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Emerging role for dysregulated decidualization in the genesis of preeclampsia

Kirk P. Conrad ^{a,*}, Maria Belen Rabaglino ^b, Emiel D. Post Uiterweer ^c

^a Departments of Physiology and Functional Genomics, and of Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL 32610, United States

^b Department of Animal Reproduction, Universidad Nacional de Rio Cuarto, Rio Cuarto, Cordoba, Argentina

^c Department of Obstetrics and Laboratory of Neuroimmunology and Developmental Origins of Disease, University Medical Center Utrecht, Utrecht, The Netherlands

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ABSTRACT

In normal human placentation, uterine invasion by trophoblast cells and subsequent spiral artery remodeling depend on cooperation among fetal trophoblasts and maternal decidual, myometrial, immune and vascular cells in the uterine wall. Therefore, aberrant function of anyone or several of these cell-types could theoretically impair placentation leading to the development of preeclampsia. Because trophoblast invasion and spiral artery remodeling occur during the first half of pregnancy, the molecular pathology of fetal placental and maternal decidual tissues following delivery may not be informative about the genesis of impaired placentation, which transpired months earlier. Therefore, in this review, we focus on the emerging *prospective* evidence supporting the concept that deficient or defective endometrial maturation in the late secretory phase and during early pregnancy, i.e., pre-decidualization and decidualization, respectively, may contribute to the genesis of preeclampsia. The first prospectively-acquired data directly supporting this concept were unexpectedly revealed in transcriptomic analyses of chorionic villous samples (CVS) obtained during the first trimester of women who developed preeclampsia 5 months later. Additional supportive evidence arose from investigations of Natural Killer cells in first trimester decidua from elective terminations of women with high resistance uterine artery indices, a surrogate for deficient trophoblast invasion. Last, circulating insulin growth factor binding protein-1, which is secreted by decidual stromal cells was decreased during early pregnancy in women who developed preeclampsia. We conclude this review by making recommendations for further prospectively-designed studies to corroborate the concept of endometrial antecedents of preeclampsia. These studies could also enable identification of women at increased risk for developing preeclampsia, unveil the molecular mechanisms of deficient or defective (pre)decidualization, and lead to preventative strategies designed to improve (pre)decidualization, thereby reducing risk for preeclampsia development.

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1. Introduction

In healthy human pregnancies, placentation is associated with invasion of the placental bed by two populations of extravillous trophoblast cells—“endovascular” and “interstitial” trophoblasts, which invade the uterine spiral arteries and interstitium,

respectively. The placental bed comprises the gestational endometrium or decidua, and inner one-third of the myometrium. Uterine trophoblast invasion occurs during the first half of gestation resulting in apoptosis of endothelial and smooth muscle cells, as well as degradation of extracellular matrix in uterine spiral arteries with deposition of fibrinoid material and incorporation of trophoblasts within the vascular walls. Through these processes, the spiral arteries transform from narrow caliber, high resistance to large caliber, low resistance vessels resulting in increased delivery of blood flow of reduced velocity to the intervillous space beginning 8–10 weeks, thus providing oxygen and nutrients to the growing placenta and fetus [1–4]. Ultimately, the successful completion of

* Corresponding author. Departments of Physiology and Functional Genomics, University of Florida College of Medicine, 1600 SW Archer Road, PO Box 100274 M552, Gainesville, FL 32610, USA.

E-mail address: kpconrad@ufl.edu (K.P. Conrad).

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this remarkable physiological chain of events hinges on the invasive potential of extravillous trophoblasts (“seed”), receptivity of the decidua including the spiral arteries (“soil”) for these invading fetal cells, and their seamless interactions.

The genesis of preeclampsia is widely believed to reside in the placental bed during early pregnancy. Namely, preeclampsia is associated with deficient uterine invasion by endovascular trophoblasts and incomplete remodeling of spiral arteries in the inner one-third of the myometrium [3–5]. The decidual spiral arteries may be similarly affected, but to a more variable extent [5–8], while interstitial trophoblast invasion has been reported to be impaired by some investigators and normal by others [5,8,9]. Ultimately, however, structural deficiency of the spiral arteries precludes the establishment of normal blood flow patterns in the intervillous space resulting in placental damage, ischemia, and ischemia-reperfusion injury [1,2,10]. Recently, this pathological chain of events has been ascribed to early- (<34 gestational weeks), but not late-onset preeclampsia, the latter being attributed to overcrowding of villous as the placenta reaches its full growth potential, thereby impeding intervillous blood flow [11]. Although deficient placentation and villous overcrowding may predominate in early- and late-onset preeclampsia, respectively, there may not be a strict, gestational age cutoff separating these two pathogenic entities. For example, in a woman who develops preeclampsia manifestations at 35 or 36 gestational weeks, some degree of spiral artery pathology may be one factor underlying her disease. Moreover, the gestational age at which disease manifestations emerge may also be determined by interaction of the deleterious factors released from the damaged placenta (e.g., anti-angiogenic growth factors, proinflammatory cytokines and syncytiotrophoblast microparticles) with maternal constitutional factors [12,13]. In this regard, it is not inconceivable that some women may have considerable placental pathology stemming from impaired placentation as described above, but are relatively resistant to the damaging effects of the circulating placental factors, thus not developing disease until later in gestation, when the placental factors ultimately become overwhelming. In contrast, other women may be relatively vulnerable due to comorbidities like obesity, metabolic syndrome or chronic hypertension, thereby developing disease earlier in pregnancy with little placental pathology. The placental and maternal constitutional etiologies of preeclampsia ultimately converge on the maternal endothelium to produce disease manifestations [13–16]. Although not mutually exclusive, other maternal factors could also contribute to the development of preeclampsia that are not necessarily related to endothelial function [17,18]. Finally, it should be noted that deficient placentation is not unique to the pathogenesis of preeclampsia, but also underlies many cases of normotensive intrauterine growth restriction, preterm labor, late sporadic miscarriage and abruptio placentae [19,20].

