Preeclampsia

Spiral Artery Remodeling and Trophoblast Invasion in Preeclampsia and Fetal Growth Restriction

Relationship to Clinical Outcome

Fiona Lyall, Stephen C. Robson, Judith N. Bulmer

See Editorial Commentary, pp 1013–1014

Abstract—Failure to transform uteroplacental spiral arteries is thought to underpin disorders of pregnancy, including preeclampsia and fetal growth restriction (FGR). In this study, spiral artery remodeling and extravillous-cytotrophoblast were examined in placental bed biopsies from normal pregnancy (n=25), preeclampsia (n=22), and severe FGR (n=10) and then compared with clinical parameters. Biopsies were immunostained to determine vessel wall integrity, extravillous-cytotrophoblast location/density, periarterial fibrinoid, and endothelium. Muscle disruption was reduced in myometrial spiral arteries in preeclampsia (P=0.0001) and FGR (P=0.0001) compared with controls. Myometrial vessels from cases with birth weight <5th percentile (P<0.001), abnormal uterine Doppler (P<0.01), abnormal umbilical artery Doppler (P<0.001), and preterm delivery (P<0.001) had less muscle destruction compared with >5th percentile. Fewer extravillous-cytotrophoblast surrounded both decidual and myometrial vessels in the normal group and preeclampsia group compared with the FGR group (P=0.001). For myometrial vessels, the normal group contained more intramural extravillous-cytotrophoblast than in preeclampsia (P=0.015). Decidual vessels in the FGR group had less fibrinoid deposition compared with controls (P=0.013). For myometrial vessels, less fibrinoid was deposited in both the preeclampsia group (P=0.0001) and the FGR group (P=0.01) when compared with controls, and less fibrinoid was deposited in the preeclampsia group when compared with FGR group (P<0.001). Myometrial vessels obtained from birth weights <5th percentile had less periarterial fibrinoid than those with >5th percentile (P<0.02). A major defect in myometrial spiral artery remodeling occurs in preeclampsia and FGR that is linked to clinical parameters. Interstitial extravillous-cytotrophoblast is not reduced in preeclampsia but is increased in FGR. (Hypertension. 2013;62:1046-1054.) ● Online Data Supplement

Key Words: decidua ▪ Doppler ultrasonography ▪ intrauterine growth retardation ▪ myometrium ▪ preeclampsia ▪ pregnancy ▪ spiral artery ▪ trophoblasts

The placental bed contains the uterine spiral arteries that supply oxygenated blood to the growing placenta and fetus. Important changes occur in the placental bed throughout pregnancy. Extravillous trophoblast (EVT) cells proliferate from anchoring chorionic villi and invade the decidualized endometrium by 2 pathways: interstitial EVT cells invade the decidualized endometrium and inner myometrium, and endovascular EVT cells invade the lumen of the spiral arteries. Some EVT become incorporated into the spiral artery wall as intramural trophoblast. The endothelium, vascular smooth muscle, and elastic lamina are destroyed and replaced by fibrinoid. By term, maternal vascular repair occurs with re-endothelialization.1 EVT cell invasion is thought to be instrumental in the transformation of the muscular spiral arteries into distended, thin-walled flaccid vessels. Remodeling is necessary for a successful pregnancy.

Preeclampsia, defined by hypertension and proteinuria, affects 3% to 7% of pregnancies, is a major contributor to maternal and fetal morbidity and mortality,2 and responsible for 50,000 deaths worldwide annually.3 Although preeclampsia is often associated with fetal growth restriction (FGR), FGR can occur in the absence of maternal features. Mothers and children of a pregnancy complicated by preeclampsia have a long-term increased risk of cardiovascular diseases and premature death.4,5 The origin of preeclampsia is not understood but is strongly associated with failure or partial failure...
of spiral artery remodeling. Originally thought to be specific for myometrial vessels, suboptimal remodeling has also been reported in decidual vessels. Unmodified/partially modified vessels can supply high or intermittent pulses of pressure flow and so damage to the placenta results from hydrostatic stress or from changes in oxygen delivery.

A 3-stage model of preeclampsia has been proposed. The first stage involves partial failure of the maternal immune tolerance to antigens expressed by paternally derived EVT. This leads to reduced invasion of fetal EVT cells and reduced remodeling of uterine spiral arteries (stage 2) and ultimately a dysfunctional uteroplacental circulation with oxidative stress and generation of factors released into the maternal circulation (stage 3).

Classical histological studies on the placental bed in preeclampsia were derived from observations on paraffin sections; interpretations were limited because of lack of antibodies to identify specific cell types and structures. Difficulties in obtaining placental bed biopsies have hampered further research. In this study, we used immunohistochemical methods applied to frozen sections of the placental bed to reveal new insights into failed physiological changes in preeclampsia and FGR, and for the first time, we link these changes to maternal and fetal clinical parameters.

