Failure of physiologic transformation of spiral arteries, endothelial and trophoblast cell activation, and acute atherosis in the basal plate of the placenta

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Abstract

Background—Failure of physiologic transformation of spiral arteries has been reported in preeclampsia, fetal growth restriction, fetal death, and spontaneous preterm labor with intact or ruptured membranes. Spiral arteries with failure of physiologic transformation are prone to develop atherosclerotic-like lesions of atherosis. There are striking parallels between preeclampsia and atherosclerotic disease, and between lesions of atherosis and atherosclerosis. Endothelial activation, identified by intercellular adhesion molecule-1 expression, is present in atherosclerotic-like lesions of heart transplantation and considered a manifestation of rejection. Similarly, endothelial activation/dysfunction has been implicated in the pathophysiology of atherosclerosis

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and preeclampsia. Intercellular adhesion molecule-1-overexpressing-activated endothelial cells are more resistant to trophoblast displacement than nonactivated endothelium and may contribute to shallow spiral artery trophoblastic invasion in obstetrical syndromes having failure of physiologic transformation.

Objective—To determine whether failure of spiral artery physiologic transformation was associated with activation of interstitial extravillous trophoblasts and/or spiral artery endothelium and presence of acute atherosis in the placental basal plate.

Study Design—A cross-sectional study of 123 placentas (19-42 weeks' gestation) obtained from normal pregnancies (n = 22), preterm prelabor rupture of membranes (n = 26), preterm labor (n = 23), preeclampsia (n = 27), intrauterine fetal death (n = 15), and small for gestational age (n = 10) was performed. Failure of spiral artery physiologic transformation and presence of cell activation was determined using immunohistochemistry of placental basal plates containing a median of 4 (minimum: 1; maximum: 9) vessels per placenta. Endothelial/trophoblast cell activation was defined by the expression of intercellular adhesion molecule-1 (ICAM-1). Investigators examining microscopic sections were blinded to clinical diagnosis. Pairwise comparisons among placenta groups were performed with the Fisher's exact and Wilcoxon rank sum tests using a Bonferroni-adjusted level of significance (.025).

Results—87% (94/108) of placentas having spiral arteries with failure of physiologic transformation (actin-positive and cytokeratin-negative) in the basal plate, and 0% (0/15) of placentas having only spiral arteries with complete physiologic transformation (cytokeratin-positive and actin-negative), had arterial endothelial and/or interstitial extravillous trophoblasts reactive with the ICAM-1 activation marker (P < .001). A significant correlation ($R^2 = 0.84$) was found between expression of spiral artery endothelial and interstitial extravillous trophoblast ICAM-1 (P < .001) in activated placentas. Lesions of atherosis were found in 31.9% (30/94) of placentas with complete and/or partial failure of physiologic transformation of spiral arteries that were ICAM-1-positive, in none of the 14 placentas with failure of physiologic transformation that were ICAM-1-negative, and in none of the 15 placentas with complete spiral artery physiologic transformation without failure (P = .001). All placentas (30/30, 100%) with atherosis were identified in placentas having concomitant spiral artery endothelial and interstitial extravillous trophoblast activation.

Conclusion—Failure of spiral artery physiologic transformation in the placental basal plate is associated with interstitial extravillous trophoblast and arterial endothelial activation along with increased frequency of spiral artery atherosis. These findings may be used to improve the characterization of different disorders of the placental bed such as in refining the existing tools for the early prediction of risk for preterm, preeclamptic, and other abnormal pregnancies.

Introduction

Remodeling of the spiral arteries during gestation leads to substantial dilation of these vessels, which at the point of discharge into the intervillous space, attain a luminal diameter 10 times larger than myometrial spiral arteries. This remodeling is associated with a 10-fold increase in blood supply to the fetoplacental unit in the third trimester compared to the non-pregnant uterus. Spiral artery remodeling, which increases total uteroplacental blood flow from a baseline value of 20-50 mL/min to 450-800 mL/min in singleton pregnancies (as measured utilizing the diffusion equilibrium principle [most often nitrous oxide] or electromagnetic flow probes placed directly on the uterine artery), is considered key to accommodate the increased blood flow to the uteroplacental circulation in the third trimester [1–7]. Initially, physiologic transformation of the spiral arteries includes endothelial vacuolation and smooth muscle swelling [8–11], and it has been attributed in part to immune processes within the decidua [4,12–18]. Subsequently, trophoblasts invading the spiral arteries destroy the smooth muscle in the media, which is replaced by fibrinoid material [1,3,19–21].

Physiologic transformation of the spiral arteries is a normal process with the degree of transformation being greater in the center of the placental bed than in the periphery [21–23]. Full conversion of both decidual and myometrial segments of the approximately 100-120 spiral arteries normally found in the placental bed into large, remodeled uteroplacental vessels is key for normal deep placentation [21,23]. Such deep placentation can be defective when remodeling of the junctional zone of the spiral arteries (arterial segments in the inner third of the myometrium and overlying endometrium) is absent or incomplete [21,24–26]. Insufficient physiologic transformation of the spiral arteries is associated with adverse pregnancy outcomes [3,21,27], including second-trimester spontaneous abortion [28–30], fetal death [30], abruptio placentae [31], preeclampsia [1,32–34], small for gestational age [32–35], preterm labor [36], preterm prelabor rupture of membranes [37,38], and maternal autoimmune diseases [39].

Failure of physiologic transformation of spiral arteries is seen in deep placentation disorders such as preeclampsia with or without intrauterine growth restriction, intrauterine growth restriction without preeclampsia, preterm labor and preterm prelabor rupture of membranes, abruptio placentae, and second-trimester abortion [21]. This failure is characterized by the absence of spiral artery trophoblastic invasion and remodeling [40]. Currently this condition can be identified only in placental bed biopsies because these specimens are required to demonstrate a defect of myometrial transformation [40]. However, physiologic transformation begins in the decidual segment of the spiral arteries during the first wave of trophoblast invasion. Therefore, failure of transformation of the spiral arteries in the decidual segment represents a more severe disorder than lack of transformation of the myometrial segment only. Abnormal transformation of the decidual portion of the spiral arteries can be detected by examining the placental basal plate [33–35,37,39,41–45]. This has practical implications because the basal plate of the placenta is available for examination in all cases while a placental bed biopsy requires a specialized procedure generally performed during a cesarean delivery. Immunohistochemistry [44] allows in-depth examination of the vessel wall through antigen detection of its different cellular components, which reduces the

subjectivity of using only conventional light microscopy for vessel recognition in the placental basal plate.

Arteries with failure of physiologic transformation of the spiral arteries (but not those with physiologic transformation) are prone to develop atherosis. Intravascular inflammation and abnormal lipid metabolism can interfere with endothelial cell function, and predispose to atherosclerosis in non-pregnant subjects [46–53]. Women with preeclampsia, the prototypic obstetrical complication characterized by failure of physiologic transformation of the spiral arteries [19,21,32,54–58] have evidence of intravascular inflammation [57,59–62] as well as changes in lipid metabolism [63–66], such as increased concentrations of triglycerides, and low-density lipoprotein, and decreases in low-density lipoprotein particle size and high-density lipoprotein [67]. Macrophage infiltration of the intima and media is identified in both atherosis and the lesions found in transplant vasculopathy [6,43,44,58,68–70]. Therefore, there are striking parallels between preeclampsia and atherosclerosis, as well as the lesions of atherosis and those observed in patients with ischemic heart disease.

