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Approaches to modeling placental function in preeclampsia in vitro and in vivo

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This paper is part of a collection of articles highlighting the breadth and depth of research being undertaken across the field of basic endocrinology by early- and mid-career researchers. The collection is published across the Journal of Endocrinology and the Journal of Molecular Endocrinology.

Abstract

Modeling preeclampsia remains difficult due to the nature of the disease and the unique characteristics of the human placenta. Members of the Hominidae superfamily have a villous hemochorial placenta that is different in structure from those of other therian mammals, including the mouse hemochorial placenta, making this common animal model less ideal for studying this disease. Human placental tissues delivered from pregnancies complicated by preeclampsia are excellent for assessing the damage the disease causes but cannot answer how or when the disease begins. Symptoms of preeclampsia manifest halfway through pregnancy or later, making it currently impossible to identify preeclampsia in human tissues obtained from an early stage of pregnancy. Many animal and cell culture models recapitulate various aspects of preeclampsia, though none can on its own completely capture the complexity of human preeclampsia. It is particularly difficult to uncover the cause of the disease using models in which the disease is induced in the lab. However, the many ways by which preeclampsia-like features can be induced in a variety of laboratory animals are consistent with the idea that preeclampsia is a two-stage disease, in which a variety of initial insults may lead to placental ischemia, and ultimately systemic symptoms. The recent development of stem cell-based models, organoids, and various coculture systems have brought in vitro systems with human cells ever closer to recapitulating in vivo events that lead to placental ischemia.

Key Words
- pregnancy
- preeclampsia
- placenta
- modelling
- trophoblast

Invited Author’s profile

Laura Clamon Schulz is an Associate Professor in the Department of Obstetrics, Gynecology and Women’s Health at the University of Missouri. After earning her Ph.D. at the University of Illinois, she completed postdoctoral fellowships at Boston University and the University of Missouri. Schulz studies the physiology of pregnancy, with a particular focus on the placenta and its role in health and disease. Current projects include studying the role of the placenta in the pathophysiology of gestational diabetes, the role of maternal myostatin in offspring growth, and development of stem cell-based models for the study of human placental development in preeclampsia. Schulz is a member of the Society for the Study of Reproduction, the Society for Reproductive Investigation and the American Association for the Advancement of Science.
Introduction

The placenta is a transient organ that provides nutrient and gas exchange between the mother and developing fetus and is critical for the development of all therian mammalian life. Placental development begins with the formation of the blastocyst. In all eutherians, this structure is made up of trophectoderm (TE), inner cell mass, and a blastocoel cavity (Knöfler et al. 2019). TE is the source of trophoblast cells which will give rise to the placenta. In human implantation, the primitive syncytium, having arisen from the TE, will invade the endometrial epithelium, establishing proper attachment (Knöfler et al. 2019).

Humans have a hemochorial placenta. The villous structure in humans and some non-human primates brings trophoblasts, which are of fetal origin, in direct contact with maternal blood and has as few as two layers of cells separating fetal blood from the maternal blood in the mature placenta. This close contact is efficient for nutrient, gas, and waste transfer. Rodents, including laboratory mice and rats, also have a hemochorial placenta, with trophoblast in direct contact with maternal blood, but the structure is labyrinthine, rather than villous (Roberts et al. 2016).

Mononuclear cytotrophoblasts (CTB) are the progenitor cells for two distinct cell populations. CTBs will fuse to form the multinucleated syncytiotrophoblast (STB) layer, which makes up the outer layer of the placental villi responsible for the exchange. CTBs will also differentiate into extravillous trophoblasts (EVT). This cell population is highly invasive and will migrate through the maternal decidua and into the myometrium. A subpopulation of these, called endovascular EVT, will remodel the uterine spiral arteries to allow for proper blood flow to the fetus (Roberts et al. 2016).

Preeclampsia (PE) is a disease of pregnancy and a leading cause of maternal and fetal death (WHO 2011). A diagnosis after 34 weeks of gestation is considered late-onset PE. A diagnosis prior to 34 weeks is considered early-onset PE (EOPE), but this can arise as early as 20 weeks of gestation (Raymond & Peterson 2011). The clinical symptoms include new onset of high blood pressure, proteinuria, and edema (Moghaddas Sani et al. 2019). The advanced condition can include hemolysis, elevated liver enzymes, low platelet count syndrome (Khalid et al. 2022). One of the most dangerous aspects of PE is the neurological symptoms (Duley 2009). Seizures during pregnancy or birth are life-threatening to both the mother and the fetus and may be associated with events such as stroke or hemorrhage in the brain (Cipolla & Kraig 2011). Although magnesium sulfate, an anticonvulsant, can reduce the risk of seizures (Duley 2009), PE often results in preterm birth, as delivery is the only way to fully resolve symptoms. Fortunately, advancements in medical technology and improved diagnostic techniques have greatly reduced the number of women who die as a result of this disease. Unfortunately, exposure to this disease in utero has adverse outcomes on the offspring later in life. These offspring have an increased risk of developing the cardiovascular, metabolic, or neurological disease (Lu & Hu 2019). Currently, the only cure for PE is to deliver the baby and placenta, regardless of the stage of pregnancy, when it becomes life-threatening.