Materials and Methods

Subjects

Samples were obtained from pregnant women at the Royal Victoria Infirmary, Newcastle upon Tyne. The study was approved by the Joint Ethics Committee of Newcastle upon Tyne Health Authority and Newcastle University. All patients gave informed consent. The principles of the Declaration of Helsinki and all government regulations were adhered to. Placental bed biopsies meeting all the criteria (listed below) were obtained from 3 groups of women: healthy pregnant women with no hypertension or FGR who served as controls (n=25), women with pregnancies complicated by preeclampsia (n=22), and women with pregnancies complicated by severe FGR in the absence of maternal hypertension (n=10). All women from the 3 groups listed in the Table provided biopsies that met the criteria for the study. preeclampsia was defined as pregnancy-induced hypertension (blood pressure ≥140/90 mm Hg) and proteinuria (≥300 mg/24 h) in women who were normotensive before pregnancy and had no other underlying clinical problems, such as renal disease.

FGR was defined ultrasonically as a fetal abdominal circumference <10th percentile with a decrease in abdominal circumference SD score (SDS) of >1.5 SDSs and an umbilical artery pulsatility index of ≥95th percentile.

We have shown previously that a fall in abdominal circumference SDS score of >1.5 SDSs is the optimal cutoff to define a group of fetuses with evidence of wasting at birth and morbidity associated with FGR. Doppler waveforms were obtained from the right and left uterine arteries as described previously. An abnormal waveform was defined as a mean pulsatility index ≥95th percentile or the presence of bilateral notching.

Birth weight percentiles were obtained from charts of the Northern Region population of England.

Sample Collection

Placental bed biopsies were obtained from women undergoing elective cesarean section as described previously. Briefly, after delivery of the infant, the position of the placenta was determined by manual palpation. Three or 4 placental bed biopsy samples (3–5 mm3) were taken under direct vision using biopsy forceps (Wolf, Wimbledon, United Kingdom). The number of suitable biopsies varied between 1 and 3 per woman. In 3 cases (2 near term preeclampsia and 1 near term FGR) the study. preeclampsia was defined as pregnancy-induced hypertension (blood pressure ≥140/90 mm Hg) and proteinuria (≥300 mg/24 h) in women who were normotensive before pregnancy and had no other underlying clinical problems, such as renal disease.

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Birth weight percentiles were obtained from charts of the Northern Region population of England.

Table. Clinical Details of Patients Recruited for Placental Bed Sampling

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Control (n=25)</th>
<th>PE (n=22)</th>
<th>FGR (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of primigravid</td>
<td>13</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Race (% white)</td>
<td>88</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>No. of vessels examined</td>
<td>Decidual (17)</td>
<td>Decidual (14)</td>
<td>Decidual (7)</td>
</tr>
<tr>
<td></td>
<td>Myometrial (33)</td>
<td>Myometrial (22)</td>
<td>Myometrial (12)</td>
</tr>
<tr>
<td>Umbilical artery Doppler</td>
<td>Normal, 25</td>
<td>Normal, 19</td>
<td>AEDF, 4</td>
</tr>
<tr>
<td></td>
<td>AEDF, 6</td>
<td>Increased Pl, 4</td>
<td></td>
</tr>
<tr>
<td>Uterine artery Doppler</td>
<td>Normal, 25</td>
<td>Abnormal, 2</td>
<td>Abnormal, 6</td>
</tr>
<tr>
<td></td>
<td>Normal, 1</td>
<td>Not recorded, 3</td>
<td></td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>31.0 (0.13)</td>
<td>28 (1.3)</td>
<td>29.7 (2.5)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>118.3 (8.2)</td>
<td>155.5 (15.3)*</td>
<td>123.0 (11.3)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73.4 (6.8)</td>
<td>105.7 (8.9)*</td>
<td>75.8 (8.2)</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>2.84 (0.19)</td>
<td>2.25 (0.20)†</td>
<td>1.37 (0.11)‡</td>
</tr>
<tr>
<td>Birth weight percentile</td>
<td>&gt;10th, 25</td>
<td>&gt;10th, 16</td>
<td>5th–10th, 3</td>
</tr>
<tr>
<td></td>
<td>5th–10th, 2</td>
<td>&lt;5th, 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;5th, 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median birth weight percentile</td>
<td>50</td>
<td>15†</td>
<td>2§</td>
</tr>
<tr>
<td>Gestation at delivery, wk</td>
<td>37.0 (0.6)</td>
<td>34.7 (0.81)†</td>
<td>34.6 (0.76)†</td>
</tr>
<tr>
<td></td>
<td>Range 26–42</td>
<td>Range 27–40</td>
<td>Range 31–37</td>
</tr>
<tr>
<td>Plasma urate, mmol/L</td>
<td>403 (13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown as mean (SD). AEDF indicates absent end-diastolic flow; BP, blood pressure; FGR, fetal growth restriction; PE, preeclampsia; and PI, pulsatility index.

