruption and FGR as

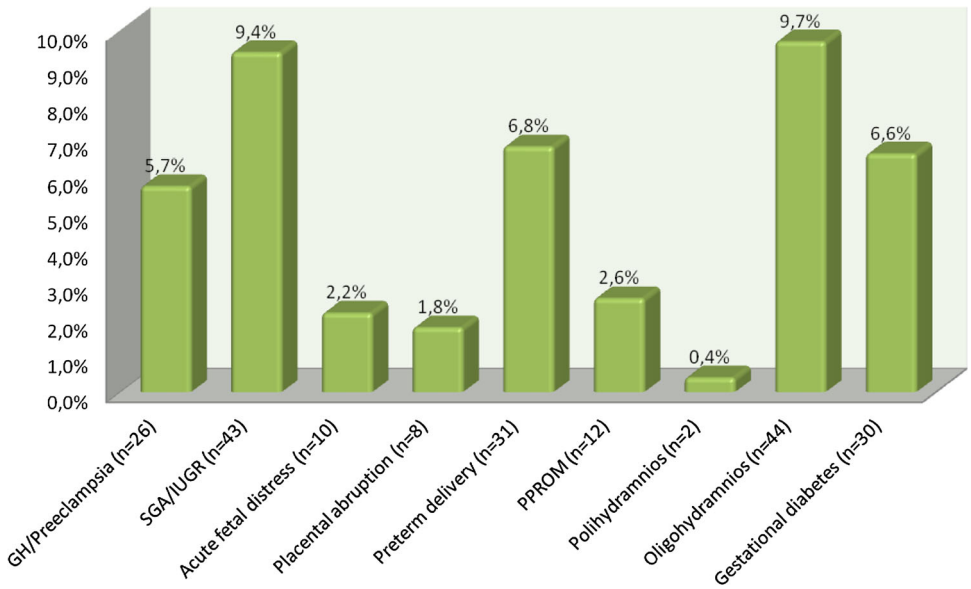

Fig. 1 Rates of observed index pregnancy complications (n = number of observed events)

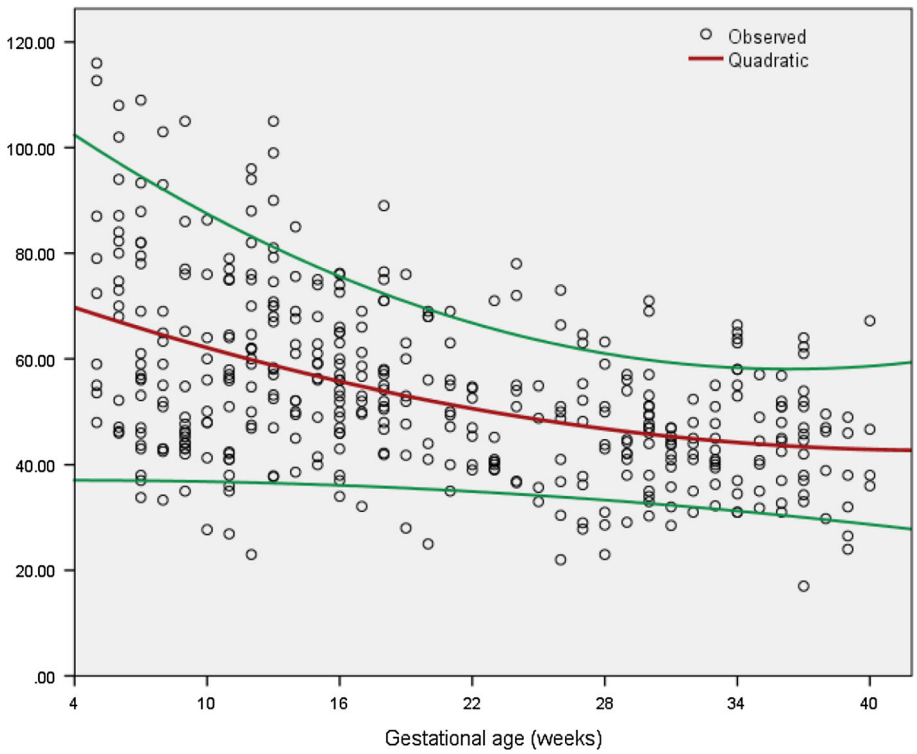

Fig. 2 Scatter plot of the free protein S levels with superimposed quadratic curves representing 10th percentile, mean, and 90th percentile

