Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways

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Introduction

Preeclampsia is recognized and currently defined as a syndrome, meaning a set of clinical signs that tend to occur together. A key feature is that the same syndrome may be recognized by varying combinations of features. Some associations may be so closely linked with a pathogenesis or cause that they may be interchangeably termed a syndrome, disease, or disorder. When the pathogenesis is known, it is better to label the condition more specifically than simply labeling it as a syndrome, which may arise from different pathogenic processes. Here, we present implications of the syndromic nature of preeclampsia. We (C.W.G.R. and A.C.S.) present a specific hypothesis that early-onset and late-onset preeclampsia use different pathophysiological pathways. It is likely that there are additional routes to the syndrome and suggestions are made as to how these subtypes might be deciphered. Together, we consider the research and clinical implications of these concepts.

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Preeclampsia evolves in 2 stages: a placental problem that generates signals to the mother to cause a range of responses that comprise the second stage (preeclampsia syndrome). The first stage of early-onset preeclampsia is poor placentation, which we here call malplacentation. The spiral arteries are incompletely remodeled, leading to later placental malperfusion, relatively early in the second half of pregnancy. The long duration of the first stage (several months) is unsurprisingly associated with fetal growth restriction.

The first stage of late-onset preeclampsia, approximately 80% of total cases, is shorter (several weeks) and part of a process that is common to all pregnancies. Placental function declines as it outgrows uterine capacity, with increasing chorionic villous packing, compression of the intervillous space, and fetal hypoxia, and causes late-onset clinical presentations such as “unexplained” stillbirths, late-onset fetal growth restriction, or preeclampsia.

The second stages of early- and late-onset preeclampsia share syncytiotrophoblast stress as the most relevant feature that causes the maternal syndrome. Syncytiotrophoblast stress signals in the maternal circulation are probably the most specific biomarkers for preeclampsia. In addition, soluble fms-like tyrosine kinase-1 (mainly produced by syncytiotrophoblast) is the best-known biomarker and is routinely used in clinical practice in many locations.

How the stress signals change over time in normal pregnancies indicates that syncytiotrophoblast stress begins on average at 30 to 32 weeks’ gestation and progresses to term. At term, syncytiotrophoblast shows increasing markers of stress, including apoptosis, pyroptosis, autophagy, syncytial knots, and necrosis. We label this phenotype the “twilight placenta” and argue that it accounts for the clinical problems of postmature pregnancies.

Senescence as a stress response differs in multinuclear syncytiotrophoblast from that of mononuclear cells. Syncytiotrophoblast irreversibly acquires part of the senescence phenotype (cell cycle arrest) when it is formed by cell fusion.

The 2 pathways converge on the common pathologic endpoint, syncytiotrophoblast stress, and contribute to preeclampsia subtypes. We highlight that the well-known heterogeneity of the preeclampsia syndrome arises from different pathways to this common endpoint, influenced by maternal genetics, epigenetics, lifestyle, and environmental factors with different fetal and maternal responses to the ensuing insults. This complexity mandates a reassessment of our approach to predicting and preventing preeclampsia, and we summarize research priorities to maximize what we can learn about these important issues.

Key words: apoptosis, autophagy, big data, cell stress, chorionic villous crowding, competing risks, endoplasmic reticulum stress, fetal death, fetal growth restriction, maternal factors, mitochondria, oxidative stress, placental senescence, pyroptosis, term stillbirths, subtypes

The Components of the Syndrome

A mandatory feature of preeclampsia is its association with pregnancy, ante-partum, intrapartum, or, over a short period, postpartum. It is usually symptomless. When symptoms occur, they can be severe and are a feature of a terminal crisis, which may be fatal to both the mother and fetus. Maternal seizures or eclampsia were recognized more than 2000 years in late pregnancy and delivery. Only recently were they distinguished from epilepsy.1 When it became possible to measure urinary
protein and blood pressure, proteinuria and hypertension were recognized, first as features of eclampsia and later as symptomless precursors of it, termed “toxemia of pregnancy” or preeclampsia. Hence, there emerged 4 components of an ill-defined maternal syndrome, pregnancy itself, hypertension, proteinuria, and eclampsia, of which the last was not mandatory. For some time, generalized edema was included but discarded 40 years ago. Until recently, these presentations were deemed primary maternal problems.