1.1. Extravillous trophoblast gene expression and function after onset of clinical disease

The extravillous trophoblast is a logical target for scientific inquiry, because uterine invasion is suboptimal in preeclampsia (*vide supra*), and the proposed paternal genetic contribution to the disease could be expressed through this fetal cell-type [21]. In pioneering work, Fisher and colleagues extensively phenotyped endovascular trophoblasts in normal and preeclamptic pregnancies *in situ* on placental bed biopsies [1,22,23], and investigated cell invasion *in vitro* [24]. These investigators reported that, as the endovascular trophoblasts invade the uterus, they normally undergo an epithelial-to-endothelial transition and increase expression of angiogenic molecules—processes which are compromised in preeclampsia [22]. (It should be pointed out that not all subscribe

to the concept of endovascular trophoblast epithelial-to-endothelial transition [9]). The invasive potential of villous cytotrophoblasts isolated from preeclamptic placentas and studied *in vitro* was also found to be restricted [25], generally consistent with the finding of deficient invasion *in situ* [3–9,26]. Moreover, the molecular and cellular defects of extravillous trophoblasts identified both *in situ* and *in vitro* were largely consistent [22,25,27].

An important caveat is that these studies are *retrospective*, insofar as extravillous trophoblast gene expression, phenotype and function were investigated after appearance of disease manifestations. Conceivably, the observed defects could have arisen in response to the myriad of factors emanating from the syncytiotrophoblast during disease that may not be only injurious to the maternal endothelium, but also to extravillous trophoblasts. Alternatively, they could have occurred as a direct consequence of the ischemia and ischemia-reperfusion injury that afflicts the preeclamptic placenta secondary to deficient placentation. In the same vein, it is unclear whether the extravillous trophoblast gene and phenotypic expression, as well as decreased invasive capacity observed after disease onset, are irreversible or not. In one study, when villous cytotrophoblasts were isolated from preeclamptic women and investigated in cell culture, defective gene and phenotypic expression, and decreased invasive capacity persisted [25]. In contrast, the expression of many genes reverted to normal levels after 48 h in culture in another study, although invasive potential was apparently not assessed [27]. If gene and phenotypic expression, and reduced invasive capacity are reversible in culture, these findings could implicate the uterine milieu as an inciting factor, i.e., the villous environment from which these trophoblast cells were isolated, perhaps modified by factors emanating from the decidua into the spiral arteries, which then travel to the intervillous space in the blood. Nevertheless, even if the observed abnormalities reverted to normal in culture, it is impossible to determine whether the injurious agents in the uterine milieu after disease onset would be the same as those in the uterine milieu months before disease onset, when the extravillous trophoblasts were invading.

In summary, elucidation of extravillous trophoblast gene and phenotypic expression in late pregnancy may bear little or no relation to early pregnancy, when these cells invade and remodel spiral arteries of the uterus—the critical time when extravillous trophoblasts presumably derail in women who will develop preeclampsia. Clearly, the abnormal gene expression and cell phenotype reported in extravillous trophoblasts during disease could impair their function at that late stage [27], and contribute to disease symptoms. But, it can be argued that, in order to unveil the initial molecular derangements underlying deficient trophoblast invasion and spiral artery remodeling in preeclampsia, *prospective* investigations of trophoblasts and decidua (*vide infra*) obtained either before or at least coincident with the unfolding of these physiological events in early pregnancy is required. Indeed, gene expression in trophoblasts and decidua may be completely different in early pregnancy bearing little or no relation to delivered tissues as recently reported [28] (and see below).

1.2. Decidua

Another relevant tissue to consider is the decidua into which extravillous trophoblasts invade. However, much less attention has been given to decidua than trophoblasts. The hypothesis that deficient or defective endometrial maturation or decidualization could contribute to the genesis of preeclampsia is a logical deduction based on the intimate proximity of extravillous trophoblasts, decidual and immune cells, and spiral arteries within the placental bed [29,30]. Further, the concept fits with the maternal inheritance

pattern of preeclampsia, which could predispose to deficient or defective (pre)decidualization as an etiological factor in the disease [21].

Briefly, uterine luminal and glandular epithelial, as well as stromal cells undergo distinct changes in morphology, gene expression and function beginning in the secretory phase (“pre-decidualization”) and continuing after implantation (“decidualization”) [31]. Spiral arteries are likewise modified by decidualization preceding invasion of extravillous trophoblasts [26,32]. Decidual changes in uterine glandular epithelium are also prerequisite to histiotrophic nutrition of the placenta and fetus before the start of intervillous blood flow and hemotrophic nutrition at 8–10 weeks [33]. In women, part and parcel of (pre)decidualization is increased number of decidual Natural Killer (dNK) cells of an immunomodulatory phenotype with reduced capacity for cytotoxicity [34]. Importantly, dNK cells likely play key roles in promoting trophoblast invasion and spiral artery remodeling [34–36]. As well, increased decidual and myometrial macrophage number of an alternatively-activated (“M2”) phenotype accumulate [37], and T regulatory cells are also believed to exert an important role in uterine immune tolerance to the fetal/placental semi-allograft [38]. Fundamentally, decidualization is preparation of the “soil” for the “seed” (embryo implantation and placentation). Thus, it is reasonable to propose that abnormalities of decidualization beginning before and continuing during early pregnancy could compromise endovascular trophoblast invasion and spiral artery remodeling. For example, deficient or defective endometrial maturation could adversely impact extravillous trophoblasts as they initially transit the decidua, thereby curtailing invasion of the underlying myometrium. Alternatively, the inner one-third of the myometrium may also undergo a kind of “decidualization”, which is compromised in women who develop preeclampsia, thereby precluding deep extravillous trophoblast invasion [29]. NK cells confined to the decidua may play a major role in orchestrating extravillous trophoblast invasion and spiral artery remodeling in the decidua (*vide supra*), while uterine macrophages also present in the inner one-third of the myometrium, could conceivably play a similar role in this region of the placental bed [37]. With impairment of decidualization, the optimal number and phenotype of these immune cells residing in the decidua and myometrium would be compromised.