Endothelial activation, identified by the expression of intercellular adhesion molecule (ICAM)-1, is present in atherosclerotic-like lesions of heart transplantation [71–76], and is considered a manifestation of rejection. Similarly, endothelial activation/dysfunction has been implicated in the pathophysiology of atherosclerosis [77–79] and preeclampsia [80–83]. Activated endothelial cells with overexpression of cell-surface ICAM-1 [84–86] are more resistant to trophoblast displacement than non-activated endothelial cells [87], and may contribute to shallow spiral artery trophoblastic invasion in obstetrical syndromes associated with failure of physiologic transformation of the spiral arteries.

The purpose of this study was to determine whether there was an association among: (1) failure of physiologic transformation of the spiral arteries in the placental basal plate; (2) endothelial and trophoblast activation; and (3) the presence of atherosis.

Materials and Methods

Study design

A cross-sectional study was performed on 123 placentas (19-42 weeks' gestation) collected at Hutzel Women's Hospital, the Detroit Medical Center, under protocols approved by the Wayne State University and *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Institutional Review Boards, and the analyses were completed by 2015 at CBL Partners for Life, Indianapolis, IN, and the California Medical Innovations Institute, San Diego, CA. Placentas were obtained from: normal pregnancies (n = 22), preterm prelabor rupture of membranes (n = 26), preterm labor (n = 23), preeclampsia (n = 27), intrauterine fetal death (n = 15), and small for gestational age (n = 10). Normal pregnancies were defined as those without medical/obstetrical complications and with birthweights adequate for gestational age (>10th percentile) at term (37 weeks of gestation). Seventeen babies were delivered vaginally and five by cesarean (none of the mothers or babies were infected). Preeclampsia was defined as new-onset hypertension >20 weeks of gestation (systolic blood pressure 140 mm Hg or diastolic blood pressure 90 mm Hg on at least two occasions, 4 hours to 1 week apart), edema, and proteinuria (300 mg in a 24-hour urine

collection or one dipstick measurement of 2+). Infants with fetal growth restriction were considered small for gestational age if they were <10th percentile for weight for their gestational age. Pregnancies were considered preterm when the gestational age was <37 weeks. Birthweight percentiles were calculated using nationwide US natality data [88]. Placentas from mothers with obesity (body mass index 30), known to be associated with a pro-inflammatory state [89], were not included in the study. All women provided written informed consent prior to the collection of placenta samples. The Institutional Review Board of the sponsoring institution approved collection and use of samples for research purposes.

Definitions

The terms used in this study follow accepted nomenclature [90]. Briefly, the placental bed is part of the decidua and adjoining myometrium that underlie the placenta and provide blood supply to the intervillous space. Extravillous trophoblasts are all trophoblasts (fetal-derived cells) located outside the placental villi. Interstitial extravillous trophoblasts are placental bed trophoblasts that have entered the decidual stroma but have not yet invaded the spiral artery walls. Extravillous trophoblasts invading spiral artery walls and replacing smooth muscle and endothelium are termed "intra-arterial trophoblasts." Physiologic transformation of the spiral arteries was defined as the complete trophoblastic invasion of the spiral arteries, partial failure of physiologic transformation of the spiral arteries as the incomplete trophoblastic invasion of the arterial muscular wall, and failure of physiologic transformation of the spiral arteries as the complete absence of trophoblastic replacement of the arterial wall.

Placental specimens and immunohistochemistry

Placental samples ($10\times10\times5$ mm) from placental septa near the center of the basal plate [3.5 (SD 0.6) per placenta] of 123 placentas obtained immediately after delivery were embedded in Tissue-Tek® optimum cutting temperature compound (Miles Inc., Elkhart, IN) and snap-frozen in liquid nitrogen. Seven serial sections (a total of seven slides) from each basal plate sample were used for the evaluation of the single-, double-, and triple-antibody techniques performed. Sample slides having basal plate spiral arteries defined immunohistochemically were included in the study, and samples without any spiral artery were excluded. A triple-antibody technique [44,73] with antibodies to α-smooth muscle actin to identify vascular smooth muscle cells, cytokeratin to identify extravillous trophoblasts and intra-arterial trophoblasts, and von Willebrand factor to identify endothelial cells was used to detect complete or partial failure of physiologic transformation and physiologic transformation of spiral arteries (median of 4 [minimum: 1; maximum: 9] vessels per placental basal plate). A triple-antibody immunohistochemistry with α-actin, cytokeratin, and ICAM-1 identified interstitial extravillous trophoblast and spiral artery endothelial cell activation [44,73,91].

Normal term placentas had cytokeratin-positive, α-actin-negative, and von Willebrand factor-positive uteroplacental arteries. Failure of physiologic transformation of the spiral arteries was characterized by cytokeratin-negative, α-actin-positive, and von Willebrand factor-positive spiral arteries. Spiral artery atherosis was cytokeratin-negative, α-actin-positive, and von Willebrand factor-positive and showed the presence of CD68^{+} macrophages in the intimal area of the spiral arteries. Serial sections (5 μm each) from each

placental block were obtained for light microscopy (hematoxylin and eosin) and single, double, and triple immunohistochemistry. The use of immunohistochemistry allowed a clear identification of the spiral arteries in all slides from the different serial sections, demonstrating 100% reproducibility. The deposition of neutral triglycerides and other lipids were examined using oil-red O to confirm atherosis. Individuals examining microscopic sections (C.A.L., H.L.D.) were blinded to clinical diagnoses.

Statistical analysis

Continuous measures were summarized using median, minimum, and maximum, and categorical measures were summarized as frequency (percent). Demographic variables were compared using a Fisher's exact test (for discrete measures) and the Kruskal-Wallis test (for continuous measures); these tests compared values for atherosis groups (atherosis versus no atherosis). Comparison of continuous measures for groups (atherosis versus no atherosis) were evaluated using the Wilcoxon rank sum test. We used analysis of variance to compare means from multiple groups. A Bonferroni-adjusted level of significance of $<.025$ was used to establish statistical significance for subsequent pairwise comparisons. Statistical analysis was performed by one of the authors (J.W.H).

Results

The various statuses of the spiral artery physiologic transformation in the placental basal plate were defined immunohistochemically as follows: physiologic transformation of the spiral arteries had complete muscular wall replacement by cytokeratin-positive trophoblasts that surrounded the endothelial lining of the vessels (Figure 1, A); spiral arteries with failure of physiologic transformation and atherosis maintained an actin-positive muscular wall, and cytokeratin-positive trophoblastic cells (interstitial extravillous trophoblasts) surrounded but did not invade the arterial walls (Figure 1, B). Spiral arteries with partial failure of physiologic transformation had a combination of smooth muscle cells and intra-arterial trophoblasts in the arterial walls (Figure 1, C). The immunohistochemical characteristics of the different statuses of spiral artery physiologic transformation and atherosis are summarized in Table 1.

Of the 123 placentas studied, 108 had spiral arteries with failure of physiologic transformation (Table 2): 79 had failure of physiologic transformation in all spiral arteries examined and 29 had both failure of physiologic transformation and physiologic transformation (Table 2). Fifteen placentas had complete spiral artery physiologic transformation without any failure of physiologic transformation (Table 2). Spiral arteries with partial failure of physiologic transformation (n = 36) had α-actin-positive cells in part of the arterial wall and cytokeratin-positive trophoblastic cells in the rest of the wall (Table 1 and Figure 1, C). Partial failure of physiologic transformation of the spiral arteries in the basal plate was associated with failure of physiologic transformation (n = 22), physiologic transformation (n = 8), or both (n = 6).

