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Immunoregulation of the decidualization program: focus on the endoplasmic reticulum stress

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Abstract

Decidualization denotes the reprogramming of endometrial stromal cells that includes the secretion of different mediators like cytokines, chemokines, and the selective recruitment of immune cells. This physiological process involves changes in the secretome of the endometrial stromal cells leading to the production of immunomodulatory factors. The increased amount of protein secretion is associated with a physiological endoplasmic reticulum (ER) stress and the resulting unfolded protein response (UPR), allowing the expansion of ER and the machinery to assist the protein folding. Notably, the signaling pathways involved in the ER stress and the UPR are interconnected with the onset of a sterile inflammatory response, as well as with angiogenesis. Both of these processes have a key role in decidualization and placentation, therefore, alterations in them could lead to pregnancy complications. In this review, we will discuss how the induction of ER stress and the UPR processes that accompanies the decidualization are associated with embryo implantation and whether they might condition pregnancy outcome. The ER stress activates/triggers sensing proteins which, among others, induces kinase/RNAse-TXNIP expression, activating the NLRP3 inflammasome. This multiprotein system allows caspase-1 activation, which catalyzes the cleavage of the inactive IL-1β proform toward the mature secretory form, with pro-implantatory effects. However, the sterile inflammatory response should be later controlled in favor of a tolerogenic microenvironment to sustain pregnancy. In accordance, alterations of the ER stress and UPR processes can be reflected in recurrent implantation failures (RIF), recurrent pregnancy loss (RPL), or complications associated with deficient placentation, such as preeclampsia (PE).
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Redefining the decidualization process

For many years, it was assumed that decidualized cells displayed a passive role during embryo implantation just associated with morphological changes of the stromal cells. Nowadays, the experimental evidence indicates that the initial inflammation associated with the embryo implantation is a physiological response that begins during the decidualization program (Boomsma *et al*. 2009, Challis *et al*. 2009, Mor *et al*. 2017). The decidualization process in humans occurs in each menstrual cycle and, unlike murine decidualization, it does not require the presence of a blastocyst (Ramathal *et al*. 2010). It is currently proposed that cyclical decidualization in the absence of pregnancy could contribute to 'preconditioning' the endometrium for receptivity. This concept is based on the fact that cyclical decidualization implies a repetitive inflammatory response with a certain degree of ischemia. Inflammation is deeply involved in fertility, from ovulation to implantation and decidualization, thus, cyclic exposure to sub-threshold tissue injury would provide protection through the induction of maternal tolerance (Brosens *et al*. 2009, Teklenburg *et al*. 2010*a*,*b*).

The decidualization program denotes changes on the secretory profile associated with the expansion of its endoplasmic reticulum (ER), a physiological response known as ER stress and the consequent unfolded protein response (UPR) commonly described as the 'integrated stress response'. This will allow decidualized cells to secrete pro-implantatory factors (Brosens *et al*. 2014). The most interesting point is that the signaling pathways involved in the ER stress and the UPR are interconnected with the onset of a sterile inflammatory response, as well as with angiogenesis (Binet & Sapieha 2015). Both of these processes have a key role in decidualization and placentation, therefore, alterations in them could lead to pregnancy complications. Depending on the severity, these alterations can be reflected in recurrent implantation failures (RIF), recurrent pregnanacy loss (RPL), or complications associated with deficient placentation as preeclampsia (PE) (Dimitriadis *et al*. 2010).

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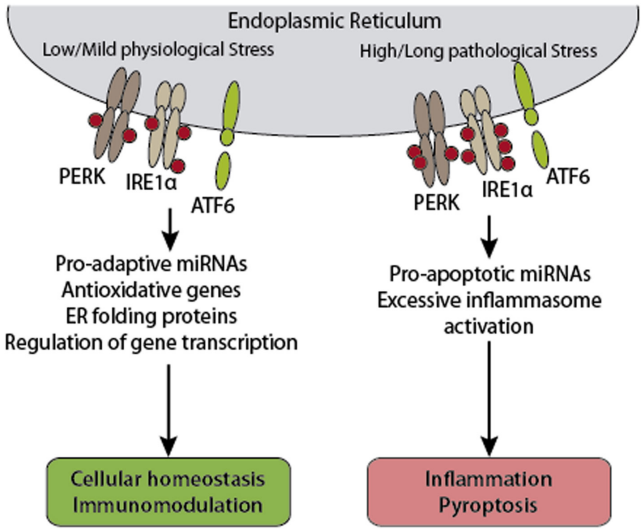

