Title

"Current model systems for the study of preeclampsia"

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Table 2. Preeclampsia *in vivo* research model systems

Models	Method	Species	Description	Advantages	Disadvantages	References
Trophoblast cell Invasion	Placental bed histological sections for identification of structural changes in uterine blood vessels, disturbed by invading trophoblastic cells in complicated pregnancies.	Human	Biopsies from the placental bed for histological evaluation of the uterine vasculature. Obtained from: - Delivered Placentas - Hysterectomy Specimens - Caesarean Section - Biopsies Under Ultrasound Guidance.	Small tissue samples are needed for the study (0.5 to 1 cm).	- Specimens acquired almost exclusively after delivery. - Marked variation in the patterns of interstitial and endovascular trophoblast invasion and in the associated modifications of the spiral arteries from the center to the margins of the placental bed. It is a challenge to extrapolate from the findings in small tissue fragments to the whole placental bed. Invasion may be irregular and vasculopathies may occur focally.	(1-4)
	Analysis of the endocrine phenotype of invasive trophoblast cells, and aspects of the regulation of trophoblast cell invasion during pregnancy of rodents with genetic deficiency of INFγ.	Rat, mouse	Sprague-Dawley, CD-1 mice, Tgε26 mice and C57BL/6 mice, IFNγ and IFNγ receptor null mutant, were used as model to investigate: - Trophoblast invasion in rats and mice. - The endocrine phenotype of these invading trophoblast cells, and the potential modulatory roles of NK cells and the NK	The rat and mouse model systems exhibit features shared with primates and expand the experimental repertoire for studying trophoblast cell invasion.	The phenotype of invasive rodent trophoblast cells is distinct from phenotypes for intraplacental trophoblast cell lineages.	(5)

			cell product IFNγ in the regulation of trophoblast invasion.			
	Evaluate the depth of endovascular trophoblast invasion and associated remodeling of spiral arteries in a transgenic model of PE in rats.	Rat	- Sprague-Dawley rats harboring the human renin (hRen) or human angiotensinogen gene (hAogen). - Fischer 344 R26-hAP rats that possess a transgene consisting of a Rosa 26 promoter driving the expression of a heat-stable human placental AP.	This technique should facilitate the discovery of endogenous regulatory mechanisms controlling trophoblast cell invasion and should represent an effective method of testing the impact of various environmental stressors on an essential part of hemochorial placentation.	The techniques are most effective when performed in conjunction with qualitative techniques used for the *in situ* identification and localization of invasive trophoblast cells.	(6, 7)
	The origin, migration routes and kinetics of invasive trophoblast cells were examined in two caviomorph species.	Guinea pig, degus	Histology and immunohistochemistry were performed on placentas from mid-gestational stages from pregnant guinea pigs and degus treated with 3 doses of BrdU (an *in vivo* marker for proliferation and for tracing of migration routes in the placenta) injected intraperitoneally.	The patterns of trophoblast invasion in caviomorphs are analogous to the situation in humans, suggesting that these rodents are appropriate animal models for the study of the dynamics of trophoblast invasion.	Not all intrauterine BrdU injections resulted in BrdU-staining of placental trophoblast, suggesting that not all intrauterine compartments are equally suitable injection sites for this tracer.	(8)
	Reduction of placental perfusion through the	Rat	Preeclamptic and pregnant control Sprague-Dawley rats	The model allows the study of dysregulated	The doxycycline is teratogenic.	(9)

