






## PARALLEL MECHANISMS BETWEEN PLACENTA/PREECLAMPSIA AND NEURODEGENERATIVE DISEASES

### ABSTRACT

This short review summarizes recent studies on placenta-preeclampsia  in the mother and/or intrauterine growth restriction  (IUGR) in the child. The ideas raised here are framed within a paradigm that favors the opening of new research lines in these themes and are focused on the outlining of early investigation and/or an adequate treatment for mothers who develop the pathology. Thus, this review focuses on those studies that categorize PE in the group of pathologies defined as "conformational diseases", as a consequence of the misfolding of proteins due to endoplasmic reticulum (ER)  stress. In this particular case, the ER stress that develops in the syncytiotrophoblast (ST) because of the oxidative stress caused in the placenta by the hypoxia that occurs as a consequence of the failure in the remodeling of endometrial arteries. This leads to an increased ST apoptosis with detachment of misfolded proteins into the maternal circulation, which in turn would be primarily responsible for the signs of PE in the mother (proteinuria, edema, and hypertension). The review also analyzes the PE-prions-placenta relationship, since the normal cell-surface protein (PrPc) is normally present in the plasma membrane of ST, but appears to be increased in cases of PE. However, although neurodegenerative disorders resulting from conformational changes in the prion protein from its normal cellular form, PrPc, to the infectious scrapie isoform, PrP (Sc) are well known , there are no studies as to whether this isoform would also be present in the ST of PE cases  in our opinion it is important to evaluate its biological implications in the normal and pathological human placentas.

### Placenta and preeclampsia

The passage of nutrients from the maternal blood to the fetus is mediated by the placenta, so the normal fetal metabolism and growth require of an adequate exchange across this organ [1]. The trophoblast is the epithelium that covers the placental fetal villous tree and during development differentiates into two layers: the syncytiotrophoblast (ST) and the cytotrophoblast (CT). The former is externally located and contains many nuclei and a continuous cytoplasm, forming a syncytium. The latter consists of a monolayer of ovoid cells immediately underlying the ST. Both structures contribute to the formation of the villi and ultimately the placenta. Villous CT fuse in order to form the ST layer that contributes to the metabolic exchange of gas and nutrients, as well as to the process of waste elimination [2, 3]. Apoptosis of the trophoblast has been observed to naturally occur in placentas of normal human pregnancies but, as expected, placentas from women with preeclampsia (PE) or intrauterine growth retardation (IUGR) show enhanced apoptosis when compared with placentas from normal pregnancies [4].

PE is a systemic pregnancy syndrome that affects about 3-5% of all pregnancies [5]. This pathology is an important contributor to maternal and perinatal morbidity and mortality worldwide. Because there is no cure other than delivery, PE is the leading cause of

45 iatrogenic preterm birth. Despite to be of unknown etiology, it is currently accepted that  
46 this pathology originates in the placenta [6] due to the fact that the maternal symptoms  
47 (high blood pressure and proteinuria) disappear once the organ has been expelled after  
48 delivery [7, 8].

49

#### 50 **Oxidative stress**

51 To date, PE has been related to the process of hypoxia due to ischemia/reperfusion  
52 experienced by the placenta as a consequence of extravillous trophoblast failure in the  
53 process of endometrial spiral arteries remodeling. The involvement of oxidative stress (OS)  
54 in the early placental hypoxia development has been previously proposed in the mechanism  
55 of the syndrome [1, 5, 8, 9]. Due to the fact that the human fetal-placental vasculature lacks  
56 autonomic innervation, it is reasonable to assume that autocrine and/or paracrine agents  
57 such as the NO radical may play an important role in the regulation of fetal-placental blood  
58 flows [9].

59 On the other hand, OS constitutes a unifying mechanism of injury involved in many  
60 types of disease. It occurs when there is an imbalance between the production of ROS and  
61 the ability of the biological system to readily detoxify these reactive oxidative species (ROS)  
62 or the tissues cannot easily repair the resulting damage [10]. In PE it has been shown that  
63 enhanced ROS generation leads to a decrease in the NO bioavailability [11]. Increased  
64 generation of superoxide anion by the placenta leads to increased peroxynitrite production,  
65 resulting in further oxidative stress and endothelial dysfunction in PE patients [8].  
66 Additionally, it has been well established that NO disrupts the mitochondrial respiratory  
67 chain in a dose dependent manner, causing changes in the mitochondrial  $Ca^{2+}$  flux that  
68 induce ER Stress in pluripotent stem cells [12]. Taking all of these evidences into account, it  
69 is plausible to assume that OS developed in the placenta by the exaggerated generation of  
70 ROS would trigger ER stress in the organ, which in turn will increase the apoptosis of the ST.