*P<0.01 vs control; †P<0.05 vs control; ‡P<0.05 vs PE; and §P<0.001 vs PE and control.
term FGR), samples were collected successfully under epidural anesthesia after vaginal delivery. These samples were taken under ultrasound guidance using biopsy forceps introduced through the cervix. Placental bed biopsy samples were included in this study if they contained decidual and/or myometrial spiral arteries and EVT cells. Samples were snap frozen in liquid nitrogen cooled methyl-2-butane (isopentane; BDH Laboratory Supplies, Poole, United Kingdom) and stored sealed at −70°C until required for immunohistochemical analysis. Cryostat sections were cut at 7 μm thickness onto 3-amino propyltriethylsilane (APES; Sigma Chemical Company, Poole, United Kingdom)—coated slides, air dried overnight, and fixed in acetone for 10 minutes at room temperature. Sections from each biopsy were stained with hematoxylin and eosin to facilitate orientation.

Immunohistochemistry
Serial sections of each biopsy (containing the same vessel) were immunostained using an avidin–biotin–peroxidase technique (Vectorstain Elite kit; Vector Laboratories, United Kingdom) for cytokeratin to identify EVT, for desmin to identify vascular and myometrial muscle, and for von Willebrand factor or CD31 (platelet endothelial cell adhesion molecule) to identify endothelium. Each monoclonal antibody was optimized before use: LP34 cytokeratins 5, 6, 18 (Novocastra, United Kingdom) 1:200; desmin (NCL-DES-DE-R-11) 1:100 (Novocastra); and von Willebrand factor (Dako, UK) 1:800 (for details, see online-only Data Supplement). Cytokeratin-immunostained sections were further stained with the periodic acid–Schiff technique to detect fibrinoid; after development of the DAB (3,3-diaminobenzidine) reaction, sections were incubated for 5 minutes in 1% periodic acid (Sigma), washed, and overlain for 10 minutes with Schiff’s reagent (Sigma). After a further wash, sections were counterstained with Mayer’s hematoxylin for 30 seconds. A total of 6 slides were used, including a hematoxylin and eosin stain.

Analysis of Structural Alterations in the Placental Bed
Structural alterations in the placental bed biopsies were graded based on a system described previously.10,11 Sections were scored by 2 observers (F.L. and J.N.B.) blinded to the sections’ identity. Agreement for grading all histological features between the 2 observers was very high (98%). Examples of scoring histological categories are shown in the Results section and in our previous publications.10,11 Sections containing decidual or myometrial vessels were analyzed separately.

Disruption of the media (vessel wall) was graded into 4 groups according to severity of disruption: preserved, separated (separation of muscular layers), disorganized (disorganization of the media with some loss of media), and absent/grossly disorganized (gross disorganization/ destruction of the media such that little or no media remained). Intimal trophoblast was defined as EVT in the decidua or myometrium surrounding but >50 μm from the spiral artery wall. After an initial screening of all slides, density of cell numbers was graded subjectively as 1 (high) or 2 (normal) based on the entire sample group. Intramural trophoblast was defined as EVT that was either within the media of the spiral artery or in the area that was occupied previously by the media; the latter being identified by the presence of fibrinoid, highlighted by periodic acid–Schiff staining. This was graded as 1 (present) or 2 (absent). Endovascular trophoblast was defined as EVT within the lumen of the spiral artery either as plugs of cells or cells lining the lumen of the spiral artery and replacing endothelial cells. This was defined as 1 (present) or 2 (absent). The presence of fibrinoid, deposited where the media vascular smooth muscle existed previously, was defined as surrounding <25% of the vessel, surrounding 25% to 75% of the vessel, or surrounding >75% of the vessel. To determine the association between the above morphological scores and clinical parameters, all cases were divided into 2 groups based on the presence or absence of a birth weight <3rd percentile, normal umbilical artery Doppler, normal uterine artery Doppler, and delivery <37 weeks of gestation (preterm delivery).

Statistical Analysis
Data were analyzed on a PC using SPSS statistical analysis package. For comparison of patient clinical details, Kruskal–Wallis ANOVA was performed followed by the Mann–Whitney U test to compare individual groups or a t test where data were normally distributed. For analysis of placental bed histological features between clinical groups, the data were analyzed using the general linear mixed model analysis with repeated measures. The model allows for repeated observations from the same patient. Odds ratios (ORs), 95% confidence intervals (CIs), and P values were obtained for comparisons between groups. Finally, each histological feature was compared with clinical parameters: birth weight, uterine artery Doppler, umbilical artery Doppler, or preterm delivery using the Mann–Whitney U test.