It is unclear when the term “preeclampsia” was first used. Eclampsia itself was so dangerous and unpredictable that it dominated management until at least the middle of the last century. Even as late as the 1930s, hypertension was considered an inconsequential feature and therefore not sought assiduously. It was only in the 1950s that it was appreciated that severe hypertension caused cerebral hemorrhages in nonpregnant cases. The systematic treatment of pregnancy hypertension began in the 1960s and remains an essential part of its management.

How Placental Involvement in Preeclampsia was First Recognized

Preeclampsia or eclampsia was originally thought to be a toxemia secondary to waste products produced by the fetus, from which the concept of eclamptogenic toxemia was derived to indicate the maternal syndrome that warned of the ensuing convulsions.

However, as early as 100 years ago, the placenta was recognized as central to this process. Very early observations indicated specific and initially unexplained placental features. Possibly the first was the association of preeclampsia with hydatidiform mole (recounted in Chesley 1978), citing the first report in 1866 and then of the discovery of trophoblastic embolism, or “deportation,” to the lungs of women dying of eclampsia (in Chesley 1978). The importance of the placenta to the occurrence of eclampsia was recognized more than 100 years ago (Figure 1). The author stated, “. . . one can be left in no doubt whatever that the disease in the placenta is secondary to the deprivation of the maternal blood supply.” Reproduced with permission from Young.


The pathology of the placental bed in preeclampsia was described in detail from cesarean hysterectomy samples. These authors found that the distal ends of the spiral arteries in normal pregnancy are remodeled and enlarged in association with invasion by extravillous cytotrophoblast derived from the base of the anchoring columns. This feature of placentation occurred from 8 to 16 weeks of pregnancy but was restricted in preeclampsia. From there, the concept was developed that malplacentation (early pregnancy), by its effect on spiral artery remodeling, could adversely affect placental perfusion and be a cause of preeclampsia. It seemed that events early in an apparently normal pregnancy determined disease much later.

Development of the 2-Stage Model of Preeclampsia

The 2-stage model of preeclampsia was then formalized. Stage 1 was composed of malplacentation, whereas stage 2 was the clinical syndrome secondary to poor uteroplacental perfusion. Numerous studies sought to determine the mechanisms of cytotrophoblast invasion and spiral artery remodeling. Although the 2-stage model has persisted, it has always been imperfect. First, the focus on trophoblast invasion restricted the interest to the end stages of placentation, whereas it represents a more profound problem of inadequate expansion of the primary extravillous cytotrophoblast, from as early as 2 weeks after conception. This leads to a spectrum...
of clinical problems from early pregnancy failure to preeclampsia and growth restriction. In the first 6 to 8 weeks of pregnancy, the spiral arteries are blocked by intraluminal extravillous cytotrophoblast so that the placenta is sustained at low oxygen tensions by histiotrophic nutrition. Unblocking of the spiral arteries begins at approximately 8 weeks and relatively quickly imposes an increased oxygen tension on the villous tree, bringing the associated risks of damage from oxidative stress. These are countered by increasing the effectiveness of antioxidant defenses with gestational maturity. However, if the arteries unplug prematurely, which is likely if the mass of extravillous cytotrophoblast is reduced, then placental growth is impaired at this critical time because of oxidative stress, not malperfusion. Hence, placenta is not just spiral artery invasion but the whole spectrum of placental growth (from the start of pregnancy), at and after implantation (Figure 2). In essence, the development of the placenta starts poorly and never catches up. This is the major reason why early-onset disease is easier to predict than late-onset condition, in which the placenta grows normally to begin with but has ended of pregnancy problems.