Figure 1 ER stress and UPR define cellular fate. The UPR is a sophisticated network of intracellular signaling pathways that has evolved to sustain an adequate folding and post-translational modifications of proteins for maintaining cellular homeostasis, which could otherwise induce autophagy and cell death (pyroptosis).

The ER stress and UPR associated to the decidualization program

As part of the reprogramming of the endometrial stromal cells, decidual cells acquire the ability to secrete a large variety of proteins (Altmäe *et al*. 2012). The increment in protein secretion levels induces the saturation of the protein folding machinery inside the ER, which leads to an accumulation of misfolded proteins. As a consequence, the cells undergo ER stress, which triggers the UPR in order to restore its cellular homeostasis (Walter & Ron 2011) (Fig. 1). These processes are evolutionarily conserved and were demonstrated in both humans and the murine model (Yamamoto *et al*. 2007, Gu *et al*. 2016, Xiong *et al*. 2016).

The UPR involves different intracellular signaling pathways generating a network to sustain the physiological folding and post-translational modifications of proteins, to reach cellular homeostasis, which could otherwise induce autophagy and cell death. The activation of the UPR occurs in a matter of minutes to hours to avoid an overload of translation in the ER. This response is mediated by the activation of three sensors:

- PERK (protein kinase RNA-like endoplasmic reticulum kinase) is activated by oligomerization and its autophosphorylation. The activated cytosolic domain causes the attenuation of the translation by direct phosphorylation of the alpha subunit that regulates the initiation of mRNA translation, eIF2. This event inhibits the translation of the protein machinery involved in cell cycle progression, resulting in G1 phase arrest and selective translation of transcription factor ATF4 that upregulates UPR target genes (Atkins *et al*. 2013).
- IRE1α (inositol-requiring enzyme 1α), whose dimerization and autophosphorylation catalyzes unconventional mRNA splicing of the transcription factor XBP1 (X-box Binding Protein 1), removing an intron of it through its endonuclease activity (Gardner & Walter 2011). The spliced and active form of this transcription factor (sXBP1) activates genes that regulate UPR.
- ATF6 (Activating Transcription Factor 6) is a basic leucine zipper transcription factor that, upon activation, is transferred to the Golgi apparatus where it is sequentially cleaved by serine proteases S1 and S2 to form an active transcription factor which induces the expression of genes that regulate UPR (Shen *et al*. 2002).

Brosens *et al*. described that, in coculture systems between decidualized endometrial cells and morphologically arrested blastocysts, the first significantly decrease the production of implantation and immunomodulatory factors. However, when these assays were performed in the presence of non-decidualized stromal cells, no changes were observed (Brosens *et al*. 2014). Therefore, after decidualization, the stromal endometrial cells acquire the ability to change their secretome according to the quality of the embryo (Salker *et al*. 2010). One of the mechanisms involved in this quality control is based on the fact that competent human embryos trigger oscillatory Ca^{2+} fluxes while non-competent embryos induce a prolonged Ca^{2+} response, which may be associated with an altered ER stress and UPR on decidualized cells and autophagy (Brosens *et al*. 2014).

The ER stress and the UPR processes as inducers of a sterile inflammatory response: focus on IL-1 system

The generation of an inflammatory response is crucial for successful embryo implantation, and it is associated with increased expression of several inflammatory cytokines and chemokines in both the endometrial cells and the blastocyst (Altmäe *et al*. 2012, Mor *et al*. 2017).