Current understanding of the pathophysiology of PE is best described by the two-stage model (Redman 1991, Figure 1). Preeclampsia is thought to result from multiple underlying causes of placental ischemia (A), which leads the placenta to secrete anti-angiogenic and inflammatory factors (B) that damage the vasculature (C), and then end organs (D). Animal models mimic various stages in this progression and are useful for understanding events downstream, but not upstream of the step being mimicked. For example, the RUPP model induces placental ischemia (A); sFLT overexpression and TNFA infusion recapitulate the placental reaction to ischemia (B); L-NAME infusion and renin-angiotensin overexpression model endothelial damage (C). These models and others all converge on late stages of preeclampsia (D). Created with https://www.biorender.com/.
Staff 2019) in which a first stage of placental oxidative stress leads to the release of factors that result in a second stage of systemic endothelial dysfunction and end-organ damage (Fig. 1). Stage 1 can result from shallow invasion by EVTs and insufficient remodeling of maternal spiral arteries, which are observed in placentas delivered from PE pregnancies (Raymond & Peterson 2011). The smooth muscle remains in control of the artery, causing inconsistent spurts of oxygenated blood instead of an open flow. Multiple potential contributors to stage 1 have been identified, including disrupted trophoblast differentiation, intrinsic invasive defects, heightened sensitivity to oxidative stress, poor oxygen sensing by trophoblasts, and inappropriate trophoblast-immune interactions (Xu et al. 2021). Proangiogenic and antiangiogenic factors, including placental growth factors (PGF), soluble VEGF (vascular endothelial growth factor) receptor sFLT (soluble fms-like kinase) and endoglin, are not appropriately expressed in PE patients, leading to endothelial damage (Moghaddas Sani et al. 2019). Endothelial dysfunction in the kidney, liver, and brain can then lead to multisystem organ failure (LaMarca 2012).

It is difficult to study PE for several reasons. First, placental tissues from an ongoing pregnancy cannot be taken and used for research. Because of this, we need model systems. This is challenging because how or precisely when the disease begins is unknown. Additionally, PE is largely a uniquely human disease. The animals whose placentas most closely resemble those of humans are a subset of non-human primates that are difficult to study for ethical and practical reasons. Mouse and rat placentas have some important similarities, but there are major structural differences. Even more challenging is finding an animal model that also spontaneously develops PE. There are reports of apes who developed PE, but these occurrences are rare and, in most cases, not confirmed. Other animal models of PE induce symptoms of the disease through surgical, pharmacological, or genetic manipulations. In contrast, in vitro models using placental cells of human origin cannot recapitulate the complex multiorgan dynamics of PE, but by allowing direct studies of human cells, including cells from PE patients, they are more likely to reveal the placentals factors initiating this uniquely human disease. Recent advancements in human stem cell technology provide multicellular models and non-cancerous cell types that can be maintained indefinitely in culture and recapitulate stages of development from early pregnancy.

Any animal model of PE should, at minimum, recapitulate the two key diagnostic features of PE. Diagnosis of PE requires new-onset hypertension after 20 weeks gestation, defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg, measured on at least 2 occasions 4 h or more apart. Additionally, patients must have proteinuria, defined as >300 mg per 24 h collection, a urine protein: creatine ratio >0.3 or a dipstick reading of 2+ (American College of Obstetricians and Gynecologists 2020). New-onset hypertension in the absence of proteinuria may still be diagnosed as PE if accompanied by new-onset thrombocytopenia, renal insufficiency, elevated liver enzymes, pulmonary edema, or new-onset medication-unresponsive headache without alternative explanation. As less than 3% of human PE cases progress to eclampsia (August & Sibai 2023), it is not required for an animal model of PE to be useful.

In addition to these defining features, the presence of other, well-documented features of PE increases confidence that any particular animal model truly recapitulates the human disease and can define which stages of PE the model represents. For example, the presence of reduced trophoblast invasion and fetal growth restriction recapitulates PE initiated by placental dysfunction in stage 1. Abnormal placental or serum sFLT, endoglin and PGF are consistent with the placental reaction to ischemia leading from stage 1 to stage 2. Animal models based on recapitulating this placental reaction, for example, by sFLT infusion, are therefore useful for understanding stage 2 and the transition from stage 1 to stage 2, but are not helpful for understanding the initial causes of placental ischemia. Evidence of renal damage, such as vacuolated endothelial cells, swollen mesangial cells, or subendothelial protein deposits, shows full progression to the symptoms of stage 2, and models with such features are particularly useful for testing treatments that may halt or reverse this progression.

In vitro models should be evaluated for their ability to phenocopy the cellular features of PE, and when generalizing results from cell culture studies, it is important to understand what stage of pregnancy and what stage of PE are being modeled. Relevant cellular features include evidence of oxidative stress and trophoblast invasion defects, as well as the altered release of angiogenic factors and cytokines. In the case of organoids or multicellular culture models, resemblance to the tissue organization of the human placenta is important for fidelity to in vivo conditions. Finally, gene expression profiles may be used to compare in vitro models to freshly obtain in vivo tissues, but the lack of suitable tissue for comparison, particularly in early pregnancy, poses a challenge.
Table 1  Animal models of preeclampsia and their preeclampsia-like characteristics.