71

#### 72 **Placental ER stress and Amyloidosis**

73 In the last few years, a number of studies suggesting that PE could be triggered by  
74 disorders in the folding of proteins in the ER of the ST, which results in amyloid deposits in  
75 this organelle [13-16] have been published. In light of this evidence, the accumulation of  
76 misfolded protein in the ER lumen has been defined as 'ER Stress' [17-20].

77 In addition, ER stress has recently been identified as a major regulator of cell  
78 homeostasis through its involvement in post-translational protein modification and folding,  
79 as well as its capacity to activate the unfolded protein response (UPR) which aims to restore  
80 the homeostatic balance within the ER [21]. If this cannot be achieved, the cell apoptotic  
81 machinery becomes consequently activated. The initial intent of the UPR is to adapt the cell  
82 to the changing environment, and reestablish normal ER function. These adaptive  
83 mechanisms involve transcriptional programs that induce expression of genes that enhance  
84 the protein folding capacity of the ER, and promote ER-associated protein degradation to  
85 remove misfolded proteins [17]. Persistent protein misfolding initiates apoptotic cascades  
86 [21] that are known to play fundamental roles in the pathogenesis of multiple human  
87 diseases, including diabetes, atherosclerosis, PE and neurodegenerative diseases [14-  
88 16,22,23], all of which have been defined as "conformational diseases".

89 The ER stress due to misfolded proteins in the ST increases placental apoptosis in this  
90 epithelial layer [16, 18]. Moreover, due to the fact that the ST establishes direct contact

91 with the maternal blood, the apoptotic process produces detachment of the syncytial  
92 infolding proteins, accumulated due to ER stress, to the maternal blood. Consequently,  
93 these particles will be mainly responsible for the development of PE symptoms in the  
94 mother. Recently, Bosco et al., [16] found that the Amyloid A (AA) was present in the ST of  
95 PE and IURG placentas, and that the degree of apoptosis of the CT regulates the amyloidosis  
96 destiny of the AA in the ST. In brief, in PE cases the misfolded proteins are expelled to the  
97 maternal blood. On the contrary, in the IURG cases they are deposited on the basal lamina  
98 of the trophoblast, without being expelled from the placenta, but also altering the  
99 mother/fetus metabolic exchange, thus producing IUGR. Moreover, Hitomi et al., [24]  
100 suggested that activation of ER-resident caspase-12 indirectly activates cytoplasmic caspase-  
101 3 and might be important in ER stress-induced neuronal apoptosis as a consequence of the  
102 presence of misfolded proteins. This is in agreement with the placental study of Bosco et al.,  
103 [16] which showed the presence of active caspase 3 in the CT of PE placentas with AA  
104 amyloidoses, but not in the CT of normal placentas.

105 It has also been reported that caspase-12-deficient mouse cortical neurons were  
106 defective in apoptosis induced by amyloid-beta protein, but not by trophic factor  
107 deprivation [25]. Thus, caspase-12 mediates an ER-specific apoptosis pathway and may  
108 contribute to amyloid-beta neurotoxicity. This idea is in concordance with Fu et al., [26]  
109 who found significantly higher caspase 12 activity in placentas of early or late severe PE. It is  
110 important to note that ER stress apoptosis can be induced by other various pathological  
111 conditions that alter the ER function. In the same line of evidence, Wang et al., [27]  
112 experimentally induced ER stress and apoptosis in placentas of pregnant rats exposed to  
113 lead, which was accompanied by an increase in the caspase-12 mRNA expression, and Xu et  
114 al., [28] found an increase in the early expression of ER stress markers, followed by  
115 increased activity of caspase 12 in placental trophoblast exposed *in vivo* and *in vitro* to *T.*  
116 *gondii*, followed by an increased apoptosis of the exposed trophoblasts. Similar results were  
117 found by Wang et al., [29] in neural stem cells exposed to this parasite. It should be  
118 emphasized that in the last three investigations no studies were carried out in order to  
119 evaluate the presence of misfolding proteins in the placentas, which would have allowed  
120 amyloidosis to be discarded. It is also important to note that in a case control study where  
121 pregnant women suspected of *T. gondii* infection were treated with spiramycin, a macrolide  
122 antibiotic administered before 18 weeks of pregnancy in order to reduce the rate of  
123 transmission of the parasite to the fetus, reported a reduced incidence of pregnancy-  
124 induced hypertension [30]. On the basis of these results, the association of *T. gondii*  
125 infection with hypertension disease during pregnancy needs to be further investigated.

