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**Efeito do soro de gestantes com pré-eclâmpsia sobre a via de estresse de  
retículo endoplasmático em células trofoblásticas**

**Versão Corrigida**

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## RESUMO

CASTRO, KR. *Efeito do soro de gestantes com pré-eclâmpsia sobre a via de estresse de retículo endoplasmático em células trofoblásticas* [tese]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2020.

É no retículo endoplasmático que ocorre a síntese, o enovelamento e a maturação de proteínas, sendo esta uma organela extremamente sensível às variações de estímulos que possam interferir na homeostase celular. Quando há um ambiente desfavorável ou maior demanda de síntese proteica, ocorre uma falha no enovelamento de proteínas e inicia-se um acúmulo de proteínas mal-enoveladas, levando a célula ao Estresse de Retículo Endoplasmático (RE). Em resposta, ocorre a ativação da via de sinalização conhecida como resposta às proteínas mal-enoveladas (do inglês, *Unfolded Protein Response*, UPR). Se a URP falhar na restauração da homeostase celular, a própria via ativa mecanismos que levam à apoptose, evitando que a célula secrete para o ambiente extracelular moléculas não-funcionais. A UPR tem sido estudada em diversas fisiopatologias como doenças neurodegenerativas e diabetes e, na gestação, a pré-eclampsia (PE) precoce. Na PE, há um desbalanço na síntese e liberação de fatores placentários associado a uma resposta materna sistêmica que consiste em inflamação vascular generalizada. Evidências mostram uma relevância neste desbalanço dos produtos placentários na patogênese da PE. Neste estudo, avaliamos o efeito do soro de gestante PE sobre a via de estresse de RE nos explantes coriônicos de placenta a termo saudáveis, tratadas com soro das gestantes saudáveis (controle) e com PE por 24h e em células HTR-8/Svneo por 3h, 6h, 12h e 24h. A razão sFlt-1/PIGF foi maior no soro das gestantes com PE ( $p=0,02$ ). Após 24h de tratamento com o soro PE, os explantes placentários apresentaram redução no metabolismo celular e viabilidade celular ( $p=0,007$ ) tiveram mais mortes quando comparados com o controle ( $p=0,01$ ). Houve um aumento significativo na expressão dos genes *GADD34* ( $p=0,02$ ), *CHOP* ( $p=0,0001$ ) e *SDF2* ( $p=0,03$ ) nos explantes, após 24h de

tratamento com soro PE quando comparado ao grupo controle. A expressão gênica de ATF4 e sXBP1 não apresentou alteração. Ocorreu aumento significativo da expressão proteica de GRP78 ( $p=0,02$ ), SDF2 ( $p=0,007$ ), p-eIF2 $\alpha$  ( $p=0,01$ ) e a razão p-eIF2 $\alpha$ /eIF2 $\alpha$  ( $p=0,0006$ ) nos explantes tratados com soro PE em comparação com o controle. Não houve alteração significativa na expressão de eIF2 $\alpha$  total. As células HTR-8/Sv-neo apresentaram mais mortes após 12h (0,007) e 24h ( $p=0,007$ ) de tratamento com soro PE quando comparadas com o grupo controle (soro saudável). A expressão gênica de GADD34 (12h:  $p=0,0001$ ; 24h:  $p=0,006$ ), CHOP (12h:  $p<0,001$ ; 24h:  $p<0,01$ ) e SDF2 ( $p<0,0001$ ) ATF4 ( $p<0,0001$ ) aumentaram significativamente após 24h de tratamento com soro PE quando comparada ao controle. Um aumento significativo na expressão proteica de eIF2 $\alpha$  total foi observado após 12h ( $p=0,016$ ) e expressão proteica de p-eIF2 $\alpha$  foi significativamente maior após 6h ( $p=0,034$ ) e 12h ( $p=0,012$ ) de tratamento do soro PE comparadas com seus respectivos controles. A razão p-eIF2 $\alpha$ /eIF2 $\alpha$  foi significativamente maior após 6h de tratamento ( $p=0,007$ ). A expressão proteica de SDF2 foi maior em 12h ( $p=0,01$ ) e 24h ( $p=0,01$ ) em relação ao grupo controle. Este estudo pode oferecer novas perspectivas na compreensão da PE, na medida em que avalia se o ambiente materno fornecendo evidências de que o soro de mulheres gestantes com PE possui fatores desencadeante de estresse de RE e de resposta a proteínas mal enoveladas (UPR) nos vilos placentários.

Palavras-chave: Placenta. Células trofoblásticas. Pré-eclâmpsia. Estresse de RE, UPR.

## ABSTRACT

CASTRO, KR. *Effect of serum from pregnant women with pre-eclampsia on the endoplasmic reticulum stress pathway in trophoblastic cells [thesis]*. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2020.

