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## **Inventory of novel animal models addressing etiology of preeclampsia in the development of new therapeutic/intervention opportunities**

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Running head: Models of preeclampsia etiology and intervention

## **Abstract**

Preeclampsia is a pregnancy-related disease afflicting 3-7 % of pregnancies worldwide and leads to maternal and infant morbidity and mortality. The disease is of placental origin and is commonly described as a disease of two stages. A variety of preeclampsia animal models have been proposed, but all of them have limitations in fully recapitulating the human disease. Based on the research question at hand, different or multiple models might be suitable. Multiple animal models in combination with in vitro or ex vivo studies on human placenta together offer a synergistic platform to further our understanding of the etiology of preeclampsia and potential therapeutic interventions. The described animal models of preeclampsia divide into four categories 1) spontaneous, 2) surgically induced, 3) pharmacologically/substance induced, and 4) transgenic. This review aims at providing an inventory of novel models addressing etiology of the disease and or therapeutic/intervention opportunities.

*Keywords:*  $\alpha$ 1-microglobulin, hypertension, placenta, proteinuria, treatment opportunities, two-stage model

## 1. Introduction

Preeclampsia is a pregnancy-related disease afflicting 3-7 % of pregnancies worldwide and leads to maternal and infant morbidity and mortality. Preeclampsia is described to have a placental origin that results in systemic effects in the mother. A variety of preeclampsia animal models have been proposed, but all of them have limitations in fully recapitulating the human disease<sup>1</sup>. The human placenta is unique among species and its function has been suggested to play a central role in the development of preeclampsia. Removal of the placenta is believed to be crucial for the resolution of the symptoms<sup>2</sup>, and has led to the theory of a placenta-derived factor as a culprit. The disease evolves in two stages<sup>3</sup>. Stage one occurs during the formation of the placenta with a defective and shallow invasion of the trophoblasts into the uterine muscle layers failing to remodel the spiral arteries<sup>4</sup>. This contributes to a reduced utero-placental blood flow, which can result in fetal intra-uterine growth restriction (IUGR), seen in one of four preeclampsia cases. Inadequate blood flow gives rise to a reduced oxygen delivery and oxidative stress, which further aggravates placental vascular dysfunction<sup>5</sup>. Stage two consists of the clinical manifestations, i.e. hypertension and proteinuria, appearing from 20 weeks of gestation onwards. Early onset preeclampsia is in general more severe than late onset, and is associated with more placenta pathology than late onset preeclampsia. As the disease progresses, angiospasm and brain edema may cause severe epileptic seizures –eclampsia<sup>6</sup>. The renal disturbances seen in preeclampsia lead to reduced glomerular filtration rate and proteinuria. Glomerular endotheliosis is pathognomonic for preeclampsia<sup>7</sup>. General endothelial dysfunction, reduced vasodilatation and increased peripheral resistance are also vascular hallmarks of preeclampsia<sup>8</sup>.

The placenta is an organ with extremely high evolutionary diversity among animal species. Hence, an animal model that fully reflects the human placenta does not exist<sup>9, 10</sup>. The majority of

described animal models, however, have placentas that are discoid hemochorial just like the human placenta<sup>9</sup>. Despite this, differences can be found in terms of anatomy, cell types and molecular composition. Few animal models aim to mimic stage one of the disease. The great majority of models instead focus on the second stage, the systemic response and symptoms present in the mother. The ideal animal model should reflect both stages of the disease.

The animal models can be divided into the following four mechanistic categories 1) spontaneous animal models of preeclampsia, 2) surgically induced animal models of preeclampsia, 3) pharmacologically/substance induced animal models of preeclampsia, and 4) transgenic animal models of preeclampsia. Over the past decade a multitude of animal models of preeclampsia have been established and they are already well described in the literature<sup>1, 11-13</sup>. This review aims at providing an inventory of novel models addressing etiology of the disease and or therapeutic/intervention opportunities (Table 1).

## **2. Animal models of preeclampsia**

### **2.1. Spontaneous animal models of preeclampsia**

There are inbred strains of mice and rats that present with spontaneous preeclampsia of various degrees. The BPH/5 mouse, an inbred strain with mildly elevated blood pressure, displays pregnancy-induced characteristics in late gestation resembling those of preeclampsia in humans, including endothelial dysfunction, glomerular lesions, proteinuria and hypertension, as well as feto-placental defects and fetal demise<sup>14</sup>. Defective trophoblast invasion, defects in maternal decidual arteries and an increase in oxidative stress in the placentas preceded the onset of the maternal symptoms<sup>15, 16</sup>. Thus, the model describes events that are linked to both stage one and

stage two of preeclampsia. Treatment with the antioxidant Tempol throughout the pregnancy improved fetal outcome and ameliorated maternal hypertension and proteinuria<sup>16</sup>. It was shown that excessive complement activation in the pregnant BPH/5 females led to increased neutrophil infiltration in the placenta followed by abnormal placental and fetal development as well as reduced vascular endothelial growth factor (VEGF) plasma levels. Inhibition of the complement activation or adenoviral delivery of VEGF early in pregnancy prevented hypertension and proteinuria, and reduced the incidence of fetal resorption<sup>17, 18</sup>. The Dahl salt-sensitive rat strain is a genetic model of kidney disease and hypertension. Females exhibit pregnancy-specific exacerbation of hypertension, proteinuria, placental hypoxia, increased levels of angiogenic factors and reduced pup and litter size<sup>19</sup>. For both these models, the major criticism is the preexisting hypertension in non-pregnant mice. However, they could be considered as models for superimposed preeclampsia where a preexisting hypertension dramatically increases the risk of developing preeclampsia during pregnancy<sup>20</sup>.