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Type</th>
<th>Hypertension</th>
<th>Proteinuria</th>
<th>Renal damage</th>
<th>Endothelial dysfunction</th>
<th>Fetal impact</th>
<th>Antiangiogenic factors</th>
<th>Ischemia</th>
<th>Trophoblast invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, Rat</td>
<td>RUPP</td>
<td>O</td>
<td>Yes</td>
<td>Yes, conditional</td>
<td>Yes (r)</td>
<td>N.R.</td>
<td>FGR</td>
<td>↑sFLT (m)</td>
<td>Yes</td>
<td>↓</td>
</tr>
<tr>
<td>Baboon</td>
<td>UPI</td>
<td>O</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>↑sFLT</td>
<td>Yes</td>
<td>↓</td>
</tr>
<tr>
<td>Mouse, rat</td>
<td>L-NAME</td>
<td>P</td>
<td>Yes</td>
<td>Inconsistent</td>
<td>No (m)</td>
<td>Yes (r)</td>
<td>FGR</td>
<td>↑NOS(r)</td>
<td>N.R.</td>
<td>N.R.</td>
</tr>
<tr>
<td>Mouse, rat</td>
<td>TNF-α infusion</td>
<td>P</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>FGR</td>
<td>No</td>
<td>↑ HIF1-a (m)</td>
<td>N.R.</td>
</tr>
<tr>
<td>Mouse</td>
<td>Lipopolysaccharide injection</td>
<td>P</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>FGR</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>eNOS KO</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>FGR</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>COMT</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>FGR</td>
<td>↑HIF-1a</td>
<td>Not affected</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Asb4 KO</td>
<td>G</td>
<td>Mild</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Notch2 cKO</td>
<td>G</td>
<td>N.R.</td>
<td>N.R.</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Apela KO</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Embryo lethality</td>
<td>N.R.</td>
<td>↑HIF-1a inflammation</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>STOX1 KO</td>
<td>G</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>↑ Mitochondria &amp; hypoxia genes, antioxidants</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>STOX1 OE</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>↑sFLT</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse, Rat</td>
<td>Renin–AT overactivation</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>FGR</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Chemerin OE</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>FGR</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>BPH/5</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>FGR</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>CBA/J × DBA/2</td>
<td>G</td>
<td>No</td>
<td>N.R.</td>
<td>Yes</td>
<td>Yes</td>
<td>FGR</td>
<td>↑sFLT</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>DOCA injection</td>
<td>P</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>LOX-1 inhibition</td>
<td>P</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td>FGR</td>
<td>↑oxLDL</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Endotoxin infusion</td>
<td>P</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>FGR</td>
<td>↑Endothelin-1</td>
<td>N.R.</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>sFLT OE</td>
<td>G</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>FGR</td>
<td>N.R.</td>
<td>Not affected</td>
<td></td>
</tr>
<tr>
<td>Chimpanzee, Patas Monkey</td>
<td>Spontaneous</td>
<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orangutan, Stuhman’s &amp; Rhesus Monkey</td>
<td>Spontaneous</td>
<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>N.R.</td>
<td>N.R.</td>
<td>N.R.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cKO, conditional knockout; G, genetic; KO, knockout; N.R., not reported; O, operative; OE, overexpression; P, pharmacologic; S, spontaneous.
Small animal models

The mouse and rat have been widely used as a model for many human diseases, including PE. These animals do not naturally develop PE so the symptoms must be induced surgically, pharmacologically, or genetically. The placenta in the mouse and rat is labyrinthine and hemochorial with two layers of STB compared to the one layer of STB in humans (Furukawa et al. 2019). Rat placenta involves deeper trophoblast invasion into the decidua than mouse placenta, which makes the rat a more relevant model for invasion (Soares et al. 2012). Additionally, the larger size of the rat placenta facilitates surgical manipulation and some visualization techniques. There are other differences in rodent and human pregnancies that may impact the fidelity of PE models. For example, rats and mice have shorter pregnancies, with more altricial young than do humans, such that late pregnancy stages of PE may not be modeled, and are both litter-bearing, which adds complex inter-placental variations.

Surgical models

The reduced uterine placental perfusion model (RUPP), previously developed in dogs, rabbits, primates, and rats (Abitbol 1982, Li et al. 2012), has more recently been incorporated into studies in the mouse (Intapad et al. 2014, Natale et al. 2018). This surgical model involves partial occlusion of the maternal arteries, either the uterine arteries alone (Natale et al. 2018), the ovarian and uterine arteries (Fushima et al. 2016), or the ovarian and uterine arteries plus the abdominal aorta near the iliac bifurcation (Intapad et al. 2014). Both sutures (Natale et al. 2018) and clips (Intapad et al. 2014) have been used. In all three approaches, fetal weight is reduced and hypertension has been demonstrated with and without aortic occlusion (Intapad et al. 2014, Fushima et al. 2016a, Natale et al. 2018). Other PE features include serum (Intapad et al. 2014) or placental sFLT elevation (Fushima et al. 2016a) and increased placental HIF1A (Fushima et al. 2016, Natale et al. 2018). In the mouse, proteinuria was observed when occluding the uterine arteries only, but not when also occluding the ovarian arteries, with or without the abdominal aorta (Intapad et al. 2014, Fushima et al. 2016). In the rat, clipping the ovarian arteries and the abdominal aorta causes hypertension, proteinuria, and fetal growth restriction (Fushima et al. 2016). These rats exhibit decreased glomerular filtration rate and renal plasma flow compared to normal pregnant rats, as well as decreased neuronal nitric oxide synthase (NOS1) in the brain (Alexander et al. 2001). Clipping the uterine and ovarian arteries without clipping the aorta solves the problem of hindlimb ischemia while still producing the preeclamptic symptoms that the original RUPP model achieves (Morton et al. 2019). Although the model is based on manipulating maternal arteries, thus mimicking a potential maternal cause of PE, it produces placental ischemia, which can result from either placental or maternal factors, and thus may to some extent model both.