Of the 108 placentas with failure of physiologic transformation of the spiral arteries in the basal plate, 100 (100/101, 99%) were from women with complicated pregnancies, and 8 (8/22, 36.4%) from normal pregnancies (Table 2). Of the 15 placentas with complete

physiologic transformation of the spiral arteries and no failure, however, only 1 (1/101, 1%) was from a woman with an abnormal pregnancy, and 14 (14/22, 63.6%) were from women with normal pregnancies (Table 2). Placentas with only physiologic transformation of the spiral arteries (Table 2) were found mostly in normal pregnancies, and placentas having only failure of physiologic transformation of the spiral arteries (Table 2) were seen predominantly in abnormal pregnancies with preeclampsia, small for gestational age, intrauterine fetal death, preterm labor, and preterm prelabor rupture of membranes (Table 2).

Of placentas from abnormal pregnancies, 99% had spiral arteries with failure of physiologic transformation in the basal plate and only 1% of them had all spiral arteries with physiologic transformation (P < .001) (Table 2). Contrarily, most placentas (63.6%) from normal pregnancies had all spiral arteries with physiologic transformation, and a smaller percentage of them (36.4%) had spiral arteries with failure of physiologic transformation (P <.001). All groups of abnormal pregnancies had a similarly high proportion of placentas with failure of physiologic transformation in all spiral arteries, which was highest in pregnancies with small for gestational age (9/10, 90%), preeclampsia (22/27, 81.5%), and intrauterine fetal death (12/15, 80%), and lowest in preterm labor (16/23, 69.6%) (Table 2). The combination of both failure of physiologic transformation and physiologic transformation of the spiral arteries was more frequently identified in preterm labor. Physiologic transformation of the spiral arteries without any failure of physiologic transformation was found predominantly in normal pregnancies (Table 2). We performed quantitative studies and evaluated the percentage of arteries with different degrees of physiologic transformation in the basal plate of the placentas of the different groups and subgroups studied (Table 3). The percentage of arteries with physiologic transformation and failure of physiologic transformation was significantly different between groups, but no differences were found in the percentage of arteries with partial failure of physiologic transformation (Table 3). The highest percentage of arteries with failure of physiologic transformation was found in placentas from fetal death and preeclampsia; the highest percentage of partial failure of physiologic transformation was found in small-for-gestational-age babies; the highest percentage of arteries with physiologic transformation was found in normal pregnancies and preterm labor; and the lowest percentage of arteries with physiologic transformation was found in small-for-gestational-age placentas (Table 3).

Of the 94 placentas with ICAM-1 expression in arterial endothelium and/or extravillous trophoblasts, all (94/94, 100%) were associated with failure of physiologic transformation (Figure 1, E, and Table 2), and none with physiologic transformation without failure (Figure 1, D, and Table 2) (P <.001). A significant correlation ($R^2 = 0.84$) was found between interstitial extravillous trophoblasts and spiral artery endothelial ICAM-1 expression (P < .001). Spiral arteries with partial failure of physiologic transformation also showed endothelial ICAM-1 expression (Figure 1, F). A high proportion of spiral arteries with endothelial and/or extravillous trophoblasts that were ICAM-1-positive (Table 2) was observed in complicated pregnancies. The combination of both failure of physiologic transformation of the spiral arteries and physiologic transformation was associated with a lower proportion of endothelial and/or extravillous trophoblasts that were ICAM-1-positive (Table 2). The percentage of arteries with endothelial ICAM-1 was significantly different between groups, being higher in small for gestational age and lower in normal pregnancies

(Table 3). Interestingly, spiral arteries with physiologic transformation had fewer CD68$^+$ macrophages surrounding extravillous trophoblasts (Figure 2, A) and spiral artery endothelium (Figure 2, C) than arteries with failure of physiologic transformation, which had increased number of CD68$^+$ macrophages around smooth muscle cells of uninvaded arteries (Figure 2, B and D), suggesting that increased inflammation is associated with arterial endothelial and/or extravillous trophoblast activation.

Atherosis, akin to placental atherosclerosis (Figure 3A), developed only in placentas with failure of physiologic transformation of the spiral arteries, and all placentas with atherosis had a high proportion of failure of physiologic transformation (Table 2) and activation of interstitial extravillous trophoblasts and spiral artery endothelium. Atherosis was identified in preterm labor (n = 6), preterm prelabor rupture of membranes (n = 9), preeclampsia (n = 7), small for gestational age (n = 3), intrauterine fetal death (n = 4), and normal (n = 1) placentas (Table 2). The percentage of arteries with atherosis lesions was significantly different between groups (P < .001); the highest percentage was found in small for gestational age and fetal death, and the lowest percentage in normal pregnancies and preterm labor (Table 3). Arteries with atherosis had numerous oil red O-positive cells, which were not observed in arteries with either physiologic transformation or failure of physiologic transformation without atherosis (Figure 3, B to D). Oil red O-positive cells were confirmed to be macrophages (Figure 3, G). Spiral arteries with atherosis were α-actin-positive (Figure 3, E and H) and cytokeratin-negative (Figure 3, F), as defined in Table 1. Lesions of atherosis were observed in 30% (30/100) of placentas with failure of physiologic transformation of the spiral arteries (Table 2) and, as expected, in none of the placentas with physiologic transformation (P = .001). Furthermore, all placentas with atherosis had concomitant spiral artery endothelial and interstitial extravillous trophoblast activation. Arteries with failure of physiologic transformation that were ICAM-1-positive had a higher frequency of atherosis than placentas without endothelial activation (P < .001). Atherosis was found in 31.9% (30/94) of placentas positive for ICAM-1 on spiral artery endothelium and/or interstitial extravillous trophoblasts; in none of the 13 placentas with failure of physiologic transformation that were ICAM-1-negative; and as expected, in none of the 15 placentas with complete physiologic transformation that were ICAM-1-negative (P = .04).

We subsequently performed a comparison among the immunohistochemically defined spiral artery phenotypes in the basal plate and the different groups of pregnancies studied, the status of spiral artery transformation, and the presence of arterial endothelial/trophoblast activation and atherosis (Table 4). As expected, abnormal pregnancies had a significantly increased proportion of spiral arteries actin-positive and cytokeratin-negative; arteries actin-positive and cytokeratin-negative were characteristic of failure of physiologic transformation, and showed increased ICAM-1 expression and atherosis (Table 4). The clustered bar chart (Figure 4) shows a summary of the immunohistochemical characteristics of the spiral arteries in the basal plate of the different pregnancy conditions studied, i.e., normal and abnormal pregnancies, physiologic transformation, partial failure of physiologic transformation, failure of physiologic transformation, placental ICAM-1 expression, and atherosis.

Comment

Principal findings of the study

First, failure of physiologic transformation of the spiral arteries in the placental basal plate had ICAM-1-positive interstitial extravillous trophoblasts. Second, placentas with failure of physiologic transformation of the spiral arteries and extravillous trophoblast activation had concomitant spiral artery ICAM-1-positive endothelium. Third, failure of physiologic transformation of the spiral arteries that were ICAM-1-positive had a higher frequency of atherosis than placentas without endothelial activation.

Activated extravillous trophoblasts found around spiral arteries with failure of physiologic transformation but not arteries with physiologic transformation suggests that the presence of ICAM-1-positive interstitial extravillous trophoblasts prevents the normal invasion of spiral arteries by those cells, possibly by enabling maternal immune cells to react against activated extravillous trophoblasts. Impaired trophoblast invasion has been associated with an increased number of macrophages around spiral arteries in preeclampsia [92] that could lead to increased trophoblast apoptosis [93] and impaired arterial invasion.