126 In another line of evidence, transthyretin (TTR) is a homotetrameric serum and  
127 cerebrospinal fluid protein. The TTR dissociation forms monomer misfolding, a variant of  
128 TTR that results in familial amyloid polyneuropathy, familial amyloid cardiomyopathy, or  
129 familial central nervous system amyloidosis [31]. TTR is also a carrier protein for thyroxin  
130 and retinol binding protein, which are secreted by trophoblast. McKinnon et al., [32] and  
131 Mortimer et al., [33] have reported that human placenta secretes TTR into the maternal and  
132 fetal circulations and that placental TTR secreted into the maternal placental circulation can  
133 be taken up by the trophoblasts and translocated to the fetal circulation, thus conforming a  
134 TTR shuttle system. This may have important implications for maternal-fetal transfer of  
135 thyroid hormones, retinol/retinol binding protein and xenobiotics, all of which bind to TTR.  
136 Additionally, Fruscalzo et al., [34] demonstrated that TTR is dysregulated in cases of IUGR

137 and severe early onset PE, and Kalkunte, et al., [13] showed the presence of amyloid  
138 aggregates of TTR in PE placentas, as well as in the serum of these patients.

139 Taken together, all these evidences allow us to postulate that by effect of the OS the  
140 placenta develops ER stress in the ST and CT, which leads to the accumulation of misfolded  
141 proteins and, if the quantity greatly increases, this will finally activate the UPR with the  
142 consequent increase of ST apoptosis and therefore the release of the misfolded proteins  
143 into the maternal blood, which in turn will trigger the symptoms of PE in the mother.

144

#### 145 **Placental**

146 The study of this prion protein was initiated due to its involvement in a number of  
147 related neurodegenerative disorders seen in various species (bovine spongiform  
148 encephalopathy in cattle, scrapie in sheep and Creutzfeldt–Jakob disease in humans). The  
149 name ‘prion’ (for Proteinaceous Infectious) was coined as the infectious agent of these  
150 diseases was found to be significantly constituted by proteins [35]. A protein with identical  
151 sequence was found to be expressed in significant quantities in the brains of non-diseased  
152 animals. Hence, a consensus was reached that the protein existed in two distinct forms: the  
153 normal cellular prion protein form (PrPc) and the diseased or scrapie form (PrPSc). However,  
154 recent evidence suggests that the scrapie form of the protein may be sufficient by itself for  
155 transmission of the disease [36]. Transmissible spongiform encephalopathies (TSE) or prion  
156 diseases are characterized by the deposition of PrPc in the structurally altered PrPSc form.  
157 While PrPc configuration is primarily  $\alpha$ -helix and susceptible to proteolysis, PrPSc instead  
158 forms fibrillar aggregates containing a high percentage of  $\beta$ -sheet and is rather resistant to  
159 proteolytic digestion [37]. TSE condition is accompanied by physiological symptoms similar  
160 to those of aging which, in turn, have been shown to be affected by divalent metal ions  
161 [38,39]. Over the past three decades, the role of metal ions in TSE has attracted  
162 considerable attention particularly since 1970s, when  $\text{Cu}^{2+}$  chelator-induced  
163 histopathological changes were documented to be similar to scrapie [38–40]. Metal ions  
164 have been implicated as potential pathogenic candidates owing to their properties of being  
165 free-radical generators and their association with metalloenzymes such as superoxide  
166 dismutases (SODs), [41]. Pathological features of TSE resemble neuronal and brain tissue  
167 loss as is observed in the case of free radical-mediated oxidative damage [42].

168 On the other hand, PrPc, a copper-binding glycoposphatidylinositol-anchored  
169 protein whose function is to protect the cells against oxidative stress and to prevent the  
170 apoptosis it is expressed in the plasma membrane of neural and not neural tissues [43-46].  
171 A number of roles in neuroprotection, cellular homeostasis, response to oxidative stress, cell  
172 proliferation and differentiation, synaptic function and signal transduction have been  
173 proposed for PrPc [43,46]. Additionally, it has been shown that the abnormal isoform of  
174 PrPSc is able to induce further PrPc  $\rightarrow$  PrPSc transition, accumulating in infected brains and  
175 forming amyloid plaques involved in prion diseases such as TSE, a disease with neuronal  
176 death and gliosis, producing extensive and sponge-like tissue vacuolization [37,38,47].  
177 Additionally, Hetz et al., [48] demonstrate that prion diseases characterized by accumulation  
178 of the misfolded protease-resistant form of the prion (PrPSc) produce neuronal death by  
179 apoptosis that also correlated with caspase 12 activation in neural mouse cells treated with  
180 PrPSc. Furthermore, it has also been reported that the hypoxia-inducible factor-1 alpha  
181 (HIF-1 $\alpha$ ), which appears to be a master regulator of the cellular response to hypoxia [49],  
182 regulates PrPc expression in order to protect against neuron cell damage [50]. In correlation