1.3. Decidual gene expression and function after onset of clinical manifestations

Several retrospective investigations of decidual basal plate in delivered placentas from women with preeclampsia have been reported. These studies demonstrated altered pro-inflammatory cytokine expression [39–43], as well as abnormal T regulatory [44,45] and macrophage number and phenotype [37,46,47] in the decidua of affected women. However, as advanced above, the argument can be made that the retrospective nature of these investigations precludes identifying these pathologic features as either cause or consequence of preeclampsia. For example, dramatically elevated levels of circulating deleterious factors emanating from the syncytiotrophoblast during pregnancy in women with preeclampsia such as sFlt1, sEng, TNF α , IL-6 and syncytiotrophoblast microparticles may not only be injurious to the endothelium (including of spiral arteries), but also to other cell-types such as decidual stromal, epithelial, and uterine immune cells. Although these decidual abnormalities reported in the basal plate of delivered placentas could impact extravillous trophoblast function at this late stage and contribute to disease symptoms, they may not be manifested before and/or during early pregnancy coincident with the critical period of trophoblast invasion and

spiral artery remodeling [28] (see **Bioinformatic Comparison of Transcriptomics in Chorionic Villous Samples vs Delivered Placental Tissues in Preeclampsia**, below).

1.4. Decidual gene expression and function before onset of clinical manifestations

The pioneering work of Cartwright and colleagues partly addresses the concern about whether decidual pathology may be because or consequence of preeclampsia [35]. They harvested NK cells from decidua of first trimester placentas obtained after elective termination. Immediately prior to elective termination, Doppler ultrasound was performed to evaluate the uterine artery resistance index. High resistance uterine artery indices were previously found to correlate with decreased endovascular trophoblast invasion of decidual spiral arteries [48]. This finding is consistent with the correlation between high uterine artery resistance index and decreased endovascular trophoblast invasion of decidual and myometrial spiral arteries in women with preeclampsia [49]. In the first trimester decidual tissues from pregnancies with high uterine artery index, Cartwright et al. identified perturbed gene expression and aberrant dNK cell phenotype, as well as impaired regulation of trophoblast function by dNK cells *in vitro* [35]. Although supportive of the concept of dysregulated decidua and dNK cells as one potential etiology of preeclampsia, these prospective data are not conclusive, because elevated resistance uterine artery indices are not highly predictive of preeclampsia [50,51].

Another unexpected line of prospective evidence supports the concept of endometrial antecedents of preeclampsia. Scattered over a period of 12 years from 1996 to 2008, six reports emerged in the literature all showing reduced levels of a decidual secretory protein, insulin growth factor binding protein-1 (IGFBP1), in the circulation of women during the first or second trimester who later developed preeclampsia [52–57] (Table 1 and Fig. 1). One interpretation of these findings is that deficient trophoblast invasion reduces the deportation of decidual IGFBP1 into the maternal circulation [52]. However, another explanation is that reduced circulating IGFBP1 reflects a primary deficiency or defect in endometrial cell maturation, and hence, IGFBP1 production [53].

Further prospective evidence supporting the concept of endometrial antecedents of preeclampsia is provided by investigations we conducted on chorionic villous samples (CVS) obtained from women who developed preeclampsia [28,58]. Previously, we conducted a number of investigations into the molecular pathology of delivered placentas from women with preeclampsia with a focus on hypoxia inducible transcription factors and downstream genes, e.g., [59–61]. However, we did not know whether the upregulation of hypoxia-inducible transcription factors and downstream genes in preeclamptic placentas was a distal or proximal event in the disease, and the only approach that we knew to address this question was to begin collection of surplus CVS. Approximately 130 specimens of 10–12 gestational weeks were snap frozen within 5–10 min of uterine abstraction from 2001 to 2005. Four of the women (~3%) developed severe preeclampsia, and we matched them with 8 women who experienced normal pregnancies. The tissues were then subjected to transcriptomic analysis. Surprisingly, the hypothesis-driven component of the project was not supported, i.e., there was no evidence for upregulation of hypoxia (or oxidative stress) regulated genes [58]. Rather, there emerged a strong signature of dysregulation of decidual gene expression, e.g., downregulation of IGFBP1, glycodefin, prolactin and IL-15 [28,58]. A synopsis of the rationale, findings and major conclusions of this study follow.

We employed systems biology approaches [28,62] to test the hypothesis that aberrant decidualization both before and during

Table 1
Lower circulating concentrations of insulin growth factor binding protein-1 (IGFBP-1) in early pregnancy is associated with the development of preeclampsia. PE, preeclampsia; NP, normal pregnancy. Average gestational age (weeks) when blood was obtained for analysis of IGFBP-1. Many of the same subjects were studied both in the 1st and 2nd trimesters (overlapping). See References for complete citations.