Placentas with failure of physiologic transformation of the spiral arteries and endothelial activation had a significantly ($P < .001$) higher frequency of atherosclerotic-like lesions of atherosis than placentas without activation, suggesting that endothelial activation is paramount in the development of the lesion. Multiple pathologic processes involving excessive decidual inflammation have been implicated in the genesis of atherosis [94–97]. Local factors may contribute to lack of trophoblast invasion and lipid deposition in spiral arteries [96]. Trophoblast migration may be suppressed by an abnormal immune interaction between maternal and fetal cells [96]. It is noteworthy that atherosis and vascular lesions in rejected kidney and heart transplants are histologically similar, suggesting that atherosis may be the result of immune dysregulation at the maternal-fetal interface [43]. In the decidua, invading trophoblasts can act as ligands for maternal uterine immune cells since trophoblasts bear paternal (foreign) HLA-C antigens that can be detected by receptors on those cells [98–101]. Under normal circumstances, immune cells do not initiate a destructive response toward trophoblasts, but this response could be triggered in complicated pregnancies and explain impaired trophoblast invasion [100,102]. We have previously proposed [43] that atherosis could have an immune origin. This hypothesis has gained support, as recently reviewed by Staff et al [96]. An immunological mismatch between mother and fetus can lead to dysregulation of decidual immunity, promote local decidual inflammation, and predispose to atherosis.

Uteroplacental ischemia can lead to the production of anti-angiogenic factors [103–113], such as sFlt-1 (soluble fms-like tyrosine kinase-1) [114–117] and endoglin [69,118], which can induce endothelial dysfunction and predispose to preeclampsia or an anti-angiogenic state [57,119–129]. In addition, an excess production of trophoblast debris can result in exaggerated intravascular inflammation and endothelial cell dysfunction [130–134]. Preeclampsia is characterized by intravascular inflammation [59–62,135] and, similar to atherosclerosis [46,136,137], is marked by increased levels of circulating pro-inflammatory cytokines (such as interleukin-6 and tumor necrosis factor-α [138–143]), C-reactive protein

[141,142,144–146], and markers of leukocyte activation [144,147]. Interestingly, pregnancy may constitute a metabolic/vascular "stress test," uncovering a subclinical endothelial cell dysfunction that manifests later in life, in mothers affected with preeclampsia or an anti-angiogenic state [148–151].

Several adverse pregnancy outcomes, such as preeclampsia [1,32–34], have characteristic disordered deep placentation [28–42]. Our findings provide an association between failure of physiologic transformation of the spiral arteries in the placental basal plate and activation of spiral artery endothelial cells and extravillous trophoblasts. By secreting both tumor necrosis factor-α and a tryptophan-depleting enzyme, excess macrophages in the placental bed of women with preeclampsia have been implicated in defective extravillous trophoblast spiral artery invasion [152]. More recently, tumor necrosis factor-α has been found to mediate fetal growth restriction during abnormal maternal inflammation [58]. Macrophages inhibit extravillous trophoblast invasion both passively, by being attracted by extravillous trophoblasts, and actively, by inducing peri-arterial extravillous trophoblast apoptosis [152]. Arterial ICAM-1 up-regulation could facilitate macrophage recruitment as shown in placental villitis of unknown etiology and massive chronic intervillositis [153,154]. Aberrant ICAM-1 expression has been described in cultured syncytiotrophoblasts pre-treated with both inflammatory cytokines, which also facilitate adhesion of monocytes to the syncytiotrophoblast [155]. Monocytes adhering to placental syncytiotrophoblasts can induce tumor necrosis factor-α-dependent apoptosis accompanied by focal trophoblast disruption [156]. Focal damage of the placenta could be a route for maternal leukocyte infiltration into the villi and a possible mechanism of villitis [156]. From our findings, we postulate that activated extravillous trophoblasts could facilitate trophoblast damage, inflammation, and subsequent inhibition of spiral artery transformation. Failure of physiologic transformation of the spiral arteries could result from ICAM-1-reactive spiral artery endothelial cells impeding the normal invasion [157], or ICAM-1-enhanced reactivity could be a consequence of failure of the physiologic transformation.

The rates of failure of physiologic transformation in our study are higher than those reported previously in women with different placental disorders [20,21,36–38,158]. The differences could be explained by the number of blocks obtained from the basal plate's placental septa [21] included in our study (a mean of 3.5 blocks per placenta) and the use of triple-antibody immunohistochemistry. The use of a triple-antibody immunohistochemical technique allows the simultaneous identification of three cell types—smooth muscle cells, trophoblasts, and endothelium—which facilitates identification not only of the type of vessel but its predominant cell type phenotypic characteristics. The immunohistochemistry technique using cytokeratin, actin, and von Willebrand factor as markers of trophoblasts, smooth muscle cells, and endothelium, respectively, allows for a more precise identification of the degree of transformation, or the lack thereof, in each vessel. Interestingly, our data in the placental basal plate are supported by recent findings of a significantly higher frequency of acute atherosis lesions in the placental basal plate than in placental bed biopsies (both decidua and myometrial segments), considering that atherosis lesions only develop in spiral arteries with failure of trophoblastic invasion [159]. As we previously discussed [36], the reasons why some women with failure of physiologic transformation of the spiral arteries develop preeclampsia, whereas others develop preterm labor, are unknown, and the

possibility still exists that women with preterm labor would have developed preeclampsia if the pregnancy had continued longer. We previously proposed that pathology of the placental bed, primarily through ischemia and/or other mechanisms such as a maternal-fetal immune response, may lead to preeclampsia, small for gestational age, preterm labor with intact or ruptured membranes, abruptio placentae, and fetal death; and why a similar insult would result in different clinical phenotypes depends on genetic and environmental factors and time of onset, duration, and extent of the ischemic insult [160]. Korzeniewski et al [161] recently demonstrated that maternal plasma angiogenic index-1 concentration ratios seem to reflect the burden of uteroplacental vasculopathy indicated by histologic examination of the placenta, regardless of clinical diagnosis. The time order between changes in plasma angiogenic index-1 concentration ratios and placental features consistent with maternal vasculopathy, however, remains to be determined. The identification of the highest proportion of arteries with complete physiologic transformation in women with preterm labor with intact membranes and preterm prelabor rupture of membranes and the lowest proportion of transformed spiral arteries in small for gestational age in our study, suggests that the difference between differing pathologies is centered in the varying proportion of spiral arteries with physiologic transformation and failure of physiologic transformation. Examining more samples or perhaps the whole placental basal plate would help clarify quantitative differences of vessel transformation in different pregnancy disorders that could explain the differing outcomes. Indeed, the presence of failure of physiologic transformation in the placental basal plate in our study was associated with a significantly lower gestational age, suggesting that abnormal placentation and the vasculopathies associated with it could predispose to an early delivery. The identification of failure of spiral artery physiologic transformation in multiple pregnancy complications in general and not only in malperfusion pathologies of the placenta in particular, suggests that inflammation leading to cell activation may be the trigger for an abnormal placentation that expresses as different pathologies of pregnancy. Finally, the frequency of atherosis in our study was higher than the frequency recently described in a large population of placentas from normal and abnormal pregnancies [159,162]. We believe this is due to the high sensitivity of triple-antibody immunohistochemistry when compared with hematoxylin-eosin used to detect failure of physiologic transformation of the spiral arteries and placental atherosis [44].