## 2.2 Surgically induced models of preeclampsia

A mechanical model, where the surgical occlusion of the uterine artery or the abdominal aorta results in reduced uterine perfusion pressure (RUPP), has been extensively used to elucidate events occurring during stage two of preeclampsia. The RUPP model has been performed in rats<sup>21</sup>, non-human primates<sup>22, 23</sup>, sheep<sup>24</sup>, rabbits<sup>25</sup>, Guinea pigs<sup>26</sup> and dogs<sup>27</sup>. The RUPP rat model has been widely used since it displays a number of typical features of stage two of human preeclampsia such as hypertension, proteinuria and increased plasma and placental levels of angiogenic markers<sup>21</sup>. This model has recently been used to test therapeutic interventions to alleviate the maternal symptoms. Treatment with sodium tanshinone IIA sulfonate (STS) led to

decreased oxidative stress, but did not improve fetal outcome or maternal blood pressure<sup>28</sup>. Treatment in late gestation with 17- $\alpha$ -hydroxyprogesterone caproate (17-HPC) resulted in decreased blood pressure, decreased levels of circulating CD4<sup>+</sup> T cells, reduced uterine artery resistance index and improved litter size<sup>29</sup>. Employing the RUPP model in baboons led to a rapid increase in blood pressure and proteinuria to levels seen in human preeclampsia<sup>23</sup>. There was a rapid rise in soluble fms-like tyrosine kinase 1 (sFlt-1) of a magnitude seen in human preeclampsia; predating the development of proteinuria but timing with the hypertensive response. The response was sustained until delivery. Given the possibility of studying pregnancy over a 4-6 week period, there is an opportunity to study the effect of reducing sFlt-1 while at the same time allowing sufficient time for the syndrome to be controlled without the inevitable delivery of the neonate.

### 2.3. Pharmacologically/substance induced models of preeclampsia

Several inducible models of preeclampsia are described and the majorities focus on the maternal systemic symptoms in stage two of the disease.

Nitric oxide (NO), a vasodilator, is synthesized by nitric oxide synthase (NOS) from the amino acid L-arginine, and is a vasodilator. Inhibition of NOS in mice or rats by injections of nitro-L-arginine methyl ester (L-NAME) at different gestational stages led to preeclampsia-like symptoms such as hypertension, proteinuria, reduced glomerular filtration rate and IUGR<sup>30, 31</sup>. Concerns have been raised regarding the validity of this model due to uncertainty of the true role of NOS in preeclampsia. However, studies in women with severe preeclampsia have shown a polymorphism in the NOS gene, with certain mutations associated with this group<sup>32</sup>. Both the L-

NAME rat and mouse preeclampsia models have been used for testing therapeutic avenues during pregnancy. Sildenafil treatment was shown to reduce hypertension, proteinuria and fetal demise in both early- and late-onset preeclampsia<sup>33-36</sup> as well as lowering the sFlt-1 and soluble Endoglin (sEng) plasma levels<sup>37</sup>. Other reports have failed to document the positive effects, both in rat and pregnant women suffering from preeclampsia<sup>38, 39</sup>. Although in both cases the treatment was given later in gestation. Resveratrol treatment in L-NAME pregnant rats reduced the hypertension and oxidative stress in placental tissue<sup>40</sup>.

Arginine vasopressin (AVP) is highly elevated throughout human preeclampsia pregnancies and as early as the 6<sup>th</sup> week of gestation it has been proposed as a predictor of preeclampsia<sup>41</sup>. AVP is a peptide hormone that regulates the body's water retention and constricts blood vessels. Thus, at high concentrations it increases the blood pressure, and it has been shown to be elevated in other hypertension disorders. AVP-infusion in mice during pregnancy resulted in both classical maternal and fetal preeclampsia symptoms such as pregnancy-specific hypertension, glomerular endotheliosis, proteinuria and IUGR<sup>41</sup>.

Abnormal fatty acid oxidation has been implicated in the pathogenesis of preeclampsia in humans<sup>42</sup>. To investigate this, pregnant mice were injected with beta 2-glycoprotein I ( $\beta$ 2GPI) prior to mating and developed preeclampsia-like symptoms such as hypertension, proteinuria and poor pregnancy outcome<sup>43</sup>.

Activin A is an anti-angiogenic factor produced by the placenta, and is strongly elevated in plasma from women with preeclampsia. Activin A has therefore been implicated in the pathophysiology of the disease<sup>44</sup>. When activin A was administered at mid-gestation to pregnant

mice it resulted in preeclampsia-like symptoms such as hypertension, endothelial oxidative stress proteinuria and IUGR, and the hypertension and proteinuria were significantly reduced by inhibiting activin A signaling by a low molecular weight activin-receptor-like kinase inhibitor<sup>44</sup>.

### *2.3.1. Fetal hemoglobin-induced model of preeclampsia in pregnant rabbit*

The preeclampsia placenta has an increased production and accumulation of cell-free fetal hemoglobin (HbF)<sup>45</sup>, resulting in damage to the placenta barrier and consequent leakage of cell-free HbF into the maternal blood circulation<sup>46</sup>. Extracellular hemoglobin (Hb) and its metabolites induce oxidative stress, which may lead to acute renal failure and vascular dysfunction seen in preeclampsia<sup>47</sup>. Cell-free HbF could be detected in the maternal circulation as early as 14 weeks of gestation in women that later developed preeclampsia<sup>48</sup>. Thereby, HbF may link the two stages of preeclampsia through damage to the placenta and eventually to the maternal endothelium<sup>49, 50</sup>. A rabbit model of HbF-induced preeclampsia-like symptoms was recently described<sup>51</sup>. By administering species-specific cell-free HbF, the model mimics the human symptoms at stage two of preeclampsia. The dams displayed disrupted placental morphology, proteinuria and renal glomerular lesions. Further examination of the placenta revealed dramatic reduction of the collagen fibers in the extracellular matrix as well as mitochondrial swelling and high levels of apoptotic bodies. The model failed to evoke any increase in blood pressure. In this model, the therapeutic effect of  $\alpha_1$ -microglobulin (A1M) was tested. The human plasma and tissue protein A1M has emerged as a potential therapeutic drug candidate in treatment or prophylaxis of diseases or conditions that are associated with oxidative stress<sup>52, 53</sup>. A1M is synthesized in the liver<sup>54</sup> and secreted to the blood<sup>55</sup>. Of high functional importance is that A1M is rapidly equilibrated between the intra- and extravascular compartments<sup>56, 57</sup>. A1M has mechanistic