	inhibition of trophoblast-induced spiral artery remodeling in pregnant rats.		were treated with doxycycline from gestational day 12 until day 18.	trophoblast invasion and vascular remodeling *in vivo* to gain important insights into PE-related mechanisms.		
Uteroplacental ischemia	Produce placental abruption by reducing its perfusion.	Dog, rabbit, rhesus monkeys, baboons	Abruptio placentae by ligating permanently or temporarily the inferior vena cava, intercotyledonary vessels or uterine arteries in pregnancy, causing progressive hypertension and proteinuria, which ceased after delivery.	Suppression of uteroplacental blood flow leads to a hypertensive state that closely resembles PE in women.	- Fibrinoid degeneration and aneurysmal dilatation were produced in the decidual vessels. - The vessels are often destroyed by the process. - The hypertensive state persisted until the postpartum period.	(10-13)
	Partially occluding the uteroplacental blood flow of pregnant animals resulted in hypertension.	Dog, rabbit, monkey	Ligating the terminal aorta to a specific degree of stricture, causing placental lesions, hypertension, proteinuria, fetal growth restriction and changes in liver and kidney.	Reductions in uteroplacental blood flow lead to a hypertensive state that closely resembles PE in women.	- The condition may lead to fetal death. - Appropriate clip size and the ideal gestational time for reducing uterine perfusion pressure were undetermined. - The degree of aortic constriction was imprecisely controlled.	(14-16)
	Produce the Reduced Uterine Perfusion Pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia.	Rat	Develop a reduced uterine perfusion pressure (RUPP) model by reducing uterine perfusion pressure in gravid Sprague-Dawley rats by placing a silver clip around the aorta below the renal arteries and on both the right and	- Reductions in uteroplacental blood flow lead to a hypertensive state that closely resembles PE in women. - Appropriate clip size and the ideal gestational time for reducing uterine	The proteinuria response was variable in the pregnant rats with reduction of uterine perfusion pressure.	(17, 18)

			left uterine arcade at the ovarian end just before the first segmental artery.	perfusion pressure were determined. - The degree of aortic constriction was precisely controlled.		
	Effects of hypoxia-inducible factor-1alpha (HIF-1α) expression on pregnant mice.	Mouse	- C57BL/6J mice with systemic administration of adenovirus expressing HIF-1α, - CITED2-knockout C57BL/6:129 mice, - Catechol-O-methyltransferase (COMT)-knockout C57BL/6J mice, - Pregnant C57BL/6JArc mice TNF-α infused.	These models showed PE-like syndrome.	- Immune response to adenoviral vector systems can cause severe complications for the animal model. - Proteinuria was present both in cases and in control groups.	(19-22)
Angiogenesis	Administration of soluble sFlt-1 and sEng.	Mouse, rat	Overexpression of angiogenic regulator factors such as sFlt-1 and sEng in pregnant mice and rats results in a PE-like phenotype.	Models mimic multiple aspects of PE (based on current ACOG criteria), including development of HELLP.	- Immune response to adenoviral and lentiviral vector systems not only impedes the delivery of genes to target cells but can cause severe complications for the animal model. - The teratogenicity of statins is an obstacle to its use.	(23-26)
	Prolonged blockade of nitric oxide synthesis.	Rat	L-nitro-arginine (a potent inhibitor of nitric oxide synthase) was infused continuously in pregnant rats.	Chronic inhibition of nitric oxide synthesis in pregnant rats leads to sustained hypertension, proteinuria, thrombocytopenia	L-nitro-arginine is a nonspecific vasoconstrictor that could inhibit the coronary vascular responses.	(27)

				and intrauterine growth retardation, providing a simple animal model for PE.		
	Angiogenesis inhibition during early placentation.	Rat	Administration of the angiogenesis inhibitor Suramin during early placentation of Sprague-Dawley rats.	The inhibition of uterine angiogenesis increases maternal blood pressure and compromises fetal and placental development.	Suramin is a nonspecific vasoconstrictor, with severe side effects.	(28)
Oxidative stress	Salted-water intake.	Rat	Increasing sodium intake (0.9% or 1.8% NaCl in drinking water) during the last week of gestation in the Sprague-Dawley pregnant rats.	Maternal insult during gestation induced an imbalance in the oxidative environment in the placenta favoring oxidation, and provides an animal model for studying the maternal manifestation of PE.	Early stages of gestations were not evaluated.	(29)
Immune response	Ultra-low-dose endotoxin infusion in pregnant rats.	Rat	Administration of an ultra-low-dose endotoxin infusion in conscious pregnant rats.	Histopathologic and clinical events mimic predominant features of human PE.	There is no evidence that human PE is caused by endotoxin.	(30)
	Administration of inflammatory cytokines.	Rat, mouse, baboons	- Sprague-Dawley rats treated with IL-6, - Papio hamadryas treated with IL-10 and TNF-α, - IL-10-knockout pregnant C57BL/6	These models showed PE-like syndrome and placental changes in molecules responding to inflammation.	The vascular endothelium releases other vasodilator substances that could influence the inflammatory cytokine activity.	(19, 31-35)