It is in the endoplasmic reticulum that protein synthesis, folding and maturation occurs, which is an organelle extremely sensitive to variations in stimuli that may interfere with cell homeostasis. When there is an unfavorable environment or greater demand for protein synthesis, a failure in protein folding occurs and an accumulation of poorly folded proteins begins, leading the cell to Endoplasmic Reticulum Stress (ER). In response, activation of the signaling pathway known as the response to malfolded proteins occurs Unfolded Protein Response (UPR). If UPR fails to restore cellular homeostasis, the pathway itself activates mechanisms that lead to apoptosis, preventing the cell from secreting non-functional molecules to the extracellular environment. UPR has been studied in several pathophysiology such as neurodegenerative diseases and diabetes and, in pregnancy, early pre-eclampsia (PE). In PE, there is an imbalance in the synthesis and release of placental factors associated with a systemic maternal response that consists of generalized vascular inflammation. Evidence shows a relevance in this imbalance of placental products in the pathogenesis of PE. In this study, we evaluated the effect of the serum of PE pregnant women on the ER stress pathway in chorionic explants of healthy term placentas, treated with serum from healthy pregnant women (control) and with PE for 24h and in HTR-8/Sv-neo cells for 3h, 6h, 12h and 24h. The sFlt-1/PIGF ratio was higher in the serum of pregnant women with PE ( $p=0.02$ ). After 24h of treatment with PE serum, placental explants showed a reduction in cell metabolism and cell viability ( $p=0.007$ ) had more deaths when compared to control ( $p=0.01$ ). There was a significant increase in the expression of the GADD34 ( $p=0.02$ ), CHOP ( $p=0.0001$ ) and SDF2 ( $p=0.03$ ) genes in the explants, after 24h of treatment with PE serum when compared to the control group. The gene expression of ATF4 and sXBP1 did

not change. There was a significant increase in GRP78 protein expression ( $p=0.02$ ), SDF2 ( $p=0.007$ ), p-eIF2 $\alpha$  ( $p=0.01$ ) and the p-eIF2 $\alpha$ /eIF2 $\alpha$  ratio ( $p=0.0006$ ) in explants treated with PE serum compared to the control. There was no significant change in the expression of total eIF2 $\alpha$ . HTR-8/Svneo cells showed more deaths after 12h (0.007) and 24h ( $p = 0.007$ ) of treatment with PE serum when compared to the control group (healthy serum). Gene expression of GADD34 (12h:  $p=0.0001$ ; 24h:  $p=0.006$ ), CHOP (12h:  $p<0.001$ ; 24h:  $p<0.01$ ) and SDF2 ( $p<0.0001$ ) ATF4 ( $p<0.0001$ ) increased significantly after 24h of treatment with PE serum when compared to the control. A significant increase in total eIF2 $\alpha$  protein expression was observed after 12h ( $p=0.016$ ) and p-eIF2 $\alpha$  protein expression was significantly higher after 6h ( $p=0.034$ ) and 12h ( $p= 0.012$ ) of PE serum treatment compared with their respective controls. The p-eIF2 $\alpha$ /eIF2 $\alpha$  ratio was significantly higher after 6 hours of treatment ( $p=0.007$ ). Protein expression of SDF2 was higher in 12h ( $p=0.01$ ) and 24h ( $p=0.01$ ) in relation to the control group. This study may offer new perspectives in the understanding of PE, as it assesses whether the maternal environment providing evidence that the serum of pregnant women with PE has triggering factors for ER stress and response to poorly folded proteins (UPR) in villi placental.

Keywords: Placenta. Trophoblastic cells. Pre-eclampsia. RE stress. UPR.

## **1 INTRODUÇÃO**

A placenta é um órgão materno-fetal transitório e multifuncional que desempenha funções respiratórias, nutritivas, excretoras e imunológicas, além de produzir hormônios necessários para o sucesso da gestação (Evain-Brion e Malassine, 2003).

O componente materno da placenta é formado por células que se diferenciam a partir dos fibroblastos da camada funcional do endométrio e que em conjunto formam a decídua basal. Embora diferenciação decidual também ocorra em outras regiões (decídua capsular e parietal, não associadas ao local de implantação embrionária) é a decídua basal que mantém contato com a porção fetal da placenta – os vilos coriônicos e o trofoblasto extraviloso - e é também denominada de placa basal (Wynn, 1967).

Os vilos coriônicos são formados por um eixo de tecido mesenquimal vascularizado e com macrófagos, revestido por células trofoblásticas individualizadas – citotrofoblasto - e uma camada sincial, - o sinciciotrofoblasto. Nas fases gestacionais mais adiantadas, há escasseamento das células do citotrofoblasto. Ao longo da gestação, os vilos coriônicos se ramificam intensamente e sua superfície sincial faz contato com o sangue materno, extravasado nos espaços intervilosos. A superfície do sinciciotrofoblasto em contato com o sangue materno é responsável pelas trocas moleculares, gasosas e nutritivas entre os organismos materno e fetal (Kaufmann, 1985).