properties, which contribute to its role as a tissue housekeeping protein and a potential drug candidate. These properties can be summarized as 1) heme-binding, 2) reductase- and 3) radical-trapping<sup>52</sup>. A1M has been shown to protect cells and tissues against internal and external chemical insult<sup>46, 51, 58-61</sup> and postulated to function as a “radical sink”. This refers to its ability to continuously clean tissues from free radicals and oxidants, including free heme and radicals generated by extracellular Hb, heme and iron, by binding, neutralizing and transporting them to the kidneys for degradation<sup>52</sup>. A1M treatment of the preeclampsia rabbits ameliorated the proteinuria and reversed the increased glomerular sieving coefficient in kidney. The A1M-treated animals also displayed a significant reduction of the structural and cellular damages seen in placenta and kidney (Figure 1).

### *2.3.2. Starvation-induced model of preeclampsia in pregnant ewes*

In a pregnant ewe animal model, starvation induces preeclampsia-like symptoms by causing hemolysis with subsequent release of cell-free Hb<sup>62</sup>. In a tailored version of the model, with a reduced time of starvation<sup>61</sup>, the exposure to Hb and its metabolites resulted in tissue damage in placenta, with an almost complete elimination of the collagen fibers as well as cellular damages. Structural damages were also observed in kidneys combined with an increase in glomerular sieving coefficient indicating a defect filtration barrier. However, the ewes did not manifest any significant elevation of blood pressure. Intravenous infusion with A1M ameliorated the structural tissue damages seen in both kidney and placenta, as well as restored the glomerular filtration rate in the kidney<sup>61</sup>.

### *2.3.3. Induced models of preeclampsia in baboons*

The use of non-human primates for study of human pregnancy is predicated on several basic principles related to physiological comparisons<sup>63</sup>. These are singleton pregnancy, upright posture, antigravity blood flow via two uterine arteries arising from the iliac circulation with no co-lateral (or ovarian) blood flow, and single disc placentas<sup>13, 63</sup>. Most importantly though, the formation of placental cell structures that relate to placental metabolic transfer and oxygenation, and likely cell signaling, are common to armadillos, guinea pigs, baboons and humans. This formation is hemomonochorial placentation, which has the lowest number of cell layers between fetal blood flow and the maternal blood-derived supply, i.e. fetal endothelium and trophoblast layer<sup>9, 12</sup>. Baboons have featured in studies of NO, Interleukin-10 (IL-10), TNF- $\alpha$ , and more recently sFlt-1 as an angiogenic pathway inhibitor in pregnancy. In pregnant baboons, treated with a NO inhibitor, the effect on blood pressure was not of the magnitude seen in other species<sup>64, 65</sup>; however, studies of cytokine imbalance have shown that anti-IL-10 caused a low grade but highly significant increase in blood pressure<sup>66</sup>, mimicking reduced IL-10 levels seen in preeclampsia<sup>67</sup>. The effect of low dose TNF- $\alpha$  infusion was proteinuria and hypertension<sup>68</sup>. These effects mimicked those seen in rodent studies, linking an inflammatory response to preeclampsia<sup>69</sup>. This was consistent with the studies of human disease, in which patients have been shown to have heightened cytokine production profiles in serum<sup>70</sup> and in placental tissue<sup>71</sup>. Therefore, the capacity to utilize this model to dissect further pathway interactions has increased comparative validity<sup>72</sup>.

#### **2.4. Genetically modified models of preeclampsia**

Several mouse knockout models display preeclampsia-like characteristics and capture events during stage one and/or stage two. Indoleamine 2,3-dioxygenase (IDO) regulates endothelial-

derived relaxing factors and T-cell activity and the IDO knockout mouse show symptoms of preeclampsia such as proteinuria, mild hypertension, IUGR and glomerular endotheliosis<sup>73</sup>. Interleukin-4 (IL-4) is an anti-inflammatory cytokine and the IL-4 deficient mice display mild preeclampsia-like symptoms during pregnancy including mild hypertension, proteinuria, increased levels of pro-inflammatory cytokines and placental inflammation<sup>74</sup>. IL-10 has been shown to support trophoblast-driven endovascular crosstalk, and pregnant IL-10 knockout mice exposed to hypoxia demonstrate a full spectrum of preeclampsia-like symptoms such as placental injury, renal pathology, proteinuria and hypertension<sup>75</sup>. High temperature requirement A1 (HtrA1) protein is expressed by trophoblast precursors in the placenta and abnormal levels have been observed in women with preeclampsia<sup>76</sup>. Pregnant HtrA1 knockout mice have reduced placental size, pathological changes to the spiral arteries and IUGR<sup>77</sup>. This model also displays impaired remodeling of the maternal arteries, which might suggest that HtrA1 plays a role in stage one of preeclampsia.

In addition to knockout models, various transgenic models in both rats and mice have been established. In a transgenic rat model, female rats transgenic for the human angiotensinogen gene are crossed with male transgenic for the human renin gene, and the pregnant females exhibit typical preeclampsia symptoms such as hypertension, IUGR and proteinuria<sup>78</sup>. This is not the case in the reverse mating. In this model, an increase in regulatory T cells by induction resulted in improved fetal outcome but had no effect on maternal proteinuria or hypertension<sup>79</sup>.