Ref.	No. Subjects	Average Gestational Age (weeks)	Circulating IGFBP-1 Concentration (ng/ml)	P value
de Groot, 1996	20NP/20PE	19	79 ± 15 vs 36 ± 6 (mean ± SEM)	= 0.02
Hietala, 2000	794NP/34PE	16	103 ± 62 vs 73 ± 43 (mean ± SD)	<0.01
Anim-Nyame, 2000	12NP/10PE	16, 20, 24, 28, 32, 36	See Fig. 1	
Grobman, 2001	24NP/12PE	22	130 ± 66 vs 84 ± 41 (mean ± SD)	<0.05
Ning, 2004	477NP/53PE	13	53 [32–83] vs 39 [19–58] Median [IQR]	<0.01
Vatten, 2008	Normal Pregnancy		Reduced IGFBP-1 during 1st and 2nd Tri. in the lowest quartile is related to higher risk of term PE: OR 4.0 (1.9–8.4) and 2.3 (1.2–4.4), respectively; but not preterm PE: OR 1.5 (0.7–3.3) and 1.7 (0.9–3.1), respectively.	
	274 1st Tri.	9	NB. Absolute values for IGFBP-1 not reported.	
	286 2nd Tri.	22		
	95 Overlapping			
	Preeclampsia (>37 wk)			
	143 1st Tri.	9		
	128 2nd Tri.	22		
	47 Overlapping			
	Preeclampsia (<37 wk)			
	107 1st Tri.	9		
	108 2nd Tri.	22		
	42 Overlapping			

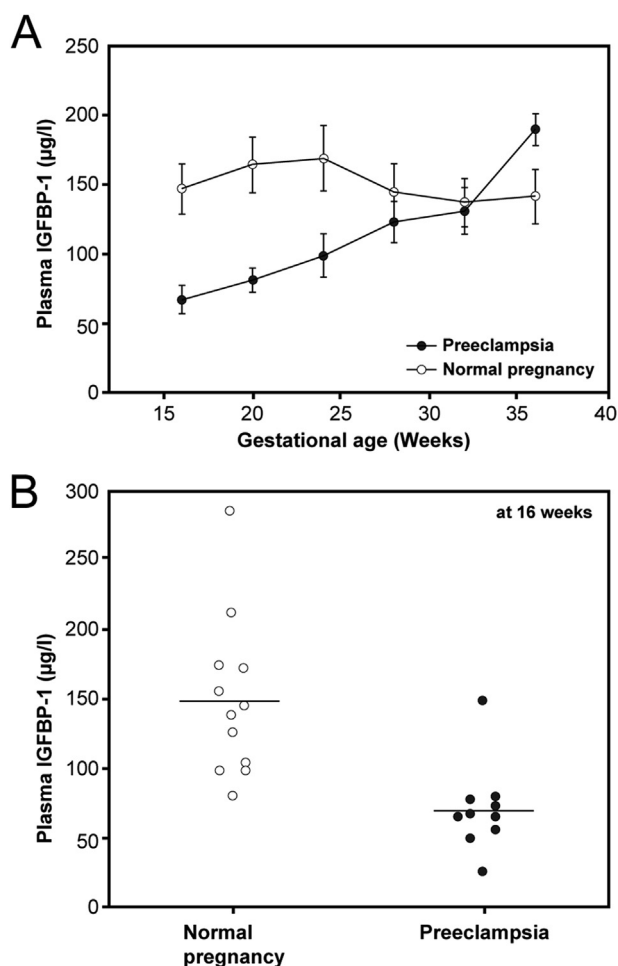


Fig. 1. Circulating IGFBP-1 concentrations in normal pregnancy and preeclampsia. (A) Serial measurements of IGFBP-1 were performed throughout pregnancy. IGFBP-1 was significantly reduced in preeclampsia compared to normal pregnancy at 16, 20, and 24 weeks of gestation ($p = 0.006$, $p = 0.001$, and $p = 0.04$, respectively). IGFBP-1 was significantly elevated in preeclampsia at 36 weeks of gestation ($p = 0.04$). (B) Scatterplot of data from 16 gestational weeks shows good separation of data points between the 2 cohorts. Anim-Nyame et al., 2000 with permission.

early pregnancy precedes preeclampsia [58]. Specifically we hypothesized that preeclampsia is antedated by dysregulated maturation of the endometrium leading to compromised local immune cell number and phenotype, i.e., sub-optimal (pre)decidualization. In turn, these endometrial defects compromise extravillous trophoblast invasion, spiral artery remodeling and placentation [30,63–66]. Because endometrial maturation during the secretory phase and early pregnancy is a biological continuum, both impaired pre-decidualization and decidualization are likely. We reasoned that, if the genes regulated in the endometrium and in dNK cells during the normal biological processes of (pre)decidualization [67–71] were changed in the opposite direction in CVS from women who developed preeclampsia (PE-CVS) relative to normal pregnancy (NP-CVS) [58], then this prospective evidence would provide the crucial linkage needed to underpin the concept of “endometrial antecedents of preeclampsia”.

We cast a wide net and included differentially expressed genes (DEG) determined by fold-change, t -test ($p < 0.05$) and J5 test for subsequent bioinformatics [28,58]. There were a total of 396 DEG between PE- and NP-CVS of which 195 were down- and 201 up-regulated in PE-relative to NP-CVS [28]. A large number, 154 or 40%, overlapped with DEG associated with various stages of normal endometrial maturation before and after implantation as identified in other microarray data sets in the public domain ($p = 4.7 \times 10^{-14}$). One-hundred and sixteen of the 154 DEG or 75% overlapped with DEG associated with normal decidualization in the absence of extravillous trophoblasts, i.e., late secretory endometrium and endometrium from tubal ectopic pregnancy ($p = 4.2 \times 10^{-9}$). Finally, 112 of these 154 DEG or 73% were changed in the opposite direction in microarray data sets related to normal endometrial maturation ($p = 0.01$). For example, 16 DEG up-regulated in decidual relative to peripheral NK cells were down-regulated in CVS from women who developed PE vs NP ($P < 0.0001$) consistent with decidual NK cell dysregulation. Overall, these results suggest that insufficient or defective maturation of endometrial and dNK cells or reduced numbers during the secretory phase and early pregnancy preceded development of preeclampsia [28].