In support of the central role played by trophoblast invasion, Kam et al [163] demonstrated that the physiological change in spiral arteries during pregnancy can only take place when interstitial trophoblasts are present. In further studies [36,38], frequent transformational failure was seen in the myometrial but not decidual spiral artery segments in preterm prelabor rupture of membranes, and increased transformational failure was seen in myometrial and decidual segments in preterm labor with intact membranes. The frequency of failure was higher in preeclampsia than in either preterm prelabor rupture of membranes or preterm labor with intact membranes. In this study, failure of physiologic transformation of the spiral arteries was highest in pregnancies with preeclampsia and lowest in preterm labor. The differences seen between the physiological responses of normal pregnancy and placental disorders may be only quantitative and not qualitative. Since the fetoplacental unit is a semi-allograft, normal pregnancy can be considered a challenge to the maternal immune system [164–169], and indeed, is a state of physiologic intravascular inflammation. We and

other investigators have reported excessive intravascular inflammation in preeclampsia [59–62], intrauterine growth restriction [170,171], preterm labor [172], preterm prelabor rupture of membranes [173], and acute pyelonephritis [174]. An exaggerated intravascular inflammatory process can impair remodeling of the spiral arteries and lead to endothelial cell activation and atherosis.

A further point that needs to be emphasized is the role of endothelial disruption in physiologic transformation of the spiral arteries and atherosis in complicated pregnancies. Interestingly, atherosis lesions can show endothelial disruption [175], and it is tempting to postulate that such disruption is no more than the development of atherosis in arteries with partial failure of physiologic transformation. Although the study did not examine smooth muscle cells, partial failure of physiologic transformation of the spiral arteries was shown immunohistochemically by complementarity staining for endothelium and endovascular trophoblast in vessels of the placental bed [176]. Our findings demonstrated that both failure of physiologic transformation and partial failure of physiologic transformation of the spiral arteries showed endothelial activation, and interestingly, partial failure of physiologic transformation had intravascular trophoblasts (Figure 1), as found in arteries with endothelial disruption. Areas with intravascular trophoblast and absence of endothelium in partial failure of physiologic transformation of the spiral arteries would not show any expression of endothelial activation markers, as found in our study.

The nomenclature used to describe disorders of vascular remodeling in the spiral arteries can be confusing and, at times, misleading. Craven et al [10] viewed vascular changes as the beginning of physiologic change that occurs without trophoblast invasion, but others [163] adopted a more narrow view of physiological change—one that not only includes trophoblast invasion but is defined by it. Pijnenborg et al [19] suggested a new nomenclature replacing the term altogether with "trophoblast-associated remodeling." We suggest the term "defective superficial placentation" to describe the failure of physiologic transformation of the spiral arteries in the placental basal plate during pregnancy. Although examination of the basal plate exclusively without availability of placental bed biopsies does not allow identification of defective deep placentation, the diagnosis of defective superficial placentation is also abnormal, because it suggests severe failure of physiologic transformation of the spiral arteries.

Although it is difficult to be certain regarding initial events during the placental development by examining third-trimester placentas, it is tempting to suggest what happens during early placentation based on our findings. The sequence of events is that trophoblasts first invade the superficial (or decidual) segment of the spiral arteries and, only later, the myometrial segment of the arteries. While the hallmark for disorders of deep placentation is failure of trophoblast invasion in the myometrial segment (which requires a placental bed biopsy), the fact that trophoblast has not invaded the decidual segment indicates that the process is more severe. This interpretation is indicative of failure of the first wave of spiral artery trophoblastic invasion [34]. Furthermore, the approach that we have proposed (examination of the basal plate of the placenta) has practical value, given that placental bed biopsies are rarely performed, and often require a cesarean delivery. In contrast, examination of the basal plate of the placenta can be undertaken in all placentas, whether the mode of delivery is

vaginal or cesarean [34]. The concept of defective superficial placentation may be used to improve the characterization of different disorders of the placental bed. It is possible that our data will be valuable in refining the existing tools for the evaluation of risk before pregnancy or in early gestation for the prediction of subsequent pregnancy outcome.

Acknowledgments

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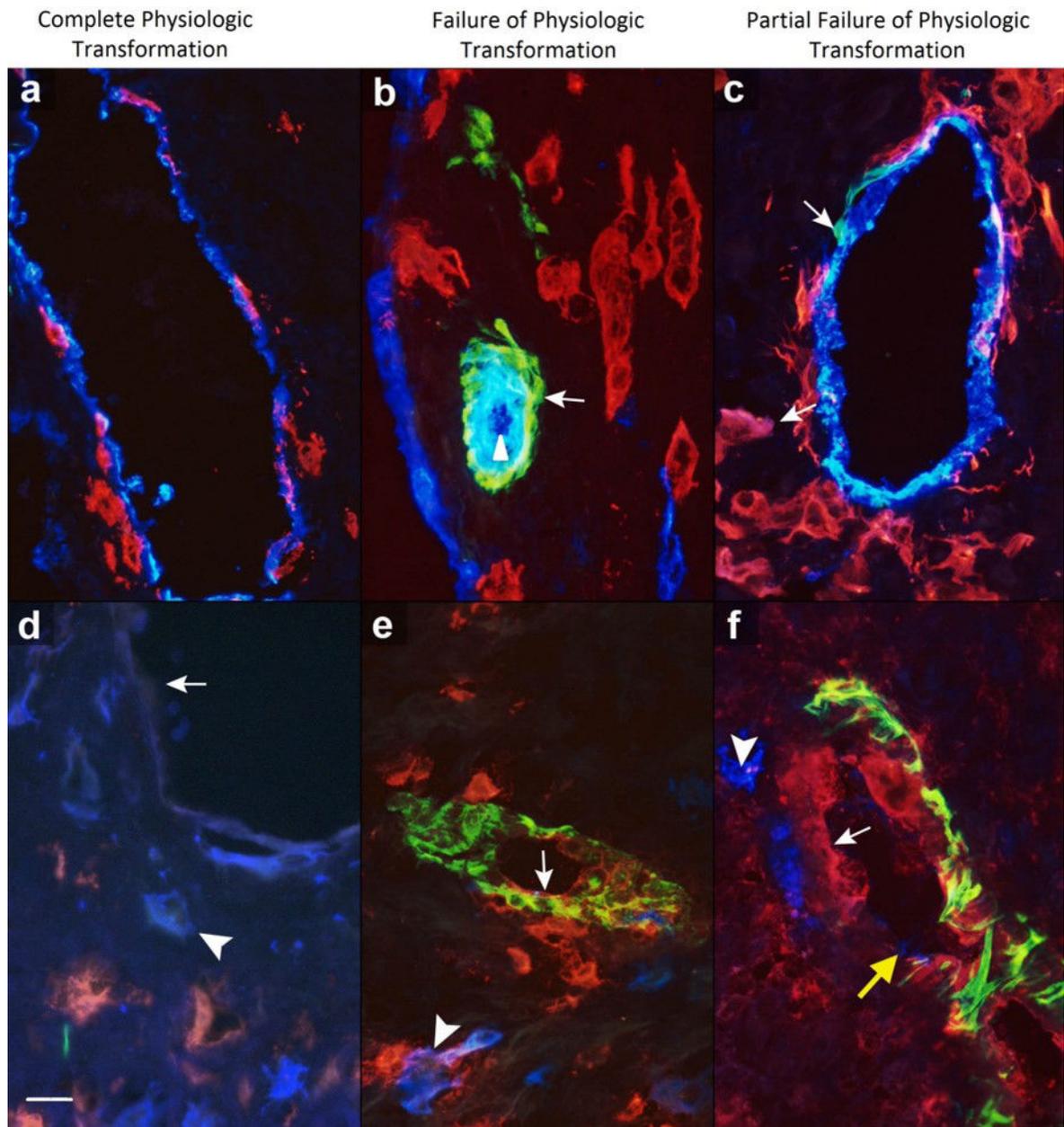
Figure 1. Different statuses of spiral artery physiologic transformation and ICAM-1 expression
Complete physiologic transformation of spiral arteries: A, presence of endothelium (blue) and trophoblast cells (red) in vessel wall; and D, absence of endothelial intercellular adhesion molecule (ICAM)-1 expression (arrow) with ICAM-1-negative trophoblast cells (blue, arrowhead). Failure of physiologic transformation of spiral arteries: B, characterized by spiral arteries with presence of endothelium (blue, arrowhead) and smooth muscle cells (green, arrow) without arterial invasion of trophoblast cells (red) as seen in artery in center (blue linings on left and lower right of figure corresponding to neighboring veins); and E, presence of endothelial ICAM-1 (red, arrow) with trophoblast cells (blue, arrowhead). Partial physiologic transformation of spiral arteries: C, presence of endothelium (blue), trophoblast cells (red, arrow), and smooth muscle cells (green, arrow) in part of vessel; and