The original 2-stage hypothesis described preeclampsia as the sequel to poor placentation. However, malplacenta cannot be the sole cause of preeclampsia because fetal growth restriction (FGR) is not a consistent part of the syndrome. In fact, most cases show little or no FGR. This issue was first addressed by the concept that preeclampsia may be maternal or placental in origin. Maternal preeclampsia typically would occur at term and result from an interaction between a healthy placenta and a mother with preexisting disorders such as cardiovascular disease (CVD) or obesity. It has always been
known that maternal medical problems contribute to the risk of preeclampsia, but the placenta is a mandatory component. The concept was consistent with reports that there was little obvious placental pathology in late-onset disease. From the counterparts than that of matched bled that of their preeclamptic disease. Placental pathology in late-onset disease was deemed distinct subtypes. In addition to FGR, early- and late-onset disease is more severe than late-onset disease in other aspects, such as maternal mortality. The threshold between the 2 types was set to be before or after 34 weeks’ gestation. In practice, the time of onset of preeclampsia is rarely known precisely, so the time of delivery serves as a surrogate. It should be remembered that the natural outcome of preeclampsia has been heavily modified by clinical practice for the last 50 to 60 years. Early features of preeclampsia at term can be quickly and safely resolved by elective delivery. Without such care, late-onset disease would be less benign than it is now. However, even today, it can still kill and does so in low-resource settings.

By the turn of the millennium, there were other advances in the 2-stage model. Even if the factors released from the dysfunctional placenta were not identified, a maternal tissue was targeted—the maternal endothelium. From the mother’s perspective, this was not just a hypertensive disorder, but one of diffuse endothelial dysfunction—later termed “vascular inflammation.” The protean features of preeclampsia could be unified mechanistically, and the association of this dysfunction with an excessive systemic inflammatory response was less surprising. In addition, what was startling was that it was also well developed in the third trimester of pregnancy in normal, pregnant, control women, whose inflammatory profile more closely resembled that of their preeclamptic counterparts than that of matched normal nonpregnant control women.

The functional link between the placenta and maternal endothelial dysfunction was clarified by the seminal discovery of the angiogenic imbalance caused by an excessive circulating soluble receptor, the vascular endothelial growth factor-1 (VEGF-1), also known as the soluble fms-like tyrosine kinase-1 (sFlt-1). A decoy factor, it inhibits the actions of the VEGF and that of its cousin, the vascular placental growth factor (PIGF), both essential for vascular endothelial health. Both sFlt-1 and PIGF are produced in large amounts by the placenta. More important to this review, they are, specifically, products of the syncytiotrophoblast (STB).

Even though this was a critical step and an increased number of villi forward, there was still a major problem. If late-onset preeclampsia is associated with neither FGR nor obvious placental pathology, can uteroplacental malperfusion be the underlying pathology? If it is not, what could be the alternative? Further progress depended on acquiring a better understanding of term and postterm pregnancies.

“Normal Pregnancy” at Term—Not as Normal as it Seems

The problems of postterm pregnancies have been documented for 70 years, first in the context of apparently unexplained stillbirths and later with what was called “dysmaturity.” The latter was a phenotype associated with postterm births, infants born with lower oxygen saturations, lower birthweights, and increased hemoglobin concentrations. At this time, the concept of FGR was not formulated, but there is no doubt that this was the problem. Its extreme forms have disappeared because of routine elective delivery of postterm pregnancies. However, milder degrees persist. Just as there is early- and late-onset preeclampsia, there is also early- and late-onset FGR. Furthermore, the incidence of late-onset preeclampsia and eclampsia increases with gestational age, being 3 times more common at 40 than at 37 weeks’ gestation. What causes these late pregnancy problems? We can learn more from ultrasound Doppler measurements of the utero- and fetoplacental circulations.

During the third trimester of pregnancy, uterine artery Doppler pulsatility changes a little, even in postterm pregnancy, which suggests that the uteroplacental circulation is stable and not likely to cast light on this problem. However, the fetal circulation clearly changes. The umbilical artery pulsatility index declines steadily throughout the second half of pregnancy compatible with a progressive increase in fetoplacental impedance. Even more interesting is that the fetal middle cerebral artery (MCA) pulsatility index peaks at approximately 30 weeks’ gestation and declines after that. The cerebroplacental ratio (CPR) between the indices of the MCA and the umbilical artery follows a similar pattern, interpreted to indicate the increasing redistribution of blood flow to the cerebral circulation and an early sign of fetal stress. A low ratio, for example, is associated with an impaired outcome, although its clinical usefulness is not yet clear.