First, it was proposed that the activation of the pattern-recognition receptors (PRRs) by endogenous intracellular molecules (DAMPs: damage-associated molecular patterns) such as ATP, high-mobility group box 1 (HMGB1), uric acid, DNA free, and IL-1α could induce a sterile inflammation at materno-placental interface that will sustain the selective recruitment of maternal immune cells (Nadeau-Vallée *et al*. 2016). These 'danger signals', also known as alarmins, could be released by necrotic cells generated during the tissue remodeling, associated to embryo implantation. However, there is another way to initiate a sterile inflammatory response that involves the induction of ER stress and UPR associated to IL-1β production.

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The IL-1 family includes 11 members that regulate inflammatory response to injuries and stressors. The main members, IL-1α and IL-1β, bind to ubiquitous IL-1R1 to trigger the expression of numerous cytokines, including itself, by the activation of the transcription factors NF-κB (Nuclear Factor Kappa B) and AP-1 (Activator Protein 1) (Dinarello 2009). Even though they display similar biological effects, both IL-1α and IL-1β are encoded by different genes and differ in their secretion pathway. IL-1α is expressed in the cytoplasm and translocates to the nucleus to regulate the expression of inflammatory genes. In opposite, IL-1β requires the inflammasome activation to be released in the active form. The inflammasome is a multiprotein complex which is activated by a wide range of stimuli from different sources, including sterile stressors. This activation ends with the cleavage and secretion of active forms of IL-1β and IL-18 (Lerner *et al*. 2012). Briefly, the complex consists of an inflammasome-sensor molecule, the adaptor protein ASC, and caspase-1. Several inflammasome-sensor molecules can trigger the formation of inflammasomes. Most of the inflammasomes that have been described contain a NOD-like receptor (NLR) sensor molecule such as NLRP3 (NOD-, LRR-, and pyrin domain-containing).

Hence, it was proposed that both, IL-1α and IL-1β, contribute to the sterile inflammation with different kinetics. Whereas IL-1α initiates the sterile inflammatory response, IL-1β amplifies the initial trigger (Nadeau-Vallée *et al*. 2016).

The IL-1 system is expressed in the endometrium and in the blastocyst and participates in the bidirectional dialogue. Regarding IL-1β's relevance, it is highly preserved in primates and has been proposed as one of the mediators in placental viviparity which increases endometrium receptivity (Paulesu *et al*. 2008, Geisert *et al*. 2012). Nowadays, the evidence points out that IL-1β contributes to the decidualization by several mechanisms: by the induction of integrins; the production of leukemia inhibitory factor (LIF) (Stewart *et al*. 1992) and leptin (Dimitriadis *et al*. 2005) and by the dissociation of the actin filamentous in human stromal cells (Strakova *et al*. 2000).

Particularly, the remodeling of the cytoskeleton is critical for the initiation of the stromal cells differentiation (Jasinska *et al*. 2006). Using inhibitors of myosin light chain kinase or myosin II, it was observed that a destabilization of the cytoskeleton and the inhibition of the decidualization induced IL-1β (Ihnatovych *et al*. 2007). Changes in actin dynamics negatively impact on decidualization and prevent the translocation of the actin-binding protein cofilin to the nucleus, an essential response to permit stromal cell differentiation (Ihnatovych *et al*. 2009).

Furthermore, IL-1β secreted by decidualized stromal cells enhances trophoblast migration, and the prevention of IL-1β treatment leads to fetal death, highlighting the relevance of this cytokine in pregnancy (Gonzalez *et al*. 2011).

Previous studies suggest that ER stress is connected with the inflammatory response, acting IRE1α as a bridge between these processes. Particularly in MCF-7 cells (a lung cancer metastasis cell line), it was reported that IRE1α interacts with the adapter protein associated with the TNF receptor factor 2 (TRAF2) through its kinase domain, promoting the activation of nuclear factor kappa β (NF-kβ) and triggering an inflammatory response (Hu *et al*. 2006). Moreover, in a model of diabetes progression using INS-1 cells (Insulin-secreting β cell line 1), it was shown that IRE1α increases the expression of TXNIP (thioredoxin-interacting protein) linked with IL-1β production in response to ER stress (Zhou *et al*. 2010, Strowig *et al*. 2012). Particularly, it was demonstrated that TXNIP binds and activates NLRP3-inflammasome (Patwari *et al*. 2006). Therefore, ER stress and UPR also contribute to sustain the sterile inflammation associated with implantation.