F, presence of endothelial ICAM-1 (red, arrow) with trophoblast cells (blue, arrowhead) and smooth muscle cells (green). Note presence of intra-arterial trophoblasts (yellow arrow) in partial physiologic transformation of spiral arteries. Scale represents 50 μm. Original magnification: ×640.

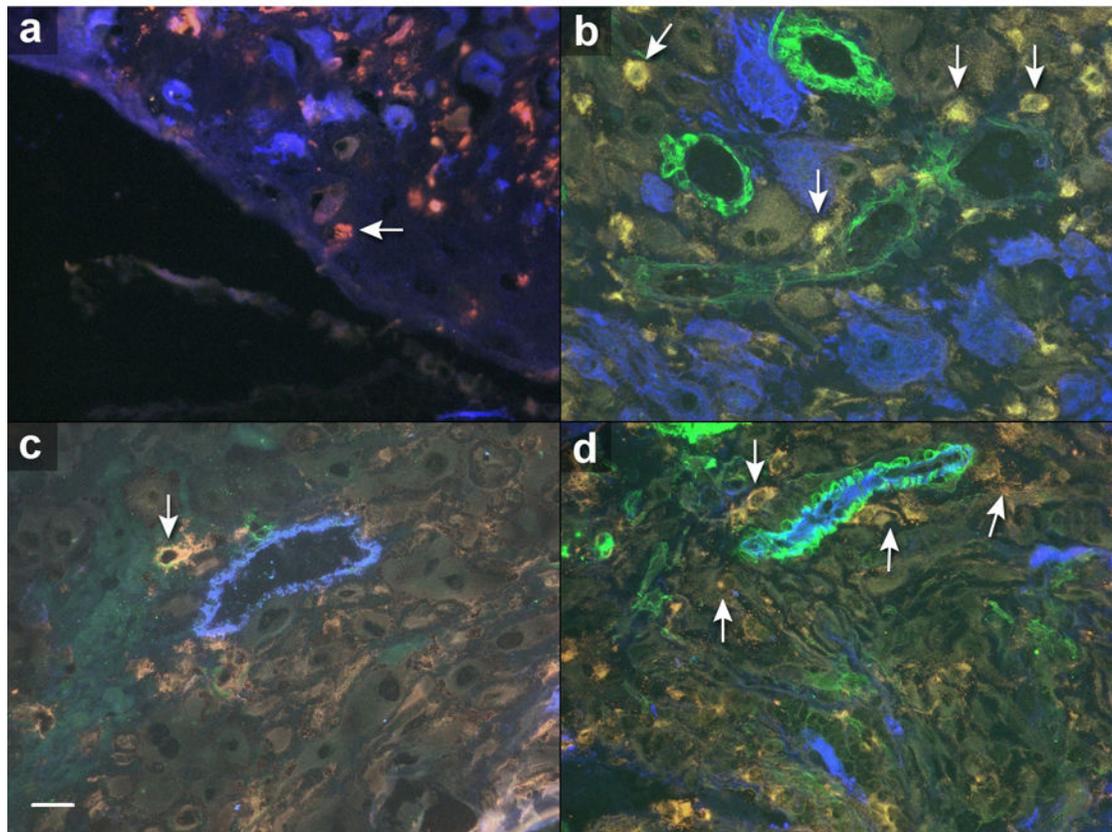Complete Physiologic Transformation   Failure of Physiologic Transformation

Figure 2. Macrophages and spiral artery physiologic transformation
Complete physiologic transformation of spiral arteries: **A and C**, few CD68^{+} macrophages
(red, arrows) around endovascular trophoblast cells (A, blue) and presence of endothelium
(C, blue) in vessels with normal trophoblastic invasion. Failure of physiologic
transformation of spiral arteries: B and D, numerous CD68^{+} macrophages (arrows) around
vessels, with presence of endothelium (D, blue), absence of intra-arterial trophoblast cells
(B, blue), and presence of smooth muscle cells (B and D, green) in spiral arteries. Scale
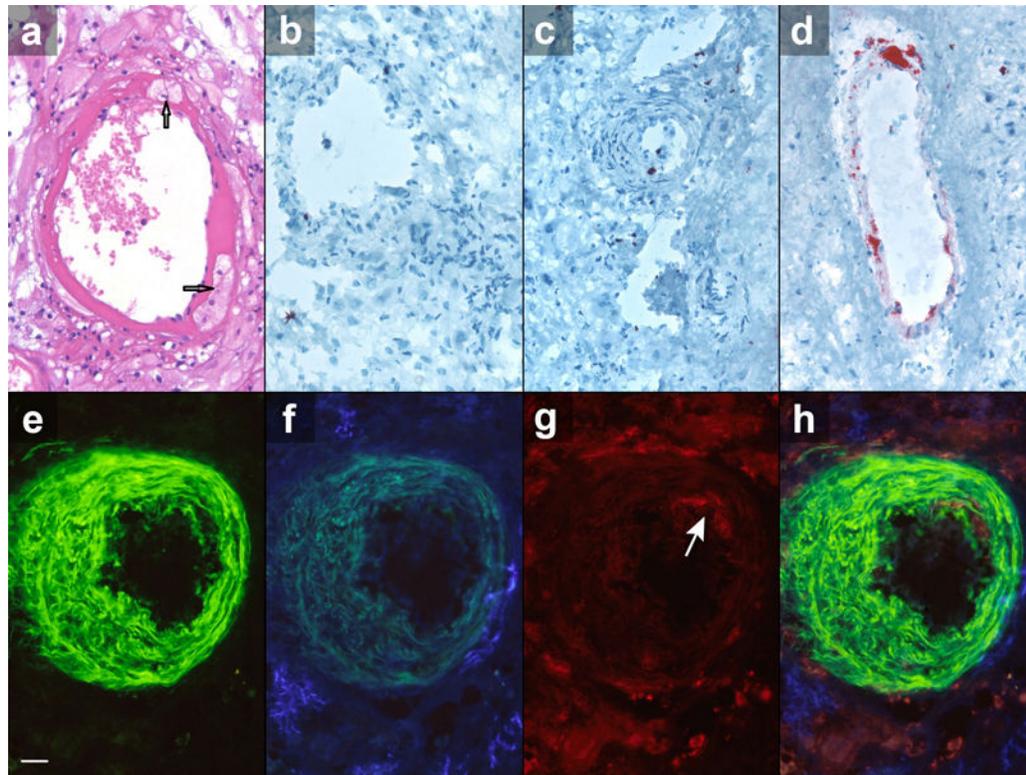represents 50 µm. Original magnification: ×640.