A variety of genetic modification is the introduction into rats or mice of adenoviral or lentiviral vectors expressing various proteins. The protein sFlt-1 is an antagonist for VEGF and increased in preeclampsia<sup>80</sup>. Overexpression of sFlt-1 via the administration of viral vectors results in

pregnancy-specific proteinuria and hypertension in mice and rats<sup>81, 82</sup>, which was alleviated by the co-administration of VEGF<sup>81</sup>. Similar to this, it was recently shown that removal of excess sFlt-1 from women with preeclampsia, by plasma apheresis, ameliorated the symptoms and prolonged the pregnancy<sup>83, 84</sup>. Mice subject to viral overexpression of sFlt1 were challenged two months post-partum with an uni-lateral carotid injury, resulting in enhanced vascular remodeling and vessel fibrosis in the preeclampsia-exposed mice<sup>85</sup>. This model could contribute to research regarding the elevated risk of cardiovascular disease seen in women who have had preeclampsia.

#### *2.4.1. STOX1 transgene mouse model*

A study of family cases of preeclampsia identified more than 20 genome regions with mutations involved in the disease, one of these has been identified in the Storkhead box 1 (STOX1) gene<sup>86</sup>. Overexpression of STOX1 altered gene expression in a trophoblast cell line, strongly correlating with the transcriptional alterations seen in the preeclamptic placenta<sup>87</sup>. In the STOX1 transgenic mouse model, pregnant female mice recapitulate the human preeclamptic phenotype with hypertension, proteinuria, and increased plasma levels of the anti-angiogenic proteins sFlt-1 and sEng<sup>88</sup>. Moreover, the mice present with alterations of the kidney structure, reminiscent of the renal endotheliosis seen in preeclampsia. Hence, this model re-capsulate both of the stages in preeclampsia, stage one by trophoblast interference in the fetoplacental unit, and stage two through its systemic effects on the mother. Since it has recently been shown that STOX1 modulates mitochondrial function, hypoxia response, and the expression of genes involved in oxidative stress, the effects of STOX1 is probably associated with an increase of the oxidative stress<sup>89</sup>. More precisely, STOX1 appears to modulate the balance between oxidative and nitrosative stress. Furthermore, recent results have also shown endothelial cell-deregulation of 2000 genes that are linked to oxidative stress, cardiac hypertrophy and down-regulation of the

cell cycle<sup>90</sup>. In summary, by covering both stages of the disease, the STOX1 transgenic mice constitute a strong model for investigating the etiology of preeclampsia, as well as testing original therapeutic avenues.

### **3. Summary**

Preeclampsia models that involve impaired trophoblast invasion and placentation are a recent contribution to the scientific literature. In the majority of cases, models inducing preeclampsia through experimental interventions fail to capture processes leading to the abnormal placentation postulated to be the core element of the pathophysiology of preeclampsia. Targeting stage two of the disease, namely the maternal symptoms, offers opportunities to evaluate therapeutic options to alleviate the maternal symptoms. However, it sheds little light on the actual etiology of preeclampsia. Based on the research question at hand, different or multiple models might be suitable. Multiple animal models in combination with *in vitro* or *ex vivo* studies on human placenta together offer a synergistic platform to further our understanding of the etiology of preeclampsia and potential therapeutic interventions.

## References

- 1 McCarthy FP, Kingdom JC, Kenny LC, Walsh SK: Animal models of preeclampsia; uses and limitations. *Placenta* 2011;**32**:413-419.
- 2 Roberts JM, Cooper DW: Pathogenesis and genetics of pre-eclampsia. *Lancet* 2001;**357**:53-56.
- 3 Roberts JM, Hubel CA: The two stage model of preeclampsia: variations on the theme. *Placenta* 2009;**30 Suppl A**:S32-37.
- 4 Brosens JJ, Pijnenborg R, Brosens IA: The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am J Obstet Gynecol* 2002;**187**:1416-1423.
- 5 Hung TH, Skepper JN, Charnock-Jones DS, Burton GJ: Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res* 2002;**90**:1274-1281.
- 6 Lipstein H, Lee CC, Crupi RS: A current concept of eclampsia. *Am J Emerg Med* 2003;**21**:223-226.
- 7 Stillman IE, Karumanchi SA: The glomerular injury of preeclampsia. *J Am Soc Nephrol* 2007;**18**:2281-2284.
- 8 Roberts JM: Endothelial dysfunction in preeclampsia. *Semin Reprod Endocrinol* 1998;**16**:5-15.
- 9 Carter AM, Pijnenborg R: Evolution of invasive placentation with special reference to non-human primates. *Best Pract Res Clin Obstet Gynaecol* 2011;**25**:249-257.
- 10 Schmidt A, Morales-Prieto DM, Pastuschek J, Frohlich K, Markert UR: Only humans have human placentas: molecular differences between mice and humans. *J Reprod Immunol* 2015;**108**:65-71.
- 11 Podjarny E, Losonczy G, Baylis C: Animal models of preeclampsia. *Semin Nephrol* 2004;**24**:596-606.
- 12 Sunderland N, Hennessy A, Makris A: Animal models of pre-eclampsia. *Am J Reprod Immunol* 2011;**65**:533-541.
- 13 Carter AM: Animal models of human placentation--a review. *Placenta* 2007;**28 Suppl A**:S41-47.
- 14 Davisson RL, Hoffmann DS, Butz GM, Aldape G, Schlager G, Merrill DC, Sethi S, Weiss RM, Bates JN: Discovery of a spontaneous genetic mouse model of preeclampsia. *Hypertension* 2002;**39**:337-342.
- 15 Dokras A, Hoffmann DS, Eastvold JS, Kienzle MF, Gruman LM, Kirby PA, Weiss RM, Davisson RL: Severe fetoplacental abnormalities precede the onset of hypertension and proteinuria in a mouse model of preeclampsia. *Biol Reprod* 2006;**75**:899-907.
- 16 Hoffmann DS, Weydert CJ, Lazartigues E, Kutschke WJ, Kienzle MF, Leach JE, Sharma JA, Sharma RV, Davisson RL: Chronic tempol prevents hypertension, proteinuria, and poor fetoplacental outcomes in BPH/5 mouse model of preeclampsia. *Hypertension* 2008;**51**:1058-1065.
- 17 Gelber SE, Brent E, Redecha P, Perino G, Tomlinson S, Davisson RL, Salmon JE: Prevention of Defective Placentation and Pregnancy Loss by Blocking Innate Immune Pathways in a Syngeneic Model of Placental Insufficiency. *J Immunol* 2015;**195**:1129-1138.
- 18 Woods AK, Hoffmann DS, Weydert CJ, Butler SD, Zhou Y, Sharma RV, Davisson RL: Adenoviral delivery of VEGF121 early in pregnancy prevents spontaneous development of preeclampsia in BPH/5 mice. *Hypertension* 2011;**57**:94-102.
- 19 Gillis EE, Williams JM, Garrett MR, Mooney JN, Sasser JM: The Dahl salt-sensitive rat is a spontaneous model of superimposed preeclampsia. *Am J Physiol Regul Integr Comp Physiol* 2015;**309**:R62-70.
- 20 Sibai BM, Lindheimer M, Hauth J, Caritis S, VanDorsten P, Klebanoff M, MacPherson C, Landon M, Miodovnik M, Paul R, Meis P, Dombrowski M: Risk factors for preeclampsia, abruptio placentae, and adverse neonatal outcomes among women with chronic hypertension. National

- Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Engl J Med* 1998;**339**:667-671.
- 21 Li J, LaMarca B, Reckelhoff JF: A model of preeclampsia in rats: the reduced uterine perfusion pressure (RUPP) model. *Am J Physiol Heart Circ Physiol* 2012;**303**:H1-8.
- 22 Cavanagh D, Rao PS, Knuppel RA, Desai U, Balis JU: Pregnancy-induced hypertension: development of a model in the pregnant primate (*Papio anubis*). *Am J Obstet Gynecol* 1985;**151**:987-999.
- 23 Makris A, Thornton C, Thompson J, Thomson S, Martin R, Ogle R, Waugh R, McKenzie P, Kirwan P, Hennessy A: Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. *Kidney Int* 2007;**71**:977-984.
- 24 Thatcher CD, Keith JC, Jr.: Pregnancy-induced hypertension: development of a model in the pregnant sheep. *Am J Obstet Gynecol* 1986;**155**:201-207.
- 25 Losonczy G, Brown G, Venuto RC: Increased peripheral resistance during reduced uterine perfusion pressure hypertension in pregnant rabbits. *Am J Med Sci* 1992;**303**:233-240.
- 26 Golden JG, Hughes HC, Lang CM: Experimental toxemia in the pregnant guinea pig (*Cavia porcellus*). *Lab Anim Sci* 1980;**30**:174-179.
- 27 Woods LL: Importance of prostaglandins in hypertension during reduced uteroplacental perfusion pressure. *Am J Physiol* 1989;**257**:R1558-1561.
- 28 Morton JS, Andersson IJ, Cheung PY, Baker P, Davidge ST: The vascular effects of sodium tanshinone IIA sulphonate in rodent and human pregnancy. *PLoS One* 2015;**10**:e0121897.
- 29 Amaral LM, Cornelius DC, Harmon A, Moseley J, Martin JN, Jr., LaMarca B: 17-hydroxyprogesterone caproate significantly improves clinical characteristics of preeclampsia in the reduced uterine perfusion pressure rat model. *Hypertension* 2015;**65**:225-231.
- 30 Ma RQ, Sun MN, Yang Z: Effects of preeclampsia-like symptoms at early gestational stage on fetoplacental outcomes in a mouse model. *Chin Med J (Engl)* 2010;**123**:707-712.
- 31 Molnar M, Suto T, Toth T, Hertelendy F: Prolonged blockade of nitric oxide synthesis in gravid rats produces sustained hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation. *Am J Obstet Gynecol* 1994;**170**:1458-1466.
- 32 Alpoim PN, Gomes KB, Pinheiro Mde B, Godoi LC, Jardim LL, Muniz LG, Sandrim VC, Fernandes AP, Dusse LM: Polymorphisms in endothelial nitric oxide synthase gene in early and late severe preeclampsia. *Nitric Oxide* 2014;**42**:19-23.
- 33 Baijnath S, Soobryan N, Mackraj I, Gathiram P, Moodley J: The optimization of a chronic nitric oxide synthase (NOS) inhibition model of pre-eclampsia by evaluating physiological changes. *Eur J Obstet Gynecol Reprod Biol* 2014;**182**:71-75.
- 34 Motta C, Grosso C, Zanuzzi C, Molinero D, Picco N, Bellingeri R, Alustiza F, Barbeito C, Vivas A, Romanini MC: Effect of Sildenafil on Pre-Eclampsia-Like Mouse Model Induced By L-Name. *Reprod Domest Anim* 2015;**50**:611-616.
- 35 Ramesar SV, Mackraj I, Gathiram P, Moodley J: Sildenafil citrate improves fetal outcomes in pregnant, L-NAME treated, Sprague-Dawley rats. *Eur J Obstet Gynecol Reprod Biol* 2010;**149**:22-26.
- 36 Rossoni G, Manfredi B, De Gennaro Colonna V, Berti M, Guazzi M, Berti F: Sildenafil reduces L-NAME-induced severe hypertension and worsening of myocardial ischaemia-reperfusion damage in the rat. *Br J Pharmacol* 2007;**150**:567-576.
- 37 Ramesar SV, Mackraj I, Gathiram P, Moodley J: Sildenafil citrate decreases sFlt-1 and sEng in pregnant L-NAME treated Sprague-Dawley rats. *Eur J Obstet Gynecol Reprod Biol* 2011;**157**:136-140.