The ER stress and the UPR processes as inducers of the sterile inflammatory response during the implantation period

Based on this evidence, we studied the role of ER stress and UPR on the induction of a sterile inflammation through IL-1β during the decidualization. In this sense, we have recently reported an increased expression of the three ER stress sensors ATF6, PERK, and IRE1α, as wells as UPR markers sXBP1 and CHOP, after *in vitro* decidualization of an endometrial stromal cell line (Grasso *et al*. 2018). In fact, we observed an increased TXNIP expression, which was previously demonstrated to bind and activate the inflammasome (Patwari *et al*. 2006). In our *in vitro* decidualization model, we detected increased expression of NLRP3, which was accompanied by the activation of caspase-1 quantified by a fluorescent probe (FAM-Flica for Caspase-1) (Fig. 2). This result was in line with the increased production of IL-1β detected by flow cytometry. Remarkably, this production was prevented by the treatment with STF-083010, an IRE1α endoribonuclease activity-inhibitor that does not affect its kinase activity. Moreover, decidualized cells pre-incubated with STF-083010 reduced the invasion index evidenced in an *in vitro* model of implantation (Grasso *et al*. 2018). Basically, this model consists of co-culturing a monolayer of decidualized stromal cells with spheroidal structures constituted by trophoblastic cells that mimic, in several aspects, the external structure of human blastocyst. These structures that differentiate into blastocyst-like spheroids (BLS) lack internal cellular mass (the embryo proper) but they conserve during 96 h a compact order with capacity of adhesion, invasion, and three-dimensional expansion (Holmberg *et al*. 2012). Since the prevention of ER stress/UPR on decidualized

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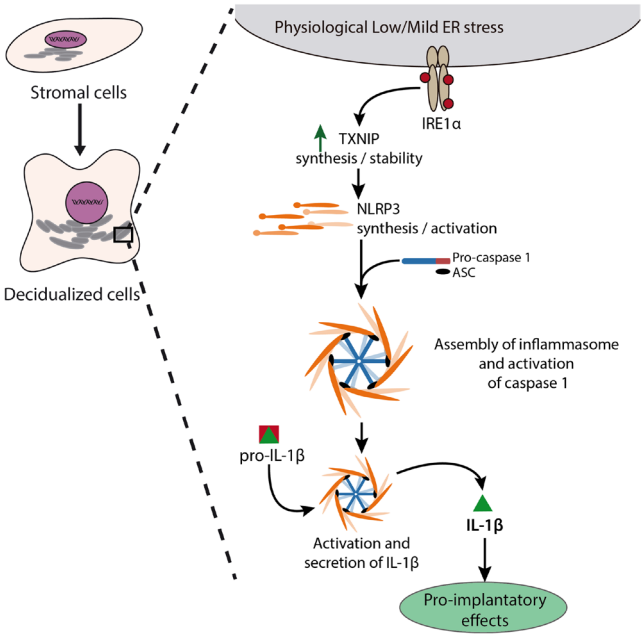

Figure 2 Impact of the ER stress and UPR in an *in vitro* model of decidualization. Human endometrial stromal cell line (HESC) after decidualization increased the ER stress-sensor IRE1α and TXNIP (thioredoxin interacting protein) associated with inflammasome activation. Particularly, increased NLRP3 (NOD-, LRR-, and pyrin domain-containing 3) expression and caspase-1 activation an IL-1β production with pro-implantatory effects.

cells reduced the ability of the BLS to invade them, we inferred that at least low/middle levels ER stress/UPR are necessary to condition the decidual cells in their receptivity.

miRNAs as regulators of the ER stress and UPR in reproduction

miRNAs are a class of short non-coding RNAs that introduce an additional level of regulation of gene expression. The fact that miRNAs selectively regulate expression at the post-transcriptional level makes them a perfect tool for the modulation of signaling pathways subsequent to RNA synthesis. The miRNAs regulate protein expression through two distinct mechanisms: repression of mRNA translation and mRNA degradation. miRNAs participate in a large number of biological processes. Particularly, the UPR modulation is a very interesting field of study: since UPR decrease cellular protein levels without modulating the corresponding mRNAs, the participation of miRNAs in this process gains relevance (Wang *et al*. 2015).