What underlies these major changes in the fetal circulation? They develop during a time of massive growth in the chorionic villous tree with an expansion of its villous tree, which is associated with a steady decline in umbilical and intervillous partial pressure of oxygen (pO2). A low pO2 was long ago discovered to be a feature of dysmaturity at term. We have confirmed this trend by analyzing umbilical arterial pO2 in elective cesarean delivery in postterm pregnancies (unpublished observations). The cause of this progressive hypoxia is unknown. Therefore, we extended our previous proposal to suggest that it arises from increasing demands of a placenta expanding into a limited space. First, expansion of the chorionic villous tree increases the resistance of the fetoplacental circulation reflected in the declining umbilical arterial pulsatility index. Second, the packing density of the chorionic villi increases with gestational age. Finally, mathematical modeling suggests that the perfusion of a placenta becomes restricted as the packing density increases beyond its optimal value, perhaps intensified by increasing maternal plasma viscosity at the end of pregnancy.

Placental Pathology

Generally, the placental pathology of early-onset preeclampsia is more severe
than that of late-onset disease, when routine histopathology is usually unhelpful. For the former, gross features include placental infarction spiral artery thrombosis or the occlusive lesions of acute atherosis, which are deemed the sequelae of malplacentation and the associated underlying spiral artery disease. They also include pathology in the intervillous space and the chorionic villi, related to hypoxia, summarized in previous reports. Associated pathologies include distal villous hypoplasia, villitis of unknown etiology, and massive intervillous fibrin deposition. The more obvious features can be ascribed to spiral artery maladaptation during placentation. However, others involving the STB compartment have been previously overlooked.

**Syncytiotrophoblast Pathology**

Classical histopathology reveals the tip of the iceberg of pathologic changes in STB. Electron microscopy or immunohistochemistry with or without confocal microscopy adds resolution and precision. Their application has revolutionized the understanding of STB in preeclampsia. The studies described below concern the STB in late pregnancy and avoid in vitro work on trophoblast cell lines or choriocarcinoma or extravillous invasive cytotrophoblast in early pregnancy.

The STB is the major interface between the mother and placenta. Its pathology is superimposed on changes in the underlying villi, which may include accelerated maturation, syncytial knots, perivillous fibrin deposition, and villous agglutination. These are considered to indicate maternal malperfusion and are typical of preeclampsia, especially of early onset.

Electron microscopy reveals patchy necrosis, distorted or scanty microvilli, swollen endoplasmic reticulum (ER) typical of ER stress, and mitochondrial swelling. These changes are considered to indicate maternal malperfusion and are typical of preeclampsia, especially of early onset.

Cellular Stress

Cellular survival is a balancing act that sustains normal functions and coexistence with neighboring cells in terms of 2-way signaling and integration with the total physiology of the organism. It is inevitably complex. Many factors constantly threaten homeostasis; if the threat is substantial, it constitutes cell stress and may lead to cell death.

There are different stresses, both internal and external, ranging from perturbations of energy supply to trauma or infection. The molecular mechanisms by which these stresses are countered to restore homeostasis constitute the cellular stress response. Because of their intricacy, they are commonly studied in a compartmentalized way to make progress. In fact, a major cellular stress will change or impede many cellular functions and stimulate various responses at the same time. There are 4 outcomes to a stress response (Figure 4): normality is restored; the cell does not recover and becomes chronically stressed, which is characteristic of senescence; or it undergoes programmed cell death or, in the most extreme case, disintegrates (necrosis).

FIGURE 3

Increasing fetal and placental hypoxia with advancing gestational age

These data were derived from blood samples taken from 200 cases using fetoscopy or cordocentesis for diagnostic purposes. Cases with fetal growth restriction, hydrops, or rhesus disease were excluded. There was no difference attributable to the method of sampling. Means (95% confidence intervals) are shown for intervillous, umbilical venous, and umbilical arterial samples, plotted by gestational age. Reproduced with permission from Soothill et al.29