- 38 Nassar AH, Masrouha KZ, Itani H, Nader KA, Usta IM: Effects of sildenafil in Nomega-nitro-L-arginine methyl ester-induced intrauterine growth restriction in a rat model. *Am J Perinatol* 2012;**29**:429-434.
- 39 Samangaya RA, Mires G, Shennan A, Skillern L, Howe D, McLeod A, Baker PN: A randomised, double-blinded, placebo-controlled study of the phosphodiesterase type 5 inhibitor sildenafil for the treatment of preeclampsia. *Hypertens Pregnancy* 2009;**28**:369-382.
- 40 Zou Y, Zuo Q, Huang S, Yu X, Jiang Z, Zou S, Fan M, Sun L: Resveratrol inhibits trophoblast apoptosis through oxidative stress in preeclampsia-model rats. *Molecules* 2014;**19**:20570-20579.
- 41 Santillan MK, Santillan DA, Scroggins SM, Min JY, Sandgren JA, Pearson NA, Leslie KK, Hunter SK, Zamba GK, Gibson-Corley KN, Grobe JL: Vasopressin in preeclampsia: a novel very early human pregnancy biomarker and clinically relevant mouse model. *Hypertension* 2014;**64**:852-859.
- 42 Bartha JL, Visiedo F, Fernandez-Deudero A, Bugatto F, Perdomo G: Decreased mitochondrial fatty acid oxidation in placentas from women with preeclampsia. *Placenta* 2012;**33**:132-134.
- 43 Ding X, Yang Z, Han Y, Yu H: Long-chain fatty acid oxidation changes in a beta2 glycoprotein I-induced preeclampsia-like mouse model. *Placenta* 2014;**35**:392-397.
- 44 Lim R, Adhikari S, Gurusinghe S, Leaw B, Acharya R, Rahman R, Ciayadi R, Potdar M, Kelso GF, Hearn MT, Wallace EM: Inhibition of activin A signalling in a mouse model of pre-eclampsia. *Placenta* 2015;**36**:926-931.
- 45 Centlow M, Carninci P, Nemeth K, Mezey E, Brownstein M, Hansson SR: Placental expression profiling in preeclampsia: local overproduction of hemoglobin may drive pathological changes. *Fertil Steril* 2008;**90**:1834-1843.
- 46 May K, Rosenlof L, Olsson MG, Centlow M, Morgelin M, Larsson I, Cederlund M, Rutardottir S, Siegmund W, Schneider H, Akerstrom B, Hansson SR: Perfusion of human placenta with hemoglobin introduces preeclampsia-like injuries that are prevented by alpha1-microglobulin. *Placenta* 2011;**32**:323-332.
- 47 Winterbourn CC: Oxidative reactions of hemoglobin. *Methods Enzymol* 1990;**186**:265-272.
- 48 Anderson UD, Olsson MG, Rutardottir S, Centlow M, Kristensen KH, Isberg PE, Thilaganathan B, Akerstrom B, Hansson SR: Fetal hemoglobin and alpha1-microglobulin as first- and early second-trimester predictive biomarkers for preeclampsia. *Am J Obstet Gynecol* 2011;**204**:520 e521-525.
- 49 Hansson SR, Gram M, Akerstrom B: Fetal hemoglobin in preeclampsia: a new causative factor, a tool for prediction/diagnosis and a potential target for therapy. *Curr Opin Obstet Gynecol* 2013;**25**:448-455.
- 50 Hansson SR, Naav A, Erlandsson L: Oxidative stress in preeclampsia and the role of free fetal hemoglobin. *Front Physiol* 2014;**5**:516.
- 51 Naav A, Erlandsson L, Axelsson J, Larsson I, Johansson M, Wester-Rosenlof L, Morgelin M, Casslen V, Gram M, Akerstrom B, Hansson SR: A1M Ameliorates Preeclampsia-Like Symptoms in Placenta and Kidney Induced by Cell-Free Fetal Hemoglobin in Rabbit. *PLoS One* 2015;**10**:e0125499.
- 52 Akerstrom B, Gram M: A1M, an extravascular tissue cleaning and housekeeping protein. *Free Radic Biol Med* 2014;**74C**:274-282.
- 53 Olsson MG, Allhorn M, Bulow L, Hansson SR, Ley D, Olsson ML, Schmidtchen A, Akerstrom B: Pathological conditions involving extracellular hemoglobin: molecular mechanisms, clinical significance, and novel therapeutic opportunities for alpha(1)-microglobulin. *Antioxid Redox Signal* 2012;**17**:813-846.
- 54 Tejler L, Eriksson S, Grubb A, Astedt B: Production of protein HC by human fetal liver explants. *Biochim Biophys Acta* 1978;**542**:506-514.