It has been reported that ER stress and UPR are directed by miRNAs which regulate the expression or stability of the transcription factors involved in their pathways (Aragón *et al*. 2009, Lerner *et al*. 2012). Related to this, the miRNAs involved in the UPR can be classified into proadaptive and proapoptotic groups. The first group helps to reduce the load of ER by increasing its folding capacity. The second group of miRNAs are involved in processes like programmed cell death event including cytoskeletal disruption, cell shrinkage, and DNA fragmentation, among others. Particularly, IRE1α can degradate the following miRNAs: miR-17, miR-34a, miR-96a, and miR-125b (Byrd & Brewer 2013).

Recently, miRNAs have gained relevance in reproduction. On one hand, different studies have reported that hormones affect the expression patterns of miRNAs in stromal endometrial cells (Kuokkanen *et al*. 2010). Moreover, it was found that several miRNAs, such as miR-21 and miR-30, were significantly modulated during the receptivity windows (Sha *et al*. 2011, Altmäe *et al*. 2013). These miRNAs are involved in the regulation of transcription, cell proliferation, and apoptosis among other processes (Sha *et al*. 2011, Altmäe *et al*. 2013). Interestingly, Tochigi *et al*. reported that the decrease of miR-542-3P is required to allow the expression of IGFBP1, PRL, and WNT4 and therefore to allow the decidualization of stromal cells, highlighting the role of miRNAs in the regulation of this process (Tochigi *et al*. 2017). On the other hand, recent studies attempted to map them ('miRNA signature') in the process of implantation and placentation (Wang 2008, Wang *et al*. 2015). Wang *et al*. analyzed the human microRNAomes between normal pregnant and miscarriage deciduas from spontaneous abortions and they found an increase in miR-199b-5p expression in the latter. miR-199b-5p was predicted to target *SGK1* (serum/glucocorticoid-regulated kinase) which regulates transport, hormone release, cell proliferation, and apoptosis, and it is important to the pregnancy maintenance (Fisher & Giudice 2011). In line with these results, the authors found a significant inverse correlation between miR-199b-5p and SGK1 *in vivo* and *in vitro* (Wang *et al*. 2015).

In RIF endometrial samples, the analysis comparing miRNA expression profiles identified 13 miRNAs differentially expressed that putatively regulate the expression of 3800 genes. Particularly, ten miRNAs such as miR-145, -23b, and -99a were overexpressed and associated with different molecular pathways such as adherens junctions, cell adhesion molecules, Wnt-signaling, p53 signaling, and cell cycle pathways (Revel *et al*. 2011). Even though RIF-associated miRNAs are promising new candidates for diagnosis of embryo implantation failures, further studies are required to evaluate the physiological role of miRNAs in the modulation of gene expression associated with endometrial receptivity.

In response to a variety of stimuli, miRNAs can be packed and released by endometrial epithelial and stromal cells. In fact, miRNAs can be found extracellularly in plasma and other body fluids and appear to mediate cell-to-cell communication. During the course of embryo implantation, extracellular vesicles

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(EVs) display different protein and miRNA cargo (Kurian & Modi 2019). Endometrial EVs could potentially control trophoblast physiology and promote cell proliferation and angiogenesis (Kurian & Modi 2019). However, the relevance of physiological alterations in EVs cargo in endometrial cells induced by different stimuli such as inflammatory mediators, ER stress, and UPR are still unknown.

It was reported that miR-141 is upregulated in preeclamptic placentas, regulating trophoblast invasion, and intercellular communication. Furthermore, elevated levels of miR-141 can be transferred from trophoblast to immune cells by release and internalization of EVs, suggesting their role in the immune regulation in both normal and pathological pregnancies. These findings also have a translational significance, since the analysis of miR-141 overexpression and EVs in maternal blood sample may serve as a noninvasive test for the detection of early serum markers of PE (Ospina-Prieto *et al*. 2016).