183 with this, a variety of studies have shown that women with PE are characterized by  
184 persistently elevated placental HIF-1 $\alpha$  levels that promote enhanced transcription of genes  
185 encoding the soluble antiangiogenic protein fms-like tyrosine kinase-1 (sFlt-1), the soluble  
186 antiangiogenic factor endoglin (sEngs) and endothelin-1 (ET-1), a powerful vasoconstrictor  
187 known to contribute to this pregnancy pathology [51-55]. Moreover, Donadio et al., [56]  
188 and Alfaidy et al., [57] reported that PrPc is highly expressed in the human placenta,  
189 especially in CT and ST, and Hwang et al., [58] found that the immunohistochemical  
190 expression of PrPc was increased in CT and ST of PE placentas versus those from the  
191 controls. Additionally, Brown et al., [59] and Brown and Besinger [60] demonstrated in  
192 mouse neurons that PrPc may directly or indirectly regulate the activity of Cu/Zn superoxide  
193 dismutase (Cu/Zn SOD). In this context, Bosco et al., [61] found a decreased activity of  
194 Cu/Zn SOD in PE placentas versus normal placentas with an increased of F2-isoprostanes, a  
195 lipid peroxidation indicator. Furthermore, Klamt et al., [47] found a decreased activity of  
196 SOD in liver, heart, hippocampus and cerebellus in PrPc knockout and wild-type mice and an  
197 oxidative damage in proteins and lipids. In addition, Anantharam et al., [46] found that PrPc  
198 plays a proapoptotic role during ER stress.

199 On the bases of the above arguments, we consider of the essential interest to carry  
200 out new research aimed at investigating the possible presence of PrPsc in ST and CT in cases  
201 of severe PE and eclampsia. This, due to the fact that poorly folded proteins form amyloid  
202 precipitates, and because in PE, a decrease in the activity of the antioxidant enzyme SOD  
203 which is regulated by PrPc [60] has been found [61]. It is noteworthy that in the cases of  
204 pregnant mothers who develop eclampsia, the maximum expression of PE, the maternal  
205 endothelial damage can lead to severe intracranial (intracerebral and subarachnoid)  
206 hemorrhage and cerebral venous thrombosis, preceded by visual hallucinations and the final  
207 appearance of convulsions and coma [62].

208 We would like to hypothetically propose that the presence of PrPSc in the ST and CT  
209 of the placenta of these mothers could be related to the increase of apoptosis in these cells  
210 and also with the significant maternal endothelial damage observed, since the release of  
211 PrPSc into the maternal blood would allow these misfolded proteins reach the blood-brain  
212 barrier. Therefore, it would be essential to perform brain biopsies of women who have died  
213 from eclampsia for the determination of amyloidosis and/or PrPSc [63]. Finally, it is  
214 important to note that in sheep placentas exposed naturally to PrPSc, the presence of PrPSc  
215 in the trophoblast has been shown by immunohistochemistry and/or ELISA essays [64].

## 216 CONCLUSIONS


217 This work revises and summarizes the latest studies showing a relationship between  
218 the presence of placental amyloidosis and PE. The amyloidosis condition may be either due  
219 to an increased ER stress in the trophoblast, or to an increase in the caspase 12 activity  
220 the ER of these cells, accompanied latter by an increase in caspase 3 activity in the CT, whose  
221 cells define the fate of the misfolding proteins in the ST.

222 We have also reviewed some studies that demonstrate the presence of the normal  
223 prion PrPc in the plasma membrane of the CT and ST, whose presence has been noticed to  
224 increase in cases of PE. However, it is important to analyze what type of response will occur  
225 if *in vitro* BeWo cells are subjected to exposure of the prion isoform PrPSc.

226

227 **PROJECTIONS**

228 Future studies are required in order to elucidate the functional role of increased  
229 amyloidosis and PrPc in the placenta of PE pregnancy, and to establish whether the  
230 determinations of amyloids [14] or PrPc [59] in urine or serum of this women could be used  
231 to prevent or predict this pregnancy pathology.

232  important to note that although diseases due to misfolding of proteins share  
233 common metabolic pathways, PE in pregnant women differs from other pathologies related  
234 to amyloidosis by ER Stress, such as diabetes, atherosclerosis, and neurodegenerative  
235 diseases, in the sense that the mother's symptoms of PE totally disappear once the placenta  
236 has been expelled, after birth.

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