Title

"Current model systems for the study of preeclampsia"

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Table 1. Preeclampsia *in vitro* research model systems

Type	Models	Method	Species	Description	Advantages	Disadvantages	References
Cell culture	- To understand trophoblast cell biology, immunology, endocrine function and placental development. - To assess the effect of differentiation and fusion on the expression and release of a PE marker. - To study the regulation of trophoblast apoptosis and placental development under hypoxic conditions.	**Trophoblast cell lines:** - ED27 - ED31 - ED77 - HP-A1 - HP-A2 - HP-W1 - HT - HT-116 - HTR-8 - HTR-8/Svneo - IST-1 - NHT - NPC - RSVT-2 - RSVT2/C - SGHPL-4 - SGHPL-5 - SGHPL-6 - SGHPL-7 - SPA-26+7 lines - TCL-1 - TL **Malignant choriocarcinoma cell lines:** - AC1 - AC1-1 - AC1-5 - AC1-9 - AC-1M32 - AC-1M46	Human	Cell cultures of trophoblast.	Isolated primary trophoblast no longer proliferates in culture. Thus, only short-term cultures can be performed with primary cells. Therefore, respective cell lines have been developed to overcome the handicap of missing proliferation of primary trophoblasts in culture.	- Although a panel of markers and phenotypic criteria have been established to characterize the cell lines so they can be considered a trophoblast cell, very few of them have been evaluated for expression of these specific factors, probably due to the availability of well-characterized reagents for evaluation. - The methods employed to extend lifespan (transfection or spontaneous immortalization/transformation) alter regulation of cell division and may impact on differentiated functions and gene expression not usually observed in trophoblast cells *in vivo* or in primary culture.	(1-3)

		- AC-1M59 - AC-1M81 - AC-1M88 - BeWo - BeWo MC-1 - BeWo MC-2 - JAR - JEG **Embryonal lines with trophoblast differentiation:** - H9 - HT-H - NCC-EC-3 - NCCIT - NCR-G3					
	To determine: - Oxidative stress. - Effects of syncytiotrophoblast-derived microparticles in the maternal circulation, serum or plasma from patients with PE on proliferation, injury and apoptosis of human umbilical vein endothelial cells. - Effects of PE biomarkers or molecules with therapeutic potentials.	Normal primary umbilical vein endothelial cells (HUVECs).	Human	HUVECs cultured with syncytiotrophoblast-derived microparticles, serum, plasma, molecules or drugs for the evaluation of proliferation, apoptosis rates of the HUVECs and therapeutic properties.	- This cell line is ideal for evaluating immune response, wound healing, cellular response to viral or bacterial infection, oxidative stress, angiogenesis, arteriolosclerosis, drug screening and tubule formation, mechanisms implicated in PE.	- HUVECs are limited to 10–15 population doublings. Continued passaging beyond 15 doublings (4–5 passages after receipt) may result in decreased growth rates and loss of biological responsiveness.	(4-8)

	To evaluate: - Basic mechanisms of gene regulation in trophoblast cell lineage differentiation. - Placenta development. - Vascularization of the placenta.	- Connexins and trophoblast cell lineage development. - Placental vascular development through ubiquitin ligase Ankyrin repeat.	Mouse	- Generation of trophoblast stem cell lines from connexin-deficient mice. This may be used to elucidate the mechanism of differentiation and the role of genes and cell types in the development of the placenta. - Differentiation of embryonic stem cell to vascular lineage through ubiquitin ligase Ankyrin repeats.	Placentation in mice involves similar cell biological events to humans, without the ethical problems of abortion and the availability of sufficient tissues for research. One major point is that the most important steps of trophoblast differentiation occur within the first weeks of gestation.	Generation of trophoblast stem cells from knockout blastocysts seems to be accompanied by more methodological problems, especially if the genes that are deleted alter the differentiation pathway.	(9, 10)
	- Human placentation and its potential in cell therapy applications. - Invasive implantation events.	- Cytotrophoblast stem (CTBS) cell lines derived from human embryonic stem cells.	Human	- Generation of cytotrophoblast cell lines isolated from embryoid bodies and selected for trophoblast enrichment by rounds of cellular aggregation and disaggregation.	- First human CTBS cell lines to be derived from human embryonic stem cells and then maintained independently without feeder cells. - Differ from immortalized placental lines in that they have multipotent capacity to differentiate to various trophoblast phenotypes including syncytiotrophoblast	- To acquire the physiology of CTBS cells, spheroidal trophoblast bodies and matrigel are required to resemble the implanting embryo and mimic the endometrial deciduas, respectively.	(11)