Figure 3. Histochemical and immunohistochemical characteristics of atherosis
Atherosis lesion recognized with hematoxylin-eosin stain: A, note presence of foam cells (arrows) reactive with oil red O, as shown in D, confirming presence of lipids, which are B, not observed in vessels with normal trophoblastic invasion or C, absence of trophoblastic invasion also reacted with oil red O. Atherosis was observed in spiral arteries using triple-antibody technique that showed arterial smooth muscle cells (E, green), trophoblast cells surrounding but not replacing vessel walls (F, blue), and presence of macrophages in vessel's intima (G, red, arrow). H, These cell subtypes are clearly shown in combined photograph. Triple-antibody immunohistochemistry for smooth muscle (alpha-actin, E), trophoblasts (cytokeratin, F), macrophages (CD68, G), and their combination (H). Scale represents 50 mm. Original magnification: ×640. A, Hematoxylin-eosin and B to D, oil red O stains.
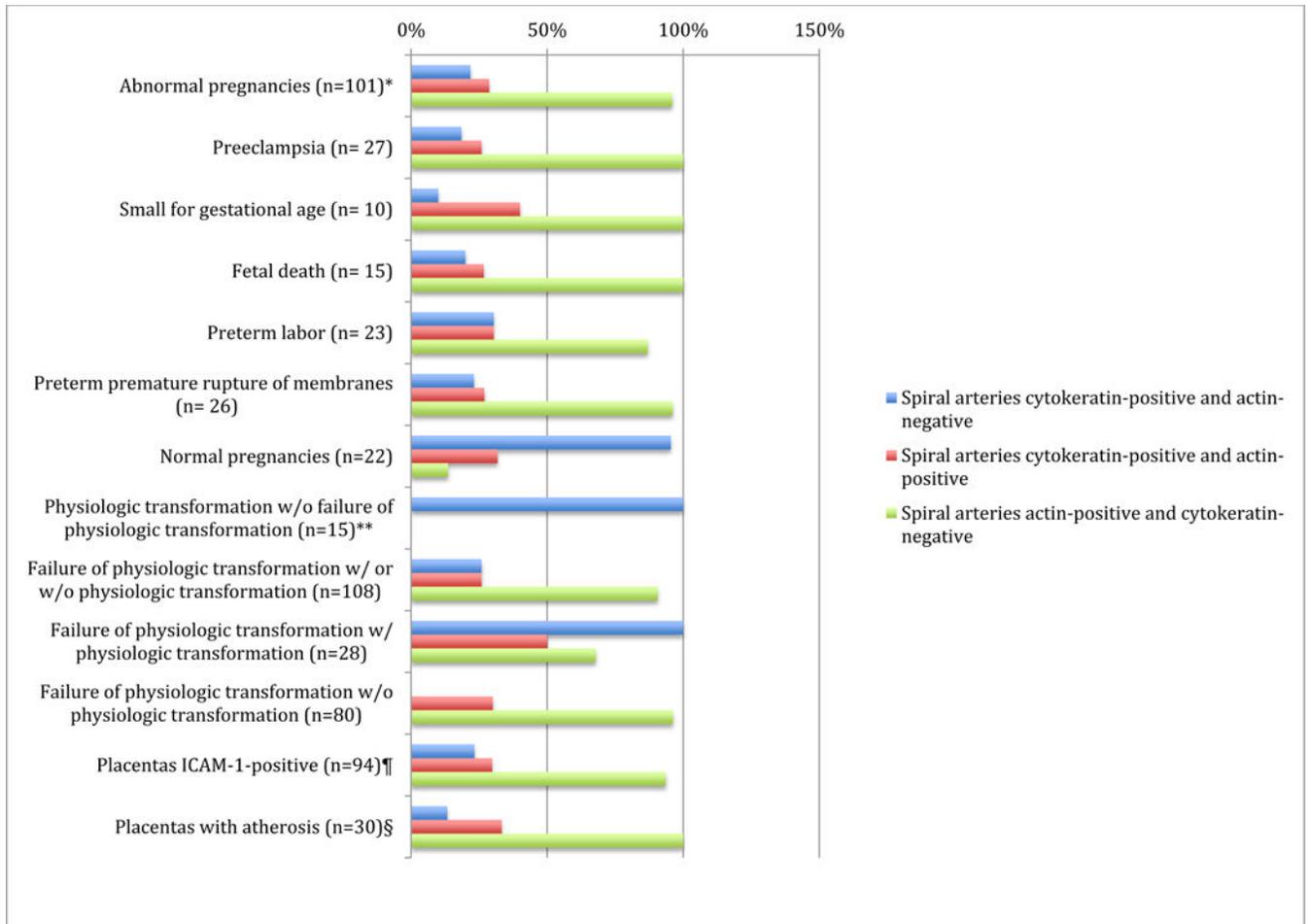
Figure 4. Summary of immunohistochemical characteristics of spiral artery physiologic transformation in basal plate of different pregnancy conditions studied

Clustered bar chart showing summary of immunohistochemical characteristics of spiral arteries in basal plate of different pregnancy conditions studied.

*Data were obtained from $n_a = 179$ arteries from $n_p = 123$ placentas;

**Failure of physiologic transformation considers complete and partial failure;

¶Placentas intercellular adhesion molecule (ICAM)-1-positive include spiral artery endothelial and/or extravillous trophoblast reactivity;

§Atherosis was identified in preterm labor ($n_p = 6$), preterm premature rupture of membranes ($n_p = 9$), preeclampsia ($n_p = 7$), small for gestational age ($n_p = 3$), fetal death ($n_p = 4$), and normal pregnancies ($n_p = 1$).

n_a, number of arteries; n_p, number of placentas.

Table 1

Immunohistochemical markers in spiral artery physiologic transformation and atherosis

Immunohistochemical characteristics	Complete physiologic transformation	Partial physiologic transformation	Failure of physiologic transformation	Atherosis
Cytokeratin	Positive	Positive/negative	Negative	Negative
Smooth muscle α-actin	Negative	Positive/negative	Positive	Positive
Von Willebrand factor	Positive	Positive	Positive	Positive
ICAM-1, endothelial	Negative	Positive/negative	Positive	Positive
ICAM-1, interstitial extravillous trophoblast	Negative	Positive/negative	Positive	Positive

ICAM-1, intercellular adhesion molecule-1.