Fig. 2 depicts \log_2 mean expression values for the DEG down-regulated in PE-CVS (relative to NP-CVS) that were: (A) uniquely upregulated in late secretory relative to proliferative endometrium (20 DEG), (B) uniquely upregulated in histologically-classified intermediate and confluent decidualized endometrium from early pregnancy (relative to non-decidualized endometrium; 13 DEG),

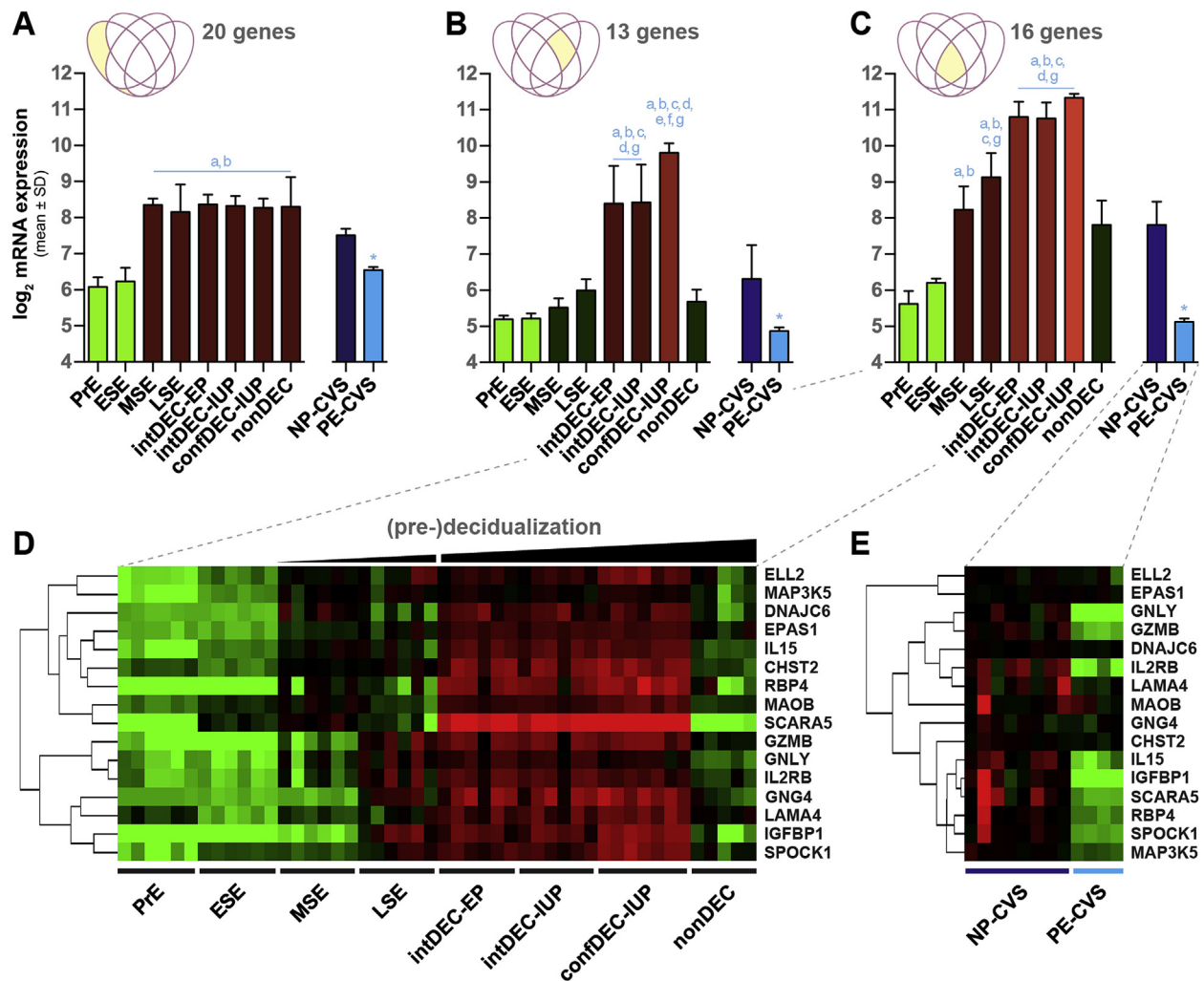


Fig. 2. Average expression levels (log base 2) of differentially expressed genes (DEG) in samples obtained from endometrium at different stages of endometrial maturation and from chorionic villous samples (CVS). PE: preeclampsia; NP-normal pregnancy. PrE, ESE, MSE, LSE: proliferative and early, middle and late secretory endometrium, respectively; IntDEC, ConfDEC and nonDEC: intermediate, confluent and non-decidualized endometrium determined histologically. Significantly different ($P < 0.05$) from: a, PrE; b, ESE; c, MSE; d, LSE; e, intDEC-EP; f, intDEC-IUP; g, nonDEC; * $P < 0.0001$ vs nonpregnant (NP)-CVS. See text for further details. Rabaglino et al., 2015 with permission.

and (C) upregulated in late secretory endometrium, intermediate and confluent decidualized endometrium (16 DEG). For the 20 DEG identified as uniquely up-regulated in late secretory endometrium (i.e., their average expression did not further increase with decidualization early in pregnancy) and down-regulated in CVS-PE, the average gene expression was significantly less in PE-CVS than in NP-CVS by ~2-fold (Fig. 2A). These findings suggest that impairment of endometrial maturation in the women who developed PE began before pregnancy in the secretory phase. The 13 DEG down-regulated in CVS-PE and up-regulated in intermediate and confluent decidualized endometrium from early pregnancy, slightly increased in late secretory endometrium, but mostly rose during decidualization in early pregnancy (Fig. 2B). Average gene expression for these 13 DEG was markedly less in PE-CVS than NP-CVS by ~3-fold. These results suggest that, in addition to a defect in pre-decidualization as described, there was also impairment of decidualization after implantation in the women who developed preeclampsia. Finally, the 16 DEG down-regulated in CVS-PE and up-regulated in late secretory endometrium, intermediate and confluent decidualized endometrium increased expression beginning in the mid-secretory endometrium and progressively rose thereafter. In this case, average gene expression of the 16 DEG was

~7-fold less in PE-CVS compared to NP-CVS (Fig. 2C and D), indicating again that pre-decidualization and decidualization were compromised in the women who developed preeclampsia.