Results
The Table shows the clinical details of the patients used in the study and the number of vessels examined. Gestational age at delivery, birth weight, and birth weight percentile were lower in the preeclampsia and FGR groups compared with the controls. Birth weight percentile was also reduced in the FGR group compared with preeclampsia group.

Examples of the different stains and scoring are shown in Figure 1. Figure 1A shows a preserved myometrial vessel from a preeclampsia case with intact media and a small lumen. The vessel was myometrial because of the presence of surrounding desmin positive muscle. Figure 1B also shows a preeclampsia fully preserved myometrial vessel. Despite the absence of endovascular EVT and spiral artery remodeling, the surrounding area has abundant interstitial trophoblast (scored high). Figure 1C shows a preserved myometrial vessel from a preeclampsia case immunostained for cytokeratin: interstitial trophoblast was scored normal. Figure 1D shows a decidual vessel from a normal pregnancy immunostained for desmin; media are absent/grossly disorganized. Figure 1E and 1F shows the same decidual vessel from a normal pregnancy case immunostained for factor VIII and cytokeratin, respectively. A flat intact endothelium is apparent in Figure 1E. Figure 1E and 1F shows endovascular EVT. Figure 1G shows a normal pregnancy decidual vessel immunostained for desmin. The vessel wall is disorganized with some areas showing no muscle, and others (indicated by the arrow) showing partial preservation of muscle. Figure 1H shows an intact myometrial vessel from a preeclampsia case immunostained for cytokeratin: the vessel is surrounded by perivascular EVT, but there is absence of intramural or endovascular EVT. Figure 1I and 1J shows 2 different decidual vessels, from normal pregnancies, immunostained for cytokeratin. Endovascular EVT is shown by broken line arrows. In a separate area of the vessel in Figure 1I, endothelial cells can be seen on the luminal side (solid arrow) with overlying intramural EVT (indicated by asterisk). In Figure 1J, endothelial cells, which appear swollen, are indicated by the arrow. On the nonluminal aspect of the endothelium, intramural EVT cells are present. Figure 1K shows fibrinoid deposition within a transformed decidual vessel.

Media Disruption
Figure 2 shows the extent of media disruption in all the spiral arteries. In the normal group, 86% of decidual vessels had absent/grossly disorganized media and in 14% media was disorganized. In preeclampsia, 53% of decidual vessels showed absent/grossly disorganized media, 23% disorganization,
12% separation, whereas the media was preserved in 11%. In FGR, 75% of decidual vessels had absent/grossly disorganized media and 25% were disorganized. In normal pregnancy, 88% of myometrial vessels had absent/grossly disorganized media and 12% showed medial separation. In contrast, in preeclampsia, only 5% of myometrial vessels showed absent/grossly disorganized media, 22% showed disorganized, and 32% separated media, whereas in 41%, the media were completely preserved. In FGR, 25% of myometrial vessels had absent/grossly disorganized, 33% disorganized, 34% separated, and 8% preserved media. There was less media disruption in myometrial spiral arteries in both preeclampsia ($P=0.0001$; OR, 0.14; 95% CI, 0.05–1.8) and FGR ($P=0.0001$; OR, 0.13; 95% CI, 0.05–0.32) compared with controls. There was no difference between preeclampsia and FGR ($P=0.93$; OR, 0.9; 95% CI, 0.85–9.6).

### Media Disruption and Clinical Parameters

Myometrial vessels from cases with a birth weight <5th percentile ($P<0.001$), abnormal uterine Doppler ($P<0.01$), abnormal umbilical artery Doppler ($P<0.001$), and preterm delivery ($P<0.001$) had less media disruption compared with normal pregnancy. There was no relationship between clinical parameters and media disruption in decidual vessels.

### Interstitial EVT

Figure 3 shows the percentage of cases with high or normal EVT in decidual (upper graph) and myometrial vessels (lower graph). There was no difference between normal pregnancy and preeclampsia in either decidual ($P=1$; OR, 1; 95% CI, 0.55–1.8) or myometrial vessels ($P=0.6$; OR, 1.2; 95% CI, 0.63–2.1). In contrast, for decidual vessels, fewer vessels scored high EVT in the normal group compared with FGR ($P=0.001$; OR, 0.01; 95% CI, 0.006–0.16) and in preeclampsia compared with FGR ($P=0.001$; OR, 0.01; 95% CI, 0.001–0.16). For myometrial vessels, there were also fewer vessels scoring high EVT in the normal group compared with FGR ($P=0.003$; OR, 0.19; 95% CI, 0.08–0.46) and in preeclampsia compared with FGR ($P=0.001$; OR, 0.16; 95% CI, 0.07–0.4). Thus, FGR was associated with more interstitial EVT for both decidual and myometrial vessels. Myometrial, but not decidual, vessels from cases with a birth weight <5th percentile were associated with

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**Figure 1.** Examples of the different staining methods used to identify cells and cellular structures in placental bed biopsies.