All these studies demonstrate that miRNAs are involved during the whole reproductive process, which shows their potential as biomarkers not only for optimizing *in vitro* fertilization treatments but also for detecting fertility and pregnancy complications.

Alterations in the decidualization process due to dysregulation ER stress and UPR condition the endometrial receptivity

Implantation represents a critical step for the success of *in vitro* fertilization. Even considering embryo quality, it has been estimated that 50% of human embryo implantations result in a failed pregnancy, highlighting the uterine contribution (Holmberg *et al*. 2012). Related to this, alterations in the decidualization process prevent the correct expression of a receptive phenotype, affecting the natural embryo selection and being associated with RIF (Salker *et al*. 2010).

Recent evidence showed that defects in decidualization could condition future pregnancies. In women with severe PE, Gómez-Garrido *et al*. demonstrated the presence of alterations in the decidua at the time of delivery that had persisted for years (Garrido-Gomez *et al*. 2017). Defective decidualization on severe PE reflects the maternal contribution to the etiology of this syndrome and is associated with a particular transcriptomic profile. This transcriptional signature could be detected before (or after) conception, which might contribute to the development of therapies focused on improving stromal decidualization.

Regarding to the relevance of the ER stress/UPR in endometrial preconditioning, we have previously evaluated the relevance of the IRE1α pathway in patients with RPL and RIF. Endometrial samples from patients with RPL displayed increased IRE1α, TXNIP, and NLRP3 expression compared with fertile women. In fact, we observed a positive correlation with IL-1β expression in endometrial cells. On the other hand, RIF patients displayed a reduction in sXBP1, TXNIP, and NLRP3 endometrial expression in comparison with fertile women (Grasso *et al*. 2018). These results highlight different endometrial profiles between RPL and RIF patients. In RPL patients, the blastocyst implants and then pregnancy is lost, while the endometrium of RIF patients is not permissive for blastocyst implantation independently of its quality. In fact, this observation is in accordance with our *in vitro* results, where the prevention of the ER stress/UPR reduced the invasion index suggesting that these processes are required for blastocyst invasion (Grasso *et al*. 2018).

Interestingly, in the same way that implantation requires inflammation yet excessive inflammation causes pathologies, and an excess of ER stress could also be associated with pregnancy complications: in placenta, Yung *et al*. provided evidence for a difference in the UPR pathway activation between patients with early PE (<34 weeks) and those with late PE (≥34 weeks) (Yung *et al*. 2014). These findings support the concept that cases of early-onset PE are associated with the activation of placental ER stress/UPR. They reported an increase in the ER stress/UPR pathways activation involving IRE1α, ATF6, XBP-1, and GRP78 in comparison with normotensive controls. In fact, hypoxia–reoxygenation can strongly induce ER stress in trophoblastic cells with an impact on the trophoblast cells proliferation in the etiology of human intrauterine growth (Yung *et al*. 2008). In this sense, using BeWo cells as a model of syncytialization, the severity of hypoxia–reoxygenation increased the activation of the ER stress/UPR pathways, displaying slow cell proliferation rate in trophoblast-like cells (Yung *et al*. 2012).

Another interesting point is the link between ER stress/UPR with angiogenesis, and hence we proposed that proper induction of these processes will allow the production of pro-inflammatory and angiogenic factors associated with a successful decidualization and the later placentation (Fig. 3). Until now, most of the studies linking UPR with angiogenic cascades have focused on VEGF-A (vascular endothelial growth factor A), the best characterized pro-angiogenic factor. The transcription factors of the three branches of the UPR have consensus sites in the VEGF A promoter inducing its production (Binet and Sapieha, 2015). Particularly, sXBP1 binds in at least two regions of the VEGF A promoter (Pereira *et al*. 2010) and ATF4 binds in the promoter region characterized by four amino acid response elements (AARE) (Roybal *et al*. 2005). In fact, other factors with vasomodulatory properties are regulated by the UPR. For example, ATF4 can transcriptionally modulate IL-8 in several endothelial cell lines from human aorta in response to oxidized phospholipids (Gargalovic *et al*. 2006). IL-8 is, among other functions, a pro-angiogenic cytokine that stimulates the proliferation of endothelial cells and the formation of capillary tubes (Li *et al*. 2003).