Pharmacological models

A popular rodent model used to induce clinical symptoms of PE is treatment with L-N^6^-Nitro arginine methyl ester (L-NAME), which inhibits nitric oxide synthase, thereby blocking the production of nitric oxide and endothelial vasodilatation (Pleifler et al. 1996, de Alwis et al. 2022). Vascular dysfunction in PE is associated with insufficient nitric oxide (Osol et al. 2017, de Alwis et al. 2022). Rats infused with L-NAME in mid-late gestation show hypertension, proteinuria, elevated sFLT, and fetal growth restriction (Yallampalli & Garfield 1993, Molnár et al. 1994, Ramesar 2011). L-NAME-treated mice also display elevated blood pressure but do not display transcriptomic evidence of kidney dysfunction, despite histological evidence of a renal inflammatory response (de Alwis et al. 2022). Some, but not all studies have observed proteinuria (de Alwis et al. 2022). At E17.5, placental weight and fetal crown to rump length are decreased compared to controls (de Alwis et al. 2022). Interestingly, fetal weight is not impacted by the treatment and the fetal-to-placental weight ratio is not different compared to controls (de Alwis et al. 2022). PE-associated factors involved in inflammatory response (CRP), anti-angiogenesis (sFlt1), and vasoconstriction (EDN1) are increased in dams given L-NAME (de Alwis et al. 2022). However, vascular reactivity remains unaffected (de Alwis et al. 2022). In contrast to women who develop PE during pregnancy, mice given L-NAME do not display long-term hypertension or maintain elevated expression of CRP and EDN1 immediately post-partum or within 10 weeks following delivery (de Alwis et al. 2022).

L-NAME treatment has been used to test the safety and efficacy of potential therapies for PE. The L-NAME-treated mouse has been used in conjunction with Sildenafil citrate, an antihypertension drug that potentiates NO action on vascular smooth muscle by inhibiting phosphodiesterase 5 (PDE5) metabolism of cGMP (Motta et al. 2015). Sildenafil improved vascular deficiencies without apparent adverse effects on the fetus, such as birth weight differences (Motta et al. 2015). Another PDE5 inhibitor, Tadalafil, reversed
placental, but not fetal, weight deficits in L-NAME mice, and restored the size of maternal blood sinuses, but not of fetal capillaries in the placenta. Renal damage was reduced. Such studies demonstrate the potential utility of mice for preclinical trials, but their ability to predict human responses is not yet known.

Consistent with the potential role of inflammation in the pathophysiology of PE, TNF-a infusion in mice results in PE-like symptoms. TNF-a was given via miniosmotic pumps starting just after mid-pregnancy, resulting in hypertension and proteinuria, but without either fetal growth restriction or elevation in sFLT (Bobek et al. 2015). However, there was evidence of hypoxia, with elevated placental HIF1A, though placental vascular development has not yet been characterized in this model (Bobek et al. 2015). A similar study was done in rats, where TNF-a was infused via miniosmotic pumps and the phenotype was comparable to the RUPP rat model (LaMacra et al. 2005). A more generalized inflammatory model of PE has been created by injection of lipopolysaccharides during gestation, borrowing from similar studies in the rat (Faas et al. 1994, Alexander et al. 2002). As with TNF-a infusion, this leads to maternal hypertension and proteinuria, renal lesions, and fetal growth restriction (Zhang et al. 2022).

Another pharmacological model of PE in rats addresses the increased extracellular fluid (ECF) or edema seen in normal human pregnancy and exaggerated in PE. In PE, the ECF increases more in the interstitial compartments than in the intravascular compartments (Ianosi-Irimie et al. 2005). Pregnant rats were given desoxycorticosterone (DOCA) by intraperitoneal injection along with 0.9% saline for drinking water and they experienced elevated systolic blood pressure, as well as an increase in urinary protein (Ianosi-Irimie et al. 2005). Another group discovered the excretion of marinobufagenin (MBG), a cardiotonic steroid, in DOCA-treated rat urine prior to the onset of hypertension symptoms (Uddin et al. 2009). Upon MBG infusion in the rat model, the vascular leak was observed in the pregnant preeclamptic rats compared to pregnant and non-pregnant controls, suggesting that vascular leak does occur in this model of PE (Uddin et al. 2009). This rat model can be used to understand the role of MBG in the pathophysiology of this preeclamptic vascular leak symptom (Uddin et al. 2009).

A rat model for high cholesterol was used to investigate the neurological effects of PE, specifically increased blood–brain barrier permeability, in women diagnosed with EOPE (Schreurs et al. 2013). Oxidized low-density lipoprotein (oxLDL), generated by oxidative stress in the placenta, will bind to the receptor OLR1 (Schreurs et al. 2013). Utilizing a OLR1 inhibitor, this study demonstrated that OLR1 activation plays a role in blood–brain barrier permeability (Schreurs et al. 2013), thus suggesting a mechanistic link between oxidative stress during PE and neurological symptoms.

In both mice and rats, endotoxin infusion in pregnancy leads to several human PE symptoms (Faas et al. 1994). This treatment causes high blood pressure, proteinuria, platelet coagulopathy, as well as glomerular fibrinogen deposits (Faas et al. 1994). Suramin injections to inhibit angiogenesis were successful in generating a pharmacologically induced PE rat model (Nash et al. 2005). The symptoms include increased blood pressure, decreased placental blood flow, increased serum Endothelin-1, and oxidative stress was evident (Nash et al. 2005). Additionally, fetal growth restriction was observed during parturition (Nash et al. 2005). Infusion of sFLT was developed in rats before being utilized in mice and has similar effects (Maynard et al. 2003).

Genetic models

Several genetic mouse models and a few genetic rat models have been developed, which successfully mimic key features of human PE. As discussed later, these include both loss-of-function and overexpression models, as well as a hybrid cross and mice selectively bred for hypertension. Single gene models are particularly powerful tools for isolating the effect of single proteins in the pathophysiology of PE and for testing hypotheses about causal mechanisms. However, homogenous genetic backgrounds and single gene causes are not general features of PE in humans, raising concern for translation to human disease. Mutations in individual genes also may not model the complex interplay of placental and maternal factors observed in women with PE (Than et al. 2018), although in some models, like the Apela null mouse, later, both the maternal and placental copies of the gene contribute to the phenotype. Regardless, these mouse genetic models may also be useful to test therapies which target shared downstream manifestations of PE, like endothelial damage and hypertension (Sato et al. 2020).