Table 2 Free protein S levels according to trimesters

	FPS	
	Mean ± SD	95 % CI of the mean
First trimester	62.48 ± 19.58[a]	59.35–65.61
Second trimester	53.36 ± 12.94[a]	51.05–55.67
Third trimester	44.61 ± 10.64[a]	42.81–46.41

[a] All three trimesters FPS mean levels were different from each other ($p = 0.001$)

the predictor variables (variables found to be associated in the correlation analysis). FGR and placental abruption were not significantly affecting the FPS level in the model. Gestational age was the only variable retaining statistically significant relation with FPS level ($\chi^2 = 0.216$, df = 3, $p = 0.001$).

Discussion

Antithrombin, protein C, and PS function as physiologic inhibitors of the coagulation and are, therefore, referred to as natural anticoagulants. Deficiencies of these proteins can lead to an imbalance in basal coagulation activity toward a prothrombotic state. The normal pregnant woman is in a prothrombotic state when compared with the age-matched non-pregnant woman. The risk for thrombosis is substantially increased in pregnant woman, and thrombosis represents the greatest cause of maternal morbidity and mortality [1]. During pregnancy increase in several of the coagulation factors can be observed, and a decrease in the natural anticoagulant system is manifested by lower levels of PS, FPS and increased resistance to APC. PS functions as a cofactor for the degradation of factors Va and VIIIa by APC. PS is also capable of directly inactivating factors Va and Xa. PS exists in equilibrium with FPS, and PS bound to a carrier protein called C4b-binding protein (C4BP). Only FPS is considered to be active and increased levels of C4BP can decrease PS activity. C4BP levels are increased during pregnancy and oral contraceptive use [22]. With advancing gestational age both PS and FPS levels decrease. It has been reported that lowest PS and FPS levels are reached in the second trimester and then a plateau is observed [4, 23, 24]. However, our study and other studies also showed that FPS levels continue to decrease modestly during the third trimester [5, 25, 26]. This difference between studies may be due to sampling interval and repeated measures design (longitudinal study) of the previous studies. In the current study, each patient contributed one value to the reference sample (cross-sectional study) in contrast to repeated measures design where each patient contributes several times. Disadvantage of longitudinal study is the loss of variability in the sample because serial measurements on an individual are highly correlated [27, 28]. In the longitudinal study of the Szecsi et al. patients

Table 3 FPS mean, 10th and 90th percentiles according to gestational age in weeks

Weeks	Mean	10th	90th
5.0	68.4	37.0	99.7
6.0	67.0	37.0	97.1
7.0	65.8	37.0	94.6
8.0	64.5	36.9	92.1
9.0	63.3	36.9	89.8
10.0	62.1	36.8	87.5
11.0	61.0	36.7	85.3
12.0	59.9	36.6	83.2
13.0	58.8	36.5	81.2
14.0	57.8	36.4	79.2
15.0	56.8	36.2	77.4
16.0	55.8	36.1	75.6
17.0	54.9	35.9	73.9
18.0	54.0	35.8	72.3
19.0	53.2	35.6	70.8
20.0	52.4	35.4	69.4
21.0	51.6	35.2	68.0
22.0	50.9	34.9	66.8
23.0	50.2	34.7	65.6
24.0	49.5	34.4	64.5
25.0	48.8	34.2	63.5
26.0	48.2	33.9	62.6
27.0	47.7	33.6	61.8
28.0	47.2	33.3	61.0
29.0	46.7	33.0	60.3
30.0	46.2	32.7	59.8
31.0	45.8	32.3	59.3
32.0	45.4	32.0	58.9
33.0	45.1	31.6	58.5
34.0	44.8	31.2	58.3
35.0	44.5	30.8	58.1
36.0	44.2	30.4	58.1
37.0	44.0	30.0	58.1
38.0	43.9	29.6	58.2
39.0	43.8	29.1	58.4
40.0	43.7	28.7	58.6

were sampled beginning from 13 weeks and authors created four antenatal periods (13–20, 21–28, 29–34, 35–42 weeks; first trimester was excluded). Szecsi et al. showed that FPS doesn't considerably change in the 29–42 weeks period. We had observed the same trend (Fig. 2), the change in the mean FPS was modest in the 29–40 weeks period. Moreover, Fig. 2 showed that decrease in the first trimester and second is prominent, which was also concordant with the previous studies.