- 55 DeMars DD, Katzmann JA, Kimlinger TK, Calore JD, Tracy RP: Simultaneous measurement of total and IgA-conjugated alpha 1-microglobulin by a combined immunoenzyme/immunoradiometric assay technique. *Clin Chem* 1989;**35**:766-772.
- 56 Wester L, Fast J, Labuda T, Cedervall T, Wingardh K, Olofsson T, Akerstrom B: Carbohydrate groups of alpha1-microglobulin are important for secretion and tissue localization but not for immunological properties. *Glycobiology* 2000;**10**:891-900.
- 57 Larsson J, Wingardh K, Berggard T, Davies JR, Logdberg L, Strand SE, Akerstrom B: Distribution of iodine 125-labeled alpha1-microglobulin in rats after intravenous injection. *J Lab Clin Med* 2001;**137**:165-175.
- 58 Olsson MG, Allhorn M, Larsson J, Cederlund M, Lundqvist K, Schmidtchen A, Sorensen OE, Morgelin M, Akerstrom B: Up-regulation of A1M/alpha1-microglobulin in skin by heme and reactive oxygen species gives protection from oxidative damage. *PLoS One* 2011;**6**:e27505.
- 59 Olsson MG, Nilsson EJ, Rutardottir S, Paczesny J, Pallon J, Akerstrom B: Bystander cell death and stress response is inhibited by the radical scavenger alpha(1)-microglobulin in irradiated cell cultures. *Radiat Res* 2010;**174**:590-600.
- 60 Olsson MG, Olofsson T, Tapper H, Akerstrom B: The lipocalin alpha1-microglobulin protects erythroid K562 cells against oxidative damage induced by heme and reactive oxygen species. *Free Radic Res* 2008;**42**:725-736.
- 61 Wester-Rosenlof L, Casslen V, Axelsson J, Edstrom-Hagerwall A, Gram M, Holmqvist M, Johansson ME, Larsson I, Ley D, Marsal K, Morgelin M, Rippe B, Rutardottir S, Shohani B, Akerstrom B, Hansson SR: A1M/alpha1-microglobulin protects from heme-induced placental and renal damage in a pregnant sheep model of preeclampsia. *PLoS One* 2014;**9**:e86353.
- 62 Talosi G, Nemeth I, Nagy E, Pinter S: The pathogenetic role of heme in pregnancy-induced hypertension-like disease in ewes. *Biochem Mol Med* 1997;**62**:58-64.
- 63 Hennessy A, Gillin A, Horvath J: Cardiovascular Research in Pregnancy - the Role of Animal-Models. *Hypertension in Pregnancy* 1993;**12**:413-437.
- 64 Hennessy A, Gillin AG, Duggin GG, Horvath JS, Tiller DJ: Low-dose nitro-L-arginine administration in baboon (*Papio hamadryas*) pregnancy. *Clin Exp Pharmacol Physiol* 1999;**26**:849-852.
- 65 Hennessy A, Gillin AG, Thompson JF, Painter DM, Waugh RD, Duggin GG, Tiller DJ, Horvath JS: Endothelin in primate pregnancy and an experimental preeclampsia-like syndrome. *Hypertension in Pregnancy* 1998;**17**:227-240.
- 66 Orange S, Rasko JE, Thompson JF, Vaughan J, Olive E, Pedler M, Horvath JS, Hennessy A: Interleukin-10 regulates arterial pressure in early primate pregnancy. *Cytokine* 2005;**29**:176-185.
- 67 Hennessy A, Pilmore HL, Simmons LA, Painter DM: A deficiency of placental IL-10 in preeclampsia. *J Immunol* 1999;**163**:3491-3495.
- 68 Sunderland NS, Thomson SE, Heffernan SJ, Lim S, Thompson J, Ogle R, McKenzie P, Kirwan PJ, Makris A, Hennessy A: Tumor necrosis factor alpha induces a model of preeclampsia in pregnant baboons (*Papio hamadryas*). *Cytokine* 2011;**56**:192-199.
- 69 Parrish MR, Murphy SR, Rutland S, Wallace K, Wenzel K, Wallukat G, Keiser S, Ray LF, Dechend R, Martin JN, Granger JP, LaMarca B: The effect of immune factors, tumor necrosis factor-alpha, and agonistic autoantibodies to the angiotensin II type I receptor on soluble fms-like tyrosine-1 and soluble endoglin production in response to hypertension during pregnancy. *Am J Hypertens* 2010;**23**:911-916.
- 70 Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y: The role of the immune system in preeclampsia. *Mol Aspects Med* 2007;**28**:192-209.
- 71 Wang Y, Walsh SW: TNF alpha concentrations and mRNA expression are increased in preeclamptic placentas. *J Reprod Immunol* 1996;**32**:157-169.

- 72 Murphy SR, LaMarca BB, Parrish M, Cockrell K, Granger JP: Control of soluble fms-like tyrosine-1 (sFlt-1) production response to placental ischemia/hypoxia: role of tumor necrosis factor- $\alpha$ . *Am J Physiol Regul Integr Comp Physiol* 2013;**304**:R130-135.
- 73 Santillan MK, Pelham CJ, Ketsawatsomkron P, Santillan DA, Davis DR, Devor EJ, Gibson-Corley KN, Scroggins SM, Grobe JL, Yang B, Hunter SK, Sigmund CD: Pregnant mice lacking indoleamine 2,3-dioxygenase exhibit preeclampsia phenotypes. *Physiol Rep* 2015;**3**:e12257.
- 74 Chatterjee P, Kopriva SE, Chiasson VL, Young KJ, Tobin RP, Newell-Rogers K, Mitchell BM: Interleukin-4 deficiency induces mild preeclampsia in mice. *J Hypertens* 2013;**31**:1414-1423; discussion 1423.
- 75 Lai Z, Kalkunte S, Sharma S: A critical role of interleukin-10 in modulating hypoxia-induced preeclampsia-like disease in mice. *Hypertension* 2011;**57**:505-514.
- 76 Inagaki A, Nishizawa H, Ota S, Suzuki M, Inuzuka H, Miyamura H, Sekiya T, Kurahashi H, Udagawa Y: Upregulation of HtrA4 in the placentas of patients with severe pre-eclampsia. *Placenta* 2012;**33**:919-926.
- 77 Hasan MZ, Ikawati M, Tocharus J, Kawaichi M, Oka C: Abnormal development of placenta in HtrA1-deficient mice. *Dev Biol* 2015;**397**:89-102.
- 78 Dechend R, Gratze P, Wallukat G, Shagdarsuren E, Plehm R, Brasen JH, Fiebeler A, Schneider W, Caluwaerts S, Vercauysse L, Pijnenborg R, Luft FC, Muller DN: Agonistic autoantibodies to the AT1 receptor in a transgenic rat model of preeclampsia. *Hypertension* 2005;**45**:742-746.
- 79 Przybyl L, Ibrahim T, Haase N, Golic M, Rugor J, Luft FC, Bendix I, Serdar M, Wallukat G, Staff AC, Muller DN, Hunig T, Felderhoff-Muser U, Herse F, LaMarca B, Dechend R: Regulatory T cells ameliorate intrauterine growth retardation in a transgenic rat model for preeclampsia. *Hypertension* 2015;**65**:1298-1306.
- 80 Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA: Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004;**350**:672-683.
- 81 Bergmann A, Ahmad S, Cudmore M, Gruber AD, Wittschen P, Lindenmaier W, Christofori G, Gross V, Gonzalves A, Grone HJ, Ahmed A, Weich HA: Reduction of circulating soluble Flt-1 alleviates preeclampsia-like symptoms in a mouse model. *J Cell Mol Med* 2010;**14**:1857-1867.
- 82 Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;**111**:649-658.
- 83 Thadhani R, Hagmann H, Schaarschmidt W, Roth B, Cingoz T, Karumanchi SA, Wenger J, Lucchesi KJ, Tamez H, Lindner T, Fridman A, Thome U, Kribs A, Danner M, Hamacher S, Mallmann P, Stepan H, Benzing T: Removal of Soluble Fms-Like Tyrosine Kinase-1 by Dextran Sulfate Apheresis in Preeclampsia. *J Am Soc Nephrol* 2015;**27**. [Epub a head of print].
- 84 Thadhani R, Kisner T, Hagmann H, Bossung V, Noack S, Schaarschmidt W, Jank A, Kribs A, Cornely OA, Kreyssig C, Hemphill L, Rigby AC, Khedkar S, Lindner TH, Mallmann P, Stepan H, Karumanchi SA, Benzing T: Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. *Circulation* 2011;**124**:940-950.
- 85 Pruthi D, Khankin EV, Blanton RM, Aronovitz M, Burke SD, McCurley A, Karumanchi SA, Jaffe IZ: Exposure to experimental preeclampsia in mice enhances the vascular response to future injury. *Hypertension* 2015;**65**:863-870.
- 86 van Dijk M, Mulders J, Poutsma A, Konst AA, Lachmeijer AM, Dekker GA, Blankenstein MA, Oudejans CB: Maternal segregation of the Dutch preeclampsia locus at 10q22 with a new member of the winged helix gene family. *Nat Genet* 2005;**37**:514-519.