A, Preeclampsia myometrial vessel immunostained for desmin (score=fully preserved). Asterisk denotes myometrium.

B, Preeclampsia fully preserved myometrial vessel immunostained for cytokeratin (interstitial extravillous trophoblast [EVT] score=1 [higher]; endovascular EVT score=2 [absent]).

C, Preeclampsia fully preserved myometrial vessel immunostained for cytokeratin (interstitial EVT score=2 [normal]; perivascular EVT score [indicated by arrow]=2 [normal]).

D, Normal pregnancy decidual vessel immunostained for desmin (media score=fully destroyed; endovascular EVT score=1 [present indicated by arrow]).

E, Normal pregnancy decidual vessel immunostained for factor VIII and parallel section immunostained for cytokeratin (F). Endovascular EVT score=1. Arrow indicates endovascular EVT plug.

F, Normal pregnancy decidual vessel immunostained for desmin (media score=disorganized, fibrinoid score<25%).

G, Preeclampsia fully preserved myometrial vessel immunostained for cytokeratin (perivascular EVT score=1 [higher]; intramural EVT score=2 [absent]; and endovascular score=2 [absent]).

H, Normal pregnancy decidual vessel immunostained for desmin (media score=fully preserved; fibrinoid score<25%).

I, Preeclampsia fully preserved myometrial vessel immunostained for cytokeratin (perivascular EVT score=1 [higher]; intramural EVT score=2 [absent]; and endovascular score=2 [absent]).

J, Normal pregnancy decidual vessel immunostained for cytokeratin (score=1). Broken arrow, Endovascular EVT plug.

K, Normal pregnancy decidual vessel stained for fibrinoid (score=25%). L indicates lumen; and M, media. The color version is available in the online-only Data Supplement (Figure S1).
increased numbers of interstitial EVT \( (P<0.001) \). No relationship was found for interstitial EVT and uterine artery Doppler, umbilical artery Doppler, or preterm delivery.

**Endovascular EVT**

Figure 4 shows the percentage of cases with or without endovascular EVT in decidual (upper graph) and myometrial (lower graph) vessels in the 3 groups. No changes were found between groups. No relationship was found between the presence or absence of endovascular EVT and clinical parameters.

**Intramural EVT**

Figure 5 shows the percentage of cases with or without intramural EVT in decidual (upper graph) and myometrial (lower graph) vessels. There was no difference between groups for decidual vessels. In contrast, more myometrial vessels from the normal group contained intramural EVT compared with preeclampsia \( (P=0.015; \text{OR}, 5.7; 95\% \text{ CI}, 1.39–23.2) \). There was no difference between the normal and FGR groups with respect to the presence of intramural EVT \( (P=0.95; \text{OR}, 0.93; 95\% \text{ CI}, 0.08–9.7) \). No relationship was found between the presence of intramural EVT and clinical parameters.

**Periarterial Fibrinoid Deposition**

The findings are shown in Figure 6. Compared with the normal group, decidual vessels from FGR had less fibrinoid deposition \( (P=0.013; \text{OR}, 0.077; 95\% \text{ CI}, 0.01–0.58) \). No difference was found between the control and preeclampsia groups \( (P=0.07; \text{OR}, 0.72; 95\% \text{ CI}, 0.45–1.03) \) for decidual vessels. For myometrial vessels, less fibrinoid deposition was found in both preeclampsia \( (P=0.000; \text{OR}, 0.13; 95\% \text{ CI}, 0.05–0.32) \) and FGR \( (P=0.01; \text{OR}, 0.28; 95\% \text{ CI}, 0.1–0.76) \) compared with controls. There was less fibrinoid deposition in the preeclampsia group compared with FGR \( (P<0.001; \text{OR}, 0.1; 95\% \text{ CI}, 0.05–0.35) \). Myometrial vessels obtained from cases with birth weight <5th percentile had less periarterial fibrinoid than those with birth weight >5th percentile \( (P<0.02) \). There was no relationship between periarterial fibrinoid deposition and birth weight for decidual vessels. No relationship was found between fibrinoid deposition and other clinical parameters.

**Discussion**

**Summary and Key Findings**

As far as we are aware, this is the first study to systematically quantify changes in EVT and spiral artery features in clearly defined groups of normal pregnancy, preeclampsia, and FGR and relate these to clinical parameters. A range of antibodies was used to identify specific cell types and structures. This is not possible with classical histology. A major defect occurred in myometrial spiral remodeling in
both preeclampsia and FGR, which was linked to clinical outcome. Although failure to destroy myometrial vessel wall smooth muscle is a feature of preeclampsia, 10% of decidual vessels in preeclampsia also retained their muscle wall. Lack of physiological change in uterine spiral arteries was not an all-or-nothing process, with some abnormal cases showing apparently normal remodeling. This may relate to the fact that not all cases of preeclampsia and FGR are attributable to failed spiral artery remodeling. Birth weight percentile was reduced in both the preeclampsia and FGR groups. Thus, reduced birth weight in the FGR group was not because of the shorter gestation.