					and extravillous cytotrophoblast.		
Explants	Trophoblast invasion	- First-trimester villous explants (to study postimplantation events, early stages of placentation and cytotrophoblast invasion).	Human	First-trimester floating villi in contact with a permissive extracellular matrix substrate stimulates cytotrophoblast proliferation and its column formation.	- Trophoblast is conditioned by co-culture with appropriate cells.	- Inability to identify the activities of individual cell types. - Drugs, oxygen, infections, nutrients, hormones, xenobiotics or cytokines can affect tissue functions and survival.	(12, 13)
		- Trans-filter cytotrophoblast migration (to study the phase of migration when single cells move through the decidual or myometrial environment)	Human, mouse	Purified primary first-trimester cytotrophoblast cell suspension.	It can be used quantitatively, providing good quality control.	- Minimal contamination by connective tissue cells may migrate or proliferate faster than trophoblast during the assay period. - Cytotrophoblasts are not unequivocally identifiable by morphological criteria.	(13, 14)
	Trophoblast invasion of spiral arteries	Spiral artery invasion and remodeling, developed using spiral artery explants, extravillous trophoblast cell lines and primary cytotrophoblasts.	Human	Fluorescently labeled trophoblasts (either primary first-trimester extravillous trophoblasts or an extravillous trophoblast cell line) are seeded on top of spiral artery segments (obtained from uterine biopsies at Caesarean section) embedded in fibrin gels (to study	It is useful for studies that lack suitable models directly examining cellular interactions during invasion.	Difficult to manipulate arteries and handle the co-culture.	(15)

				interstitial invasion) or perfused into the lumen of arteries (to study endovascular invasion). Both interstitial and endovascular interactions/invasion can be detected and immunohistochemical analyses carried out.			
	Probe preventive and therapeutic agents for PE	Extravillous trophoblast.	Human	Chorion from term placentas was digested and extravillous trophoblast isolated, then it was used to investigate the effect of unfractionated, low-molecular-weight heparin and aspirin on *in vitro* extravillous trophoblast differentiation.	- Although the model was developed to study thrombophilia, it is useful to apply in the study of PE. - It is a useful tool for demonstrating any effect of putative therapeutics on extravillous trophoblast, especially during very early placentation since defects in extravillous trophoblast function could play an important role in the etiology of pregnancy disorders such as PE.	- It has been reported that these kinds of cells are only partly involved in the release of any of the predictive biomarkers available today. - They no longer proliferate in culture. Hence, only short-term cultures for some days can be performed. At the same time these cells spontaneously syncytialize in culture and thus may be of use to study the release of syncytial fragments and factors *in vitro*.	(16)
	Oxidative stress	Villous trophoblast and amniotic tissue	Human	Term placenta was digested and villous	This culture represents a useful	- They no longer proliferate in culture. Hence, only short-term	(8, 17)

		cultures.		cytotrophoblasts isolated, then they were used to evaluate the effects of antioxidant vitamins C and E on trophoblast in culture. Villous and amniotic tissue cultures and stimulation with 4-hydroxy-nonenal, natrium fluoride and xanthine/xanthine oxidase.	*in vitro* model system to assess drug effectiveness.	cultures for some days can be performed. - At the same time these cells spontaneously syncytialize in culture and thus may be of use to study the release of syncytial fragments and factors *in vitro*.	
	- Placental metabolism and syncytiotrophoblast death. - Effect of anti-hypertensive drugs on placental hormones and angiogenic protein synthesis, known to be altered in PE.	Villous explants.	Human	- First- and third-trimester human placental explants were cultured with antiphospholipid antibodies, and several metabolites with important roles in cell death regulatory pathways were analyzed. - Placental villous explants from late onset PE were cultured at 20% oxygen with variable doses of anti-hypertensive drugs. The levels of different molecules were measured in explant culture media.	Useful to study various facets of the materno-fetal interface, including analysis of placental endocrine function, metabolism, transport and to dissect cellular processes such as proliferation, differentiation, apoptosis and syncytial fusion.	- Deficient availability of fresh material for explant cultures. - Villous explants are not regularly stored frozen. Hence, placental tissues can only be used directly after delivery, and explant cultures using tissues from a single placenta cannot be repeated at a later date. So to overcome this problem a larger number of villous explants derived from a single placenta could be explored.	(18, 19)
Co-	Immune response	- Macrophage-	Human	- Isolation and cell	None of the co-	- The type of cytokines	(20-24)