			mice exposed to low levels of oxygen or Toll-like receptor 3, - And pregnant C57BL/6JArc mice TNF-α infused.			
	Placental Toll-Like Receptor 3 and Toll-Like Receptor 7/8 activation.	Mouse	Treated pregnant C57BL/6J mice with the TLR3 agonist polyinosinic-polycytidylic acid (poly I:C), the TLR7-specific agonist imiquimod (R-837) and the TLR7/8 agonist CLO97 causing PE-like syndrome.	Treatment of mice with poly I:C, R-837, or CLO97 caused pregnancy-dependent hypertension, endothelial dysfunction, splenomegaly and placental inflammation.	Placental activation of LR3/7/8 by dsRNA and ssRNA could arise from latent viruses, viruses acquired during gestation, as well as excessive cellular necrosis/apoptosis resulting from aberrant implantation, placentation, placental hypoxia and/or trophoblast invasion.	(36)
	Adoptive cell transfer in pregnant mice.	Mouse	Adoptively transferring activated BALB/c Th1-like splenocytes into allogeneically pregnant BALB/c female mice during late gestation.	Cell transfer provoked PE symptoms (increased blood pressure and glomerulonephritis accompanied by proteinuria).	Adoptive cell transfer further affected pregnancy outcome by increasing fetal rejection through an inflammatory profile of uterine immune cells.	(37)
	Effect of angiotensin receptor agonistic autoantibodies in pregnant rodents.	Rat, Mouse	Infusion of activating antibodies against angiotensin II type 1 receptor (AT(1)-AB) alone or combined with angiotensin II into pregnant Sprague-Dawley and C57BL/6J pregnant mice induced PE-like syndrome.	- Key features of PE, placental abnormalities and small fetus size appeared in pregnant rodents after injection with AT(1) agonistic autoantibodies.	- No progression to eclampsia. - Not all women with PE have antibodies. - The effects could be prevented by co-injection with losartan, an AT (1) receptor antagonist, or by an antibody neutralizing seven-amino-acid epitope peptide.	(38, 39)
	Low-dose cadmium	Rat	Intraperitoneal	Key features of PE	Pregnant women are more	(40)

	chloride (CdCl2) infusion in pregnant rats.		administration of cadmium chloride (CdCl2) in Wistar rats on gestational days 9–14.	appeared in pregnant rats after the administration of low dose of CdCl2.	vulnerable to Cd because of the greatly increased absorption and retention of Cd caused by nutritional deficiencies during pregnancy.	
Other	Chronic administration of arginine vasopressin (AVP).	Mouse	Chronic infusion of AVP in C57BL/6J mice during pregnancy is sufficient to phenocopy PE.	AVP constitutes a novel very early human pregnancy biomarker and clinically relevant mouse model of PE.	AVP is known to be affected and is an effector of multiple immune cells.	(41)
	Spontaneous PE.	Mouse, rat, caviomorph, patas monkey.	Reports of spontaneous preeclamptic symptoms in BPH/5 mice, Dahl salt-sensitive S and spontaneously hypertensive (SHR SHR/NHsd or SHR) rats, guinea pigs and Erythrocebus patas.	- Might share etiology with human PE. - Disease severity is similar to that seen in humans. - Resembled PE in humans more closely than the experimentally induced disease in other animals.	- Cost and availability of these animals are limiting. - Disease has not been characterized based on the current diagnostic criteria for humans (ACOG, 2013).	(42-46)

References

1. Robertson WB, Khong TY, Brosens I, De Wolf F, Sheppard BL, Bonnar J. The placental bed biopsy: review from three European centers. American journal of obstetrics and gynecology. 1986;155(2):401-12.
2. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. British journal of obstetrics and gynaecology. 1994;101(8):669-74.
3. Dixon HG, Robertson WB. A study of the vessels of the placental bed in normotensive and hypertensive women. The Journal of obstetrics and gynaecology of the British Empire. 1958;65(5):803-9.
4. Pijnenborg R, Ball E, Bulmer JN, Hanssens M, Robson SC, Vercruysse L. In vivo analysis of trophoblast cell invasion in the human. Methods in molecular medicine. 2006;122:11-44.

5. Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: novel endocrine phenotype and regulation. Developmental biology. 2003;260(1):176-90.
6. Arroyo JA, Konno T, Khalili DC, Soares MJ. A simple in vivo approach to investigate invasive trophoblast cells. The International journal of developmental biology. 2005;49(8):977-80.
7. Geusens N, Verlohren S, Luyten C, Taube M, Hering L, Vercruysse L, et al. Endovascular trophoblast invasion, spiral artery remodelling and uteroplacental haemodynamics in a transgenic rat model of pre-eclampsia. Placenta. 2008;29(7):614-23.
8. Mess A, Zaki N, Kadyrov M, Korr H, Kaufmann P. Caviomorph placentation as a model for trophoblast invasion. Placenta. 2007;28(11-12):1234-8.
9. Verlohren S, Geusens N, Morton J, Verhaegen I, Hering L, Herse F, et al. Inhibition of trophoblast-induced spiral artery remodeling reduces placental perfusion in rat pregnancy. Hypertension. 2010;56(2):304-10.
10. Cavanagh D, Rao PS, Tung KS, Gaston L. Eclamptogenic toxemia: the development of an experimental model in the subhuman primate. American journal of obstetrics and gynecology. 1974;120(2):183-96.
11. Haynes DM. Experimental abruptio placentae in the rabbit. American journal of obstetrics and gynecology. 1963;85:626-45.
12. Howard BK, Goodson JH. Experimental placental abruption. Obstetrics and gynecology. 1953;2(5):442-6.
13. Myers RE, Fujikura T. Placental changes after experimental abruptio placentae and fetal vessel ligation of rhesus monkey placenta. American journal of obstetrics and gynecology. 1968;100(6):946-51.
14. Abitbol MM, Driscoll SG, Ober WB. Placental lesions in experimental toxemia in the rabbit. American journal of obstetrics and gynecology. 1976;125(7):942-8.
15. Abitbol MM, Ober MB, Gallo GR, Driscoll SG, Pirani CL. Experimental toxemia of pregnancy in the monkey, with a preliminary report on renin and aldosterone. The American journal of pathology. 1977;86(3):573-90.
16. Abitbol MM, Pirani CL, Ober WB, Driscoll SG, Cohen MW. Production of experimental toxemia in the pregnant dog. Obstetrics and gynecology. 1976;48(5):537-48.
17. Granger JP, LaMarca BB, Cockrell K, Sedeek M, Balzi C, Chandler D, et al. Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia. Methods in molecular medicine. 2006;122:383-92.
18. Douglas J. Eder, McDonald MT. A Role for Brain Angiotensin II in Experimental Pregnancy-Induced Hypertension in Laboratory Rats. Clinical and Experimental Hypertension Part B: Hypertension in Pregnancy. 1987;6(3):431-51.
19. Bobek G, Surmon L, Mirabito KM, Makris A, Hennessy A. Placental Regulation of Inflammation and Hypoxia after TNF-alpha Infusion in Mice. Am J Reprod Immunol. 2015;74(5):407-18.
20. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. Nature. 2008;453(7198):1117-21.
21. Tal R, Shaish A, Barshack I, Polak-Charcon S, Afek A, Volkov A, et al. Effects of hypoxia-inducible factor-1alpha overexpression in pregnant mice: possible implications for preeclampsia and intrauterine growth restriction. The American journal of pathology. 2010;177(6):2950-62.