The surface of the placenta. Its cellular covering the entire folded microvillous structures can redirect metabolic programs or change their overall endpoints. As far as it is known, STB is 1 huge cell that has been demonstrated to involve the syncytium in relation to preeclampsia. We focus on the pathology of placental tissue (minced) does not resolve STB from other cell types. Immunohistochemistry with confocal microscopy allows higher resolution to demonstrate specific DNA, RNA, or proteins in subcellular organelles. These can be confirmed in experiments with cultured placental explants that are exposed to specific stresses, such as hypoxia-reoxygenation or molecular inducers of ER stress. A major challenge is to be able to distinguish cytotoxicity from STB. The latter is formed by fusion with the former, which may donate markers to STB, which re-occurs from STB. The mitochondria and ER are, respectively, the powerhouse and protein factories of the cell, and both generate reactive oxygen species (ROS). ROS are unstable, highly reactive free radicals that can be induced in vitro by hypoxia or reactive oxygen species. How they are formed and what their pathophysiological function are unknown. Some consider that they are a type of STB apoptosis (programmed cell death—see the section ‘Autophagy, Apoptosis and Necrosis’ below). Others consider that they have other functions because some knots are transcriptionally active and that the STB nuclei do not turn over but accumulate in the syncytiotrophoblast of preeclampsia. Syncytial knots are important to this review because they clearly demonstrate that placental dysfunction and pathology are not confined to early-onset preeclampsia. Because they increase with gestational age and are commonplace in term placentas without preeclampsia or FGR, they have, for many years, been disregarded as “normal.” In fact, they are important as indicators of the hidden stresses of an apparently uncomplicated term pregnancy.

### Syncytiotrophoblast Stress

#### Sprouts and knots—unique syncytial features

Syncytial sprouts and knots are 2 types of syncytiotrophoblast multinuclear aggregates that are readily seen by simple microscopy. The former are composed of outgrowths from the syncytial surface, are transcriptionally active and normal structures, and are present from the early stages of pregnancy. Syncytial knots are clusters of transcriptionally inactive nuclei (a marker of stress) that increase with gestational age and with disorders of maternal malperfusion, such as preeclampsia. They can be induced by hypoxia or reactive oxygen species.
respond quickly to stress, ROS levels can increase dramatically and oxidatively damage its contents.

Cells are powered by oxidative phosphorylation to produce adenosine triphosphate (ATP), a process that is expedited by high energy electron transfers within the mitochondria. Electrons are agents of redox reactions. If a molecule loses an electron, it is oxidized; if it gains an electron, it is reduced. This is the basis of oxidative phosphorylation. Because of their high energy, electrons must be contained within the mitochondrial matrix, shielded from other cell contents by a double membrane. Free radicals, containing unpaired electrons, are powerful oxidants by virtue of electron donation. If they leak out of the safe confines of the mitochondria, they can damage cell molecules in a relatively indiscriminate way. This may reduce or enhance molecular functions or even induce new (and unwanted) functions. This is oxidative stress. It is not simply hypoxia. As is well known, cellular antioxidants neutralize these activities, but when overwhelmed, oxidative stress occurs. Nitric oxide is another hyperreactive free radical that can cause damage by nitrosative stress, analogous to oxidative stress.

**Syncytiotrophoblast, Organellar Stress, and Preeclampsia**

The concept of oxidative stress in preeclampsia began to evolve 30 years ago and was expanded as the techniques for its study improved. Oxidative stress of the placenta will extend to all cells in the chorionic villus and, in most instances, include the STB. Thus, one of the first studies showed the content of lipid peroxides (products of oxidative stress) in STB membranes as measured by its marker, malondialdehyde, to be higher in preeclampsia samples at term than in normal control samples. Expanded interest lead to various therapeutic trials of antioxidants to counter the stress and help prevent the disorder. Now, oxidative stress is well documented as part of the pathology of the placenta. What is important for this review is that STB is affected. This is shown in terms of several markers, such as 4-hydroxynonenal, which indicates oxidative stress in DNA, mostly in the syncytial nuclei or, finally, advanced glycation end products, which are formed by oxidative stress in the context of hyperglycemia. The causes of such stress are likely to include intermittent oxygenation intrinsic to the complexities of perfusion through the crevices of the intervillous space in which the placental villi are packed at increasing density as gestation progresses.