Table 2

Spiral artery physiologic transformation and characteristics of the study population

Characteristics	Physiologic Transformation w/o Failure of Physiologic Transformation (n=15)	Failure of Physiologic Transformation (n=108)[a]			P-value[b]
		Failure of Physiologic Transformation w/or w/o Physiologic Transformation (n=108)	Failure of Physiologic Transformation w/ Physiologic Transformation (n=29)	Failure of Physiologic Transformation w/o Physiologic Transformation (n=79)	
Gestational age at delivery (weeks)[c]	39.5 (26.1, 41.6)	31.2 (18.5, 41.2)	31.3 (18.5, 40.4)	31.2 (19.2, 41.2)	<0.001
Birthweight (percentile)[c]	62.5th (7.5th, 99th)	25.5th (1st, 99th)	47th (1st, 99th)	17.5th (1st, 99th)	<0.001
Abnormal pregnancies (n=101)[d]	1 (1%)	100 (99%)	22 (21.8%)	78 (77.2%)	<0.001
Preeclampsia (n=27)	0 (0%)	27 (100%)	5 (18.5%)	22 (81.5%)	
Small for gestational age (n=10)	0 (0%)	10 (100%)	1 (10%)	9 (90%)	
Fetal death (n=15)	0 (0%)	15 (100%)	3 (20%)	12 (80%)	
Preterm labor (n=23)	0 (0%)	23 (100%)	7 (30.4%)	16 (69.6%)	
Preterm prelabor rupture of membranes (n=26)	1 (3.8%)	25 (96.2%)	5 (19.2%)	20 (76.9%)	
Normal pregnancies (n=22)	14 (63.6%)	8 (36.4%)	7 (31.8%)	1 (4.5%)	<0.001
Placentas ICAM-1-positive (n=94)[e]	0 (0%)	94 (100%)	21 (22.3%)	73 (77.7%)	<0.001
Abnormal pregnancies (n=92)	0 (0%)	92 (100%)	20 (21.7%)	72 (78.3%)	
Preeclampsia (n=23)	0 (0%)	23 (100%)	5 (21.7%)	18 (78.3%)	
Small for gestational age (n=9)	0 (0%)	9 (100%)	1 (11.1%)	8 (88.9%)	
Fetal death (n=14)	0 (0%)	14 (100%)	3 (21.4%)	11 (78.6%)	
Preterm labor (n=23)	0 (0%)	23 (100%)	7 (30.4%)	16 (69.6%)	
Preterm prelabor rupture of membranes (n=23)	0 (0%)	23 (100%)	4 (17.4%)	19 (82.6%)	
Normal pregnancies (n=2)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	
Placentas with atherosis (n=30)	0 (0%)	30 (100%)	6 (20%)	24 (80%)	<0.001
Abnormal pregnancies (n=29)	0 (0%)	29 (100%)	6 (20.7%)	23 (79.3%)	
Preeclampsia (n=7)	0 (0%)	7 (100%)	2 (28.6%)	5 (71.4%)	
Small for gestational age (n=3)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	
Fetal death (n=4)	0 (0%)	4 (100%)	0 (0%)	4 (100%)	
Preterm labor (n=6)	0 (0%)	6 (100%)	2 (33.3%)	4 (66.7%)	
Preterm prelabor rupture of membranes (n=9)	0 (0%)	9 (100%)	2 (22.2%)	7 (77.8%)	
Normal pregnancies (n=1)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	

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[a] Failure of physiologic transformation includes complete and partial failure.

[b] P-values compare the listed characteristic for the group of physiologic transformation (n=15) and the group of failure of physiologic transformation (n=108).

[c] Values are expressed as median (minimum, maximum) according to nationwide United States Natality data [88].

[d] Values in both columns are expressed as number (percentage) of each clinical/immunopathological group.

[e] ICAM-1-positive placentas include endothelial and/or extravillous trophoblast reactivity.

ICAM-1, intercellular adhesion molecule-1.

Table 3

Physiologic transformation, endothelial activation, and atherosis quantification in the study groups

Characteristics	Abnormal pregnancies (n=101)						Normal pregnancies (n=22)	P-value[a]
	Total (n=101)	Preeclampsia (n=27)	Small for gestational age (n=10)	Fetal death (n=15)	Preterm labor (n=23)	Preterm prelabor rupture of membranes (n=26)		
Percent arteries with physiologic transformation[†]	8.1±18.9	7.7±19.4	1.4±4.5	5.1±11.9	11.8±20.4	9.6±23.3	81.7±29.7	<0.0001
Percent arteries with partial failure of physiologic transformation	8.3±15.1	5.3±10.5	15.5±22.8	5.6±10.2	10.4±17.2	8.5±16	12.4±21.8	0.30
Percent arteries with failure of physiologic transformation	83.5±25.7	87.0±23	83.1±25.9	89.3±16.5	77.8±33.9	81.9±25	6.0±17.4	<0.0001
Percent arteries with endothelial ICAM-1	81.8±35.1	77.5±38.1	88.6±31.4	84.4±34.8	81.2±34.1	82.7±36.4	4.5±21.3	<0.0001
Percent arteries with atherosis	10.4±21.7	10.7±19.9	18.3±33.7	16.0±30.5	4.3±7.7	9.2±20.4	2.3±10.7	<0.0001

[†] Values are expressed as mean ± SD for each clinical group/subgroup. All percentages were calculated by dividing the number of vessels with a particular characteristic by the total number of vessels × 100.

[a] P-values are for comparison of total abnormal results (n=101) versus normal results (n=22).

ICAM-1, intercellular adhesion molecule-1.

Table 4

Immunohistochemistry of spiral artery physiologic transformation in the different study groups

| Characteristics | Spiral artery immunohistochemistry (n_p= 123)[a] | | | |
	Spiral arteries *cytokeratin-positive* and actin-negative (n_a=43)	Spiral arteries *cytokeratin-positive* and *actin-positive* (n_a=36)	Spiral arteries *actin-positive* and cytokeratin-negative (n_a=100)	*P*-value[b]
Abnormal pregnancies (n_p=101)[a]	22 (21.8%)	29 (28.7%)	97 (96.0%)	<0.001
Preeclampsia (n_p= 27)	5 (18.5%)	7 (25.9%)	27 (100%)	
Small for gestational age (n_p= 10)	1 (10%)	4 (40%)	10 (100%)	
Fetal death (n_p= 15)	3 (20%)	4 (26.7%)	15 (100%)	
Preterm labor (n_p= 23)	7 (30.4%)	7 (30.4%)	20 (87%)	
Preterm prelabor rupture of membranes (n_p= 26)	6 (23.1%)	7 (26.9%)	25 (96.2%)	
Normal pregnancies (n_p=22)	21 (95.5%)	7 (31.8)	3 (13.6%)	<0.001
Physiologic transformation w/o failure of physiologic transformation (n_p=15)[c]	15 (100%)	0 (0%)	0 (0%)	
Failure of physiologic transformation w/or w/o physiologic transformation (n_p=108)	28 (25.9%)	28 (25.9%)	98 (90.7%)	
Failure of physiologic transformation w/physiologic transformation (n_p=28)	28 (100%)	14 (50%)	19 (67.9%)	
Failure of physiologic transformation w/o physiologic transformation (n_p=80)	0 (0%)	24 (30%)	77 (96.3%)	
Placentas ICAM-1-positive (n_p=94)[d]	21 (22.3%)	28 (29.8%)	88 (93.6%)	<0.001
Placentas with atherosis (n_p=30)[e]	4 (13.3%)	10 (33.3%)	30 (100%)	<0.001

[a] Data were obtained from n_a=179 arteries from n_p=123 placentas.

[b] P-values compare the listed characteristic for the groups with spiral arteries that are cytokeratin-positive and actin negative (n_a =43), cytokeratin-positive and actin-positive (n_a =36), and cytokeratin-negative and actin-positive (n_a =100).

[c] Failure of physiologic transformation considers complete and partial failure.

[d] Placentas ICAM-1-positive include spiral artery endothelial and/or extravillous trophoblast reactivity.

[e] Atherosis was identified in preterm labor (n_p =6), preterm prelabor rupture of membranes (n_p =9), preeclampsia (n_p =7), small for gestational age (n_p =3), fetal death (n_p =4), and normal pregnancies (n_p =1).

ICAM-1: Intercellular adhesion molecule-1.