22. Withington SL, Scott AN, Saunders DN, Lopes Floro K, Preis JI, Michalicek J, et al. Loss of Cited2 affects trophoblast formation and vascularization of the mouse placenta. Developmental biology. 2006;294(1):67-82.
23. Karumanchi SA, Stillman IE. In vivo rat model of preeclampsia. Methods in molecular medicine. 2006;122:393-9.
24. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, et al. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(4):1451-5.
25. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. The Journal of clinical investigation. 2003;111(5):649-58.
26. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nature medicine. 2006;12(6):642-9.
27. 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. American journal of obstetrics and gynecology. 1994;170(5 Pt 1):1458-66.
28. Carlstrom M, Wentzel P, Skott O, Persson AE, Eriksson UJ. Angiogenesis inhibition causes hypertension and placental dysfunction in a rat model of preeclampsia. Journal of hypertension. 2009;27(4):829-37.
29. Beausejour A, Bibeau K, Lavoie JC, St-Louis J, Brochu M. Placental oxidative stress in a rat model of preeclampsia. Placenta. 2007;28(1):52-8.
30. Faas MM, Schuiling GA, Baller JF, Visscher CA, Bakker WW. A new animal model for human preeclampsia: ultra-low-dose endotoxin infusion in pregnant rats. American journal of obstetrics and gynecology. 1994;171(1):158-64.
31. Chatterjee P, Chiasson VL, Kopriva SE, Young KJ, Chatterjee V, Jones KA, et al. Interleukin 10 deficiency exacerbates toll-like receptor 3-induced preeclampsia-like symptoms in mice. Hypertension. 2011;58(3):489-96.
32. Lai Z, Kalkunte S, Sharma S. A critical role of interleukin-10 in modulating hypoxia-induced preeclampsia-like disease in mice. Hypertension. 2011;57(3):505-14.
33. Orange S, Rasko JE, Thompson JF, Vaughan J, Olive E, Pedler M, et al. Interleukin-10 regulates arterial pressure in early primate pregnancy. Cytokine. 2005;29(4):176-85.
34. Orshal JM, Khalil RA. Interleukin-6 impairs endothelium-dependent NO-cGMP-mediated relaxation and enhances contraction in systemic vessels of pregnant rats. American journal of physiology Regulatory, integrative and comparative physiology. 2004;286(6):R1013-23.
35. Sunderland NS, Thomson SE, Heffernan SJ, Lim S, Thompson J, Ogle R, et al. Tumor necrosis factor alpha induces a model of preeclampsia in pregnant baboons (Papio hamadryas). Cytokine. 2011;56(2):192-9.
36. Chatterjee P, Weaver LE, Doersch KM, Kopriva SE, Chiasson VL, Allen SJ, et al. Placental Toll-like receptor 3 and Toll-like receptor 7/8 activation contributes to preeclampsia in humans and mice. PloS one. 2012;7(7):e41884.
37. Zenclussen AC. A novel mouse model for preeclampsia by transferring activated th1 cells into normal pregnant mice. Methods in molecular medicine. 2006;122:401-12.

38. Wenzel K, Rajakumar A, Haase H, Geusens N, Hubner N, Schulz H, et al. Angiotensin II type 1 receptor antibodies and increased angiotensin II sensitivity in pregnant rats. Hypertension. 2011;58(1):77-84.
39. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, et al. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. Nature medicine. 2008;14(8):855-62.
40. Zhang Q, Huang Y, Zhang K, Yan Y, Wang F, Wu J, et al. Cadmium-induced immune abnormality is a key pathogenic event in human and rat models of preeclampsia. Environ Pollut. 2016;218:770-82.
41. Santillan MK, Santillan DA, Scroggins SM, Min JY, Sandgren JA, Pearson NA, et al. Vasopressin in preeclampsia: a novel very early human pregnancy biomarker and clinically relevant mouse model. Hypertension. 2014;64(4):852-9.
42. Ganaway JR, Allen AM. Obesity predisposes to pregnancy toxemia (ketosis) of guinea pigs. Laboratory animal science. 1971;21(1):40-4.
43. Gillis EE, Williams JM, Garrett MR, Mooney JN, Sasser JM. The Dahl salt-sensitive rat is a spontaneous model of superimposed preeclampsia. American journal of physiology Regulatory, integrative and comparative physiology. 2015;309(1):R62-70.
44. Davisson RL, Hoffmann DS, Butz GM, Aldape G, Schlager G, Merrill DC, et al. Discovery of a spontaneous genetic mouse model of preeclampsia. Hypertension. 2002;39(2 Pt 2):337-42.
45. Dokras A, Hoffmann DS, Eastvold JS, Kienzle MF, Gruman LM, Kirby PA, et al. Severe feto-placental abnormalities precede the onset of hypertension and proteinuria in a mouse model of preeclampsia. Biology of reproduction. 2006;75(6):899-907.
46. Palmer AE, London WT, Sly DL, Rice JM. Spontaneous preeclamptic toxemia of pregnancy in the patas monkey (Erythrocebus patas). Laboratory animal science. 1979;29(1):102-6.