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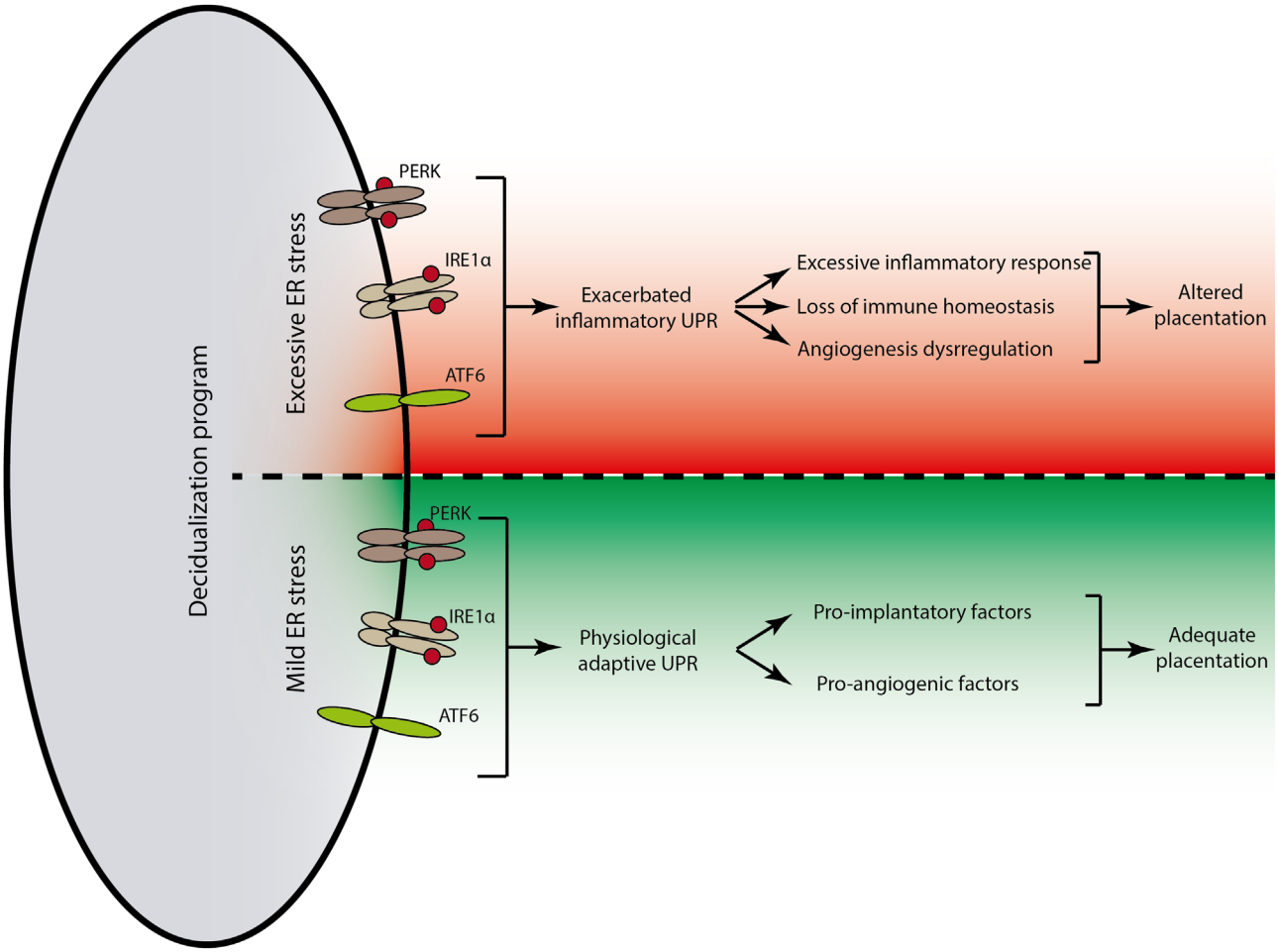

Figure 3 ER stress and UPR levels could condition decidualization and placentation: A proposed model. Since ER stress and UPR are linked with the induction of a sterile inflammation and angiogenesis, we propose that proper induction of these processes will allow the production of pro-inflammatory and pro-angiogenic factors associated with a successful decidualization and later placentation.