cultures		trophoblast interactions. - Trophoblast-derived cell line co-cultured with peripheral blood mononuclear cells under hypoxic conditions. - Maternal neutrophils co-cultured with the syncytiotrophoblast microvillous membranes. Factors in plasma of PE women activate endothelial cells to produce IL-8 resulting in transendothelial migration of neutrophils. - Interaction between natural killer cells and villous explants from concordant first-trimester pregnancies co-cultured in a collagen model of placentation.		culture of macrophages, primary trophoblasts, peripheral blood mononuclear cell, neutrophils and natural killer cells. - Co-culturing of macrophages-trophoblast, trophoblast-peripheral blood mononuclear cells, neutrophils-syncytiotrophoblast microvillous membranes, and natural killer-villous explants. - Evaluating the co-culture interactions.	cultured cells affect proliferation, apoptosis and cell column formation.	produced by a macrophage depends on its activation state. - The type of effect in the inflammatory response depends upon the state of trophoblast. - The co-cultures need a scaffold that allows a 3D conformation leading to cell-cell interaction and column formation.	
	Trophoblast proliferation, migration, invasion and endothelial cell interactions.	HTR-8/SVneo trophoblast cells or trophoblast-derived JEG-3 cells co-cultured with	Human	Trophoblast migration and interactions with endometrial endothelial cells were	- To evaluate the effect of each cell line co-cultured on the proliferative and invasive	- These models only identified the maternal physical properties of the invasion. - The difficulty in obtaining the samples on a regular basis also	(25-27)

		human uterine myometrial microvascular endothelial cells or with human endometrial endothelial cells into formation of capillary-like cellular networks.		measured using Transwell permeable support or co-cultured in matrigel.	properties of the other. - To evaluate antihypertensive and anti-inflammatory drugs that can modulate the interaction between trophoblast and endothelial cells. - These are 3D models (cellular networks) that likely mimic the trophoblast interaction with endothelium during remodeling.	prohibited these models' widespread use.	
	Cytotrophoblast invasion	Cytotrophoblast co-cultured with human uterine spiral artery smooth muscle cell.	Human	Transwell migration assay was used to detect the invasive ability of cytotrophoblast co-cultured with human uterine spiral artery smooth muscle cell, incubated with normal or preeclamptic serum.	Cytotrophoblast cultured alone with normal or preeclamptic serum was lower than cytotrophoblast cultured with human uterine spiral artery smooth muscle cells.	Because placental immunological tolerance is regulated by many factors, this *in vitro* model might not fully represent the *in vivo* situation of uterine spiral artery remodeling in PE.	(28)
Placental organ culture	Placenta biology	Placenta-on-a-Chip.	Human	A 'Placenta-on-a-Chip' is a microsystem that consists of two polydimethylsiloxane microfluidic channels separated by a thin extracellular matrix	- Replicates the architecture and function of the placenta. - Provides new opportunities to simulate and analyze critical	- Although the microfluidic culture system provides a greater physiological cell culture environment than conventional static cultures, the levels of shear stress generated in the model are substantially lower than those in fetal	(29)

				membrane. To reproduce the placental barrier, human trophoblasts (JEG-3) and human umbilical vein endothelial cells (HUVECs) are seeded on the opposite sides of the membrane and cultured under dynamic flow conditions to form confluent epithelial and endothelial layers in close apposition.	physiological responses of the placental barrier. - It is a low-cost experimental platform with a broad range of applications. - Overcomes the limitations that the current *in vitro* models have in recapitulating the organ-specific structure and key physiological functions of the placenta.	capillaries. This is mainly due to the large size of the cell culture chambers. - It has only been proved by employing the JEG-3 cell line and HUVECs to represent trophoblast and endothelial cells, respectively. It could be better if it employs primary human trophoblasts and fetal capillary endothelial cells.	

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