Limitations of Classical Histological Studies

The classical descriptive studies have several limitations: antibodies were not available to identify specific cells, vessels were typically described as showing physiological change or not, with no consistent definition, and no attempts were made to score changes in individual features. Scoring and statistical methods were used rarely, and subject groups were defined poorly (eg, cases with small-for-gestational age infants or chronic hypertension were not analyzed separately). Many small-for-gestational age infants are constitutionally small; we studied clearly the defined growth-restricted cases. Sheppard and Bonnar were the first to consider the impact of spiral artery changes on birth weight (for details, see online-only Data Supplement). Their findings were different from those of Brosens et al who reported that physiological changes were never observed in myometrial vessels in preeclampsia. Gerretsen et al and Khong et al also reported their findings from a mixed group of patients (see online-only Data Supplement).

Specific Features

We found interstitial EVT density to be similar in normal and preeclampsia pregnancy but increased in FGR. This was a surprising finding. Whether this relates to more apoptosis of EVT in the normal group, a compensatory response or some other reason remains to be elucidated. These findings suggest that failed spiral artery remodeling in preeclampsia and FGR is not because of a paucity of interstitial EVT. This is important because in vitro models of trophoblast invasion assume that all EVT cell invasion is impaired in preeclampsia. It is worth noting that comparing the amount of EVT between groups does not necessarily imply the function of EVT is similar between pregnancy groups. The number of interstitial EVT could relate to inherent differences in EVT generation/proliferation from columns or could be secondary to local decidual factors that limit or prevent further invasion or apoptosis of interstitial EVT. Our findings are consistent with.

Figure 4. Percentage of cases with or without endovascular extravillous trophoblast (EVT) in decidual vessels (A) or myometrial vessels (B) for normal pregnancy, preeclampsia (PE), and fetal growth restriction (FGR).

Figure 5. Percentage of cases with intramural trophoblast in decidual vessels (A) or myometrial vessels (B) for normal pregnancy, preeclampsia (PE), and fetal growth restriction (FGR). More myometrial vessels from the normal group contained intramural extravillous trophoblast compared with PE (P=0.015).
appear. This is a limitation of the present and any study examining endovascular EVT at term. In this study, myometrial vessels in the preeclampsia group, but not FGR, contained significantly fewer intramural EVT than the normal group. Thus, if the assumption holds true, then preeclampsia is associated with reduced endovascular EVT. No relationship existed between intramural EVT and clinical parameters. The localization of the EVT in the decidual and myometrial tissue observed in this study at delivery (third trimester) does not reveal the invasion route that these EVT have undergone, as theoretically an intramural EVT might have invaded interstitially or may have invaded by the endovascular route. The same could be said about the interstitial EVT identified; we cannot conclude what their invaded route was originally.

Hustin et al.32 and Meekins et al.19 reported findings on cytotrophoblast (CTB) invasion (for findings and limitations, see online-only Data Supplement).

**Other Features**

Fibrinoid is deposited where the muscle wall has been destroyed. Overall, myometrial vessels in both preeclampsia and FGR were associated with less fibrinoid deposition, but we observed some cases showing little fibrinoid and little muscle and vice versa, consistent with Pijnenborg et al.25 who reported that 10 of 20 cases in a preeclampsia group showed fibrin deposits in myometrial vessels versus 3 of 6 in a normal group. In our study, reduced fibrinoid was also related to a birth weight <5th percentile.

**Doppler and the Placental Bed**

We related changes in spiral arteries to Doppler assessment because of the relationship between Doppler and risk of preeclampsia. Increased pulsatility index with notching is the best predictor of preeclampsia. Increased pulsatility index with notching is the best predictor of overall- and severe intrauterine growth restriction among low-risk patients.33 Matijevic et al.14 reported higher resistance index and pulsatility index in women with preeclampsia; however, Kingdom15 questioned the pathophysiological significance of measuring 8% of uteroplacental blood flow. Other reports involving Doppler used mix patient groups (see online-only Data Supplement).

It has been suggested that CTB acquire an endothelial phenotype at term characterized by the expression of platelet endothelial cell adhesion molecule-1 and other endothelial cell adhesion molecules and that failure to express this phenotype impairs CTB invasive potential.37 It was also suggested that the CTB replace the endothelial cells at term. However, spiral arteries undergo repair and are re-endothelialized by term.1 Other published studies have reported that CTB do not acquire an endothelial phenotype.1,38 Furthermore, because CTB interstitial invasion does not fail, it is difficult to understand statements linking adhesion molecules to failed CTB invasion.