In that sense, it was recently shown that basal decidua tissues of patients with RPL contained more vessels (arteries, veins, and lymphatic vessels) than fertile women (Windsperger *et al*. 2017). On the other hand, a higher density of blood vessels was also identified in the secretory endometrium of non-pregnant patients with RPL (Quenby *et al*. 2009). In line with these results, angiogenic factors expressed were also elevated both in the secretory endometrium and in the basal decidua (Plaisier *et al*. 2008). Therefore, it was consistent that an increased angiogenesis in the secretory phase higher density of vessels in the endometrium would compromise the ability of the endovascular trophoblast to invade the luminal structures of the uterus in patients with RPL. Even though there is an association between alterations in the vascular pattern of the decidua from RPL patients and ER stress and UPR processes, the mechanisms involved are still unknown.

Finally, since some ER stress and UPR pathways contribute to a range of diseases, there is growing interest in developing new therapeutic strategies aimed at interfering with these processes (Binet & Sapieha 2015). In recent years, several classes of small-molecule drugs have been designed to modulate UPR signaling as repressors of the pro-apoptotic arm of the UPR for treatment of chronic diseases (Ozcan *et al*. 2006, Moreno *et al*. 2013) and suppressors of the UPR's pro-survival properties for treatment of cancer (Mimura *et al*. 2012, Atkins *et al*. 2013). Pharmacological modulators of the UPR offer new prospective therapies in models of chronic neurodegenerative disease and as a anti-tumor chemotherapeutic agent (Papandreou *et al*. 2011, Moreno *et al*. 2013). Considering that ER stress and UPR might condition endometrium for implantation, UPR modulators might be promising; however, further basic research and validation of decidualization *in vitro* models will be required to determine their benefits.

Translational impact

The ability of the human endometrium to generate an adequate decidual response based on successive

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inflammatory events might contribute to a sensitization of the uterine tissues. Under this hypothesis of repeated inflammatory events, a tight immune homeostatic control prior to implantation is required (Kwak-Kim *et al*. 2009, Weiss *et al*. 2009).

Research in the last 10 years provided a better understanding of the decidua-blastocyst crosstalk; however, there are a number of unanswered questions in many aspects as the earliest triggers of the decidualization, the nature and modulation of embryo signals that modulate the decidual secretome, and the intercellular network between decidualization, inflammation, and angiogenesis.

The possibility of deepening into regulatory mechanisms associated with decidualization and embryo implantation could contribute from basic research to the identification of biomarkers, to develop novel therapeutic strategies as well as the optimization of treatments currently used in assisted reproduction. In particular, the identification of biomarkers focussing on the 'miRNA signature' of the decidualization and its modulation in patients with reproductive failures might explain the post-transcriptional regulation of the ER stress/UPR and the molecular processes that prevent implantation. Getting deeper into immunological mechanisms involved in embryo implantation would have major implications for patients with reproductive failures; however, further clinical studies are required.

Finally, although many implantation factors are evolutionarily conserved, there are differences between species which highlights the importance to develop new *in vitro* models for the study of the earliest events during implantation processes that condition the development of pregnancy (Ramathal *et al*. 2010, Xiong *et al*. 2016, Zhao *et al*. 2017). We hope that future advances in research models will elucidate the molecular mechanisms of pathologies associated with implantation and placentation, and thus, obtain potential biomarkers, as well as improve therapeutic strategies for reproductive failures.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

C P L and R R designed the study, supervised the experimental work, and wrote the manuscript. E S, E G, and S G and carried out all the experiments with the decidualizated cells. L F and L G performed the inflammasome activity-assays. L G and V H processed experiments in mice (VIP-KO, VIP-HT, and WT mice). E S, E G, D P, and S G did the RT-PCRs data analysis and interpretation. C P L and R R supervised the whole study. All authors read and approved the final manuscript.

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