Loss of function models

Mouse models that mimic key features of PE include the endothelial nitric oxide synthase (NOS3) knockout that targets the same pathway as the L-NAME pharmacological described earlier (Kusinski et al. 2012). Loss of NOS3
results in reduced uterine artery relaxation and impairs placental amino acid transport. They display both pre-
gerstational and gestational hypertension, with fetal growth restriction and proteinuria (Kusinski et al. 2012). Catechol-O-methyltransferase (COMT) is an enzyme that metabolizes catecholamines and related structures, including catalyzing the methylation of 2-hydroxyestradiol to 2-methoxyestradiol (2-ME) in the placenta (Iqbal et al. 2021). Mice with Comt knockout have proteinuria, elevated blood pressure, HIF1A and sFLT in late pregnancy, effects reversed by 2-ME treatment (Kanasaki et al. 2008), as well as reduced fetal weight (Poudel et al. 2013). However, Comt null rats have normal placental morphology and normal placental and fetal weights under control and hypoxic conditions and human gene association studies are mixed (Iqbal et al. 2021). Asb4 knockout mice also have mild hypertension and proteinuria, key PE symptoms, along with reduced litter sizes, due to embryonic lethality (Townley-Tilson et al. 2014, Li et al. 2016). Asb4 is a broadly expressed ubiquitin E3 ligase, particularly important in endothelial development but also suppresses trophoblast stem cell (TSC) markers in JAR choriocarcinoma cells and disrupts placental morphology (Townley-Tilson et al. 2014), making the mechanisms of action difficult to pinpoint precisely. Conditional knockout in mice of Notch2 in the Tpiba lineage, which gives rise to spongiform trophoblast, and trophoblast giant cells and glycogen cells responsible for endothelial invasion in the mouse, also leads to a PE-like placental phenotype (Hunkapiller et al. 2011). The number of invaded trophoblast and trophoblast-lined canals supplying blood to the placenta were reduced. However, neither proteinuria nor blood pressure was assessed. Apela (also called Ela or Elabela) encodes a hormone that binds to the apelin receptor and is critical to cardiovascular development, with mouse knockout resulting in approximately 50% embryonic lethality (Freyer et al. 2017). Surviving Apela null females develop hypertension and proteinuria with renal damage specific to pregnancy (Ho et al. 2017). Apela is also expressed by STB, and null murine placentas are poorly vascularized with reduced STB development, elevated HIF1A expression, and inflammatory gene expression, defects which appear to precede embryonic cardiovascular phenotypes (Freyer et al. 2017, Ho et al. 2017). The transcription factor Stox1 was first implicated in PE in 2013 through gene association studies in the Netherlands (van Dijk et al. 2005), and a mouse model was attempted based on the potential clinical significance of this loss-of-function allele. Mice lacking functional Stox1 develop gestational hypertension and altered placental vascularity, but blood pressure may be normalized by inhibition of renin–angiotensin signaling, and other signs of PE like proteinuria, renal damage, and elevated sFLT are absent (Parchem et al. 2021).

**Gain of function models**

Unlike Stox1 knockout mice, pregnant mice carrying embryos with constitutive Stox1 overexpression in the placenta and embryonic tissues do display multiple features of PE (Doridot et al. 2013). Fetoplacental Stox1 overexpression leads to hypertension, proteinuria, renal pathology, and sFLT elevation, with largely normal placental morphology (Doridot et al. 2013). Mitochondrial mass, mitochondria-related gene expression, response to hypoxia, and antioxidants are altered, although HIF1A expression is not (Doridot et al. 2014). Although the dams themselves do not carry the mutant Stox1, they show signs of cardiac hypertrophy and endothelial inflammation 6 months postpartum, suggesting the utility of the model for studying the long-term consequences of PE for maternal health (Miralles et al. 2019). Following a similar protocol in the rat (Maynard et al. 2003), several studies have used transient overexpression of the soluble (inhibitory) isoform of the VEGF receptor in pregnant mice by injecting an adenoviral vector containing sFLT at days 8–9 of gestation. Overexpression results in hypertension, fetal and placental growth restriction (Lu et al. 2007), proteinuria (Suzuki et al. 2009), renal endotheliosis, and impaired placental vascularization (Yang et al. 2022). Placental metabolism shifts to greater fatty acid oxidation, greater glycolysis, and reduced ATP production (Sato et al. 2020). A similar PE-like phenotype is caused by sFLT overexpression specifically in the placenta, driven by a lentiviral vector (Kumasawa et al. 2011). A strength of this model is the consistent finding of sFLT overexpression in women with PE, suggesting its clinical relevance (Levine et al. 2004).

Overactivation of the renin–angiotensin pathway specifically in pregnancy is achieved by mating mouse or rat males expressing human renin with females expressing human angiotensinogen (Bohlender et al. 2000). The maternal exogenous angiotensinogen and placently secreted human renin interact with one another but not with their murine homologs (Takimoto et al. 1996). This results in hypertension, proteinuria, and renal damage in mid-late pregnancy (Takimoto et al. 1996). Placental edema and necrosis were observed, along with fetal growth restriction (Saito et al. 2004). A related model in which the females overexpress both human angiotensinogen

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and renin allows examination of PE superimposed on preexisting hypertension (Falcao et al. 2009).