This study further highlights that preeclampsia is a heterogeneous disease with failed spiral artery conversion probably being one of several underlying causes. Our study also highlights the importance of using correct definitions and diagnosis that not all older studies had adhered to.39

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**Figure 6.** Percentage of cases with <25%, 25% to 75%, or 75% to 100% periarterial fibrinoid in decidual vessels (A) or myometrial vessels (B) for normal pregnancy, preeclampsia (PE) and fetal growth restriction (FGR). Compared with the normal group, decidual vessels from FGR had less fibrinoid deposition (P=0.013). For myometrial vessels, less fibrinoid deposition was found in both PE (P=0.000) and FGR (P=0.01) compared with controls. Less fibrinoid was deposited in the PE group compared with FGR (P<0.001).

Pijnenborg et al.29 and Gerretsen et al.28 who reported higher concentrations of interstitial trophoblast giant cells surrounding noninvaded junctional spiral arteries in preeclampsia. In contrast, 2 groups have reported reduced interstitial invasion in preeclampsia.30,31 The differences may relate to the fact that Kadyrov et al.30 studied a small number of archived cesarean hysterectomy specimens that are known to lose their ability to stain for antibodies, and placental bed was defined by the presence of trophoblast alone. Furthermore, intramural and endovascular EVT were grouped together and termed endovascular trophoblast. Media destruction was not assessed. Naicker et al.31 eliminated women who had diastolic blood pressure <110 mm Hg and samples with mixed nature of the physiological conversion of the spiral artery.

**Endovascular Trophoblast**

The lack of difference in endovascular EVT between groups at first seems surprising because it is repeatedly stated in the literature that preeclampsia is associated with failed endovascular invasion. Without specific antibodies it is easy to misinterpret the cells that are being examined or indeed to miss cells totally. However, by term, most of the vessels have a new endothelium. Thus, a major assumption made in all the classical studies is that the intramural EVT at term was previously endovascular EVT before a new endothelium
In recent times, it has been suggested that early and late onset preeclampsia may differ as well as the classical idea of placental versus maternal preeclampsia; however, how these, and other possible subclasses of preeclampsia, are defined clinically has been a challenge. Early onset disease is more often associated with severe complications for both the mother and the baby both short-term and long-term risk of cardiovascular disease. The vast majority of patients with preeclampsia in this study delivered before 37 weeks. In the few cases that delivered after 37 weeks, failure to modify myometrial vessels was noted in half of these. Although the small number of cases studied in the late onset group does not allow a statistical comparison between early and late onset disease, failed spiral artery conversion was not restricted to delivery before 37 weeks.

Limitations
Clearly, there are limitations to studying small placental bed biopsies; although efforts were made to sample the center of the placental site, it is clear that this is not always achieved and histological features may vary between the center and the periphery of the placental bed. Spiral artery remodeling, which was assessed using an arbitrary score, is qualitative but nevertheless highly reproducible. Describing the physical changes in trophoblast populations and spiral arteries provides no insight into the mechanisms of failed remodeling. In vitro models using spiral artery explants may help to provide insight into the mechanisms although it remains a challenge to replicate the 2 different pathways of EVT invasion, create the correct physiological environment both in oxygen and extra-cellular matrix environment, and allow for absence of other cells normally present within the decidua.

Perspectives
This study focuses on a critical early step in the development of pregnancy complications, preeclampsia, and FGR, namely the essential remodeling of uteroplacental spiral arteries. Remodeling involves both fetal-derived cells (trophoblasts) and maternal cells (spiral artery and uterine wall cells, including important specialized maternal immune cells). Without remodeling a normal pregnancy outcome is not achieved. FGR and preeclampsia affect, in total, >10% of all pregnancies. Several studies have shown that experiencing preeclampsia or FGR predicts increased future risk for cardiovascular disease, for both mother and child. This study (1) provides more clarity into how remodeling is disturbed, (2) is vital for future more in vitro mechanistic insights that should distinguish endovascular versus interstitial invasion, and (3) may help identify clinical preventive or intervention measures. The findings may extend into understanding hypertensive disorders in women and associated cardiovascular diseases, as well as expanding the understanding of in utero programming of offspring adult diseases. The study includes a detailed description of EVT position (interstitial or endovascular) in relation to the arterial wall anatomy and media disruption grading (both are important features of the remodeling process and vessel pathology). Importantly, growth-restricted pregnancies are carefully described clinically, often lacking in other studies.

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Disclosures
None.

References

**Novelty and Significance**

What Is New?
- A systematic analysis of the morphological features of human placental bed biopsies was performed and related to clinical features and pregnancy outcome, namely preeclampsia and fetal growth restriction.
- Pregnancies resulting in low birth weight, preterm delivery, or abnormal Doppler of uterine and umbilical arteries were associated with placental bed spiral artery vessels that retained the muscle wall at term.
- Poor pregnancy outcome was not related to a paucity of invasive trophoblast cells as previously assumed.

