Immunoblot for PAPP-A2 of pregnancy sera (10uL; diluted 1:10 in PBS) from pregnancies at stated gestational ages (weeks+days) from normotensive (n=6; CTL) or preeclamptic (n=7; PE) pregnancies. Serum from non-pregnant female and male serve as negative controls and serum from pregnant female serves as positive control. Samples ordered by decreasing age (39 to 25 weeks gestation) within each category. Non-specific band (*) at ~60kDa consistent with albumin shows protein loading. The 36-week CTL sample is clearly underloaded. (Image of full blot provided as supplemental data; Fig. S4)

Figure 6: PAPP-A2 is not detectable in cord blood serum.

Immunoblot for PAPP-A2 on venous cord blood serum (12 µl; 1:10 dilution with 1x PBS) from normotensive (n= 5) and preeclamptic (n=6) pregnancies with maternal serum from some of the same pregnancies (Mat; n=4). Gestational age at delivery noted as weeks + days above lane. Non-pregnant and pregnant sera serve as negative and positive controls. Non-specific band (*) at ~60kDa consistent with albumin reflects protein loading. An overexposed film is shown to clarify lack of detection of PAPP-A2 in cord blood sera. (Image of full blot provided in supplemental data; Fig. S5).

Supplemental Data

Table S1: Semi-quantitative PAPP-A2 Immunoreactivity

Formalin-fixed serial tissue sections from across gestation were stained for cytokeratin-7 (CK-7) to identify all trophoblasts, HLA-G to identify invasive CTBs, and PAPP-A2. The CK-7 and HLA-G staining identified trophoblast populations and served as control for staining quality of the tissue. PAPP-A2 immunoreactivity by cell type, as assessed by morphology and trophoblast staining, was scored as follows: (-) =no immunoreactivity,

+=light, ++=moderate, and +++ =strong. Number of placentas with each degree of staining listed in ( ). The variability of staining is represented by range of staining intensity. STB: synctiotrophoblasts, vCTB:villous cytotrophoblast,, CC: cell column, iCTB: invasive cytotrophoblast.

Figure S1: Placental PAPP-A2 Expression Changes over Gestation. Immunoblot of PAPP-A2 expression in chorionic villi in the second trimester. Image of the full blot presented in Fig. 3C. Image is overexposed to reveal all immunoreactive bands. Of interest the band pattern is different between the serum and the placental tissue lysates. Non-specific bands are present at ~50kDa and 75kDa in the placental lysates. Several samples have additional bands (15+5, 18+0 and 19+5) which may reflect alternative isoforms or degradation products at ~45kDa and ~60kDa. Of interest is the 23+3 week samples, which had minimally detectable ß-actin (Fig.3C), does show similar levels of the ~50kDa non-specific band to the other samples.

Figure S2: Labor does not effect PAPP-A2 expression in the placenta.
Densitometry in relative units of PAPP-A2 levels normalized to ß-actin determined by Immunoblot on basal plate (BP) protein lysates (Panel A) or chorionic villi (CV) protein lysates (Panel B) from term pregnancies delivered by scheduled cesarean section (n=8; non-labored) or vaginal delivery (n=8: labored). Data presented as mean and standard error. Statistical analysis performed using Student's t-test. There was no statistical difference in PAPP-A2 in labored (43.70 ± 2.71) and unlabored (34.00 ± 2.45) BP samples, p=0.25. There was no statistical difference in PAPP-A2 in labored (48.94 ± 3.06) and unlabored (62.37 ± 7.33) CV samples, p=0.52.

Figure S3: Placental PAPP-A2 expression levels for early onset and late onset preeclampsia (PE). Data is that presented in Figure 4 broken out by early (24-34 weeks) and late onset (35-40 weeks) PE and matched controls (CTL). Relative PAPP-A2 levels normalized to ß-actin determined by immunoblot analysis of basal plate (A) and chorionic villi (B) protein lysates. Early onset are circles and late onset are triangles. CTLs are open symbols and PE is closed symbols. Statistical analysis by Kruskal-Wallis with Dunn's Post test showed only preterm CTl versus late PE were significantly different (p=<0.01). Small sample sizes (n=4) likely contributes to the lack of statistical differences.

Figure S4: Serum levels of PAPP-A2 are higher from pregnancies complicated by preeclampsia. Image of the full immunoblot presented in Fig. 5 that has been overexposed to show all immunoreactive bands. PAPP-A2 is the ~220kDa band. Arrow points to band that may represent the alternative spiced form of PAPP-A2 reported by Kloverpris *et al.* ; although this band migrates at a higher molecular weight (>100kDa compared to ~70kDa). The broad band at ~60kDa (*) likely represents non-specific binding of antibody to albumin based on Ponseau S staining of the membrane (data not shown).

Figure S5: PAPP-A2 is not detectable in cord blood serum. Image of the full immunoblot presented in Fig.6 that has been overexposed to show all immunoreactive bands. PAPP-A2 is the ~220kDa band. Arrow points to band that may represent the alternative spiced form of PAPP-A2 reported by Kloverpris *et al*. Although this band migrates at a higher molecular weight (>100kDa compared to ~70kDa). The broad band at ~60kDa (*) likely represents non-specific binding of antibody to albumin based on

Ponseau S staining of the membrane (data not shown). The single band in sample Cord 39 (between 75 and 100kDa) is not seen in any other sample and identity has not been determined.