The adipokine chemerin has also been found to be elevated in PE, and in a recently developed model, overexpression in trophoblasts by lentiviral transduction of blastocysts, results in reduced fetal weights, maternal hypertension and proteinuria, and glomerular endothelial damage (Tan et al. 2022a).

Multigenic models

The BPH/5 mouse model has been described as a model for the spontaneous development of PE (Davisson et al. 2002). This mouse model is derived from the genetically hypertensive BPH/2 (Schlager & Sides 1997) mouse and demonstrates the long-term elevation in blood pressure that is seen in women who developed PE during pregnancy. These mice exhibit late gestation hypertension, proteinuria, endothelial dysfunction, and renal glomerulosclerosis, which resolve following parturition (Davisson et al. 2002, Dokras et al. 2006). Apart from slightly elevated blood pressure, these mice do not demonstrate any of the additional symptoms prior to pregnancy (Davisson et al. 2002, Dokras et al. 2006). Notably, the BPH/5 mouse model uniquely does not require further intervention to achieve the full spectrum of maternal symptoms (Davisson et al. 2002, Dokras et al. 2006). Fetal outcomes of PE, such as fetal growth restriction, low birth weight, and death, are also present in this model (Davisson et al. 2002, Dokras et al. 2006). In addition to recapitulating key features of PE, these mice represent a multigenic model of PE, having been created by crossing 8 strains of mice and selecting for hypertension across 23 generations (Sones et al. 2021), although the resulting strain is highly inbred.

Hybrid crosses of female mice of the CBA/J strain with males of the DBA/2 strain lead to renal endotheliosis, fetal growth restriction, sFLT elevation, and significant embryonic losses, although there is not a statistically significant increase in blood pressure (Ahmed et al. 2010). Strikingly, embryonic losses and growth restriction are limited to first pregnancies in this mouse model, just as PE occurs most often during first pregnancies in women. The bias toward first pregnancies has been hypothesized to result from immune incompatibility in women, and the CBA/J × DBA/2 model provides support for this hypothesis, as embryonic losses are attributable to immune activation, including cytokine release, natural killer cell and macrophage activation, and activation of complement (Clark et al. 1998). However, the lack of conclusive evidence for hypertension suggests that immune incompatibility alone may not be sufficient to reproduce PE.

Non-human primates

Non-human primates, particularly apes and old-world monkeys, have the most similar pregnancies to humans. Non-human primate models have been used to study many aspects of pregnancy, including placentation (Grigsby 2016). Gestation length varies among species, with the chimpanzee being the closest to human gestation length at 238 days (vs 268 days in humans (Casarini et al. 2018)). Like humans, most other primate species have a discoid hemochorial placenta with fetal tissues in direct contact with maternal blood. While implantation is superficial in most primates, the blastocyst in humans and other apes, such as chimpanzees and gorillas, will invade entirely into the endometrium (Carter et al. 2015, Carter 2021). This deep trophoblast invasion shared by apes and humans has been hypothesized to play a role in the onset of pregnancy disorders like PE, thus making apes particularly apt models for studying PE (Carter 2011, 2021). In addition to placental structure, hormone secretion is very similar amongst primates. For example, anthropoid primates (new and old world monkeys and apes) all rely on trophoblast secretion of hCG for endometrial receptivity (Maston & Ruvolo 2002, Banerjee & Fazleabas 2010, Casarini et al. 2018).

Various non-human primate species are used to study specific aspects of human pregnancy. For example, the baboon is an established model for uterine receptivity, due to hormonal regulation that operates in parallel to humans (Fazleabas et al. 1999, Banerjee & Fazleabas 2010). Non-human primate models for placentation include the yellow baboon and the olive baboon, though the chimpanzee and gorilla maternal–fetal interfaces most accurately resemble humans (Carter et al. 2015). Further, EVTs invade interstitially through the endometrium and into the myometrium in the gorilla and chimpanzee as they do in humans, but not in gibbons or old-world monkeys (Carter et al. 2015).

PE is a disease of pregnancy that appears to be unique to humans. However, there have been reports of spontaneous PE in chimpanzees, orangutans, a Stuhlman’s monkey, a rhesus monkey, and a patas monkey (Krugner-Higby et al. 2009), and there may just be insufficient information on its rate of occurrence in humans‘ close relatives. A pregnant chimpanzee presented with glomerulonephritis, which is a pathology observed in...
human preeclamptic pregnancy (Stout & Lemmon 1969, Carter 2021). Baboons have been surgically manipulated to mimic some aspects of PE through uteroplacental ischemia (UPI) by ligation of a uterine artery, like the rodent RUPP models described earlier. In the baboon, this also produced PE-like symptoms such as hypertension, proteinuria, and an increase in antiangiogenic factors (Makris et al. 2007, Bakrania et al. 2022). The uterine artery ligation technique limits the flow of oxygen to the fetus and that alone may be the cause of PE symptoms (Makris et al. 2007, Carter 2021). Interestingly, in this UPI model, treatment with rhPGF can improve the symptoms that the animal develops (Makris et al. 2016, Bakrania et al. 2022). The use of non-human primates as models to study human pregnancy is critical because of the closely related placental structure and function.

A major drawback to using non-human primates as models is the expense and ethical considerations required to house and care for the animals. Facilities must have a veterinarian on site for general care, and some facilities require specialists in animal behavior (Coleman 2011). These animals have the ability to process complex emotions, so it is essential that they are taken care of appropriately (Coleman 2011). There are only seven national primate research centers in the United States and investigators must conduct the research in one of these facilities, as moving animals out of the centers is not permitted.