Paidas et al. [5] compared patients with pregnancy complications and normal outcome according to PS and protein Z levels. They showed that for all trimesters, patients with adverse pregnancy outcome had significantly lower PS and protein Z levels. When they evaluated adverse pregnancy outcomes individually, patients with FGR had the lowest mean protein Z levels and patients with PPROM had the highest mean protein Z levels. However, they were not able to show any statistically significant difference for PS. From their data Paidas et al. [5, 6] proposed cut-off values for FPS. Authors identified 30 and 25 % cut-off values for FPS in the second and third trimesters, respectively. Reference curves that we had obtained from our cohort also indicated similar cut-off values (Fig. 2; Table 3). Hojo et al. [29] compared maternal PS levels between gestations complicated with FGR and uncomplicated gestations. PS activity in women with FGR was significantly decreased in both second trimester (36.6 ± 13.2 %) and third trimester (30.2 ± 12.2 %) compared with the control group levels. In our study, there was weak positive correlation between placental abruption with FPS levels and weak negative correlation between FGR with FPS levels. However, when the effect of gestational age was removed these weak correlations lost their significance. Very recently, association between placental abruption and anticoagulant system was investigated by Ananth et al. [30] in postpartum patients within 72 h of delivery. In this study decrease in PS was not associated with placental abruption.

In conclusion, FPS levels decrease significantly throughout gestation in gravidas without hereditary and/or acquired thrombophilias. Accordingly it should not be ordered during pregnancy for diagnostic purposes. In patients without thrombophilias FPS levels are not associated with adverse pregnancy outcomes; therefore it does not require any treatment. The obtained reference intervals may be useful for the clinicians ordering FPS during pregnancy.

Conflict of interest The authors declare that there are no conflicts of interests.

Ethics Approval The study was approved by the ethics committee of the Hacettepe University, School of Medicine, Ankara, Turkey (Protocol FON05/33).

References

1. Lockwood CJ (2002) Inherited thrombophilias in pregnant patients: detection and treatment paradigm. Obstet Gynecol 99(2):333–341
2. Robertson L, Wu O, Langhorne P, Twaddle S, Clark P, Lowe GD, Walker ID, Greaves M, Brenkel I, Regan L, Greer IA (2006) Thrombophilia in pregnancy: a systematic review. Br J Haematol 132(2):171–196. doi:10.1111/j.1365-2141.2005.05847.x
3. Said JM, Higgins JR, Moses EK, Walker SP, Borg AJ, Monagle PT, Brennecke SP (2010) Inherited thrombophilia polymorphisms