- 87 Rigourd V, Chauvet C, Chelbi ST, Rebourcet R, Mondon F, Letourneur F, Mignot TM, Barbaux S, Vaiman D: STOX1 overexpression in choriocarcinoma cells mimics transcriptional alterations observed in preeclamptic placentas. *PLoS One* 2008;**3**:e3905.
- 88 Doridot L, Passet B, Mehats C, Rigourd V, Barbaux S, Ducat A, Mondon F, Vilotte M, Castille J, Breuiller-Fouche M, Daniel N, le Provost F, Bauchet AL, Baudrie V, Hertig A, Buffat C, Simeoni U, Germain G, Vilotte JL, Vaiman D: Preeclampsia-Like Symptoms Induced in Mice by Fetoplacental Expression of STOX1 Are Reversed by Aspirin Treatment. *Hypertension* 2013:662-668.
- 89 Doridot L, Chatre L, Ducat A, Vilotte JL, Lombes A, Mehats C, Barbaux S, Calicchio R, Ricchetti M, Vaiman D: Nitroso-redox balance and mitochondrial homeostasis are regulated by STOX1, a preeclampsia-associated gene. *Antioxid Redox Signal* 2014;**21**:819-834.
- 90 Ducat A, Doridot L, Calicchio R, Mehats C, Vilotte JL, Castille J, Barbaux S, Couderc B, Jacques S, Letourneur F, Buffat C, Le Grand F, Laissue P, Miralles F, Vaiman D: Endothelial cell dysfunction and cardiac hypertrophy in the STOX1 model of preeclampsia. *Sci Rep* 2016;**6**:19196.

**Table 1. Four mechanistic categories of animal models of preeclampsia addressing etiology of the disease and or therapeutic/intervention opportunities**

<b>Mechanism</b>	<b>Species</b>	<b>Stage 1</b>	<b>Stage 2</b>	<b>Therapeutic intervention</b>
<b>Spontaneous</b>	Mouse	BPH/5		Tempol
	Mouse	BPH/5		Inhibition of complement
	Mouse	BPH/5		VEGF
	Rat	Dahl S		
<b>Surgical</b>	Rat		RUPP	STS
	Rat		RUPP	17-HPC
	Baboon		RUPP	
<b>Pharmacological</b>	Mouse		L-NAME	Sildenafil
	Rat		L-NAME	Sildenafil
	Rat		L-NAME	Resveratrol
	Mouse		AVP	
	Mouse		$\beta$ 2GPI	
	Mouse		Activin A	Inhibitor
	Rabbit		HbF	A1M
	Sheep		Starvation	A1M
	Baboon		NO inhibitor	
	Baboon		Anti-IL-10	
	Baboon		TNF $\alpha$	
	<b>Genetic</b>	Mouse		IDO (ko)
Mouse			IL-4 (ko)	
Mouse		IL-10 (ko)		
Mouse		HtrA1(ko)		
Rat			Angio/Renin(tg)	Induction of T-reg
Mouse		STOX1(tg)		
Mouse			sFlt1 (vector)	VEGF
Rat			sFlt1 (vector)	

$\beta$ 2GPI, beta-2-glycoprotein I; A1M,  $\alpha$ <sub>1</sub>-microglobulin; AVP, arginine vasopressin; HtrA1, high temperature requirement A1; HbF, fetal hemoglobin; 17-HPC, 17- $\alpha$ -hydroxyprogesterone caproate; IDO, indoleamine 2,3-dioxygenase; L-NAME, nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; RUPP, reduced uterine perfusion pressure; sFlt-1, soluble fms-like tyrosine kinase 1; STOX1, Storkhead box 1; STS, sodium tanshinone IIA sulfonate; VEGF, vascular endothelial growth factor.

## Figure legends

### **Figure 1. A1M treatment ameliorates the structural damages caused by cell-free HbF in rabbit placenta**

Transmission electron microscopy of placental tissue from HbF-infused pregnant rabbits. (A) Control rabbits showing normal placental tissue with extracellular matrix filled with dense bundles of collagen fibers. (B) HbF-infusion causes loss of collagen fibers (indicated by arrows) together with severe damage to the extracellular matrix, extracellular apoptotic bodies, cell debris and a lot of empty extracellular space (indicated by stars). (C) The structural damages were significantly normalized by A1M treatment, with normal bundles of collagen fibers, normal electron dense barrier and reduced numbers of apoptotic bodies in the extracellular space. Scale bar 500 nm. Image modified from Naav et al. (2015).<sup>51</sup>