Decidual macrophages may also have been dysregulated during early pregnancy in the women who developed preeclampsia. Gustafsson and colleagues investigated gene expression in CD14⁺ decidual macrophages isolated from first trimester placenta acquired through elective terminations and the CD14⁺ macrophages isolated from the corresponding peripheral blood [72]. We reanalyzed the DNA microarray data available in the Gene Expression Omnibus (GEO) database (accession number GSE10612) using fold-change > 2.0 and $p < 0.05$ as criteria for selecting DEG. There was a total of 1078 DEG between decidual and peripheral blood CD14⁺ macrophages, of which 54 overlapped with the 396 DEG in PE-CVS ($p = 7.0 \times 10^{-20}$). Of these 54 overlapping DEG, 37 or 69% changed in the opposite direction. Specifically, there were 12 DEG upregulated in PE-CVS that were downregulated in CD14⁺ decidual macrophages ($p = 1.1 \times 10^{-6}$) and 25 DEG downregulated in PE-CVS and upregulated in CD14⁺ decidual macrophages ($p = 9.5 \times 10^{-13}$). Thus, in addition to NK cells (*vide supra*), taking a similar bioinformatics approach reveals the possibility that macrophages may also have been abnormal in the decidua of women

who later developed preeclampsia.

1.5. Bioinformatic Comparison of Transcriptomics in Chorionic Villous Samples vs Delivered Placental Tissues in Preeclampsia

To reinforce the concept that the molecular pathology of delivered placental tissues—villous, decidua or isolated villous cytotrophoblasts—apparently bear little resemblance to the placenta in early pregnancy (CVS) from women who developed severe preeclampsia as discussed above, we performed further bioinformatical analyses (Table 2A and B). The results demonstrate little or no overlap of DEG changing in the same direction between CVS and delivered placental tissues—chorionic villous, isolated villous cytotrophoblasts or decidual basal plate [27,73–78].

1.6. Potential mechanisms of impaired decidualization in preeclampsia

Fig. 3 summarizes our current thinking about the concept of endometrial antecedents of preeclampsia and potential etiological factors. Several diseases have been reported to both impair decidualization and predispose to preeclampsia including polycystic ovarian syndrome [79–84], obesity [79,80,85,86], diabetes mellitus [87–89], and endometriosis [90–92]. These comorbidities may exert their disruptive action on the endometrium through anomalous inflammation, exaggerated production of androgens, insulin, and homocysteine, as well as pathological epigenetic modifications [79,91,93–96]. Indeed, epigenetic dysregulation is

another likely instigating factor, insofar as much of the normal biological process of decidualization is epigenetically regulated [97–103]. Genetic factors include certain pairings of highly polymorphic KIR receptors on dNK cells with HLA-C ligands on extravillous trophoblasts that increase the risk for preeclampsia [34]. Seminal plasma has been reported to modulate immune cell number and function in the uterus. In particular, seminal fluid was shown to stimulate CCL19 expression by glandular and luminal epithelial cells that, in turn, recruited T regulatory cells into the uterus via the CCR7 receptor prior to embryo implantation in mice [104]. Thus, it is not inconceivable that lack of timely or sufficient exposure to seminal plasma may predispose to preeclampsia [105]. We further postulate that the increased risk of preeclampsia in donor egg recipient [106] and frozen embryo transfer [107–109] pregnancies may in part stem from lack of fine tuning of decidualization by corpus luteal products like relaxin (and perhaps other, as of yet, undiscovered corpus luteal factors), which is missing, if embryo transfer occurs in the absence of a corpus luteum. Finally, with the revelation of intimate host-microbial interactions in the gut and other organs [110–112], the existence of an endometrial microbiome is a distinct possibility, which has recently gained support [113–115]. Whether a normal endometrial microbiome contributes to the biological process of (pre)decidualization possibly through influencing epigenetic events [116], and whether an abnormal microbiome impairs (pre)decidualization, thus predisposing to preeclampsia, are obvious questions that need to be addressed in future investigations.

Table 2A

Bioinformatics analysis reveal little overlap of differentially expressed genes changing in the same direction in chorionic villous samples from women who later developed preeclampsia and differentially expressed genes in villous or isolated villous cytotrophoblast of delivered placentas from women who experienced preeclampsia. PE, preeclampsia; NP, normal pregnancy; CVS, chorionic villous samples; DEG, differentially expressed genes between women who experienced preeclampsia vs normal pregnancy. No. DEG (CVS) from Rabaglino MB et al. Hypertension 65:421–9, 2015. See References for complete citations.

Publication	No. DEG (CVS)	No. DEG (Delivered Placenta)	No. Overlapping DEG (In Same Direction)	Comments
Zhou Y et al. JCI 123:2862–2672, 2013	396	907	3 upregulated 5 downregulated	<i>Isolated Cytotrophoblast.</i> 5 subjects with severe PE and 5 with preterm labor without signs of infection. Data downloaded from GEO#GSE40182, and reanalyzed. Only freshly isolated CTB samples were included in the analysis.
Meng T et al. OMICS 16: 301, 2012	396	860	3 upregulated 5 downregulated	<i>Villous.</i> 6 subjects each for preeclampsia and normal pregnancy. Data abstracted from Supplementary Material 1.
Vaiman D et al. PLOS One e65498: 1–14, 2013	396	98	0	<i>All villous except 1 basal plate decidua sample.</i> Meta-analysis of 6 datasets, which included a total of 79 PE and 96 NP subjects (Supplementary Tables 1 and 2).
Van Uiter M et al. PLOS One http://dx.doi.org/10.1371/journal.pone.0132468 : 1–15, 2015	396	388	1 upregulated 1 downregulated	<i>All villous except 1 basal plate, 1 villous + basal plate, and 1 unspecified sample.</i> Meta-analysis of 11 datasets, which included a total of 116 PE and 139 NP subjects (Supplementary Table 1).