**Human cell culture models**

While animal models are particularly useful for studying inter-organ communications in PE and testing treatments, human cell culture models are needed to fully understand PE pathophysiology. The cell types of the human placenta, particularly the invasive extravillous CTB, have analogies in rodents, but not true counterparts, thus requiring the direct study of human cells. Secondly, as PE appears to only spontaneously arise at a relatively high frequency in humans, cells derived from pregnancies complicated by PE are needed to uncover the events that initiate the condition *in vivo*. Finally, while animal models may be genetically or pharmacologically manipulated to test mechanistic relationships, this cannot be ethically done in humans; thus, mechanistic studies of human placental cell function must be performed *in vitro*.

Placental tissues can be recovered following delivery for brief culture, either as isolated cells or as tissue explants. Placental explant cultures have been used for a variety of studies including hormone secretion, transport, growth, pharmacological purposes, etc. Both first-trimester and third-trimester villous tissues can be cultured in suspension with or without a matrix coating in a plastic dish, making this a fairly simple culture system with many potential applications (Miller et al. 2005). CTBs isolated from term placental tissue will spontaneously syncytialize in a culture dish and can survive up to 96 h (Kliman et al. 1986). This model is good for understanding syncytialization of mature trophoblasts, but not ideal for answering questions about early placental functions like trophoblast invasion. Obtaining cells from earlier stages of pregnancy is difficult, as these cells are needed for the placenta to continue developing. Miscarriage is sometimes a source of these tissues. Though it is no longer common due to advancements in cell-free DNA techniques, chorionic villous sampling has been used in research to examine placental tissue from patients undergoing prenatal genetic screening that later went on to develop PE (Rabaglino et al. 2015). The biggest obstacle to studying the onset of PE by using primary cells is that the symptoms do not manifest until 20 weeks of pregnancy at the earliest. Thus, even when placental tissues can be obtained from pregnancies that end in the first trimester, it is not possible to know whether the pregnancies would have been diagnosed with PE.

Placental cells are also commonly modeled with cell lines, either generated from human choriocarcinomas or by transfection of first-trimester placental cells with the SV40 antigen (Lala et al. 2021). These cell models are useful when access to primary cells is difficult or impossible. The most used choriocarcinoma-derived cell lines are BeWo, JEG-3, and JAR, while HTR-8/SVneo cells are immortalized (Abou-Kheir et al. 2017, Rothbauer et al. 2017). BeWo cells are useful for syncytialization studies, while JEG-3, JAR, and particularly HTR-8 cells have invasive properties similar to EVTs (Rothbauer et al. 2017). While these cell lines can be trophoblast-like in many ways such as invasion and elevation of hCG, they are cancer cells or have been transformed (Pavan et al. 2003) and are fundamentally not normal trophoblast (Lee et al. 2016a).

Stem cells are also used to model PE in humans (Roberts et al. 2018, Dong et al. 2020, Horii et al. 2021). *In vitro* tissue culture will naturally not be the same as the multisystem environment *in vivo*. However, an advantage of stem cells is that they can be derived from the diseased human tissue (Marshall et al. 2018, Gatford et al. 2020) and therefore possess the multifactorial, intrinsic features of human diseased cells, rather than placental ischemia imposed by surgery, pharmaceutical, or single gene manipulation.
For example, umbilical cord fibroblasts have been isolated at delivery from normal pregnancies and those complicated by EOPE and reprogrammed using the Yamanaka factors (Takahashi & Yamanaka 2006, Sheridan et al. 2019, Horii et al. 2021) to recapitulate the pluripotent cells of early pregnancy. Treatment with a cocktail of BMP4, A83-01, and PD173074 directs these cells toward a mixed population of CTB, EVT, and STB (Yang et al. 2015, Yabe et al. 2016, Jain et al. 2017, Roberts et al. 2018). These cells recapitulate key phenotypes of trophoblasts from preeclamptic pregnancies. They are more sensitive to oxidative stress than trophoblasts derived from control pregnancies, exhibiting reduced trophoblast invasion (Sheridan et al. 2019). However, the inability to investigate the behaviors of individual trophoblast subtypes is a drawback. For example, in invasion studies done with iPSCs, it is difficult to distinguish invasive primitive STB from EVT.

Induced trophoblast stem cells (iTSC) offer an advantage, as they can be passaged indefinitely as trophoblast, and be directed to differentiate to specific lineages, such as EVT and STB separately (Okae et al. 2018), yet like iPSC, may be derived from disease-specific tissues. The iTSC can be derived from differentiated cells or converted from pluripotent stem cells (Liu et al. 2020, Jang et al. 2022, Tan et al. 2022b). The initial process is similar to the derivation and reprogramming of fibroblasts to develop iPSCs. The key difference is the growth medium, which contains components that will activate the Wnt and EGF signaling pathways and simultaneously inhibit TGF-β, ROCK, and HDAC activity (Okae et al. 2018, Tan et al. 2022b). Following several passages, the cell population will be entirely proliferative TS cells. The initial stage of differentiation to EVT can be achieved by removing the Wnt activator and adding NRG1, A83-01, and Matrigel (Okae et al. 2018). To continue the differentiation process, slight adjustments are made to the growth medium and differentiation will be completed by day 8 of culture. Differentiation of TS cells to STB requires forskolin treatment to encourage fusion and formation of large syncytial patches (Okae et al. 2018).