What Is Relevant?
- Our findings contribute to improve our knowledge of the complex relationship between failure to modify the small spiral arteries of the placental bed during early pregnancy and adverse pregnancy outcome as well as long-term risk of cardiovascular disease.
- When considering ways of preventing preeclampsia, consideration has to be given to the fact that therapeutic targets need to be aimed at the placental bed in the early stages of pregnancy in a high-risk, subset of women subsequently develop the condition.

**Summary**
In the small spiral arteries of the human placental bed obtained from cases of preeclampsia and fetal growth restriction, there is an association between failure to lose the muscle wall by term and adverse clinical features, namely abnormal Doppler of uterine and umbilical arteries, preterm delivery, and low birth weight.
Spiral Artery Remodeling and Trophoblast Invasion in Preeclampsia and Fetal Growth Restriction: Relationship to Clinical Outcome
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Spiral artery remodeling and trophoblast invasion in pre-eclampsia and fetal growth restriction: relationship to clinical outcome:

Online Supplement

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Figure S1: Color version
Immunohistochemistry

Following rehydration in Tris-buffered saline (TBS; 0.1M Tris, 0.05 M saline, pH 7.6) sections were overlain for 20 min with normal horse serum to block non-specific binding sites. Sections were next incubated with the primary antibody for 30 min, washed in TBS and incubated for 30 min with biotinylated horse anti-mouse IgG supplied in the kit. After further washes sections were overlain with horseradish peroxidase-labelled Vectastain Elite ABC Reagent for 30 min. After further washes, the reaction was developed with 1 mg/ml 3,3-diaminobenzidine (DAB, Sigma) containing 0.02% hydrogen peroxide for 1 min. Sections immunostained for desmin and von Willebrand factor (vWF) were counterstained with Mayer’s haematoxylin, dehydrated, cleared, and mounted in synthetic resin.

Discussion

Limitations of classic histology papers:

Sheppard and Bonnar [27] were the first to consider the impact of spiral artery changes on birthweight. Using archived samples they reported that myometrial vessels from 10/11 cases of SGA babies were morphologically similar to myometrial vessels in the non-pregnant uterus. For PE (n=28) half of the myometrial vessels examined were also morphologically similar to myometrial vessels in the non-pregnant uterus. Within the PE group changes were similar regardless of birthweight (<10th centile (n=11). No scoring or formal analysis of birthweight and media changes was undertaken [24]. Their findings were different from those of Brosens et al [23] who reported that physiological changes were never observed in myometrial vessels in PE.

Gerretsen et al 1981 [28] reported physiological changes in junctional myometrial vessels. All but one of the 30 PE cases had absent physiological changes regardless of birthweight. Khong et al [6] reported that absence of physiological change could also occur in decidual vessels in some cases of PE and SGA (<10th centile). Full physiological change was reported in all normal cases in both decidual and myometrial vessels. In hypertensive disorders (a mixed group some of which had PE) without SGA 8/14 showed no change in decidual vessels and 2 showed partial change. When SGA was also present with hypertensive disorders 31/32 showed no change and 1 showed partial changes. For SGA without hypertension 16/24 showed no change and 3 showed partial change. In SGA without hypertension 8/24 showed no change and 2/24 partial change. For myometrial vessels all 11 cases of hypertensive disorders without SGA lacked physiological change; when SGA was present with hypertension 31/32 showed no change and 1 showed partial changes. For SGA without hypertension 16/24 showed no change and 3 showed partial change while for decidual vessels 8/24 showed no change and 2 showed partial change.

Hustin et al [32] reported that “Cytotrophoblast vascular invasion which was evident up to the myometrial junction appeared to be reduced in preeclampsia and normotensive fetal growth retardation cases”. No trophoblast specific antibodies were used. Meekins et al [19], with the aid of a cytokeratin antibody, studied intramural trophoblast (although they referred to this as ‘endovascular trophoblast’). Trophoblast invasion was seen in all decidual vessels in normal pregnancy and in 46% of decidual vessels in PE: our study showed a trend for fewer EVT in the vessel wall in decidual
vessels in PE. In the Meekins study 68% of myometrial vessels in normal pregnancy were graded as showing complete trophoblast invasion compared with 18% in PE.

Madazli et al [36] reported the incidence of placental bed pathology defined by ‘inadequate endovascular CTB invasion, acute atherosis, thrombosis or luminal obliteration of spiral arteries’ was higher in FGR with abnormal uterine artery Doppler compare to those with normal Doppler. Trophoblast markers were not used and media destruction was not assessed. No comment was made to whether decidual or myometrial were examined.