Table 2B

Bioinformatics analysis reveals little overlap of differentially expressed genes changing in the same direction in chorionic villous samples from women who later developed preeclampsia and differentially expressed genes in decidua basalis of delivered placentas from women who experienced preeclampsia. PE, preeclampsia; NP, normal pregnancy; CVS, chorionic villous samples; DEG, differentially expressed genes between women who experienced preeclampsia vs normal pregnancy. No. DEG (CVS) from Rabaglino MB et al. Hypertension 65:421–9, 2015. See References for complete citations.

Publication	No. DEG (CVS)	No. DEG (Delivered Placenta)	No. Overlapping DEG (In Same Direction)	Comments
Winn VD et al. Pregnancy Hypertens. 1:100–108, 2011	396	116	0 upregulated 3 downregulated ($p = 0.01$)	<i>Basal plate decidua.</i> 12 women with severe preeclampsia and 11 with preterm labor without signs of infection. Data from Supplemental Figure S1.
Yong HEJ et al. Plos One 2015.10 (5): p. e0128230	396	407	2 upregulated 1 downregulated	<i>Basal plate decidua.</i> 60 women with PE and 65 with normal pregnancies (Supplemental Table 4).
Loset M et al. Am J Obstet Gynecol. 204:84.e1–e27, 2011	396	454	0	<i>Basal plate decidua.</i> 43 women with preeclampsia and 59 with normal pregnancies (Table 2).

Endometrial antecedents of preeclampsia: A pathway to disease?

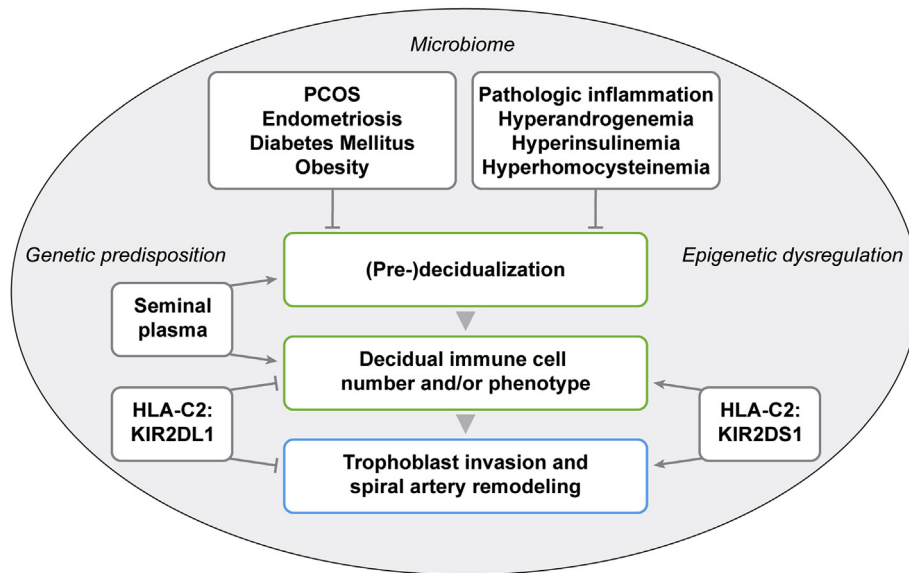


Fig. 3. Aberrant decidualization in the late secretory phase and during early pregnancy may play a role in the development of preeclampsia for some women. See text for details.

1.7. Future investigations

Although fraught with formidable logistical challenges and inherent shortcomings, additional prospective studies are needed to reinforce the concept of endometrial antecedents of preeclampsia. To this end, further “omics” analyses of surplus CVS are needed in larger cohorts of women who develop preeclampsia. The several institutions around the world still performing CVS in sufficient numbers could collaborate and coordinate collection of surplus CVS to be processed for RNA (and other analyses) immediately after uterine extraction. Clearly, the logistical challenges include the recent emergence of non-invasive prenatal diagnosis, which is dramatically reducing CVS procedures, the requirement for rapid processing of the surplus CVS tissue to preserve high quality RNA, and the need for obtaining obstetrical outcomes. Nevertheless, to our knowledge, there is no other way to obtain decidua from early pregnancy and the obstetrical outcome, which are both essential for testing the endometrial antecedents of preeclampsia.

Because our transcriptomic and bioinformatics analyses of surplus CVS suggested deficiency of both pre-decidualization and decidualization in women who developed preeclampsia [28,58] (Fig. 2), we have been obtaining endometrial biopsies during the late secretory phase of women who experienced severe preeclampsia in the previous pregnancy. The overall goal is to determine whether there is dysregulation of pre-decidualization in a non-conceptive cycle as assessed by “omics” and functional analyses of cultured endometrial stromal cells derived from the biopsy. If so, then these findings would corroborate our transcriptomic and bioinformatics analyses of surplus CVS, which indicated impairment of decidualization actually begins before pregnancy [28] (Fig. 2). Potential downsides to this approach include: (i) preeclampsia *per se* in the index pregnancy may adversely affect endometrial maturation in subsequent menstrual cycles, (ii) the quality and extent of pre-decidualization in the conceptive cycle preceding the index preeclamptic pregnancy may not be the same in subsequent non-conceptive cycles, and (iii) preeclampsia recurrence is only ~ 20% [117]. With respect to the first of these