Trophoblast organoids are self-organizing, three-dimensional structures made from human TSCs (Haider et al. 2018, Okae et al. 2018, Pascual 2022) or first-trimester placental cells (Turco et al. 2018). There is hope that such 3D models will be useful for studying cell-cell interactions, for example, in implantation studies, especially in combination with endometrial organoids derived from uterine biopsy (Fitzgerald et al. 2019). One caveat to trophoblast organoids is that it is difficult to produce them in the proper orientation. These structures contain CTB-like cells, which differentiate into STB and EVT. Interestingly, the EVT population migrates out from the rest of the structure once they have been fully differentiated. Several groups have described trophoblast organoids with CTB and EVT on the outside and CTB alternating with clusters of STB that surround lacunae-like structures on the inside. However, recent reports describe methods for trophoblast organoid establishment in the proper orientation with the CTB on the inside and the STB on the outside (Zhou et al. 2022, Yang et al. 2023). Production of hormones and cytokines is increased in the properly polarized trophoblast organoids compared to those that have CTB on the outside, which makes them a more physiologically relevant model. Additionally, the large continuous, patches of syncytium enable examination of ways by which proteins and pathogens cross the placental barrier, and the fetus is protected in the event of infection during pregnancy (Yang et al. 2023).

Trophoblast organoids can also be induced to generate functional, invasive EVT (Turco et al. 2018, Sheridan et al. 2020, Zhou et al. 2022). Organoid models could be used to study placental development in cells derived from preeclamptic as well as normal pregnancies.

Pluripotent stem cells, when treated with the proper cocktail of inhibitors, can also form a blastoid (Kagawa et al. 2022). This structure contains TE, epiblast, and primitive endoderm, which make up the early blastocyst (Kagawa et al. 2022). Specifically, the inhibition of the Hippo pathway is essential for TE lineage commitment, but inhibition of TGFβ and ERK pathways is necessary for the pluripotent stem cells to transition to TE. Upon hormone stimulation of endometrial cells, the blastoid will attach, mimicking implantation (Kagawa et al. 2022). While blastoids allow the study of early events in placental development without the use of human embryos, they remain controversial and are not currently permitted in NIH-sponsored research. Ethical concerns limit how far into placental development they may be followed.

Coculture studies with multiple placental cell types and organoids represent an emerging field of study, such that interactions between maternal and fetal tissues could be followed in vitro and various bioengineering approaches are being developed to better mimic in vivo conditions. A gel-patterning microfluidic chip is one approach that has been successful in trophoblast invasion studies (Ko et al. 2022). This chip has the capacity to allow HTR8/SVneo cells to invade through HUVECs in order to examine trophoblast invasion of endothelial cells, mimicking artery remodeling (Ko et al. 2022). ‘Placenta-on-a-chip’ has been developed for a similar purpose, to investigate cell interactions, for example, in implantation studies, especially in combination with endometrial organoids derived from uterine biopsy (Fitzgerald et al. 2019). One caveat to trophoblast organoids is that it is difficult to produce them in the proper orientation. These structures contain CTB-like cells, which differentiate into STB and EVT. Interestingly, the EVT population migrates out from the rest of the structure once they have been fully differentiated. Several groups have described trophoblast organoids with CTB and EVT on the outside and CTB alternating with clusters of STB that surround lacunae-like structures on the inside. However, recent reports describe methods for trophoblast organoid establishment in the proper orientation with the CTB on the inside and the STB on the outside (Zhou et al. 2022, Yang et al. 2023). Production of hormones and cytokines is increased in the properly polarized trophoblast organoids compared to those that have CTB on the outside, which makes them a more physiologically relevant model. Additionally, the large continuous, patches of syncytium enable examination of ways by which proteins and pathogens cross the placental barrier, and the fetus is protected in the event of infection during pregnancy (Yang et al. 2023).

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endothelial–trophoblast interactions (Lee et al. 2016b). The maternal–placental interface has also been explored using a microfluidic chip by initiating a coculture of BeWo cells and mouse embryonic stem cells (Boos et al. 2021) and a 3D bioprinted system was developed to recapitulate the placental barrier (Kreuder et al. 2020). Another system includes BeWo layered on top, mesenchymal stroma with fibroblasts in the gel layer, and human placental vascular endothelial cells lining the bottom of the upper chamber to model placental villi (Kreuder et al. 2020). A deeper understanding of the interactions of invasive trophoblast with luminal and glandular epithelium at implantation and with uterine stroma and vasculature in later placentaion is also an important aim for understanding the pathophysiology of PE. This will require further development of culture environments hospitable to the multiple cell types involved.

Discussion

In this review, we have discussed many different models of PE across multiple species as well as in vitro models. Each has advantages and disadvantages, with species differences limiting the interpretation of animal models, and a limited number of cell and tissue types limiting cell culture models. The use of mouse and rat models to study PE is problematic, due to the major differences in gestation length compared to humans, placental structure, and the fact that these animals do not develop PE naturally. Non-human primate models could be considered more useful since there are more similarities to humans, but there are many drawbacks to using these animals for research, including ethical concerns, expense of care, and the development of PE is extremely rare. Cell culture models cannot account for the interactions between tissues like an in vivo model. However, the ability to take samples from patients diagnosed with PE and reprogram them to recapitulate earlier stages of pregnancy has led to deeper insights into these specialized placental cells. 3D cultures can further demonstrate the behavior of these cells and will lead to understanding the underlying mechanisms that control the development of disease. The two-stage model of PE proposes that the disease begins in utero prior to the onset of symptoms. Thus, it is essential to discover the causes of stage 1, and to utilize the optimal models for experimentation, to ultimately prevent stage 2. The goal of further understanding PE continues to lead to the development of new techniques and technologies, with stem cell, organoid, and bioengineering approaches providing ever more sophisticated approaches to these challenges.

Declaration of interest

The authors have no conflicts of interest that could be perceived as prejudicing the impartiality of the manuscript.

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Author contribution statement

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