On the left are lists of some of the many stresses to which cells must respond to maintain homeostasis. On the right are the final outcomes that may ensue.
DNA damage (nuclear stress) is typically caused by oxidative stress and may be lethal. If the cell cannot repair the DNA, it loses its ability to divide (cell cycle arrest) and its normal function to renew the tissue of which it is a part. Alternatively, the cell may survive with a malignant mutation that could kill the organism. Hence, the DNA damage response is critical. It is well characterized particularly in terms of the tumor suppressor factors, of which p53 is the best known. Their main function is to stop the faulty cell from proliferating (cell cycle arrest) or to remove the cell by apoptosis.

Tumor suppressor proteins are a subset of transcription factors that bind to one or several DNA sequences, in 1 or more genes, to regulate their transcription, frequently coordinated with other transcription factors regulating the same gene. They can therefore promote pleomorphic responses that vary according to context. By inhibiting cell division and promoting apoptosis, they limit the development of mutated malignant cells. These attributes are demonstrated by p53, which apart from inhibiting cell proliferation can promote apoptosis, autophagy, or other parts of the stress response and is up-regulated in STB under stress conditions.

Apart from the UPR, the increasingly stressed cell may resort to autophagy or, in extreme cases, undergo apoptosis.

**Autophagy, Apoptosis, and Necrosis**

As part of a rescue program, the ER or mitochondrial stress can stimulate autophagy, which is a form of cellular retrenchment to cope with troubled times. It is an intracellular process. Damaged organelles and other dysfunctional protein structures are removed through degradation in lysosomes. These can be recycled to conserve energy or excreted from the cell. Autophagy has been demonstrated in the STB of preeclamptic placentas and in the related condition of normotensive FGR. It is therefore part of the syncytial stress response. Mitophagy, autophagy of the mitochondria, is an important subset of
autophagy, which has not yet been fully investigated in relation to normal or preeclamptic pregnancies.

If normality cannot be restored, then the cell may be directed to apoptosis, as summarized elsewhere, which is a type of programmed cell death that may be triggered by extracellular or intracellular mechanisms. Apoptosis involves a cascade of proteolytic enzymes (caspases) that deconstruct the cell in a controlled way into subcellular membrane-bound vesicles (including pieces of fragmented nuclear DNA) that are released as apoptotic bodies. Because they retain the outer cell membrane, they are relatively noninflammatory, which facilitates simple removal by phagocytic cells. Apoptosis needs energy to be completed. In extremis, if the energy supply fails, then the cell falls apart in an uncontrolled way (necrosis), spilling its remnants into its extracellular environment.

ER and mitochondrial stress are potent causes of apoptosis. This involves the intrinsic pathway of apoptosis and is mediated by what is called the mitochondrial permeability transition. The mitochondria swell, and the outer membrane may rupture with the loss of cytochrome C into the cytoplasm. This is a strong apoptotic signal, especially as it is combined with depletion of ATP because of loss of mitochondrial function.

Syncytiotrophoblast and Apoptosis

It seems counterintuitive that one vast syncytiotrophoblast could fuse with, and deliver apoptotic markers to, its syncytial neighbor, inappropriately suggesting primary apoptotic activity in STB. The current evidence is that the STB displays markers of apoptotic activity, such as neoantigen M30, positive TUNEL labeling, apoptosis protease activating factor-1 (APAF-1), caspases 3 and 9, and electron microscopic evidence of nuclear fragmentation. Such activity becomes more evident toward term in normal placentas or with pre-eclampsia. There are different forms of programmed cell death other than apoptosis. They include pyroptosis, netosis, and oncosis, all of which concern the fate of single mononuclear cells. Of these variants only pyroptosis has been studied in pre-eclampsia in relation to STB. Markers of pyroptosis are up-regulated in early-onset preeclampsia, can be induced by oxidative stress experimentally, and are correlated also with STB and ER stress in terms of an UPR. The question of how the pyroptotic program might be executed in a trophoblastic syncytiotrophoblast is not addressed.