potential pitfalls, a positive study outcome could be followed up in future investigations of banked, formalin-fixed paraffin embedded (FFPE) endometrial biopsies that were obtained *prior* to assisted reproductive cycles (“mock” cycles) starting with identification of obstetrical outcomes and followed by gene expression analysis (technology for extracting sufficient quality RNA from FFPE tissues for gene expression analysis is emerging). However, this undertaking would be huge, and the approach involving endometrial biopsies after the index pregnancy of preeclampsia may indeed be more feasible as the first step. The second potential pitfall is difficult to address except by obtaining a positive outcome to the study. Regarding the last of the potential pitfalls, it is not inconceivable that the same impairment of pre-decidualization persists in subsequent menstrual cycles after the index preeclamptic pregnancy, but that compensatory maternal responses or compensation by extravillous trophoblasts mitigates preeclampsia recurrence. Alternatively, inadequate pre-decidualization may be a predisposing factor, and a “second hit” is needed to initiate the disease process. Although these potential pitfalls are all valid criticisms, they should not derail pursuit of obtaining endometrial biopsies, because the rewards, in the event of positive results, would be enormous. Moreover, specific molecular deficiencies or defects could conceivably be identified, and preventative or therapeutic countermeasures designed and tested to improve (pre) decidualization.

Finally, as depicted in Table 1, there may be circulating biomarkers of (pre)decidualization that could identify women who are at increased risk of developing preeclampsia. These biomarkers could be informed by the differential gene and protein expression in the endometrial biopsies as discussed above. Or, they could be informed by transcriptomic analyses available in the public domain of normal decidualization from early ectopic and intrauterine pregnancies [67]. Of course, all of these prospective approaches could be applied to investigating the role of aberrant (pre)decidualization in other adverse obstetrical outcomes that may arise from deficient placentation, too, such as preterm labor and normotensive intrauterine growth restriction.

1.8. Perspectives

The general premise presented in this treatise is that insufficient or defective maturation of endometrium before and during early pregnancy compromises immune cell number and/or phenotype leading to impaired trophoblast function, spiral artery remodeling, and consequently, the development of preeclampsia. Fig. 4 depicts this concept in the context of the classic 2 or 3 step model for the pathogenesis of preeclampsia [12]. Although logistically challenging, we believe that this hypothesis is further testable (*vide supra*). For example, unveiling aberrant endometrial gene expression on endometrial biopsy of women in late secretory phase who were affected by severe preeclampsia in a prior pregnancy could inform targeted investigation and discovery of protein biomarkers in blood, urine or uterine fluid even before conception that eventually could constitute a diagnostic panel. Ultimately, designing interventions that improve endometrial maturation to facilitate normal placentation and reduce preeclampsia risk might be a logical therapeutic course of action [118].

The hypothesis that preeclampsia may arise from defective or insufficient endometrial maturation may not be particularly new [29], and might even be considered as self-evident or intuitive in light of the close proximity of decidual stromal, epithelial, and immune cells with trophoblast at the maternal-fetal interface. Nevertheless, prospective evidence (i.e., obtained during pregnancy months before disease onset or even before pregnancy), which is critically needed to lend credence to the concept has been missing until recently. Clearly, study of trophoblast and decidua from delivered placentas has revealed valuable insights into our understanding of preeclampsia pathogenesis, but perhaps not so much about etiology due to the retrospective timing of tissue acquisition. In this review, we bring together the new and emerging, prospective evidence supporting the concept that deficiency or defects in (pre)decidualization during the secretory phase and early pregnancy including dysregulation of uterine immune cell number and/or phenotype antedate preeclampsia at least in a

subset of women who develop the disease. Indeed, the degree of impairment in (pre)decidualization likely impacts the severity of pregnancy outcome. Thus, implantation failure, miscarriage, pre-term labor, intrauterine growth restriction and preeclampsia may arise from a spectrum of (pre)decidual insufficiency ranging from most to least severe. In support of this concept, we recently reported significant overlap of the transcriptomes in CVS from women who developed preeclampsia with secretory endometrium from women who experienced recurrent implantation failure or miscarriage [119].

Conflict of interest

None.

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Note

Garrido-Gomez et al. recently reported impairment of *in vitro* decidualization of endometrial stromal cells isolated and cultured from mid-secretory endometrial biopsies of women who were affected by severe preeclampsia (Reprod. Sci. 24 No.1 (Suppl.): 90A, 2017 (abstr.)). These findings support the concept of endometrial antecedents of preeclampsia as advanced in this article.

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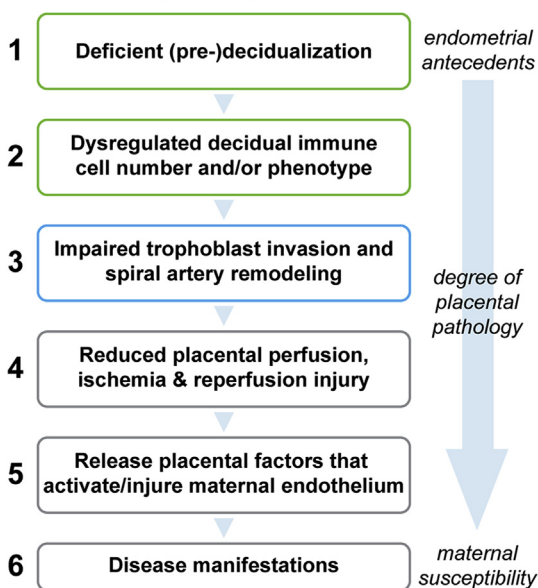


Fig. 4. Composite model of preeclampsia that includes the concept of dysregulated decidual and immune cell function in the genesis of impaired placentation (steps 1–3) prior to the traditional 2 or 3 stage model of disease pathogenesis (steps 4–6).

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