and pregnancy outcomes in nulliparous women. Obstet Gynecol 115(1):5–13. doi:10.1097/AOG.0b013e3181c68907
4. Malm J, Laurell M, Dahlback B (1988) Changes in the plasma levels of vitamin K-dependent proteins C and S and of C4b-binding protein during pregnancy and oral contraception. Br J Haematol 68(4):437–443
5. Paidas MJ, Ku DH, Lee MJ, Manish S, Thurston A, Lockwood CJ, Arkel YS (2005) Protein z, protein S levels are lower in patients with thrombophilia and subsequent pregnancy complications. J Thromb Haemost 3(3):497–501
6. Practice bulletin no. 138: inherited thrombophilias in pregnancy (2013). Obstet Gynecol 122(3):706–716. doi:10.1097/01.AOG.0000433981.36184.4e
7. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, PG DEG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA (2006) International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 4(2):295–306. doi:10.1111/j.1538-7836.2006.01753.x
8. Bakketeig LS, Butte N, de Onis M, Kramer M, O'Donnell A, Prada JA, Hoffman HJ (1998) Report of the IDECG Working Group on definitions, classifications, causes, mechanisms and prevention of IUGR. Eur J Clin Nutr 52(Suppl 1):S94–S96
9. Tan TY, Yeo GS (2005) Intrauterine growth restriction. Curr Opin Obstet Gynecol 17(2):135–142
10. Miller J, Turan S, Baschat AA (2008) Fetal growth restriction. Semin Perinatol 32(4):274–280. doi:10.1053/j.semperi.2008.04.010
11. Turan S, Miller J, Baschat AA (2008) Integrated testing and management in fetal growth restriction. Semin Perinatol 32(3):194–200. doi:10.1053/j.semperi.2008.02.008
12. Report of the national high blood pressure education program working group on high blood pressure in pregnancy (2000). Am J Obstet Gynecol 183(1):S1–S22
13. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM (2001) The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the international society for the study of hypertension in pregnancy (ISSHP). Hypertens Pregnancy 20(1):IX–XIV. doi:10.1081/PRG-100104165
14. National Institute for Health and Clinical Excellence (2011) Hypertension in pregnancy: the management of hypertensive disorders during pregnancy [CG107] (CG107 January 2011). London: National Institute for Health and Clinical Excellence
15. Ananth CV, Vintzileos AM (2006) Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med 19(12):773–782. doi:10.1080/14767050600965882
16. Simhan HN, Canavan TP (2005) Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. BJOG 112(Suppl 1):32–37. doi:10.1111/j.1471-0528.2005.00582.x
17. Schrimmer DB, Moore TR (2002) Sonographic evaluation of amniotic fluid volume. Clin Obstet Gynecol 45(4):1026–1038
18. Hollander MH, Paarlberg KM, Huisjes AJ (2007) Gestational diabetes: a review of the current literature and guidelines. Obstet Gynecol Surv 62(2):125–136. doi:10.1097/01.ogx.0000253303.92229.59
19. National Institute for Health and Clinical Excellence (2008) Diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period [CG63] (CG63 July 2008). London: National Institute for Health and Clinical Excellence
20. Elsasser DA, Ananth CV, Prasad V, Vintzileos AM (2009) Diagnosis of placental abruption: relationship between clinical and histopathological findings. Eur J Obstet Gynecol Reprod Biol. doi:10.1016/j.ejogrb.2009.10.005
21. Deffert C, Esteve F, Grimaux M, Gouault-Heilmann M (2001) A direct, automated, immuno-turbidimetric assay of free protein S antigen in plasma. Blood Coagul Fibrinolysis 12(2):137–141
22. Buchanan GS, Rodgers GM, Ware Branch D (2003) The inherited thrombophilias: genetics, epidemiology, and laboratory evaluation. Best Pract Res Clin Obstet Gynaecol 17(3):397–411
23. Szecsi PB, Jorgensen M, Klajnbard A, Andersen MR, Colov NP, Stender S (2010) Haemostatic reference intervals in pregnancy. Thromb Haemost 103(4):718–727. doi:10.1160/TH09-10-0704
24. Hansen AT, Andreasen BH, Salvig JD, Hvas AM (2011) Changes in fibrin D-dimer, fibrinogen, and protein S during pregnancy. Scand J Clin Lab Invest 71(2):173–176. doi:10.3109/00365513.2010.545432
25. Faught W, Garner P, Jones G, Ivey B (1995) Changes in protein C and protein S levels in normal pregnancy. Am J Obstet Gynecol 172(1 Pt 1):147–150
26. Kurasawa G, Kotani K, Ito Y, Saiga K, Iijima K (2007) Reduction in protein S activity during normal pregnancy. Aust N Z J Obstet Gynaecol 47(3):213–215. doi:10.1111/j.1479-828X.2007.00720.x
27. Altman DG, Chitty LS (1993) Design and analysis of studies to derive charts of fetal size. Ultrasound Obstet Gynecol 3(6):378–384. doi:10.1046/j.1469-0705.1993.03060378.x
28. Royston J (1993) Calculation of cross-sectional, longitudinal and conditional reference intervals from longitudinal measurements. Submitted
29. Hojo S, Tsukimori K, Kinukawa N, Hattori S, Kang D, Hamasaki N, Wake N (2008) Decreased maternal protein S activity is associated with fetal growth restriction. Thromb Res 123(1):55–59. doi:10.1016/j.thromres.2008.01.016
30. Ananth CV, Nath CA, Philipp C (2010) The Normal anticoagulant system and risk of placental abruption: protein C, protein S and resistance to activated protein C. J Matern Fetal Neonatal Med 23(12):1377–1383. doi:10.3109/14767051003710284