Na extremidade distal dos vilos coriônicos, células citotrofoblásticas proliferam, rompem o revestimento sincial e passam a interagir diretamente com os componentes deciduais. Estas células trofoblásticas assumem diferentes morfologias e funções, caracterizando subtipos de células denominadas de citotrofoblásticas extravilosas e são consideradas de fundamental importância para a homeostase metabólica, imunológica e funcional da gestação (Tarrade et al., 2001).

Em um primeiro momento, as células citotrofoblásticas bloqueiam as extremidades das artérias uterinas espiraladas, impedindo o fluxo sanguíneo arterial nos espaços intervilosos (Jaffe, Jauniaux e Hustin, 1997). Neste período, estas

artérias passam por extenso processo de remodelamento das quais participam células citotrofoblásticas extravilosas e outros componentes endometriais como as abundantes células NK presentes no endométrio (Rätsep et al., 2015). As células do citotrofoblasto extraviloso substituem as células endoteliais e musculares vasculares formando canais vasculares que levam a uma circulação placentária de alto fluxo e baixa resistência, garantindo uma perfusão mais abundante e regular no espaço interviloso (Granger et al., 2001). A abertura destes canais no espaço interviloso ocorre ao redor da 12<sup>a</sup> semana de gestação.

Especialmente a partir do 2º trimestre de gestação, o fluxo sanguíneo adequado na interface materno-fetal está intimamente associado à vasodilatação da circulação uterina, que em parte é assegurada também pelos canais vasculares trofoblásticos (Brosen et al.; Robertson, Brosen e Dixon, 1967). A produção de fatores vasodilatadores por células endoteliais uterinas e de toda a circulação sistêmica é aumentada durante a gestação, assim como a resposta a estes fatores também parece ser mais efetiva (Poston et al., 1995, Bird, Zhang e Magness, 2003). Falhas nos mecanismos de vasodilatação e nas funções endoteliais são encontradas em doenças hipertensivas gestacionais (Morton, Care e Davidge, 2017).

As consequências destas doenças se fazem sentir tanto no feto como na gestante. Em geral, acredita-se suas implicações fetais incluem hipóxia fetal, restrição de crescimento fetal (RCF), nascimento prematuro e até óbito fetal (Sibai, 2002; Bramham et al., 2014).

Várias classificações são descritas para os distúrbios hipertensivos na gravidez, entre elas, destacamos aqui a pré-eclâmpsia (PE), a eclampsia e a síndrome HELLP (hemólise, enzimas hepáticas elevadas, baixa contagem de plaquetas), por serem as patologias gestacionais que causam os maiores riscos de mortalidade e morbidade materna e perinatal (ACOG Practice Bulletin, 2019). Estas desordens de incidência mundial, também podem limitar a saúde materna e determinar graves consequências ao feto e ao recém-nascido. No Brasil, as síndromes hipertensivas da gestação são as principais causas de morte materna

principalmente quando apresentadas em suas formas mais graves: eclampsia e síndrome HELLP (Queiroz, 2018).

Neste estudo, abordamos apenas a PE. Estima-se que esta desordem afete 4% a 5% das gestantes em todo o mundo (Phipps et al., 2019) e no Brasil, 1,5% (Abalos et al., 2013). Além da hipertensão arterial, a PE se caracteriza também por proteinúria, ocorrendo na segunda metade da gestação, associada ao aumento de inflamação sistêmica (Redman e Sargent, 2004). A forma mais grave da PE ocorre em cerca de 0,5% das gestações. Se expressa precocemente, podendo ser diagnosticada após a 20<sup>a</sup> semana de gestação e está associada à restrição do RCF (Staff et al., 2013).

Ainda há necessidade de muitos estudos para que possamos entender as origens da PE; no entanto há um consenso de que as causas devem ser multifatoriais (Redman e Sargent, 2005). A remoção da placenta é a única forma conhecida de abrandar os sintomas, sugerindo que fatores liberados pelo trofoblasto atuam sobre o organismo materno de gestantes suscetíveis (Huppertz, 2008; Roberts e Escudero, 2012; Huppertz, 2015).

Principalmente a PE de início precoce tem sido atribuída à invasão superficial do trofoblasto extraviloso na decídua, levando a uma remodelação das arterias uterinas de forma deficitária e consequentemente a um fluxo sanguíneo útero-placentário inadequado. A remodelação deficiente das artérias espirais uterinas por sua vez, causam perfusão errática na interface materno-fetal, com ondas de isquemia e reperfusão, levando a estresse oxidativo (Osol, Ko e Mandalà, 2017) e desbalanço na produção de fatores antiangiogênicos pelo trofoblasto (Sankaralingam et al., 2006), o que se acredita que seja um fator crucial para a disfunção endotelial e inflamação no organismo materno.

Distúrbios como hipóxia e deprivação de nutrientes, como os que acontecem na pré-eclâmpsia também podem afetar a síntese protéica, alterando a estruturação de proteínas no retículo endoplasmático, evento celular conhecido como estresse do retículo endoplasmático (RE) (Walter e Ron, 2011). Em geral, insultos celulares com comprometimento do RE levam à produção de proteínas mal-enoveladas no lúmen do RE, que por suas alterações não prosseguem seu destino de secreção,

acumulando-se neste lúmen (Walter e Ron, 2011). O estresse do RE ativa multiplas respostas celulares com o intuito de resgatar a homeostasia celular ou induzir a apoptose da célula injuriada, impedindo a secreção de proteínas inativas ou corrompidas (Xu, Bailly-Maitre e Reed, 2005). Esta resposta em conjunto é denominada de “resposta a proteínas mal enoveladas” (UPR, do inglês, *unfolded protein response*) (Schröder e Kaufman, 2005; Walter e Ron, 2011) e inclui a inibição da síntese proteica, diminuição da proliferação celular, ativação de respostas pró-inflamatórias e morte celular com prejuízo das funções orgânicas e teciduais (Yung et al. 2008; Sano e Reed, 2013). Estudos prévios mostraram que ocorre ativação da via UPR em placenta de mulheres com PE de início precoce, portanto grave (Yoshida, 2007; Burton e Yung, 2011). Estudos recentes também mostraram que a proteína SDF2 está estreitamente associada à via UPR (Lorenzon et al., 2010), particularmente quando esta via ativa processos de morte celular (Lorenzon et al., 2014). Além disso, também se observou aumento de sua expressão nos casos mais severos desta doença (Lorenzon et al., 2020).

A fisiopatologia da PE tem sido bastante discutida, assim como os fatores que agravam o estado inflamatório e de saúde geral da gestante. Estudos prévios em nosso laboratório mostraram que o soro de gestantes com PE altera o perfil de expressão gênica dos fatores angioativos como o fator de crescimento placentário (PIGF) e da tirosina 1 semelhante a fms solúvel (sFlt-1) por vilos coriônicos de gestantes saudáveis (Prado, 2019). A alteração de expressão e secreção destes fatores tem sido considerados biomarcadores da PE (Monte, 2011; Mathur et al., 2016; Tomimatsu et al., 2019). Neste contexto, estes resultados parecem indicar que pode haver um mecanismo de retroalimentação crônico como fator de agravamento da doença. Não está claro, no entanto, se fatores circulantes maternos gerados durante a PE grave podem atuar nas células da placenta, ativando mecanismos de estresse e levando à um agravamento da PE. Propomos que este seja um mecanismo que pode contribuir para os sintomas clínicos de agravamento da pré-eclâmpsia. Mais especificamente, este estudo tem como proposta avaliar se fatores presentes no soro materno exercem efeito sobre as células trofoblásticas ativando a via de estresse de RE e desequilibrando a expressão dos produtos

vasoativos que caracterizam o ambiente pré-eclâmptico. Uma vez ativada, essa via exacerba a resposta inicial desencadeante da patologia, fazendo com que a resposta ao insulto patológico seja ao mesmo tempo indutor e agravante da PE.

## **9 CONCLUSÕES**

A partir dos ensaios realizados neste estudo, pudemos concluir que o soro de mulheres com pré-eclâmpsia:

Reduz a atividade metabólica e a viabilidade celular nos explantes coriônicos e nas células trofoblásticas da linhagem HTR-8/Sv-neo, indicando uma interferência negativa sobre estas células.

Possui fatores capazes de desencadear estresse no retículo endoplasmático das células dos vilos coriônicos provenientes de gestações saudáveis e em células trofoblásticas da linhagem HTR-8/Sv-neo, sugerindo que este soro interfere com a homeostasia destas células.

Ativa uma resposta ao estresse de RE (UPR) via o sensor PERK, que se reflete por um lado na expressão de GADD34, o que indica uma tentativa de restabelecimento da homeostasia celular.

Também induz uma resposta da via UPR mediada por SDF2 e CHOP, o que sugere um destino voltado a morte celular e, portanto, de destruição de células com sistema de síntese incapaz de ser regenerado pela injuria causada pelo soro PE.

Em conjunto, nos ensaios in vitro realizados neste estudo, o soro de gestantes com PE comprometeu a homeostasia dos vilos coriônicos (células trofoblásticas) sugerindo que in vivo, mecanismos semelhantes podem estar ocorrendo nas gestantes com PE.

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