Syncytiotrophoblast, Apoptosis, and Fibrin Deposition

In an ex vivo model of STB apoptosis, the importance of limiting the process was demonstrated. In this context, a single focus of apoptosis spreads centrifugally and stops only when it reaches the edges of the syncytial structure. In vivo, such unrestrained STB apoptosis could cause widespread damage to the chorionic villi. However, small syncytial breaks are not uncommon in term placentas, so something limits their spread. This seems to be achieved by perivillous deposits of fibrin or fibrinoid with which the breaks are associated. They cordon off the damage to allow the STB to reestablish its continuity. The association of fibrinoid with STB showing apoptotic features without breaks has been shown by other investigators. In summary, limited apoptosis of the STB is possible because it is restricted behind a barrier of fibrin or fibrinoid.

The involvement of apoptosis with fibrin or fibrinoid has been confirmed by EM histology. However, apoptosis, as it is classically defined, does not occur. A break in the syncytiotrophoblast implies that its interior has been briefly exposed to the extracellular environment. This is an extreme form of cellular stress associated with a rapid influx of Ca2+ ion from the extracellular fluid that activates many stress signals. Membrane repair has been documented in other cell systems. It depends on annexins. Annexin A5 (ANXA5) fills this specific role in STB. Apart from apoptosis, it is also possible that syncytium is damaged when a multinuclear aggregate (sprout or knot) breaks from the villous surface. Syncytial breaks have not been assessed in preeclampsia cases. However, because apoptosis is more prominent in preeclampsia placentas, it would be predicted that the occurrence of breaks would be proportionally more common. An alternative possibility is that they are secondary to maternal immune attack. In favor of this is their association with complement deposition. Not in favor is their relatively rare focal distribution. Maternal immune attack on the placental would likely be more widespread and destructive.

During apoptosis, phosphatidylinerine is exteriorized in the plasmalemma. It is strongly procoagulant but binds to ANXA5, which is an anticoagulant and uniformly present in the plasma membrane of normal STB. ANXA5 forms an anticoagulant protective surface. In preeclampsia, STB labeling for ANXA5 is reduced, implying that the STB is less well protected, possibly as a consequence of its stress.

Secondary Pathology of Syncytiotrophoblast Stress with Apoptosis

Perivillous fibrin deposition is a well-documented placental lesion. It has been classified as part of the pathology of maternal placental malperfusion and is associated with early-onset preeclampsia. Stasis in intervillous blood flow and maternal hypercoagulability may contribute, but it is reasonable to speculate that the fibrin deposition that
seals off focal syncytial damage, secondary to syncytial stress, also contributes. In contrast, massive perivillous fibrin deposition (maternal floor infarction) seems to be an unrelated condition, not specifically associated with preeclampsia but with severe FGR. There is evidence that it represents maternal immune rejection of the fetus. 39

The STB is an immune frontier that separates a competent maternal immune system from her alloimmune child. Immunohistochemistry has demonstrated that, if it is breached, maternal leukocytes can gain access to the stroma of the chorionic villus and its resident fetal macrophages (Hofbauer cells). 109

The alloimmune reactivity that ensues is the basis of villitis of unknown etiology. The sequence of events is under discussion in relation to the role of complement. Complement attack might be the primary cause of syncytiotrophoblast (STB) stress. Their numbers are increased in preeclampsia, as previously summarized. 114 They are potential circulating biopsies of the STB but are a minor part of the total circulating ECVs, of which most are derived from maternal platelets. Although the technology to isolate them from peripheral blood samples is improving, it is still unreliable. A unique feature of pregnancy is the release of multinuclear syncytial vesicles. These are not well studied because they are retained in the maternal pulmonary vasculature. Apart from postmortem studies, they can be detected in uterine vein blood samples taken at cesarean delivery. They may represent deported syncytial sprouts (which are physiological) or detached syncytial knots (indicators of STB stress). Their numbers are increased in preeclampsia and include vesicles with the phenotype of syncytial knots. This is important because it may indicate the shedding of unwanted materials for disposal elsewhere and reflect STB stress.

The potential for STB ECVs to signal to maternal tissues is large. Current research touches only a small fraction of an issue of undiscovered complexity. They can deliver their cargoes to endothelial cells. 117 For example, we find that in preeclampsia, the STB ECVs bear reduced nitric oxide synthase; increased nephrilysin; a peptidase that can cause hypertension, increased trophoblast glycoprotein, which is an example of an oncofetal protein; and, finally, a novel family of non-coding RNA, namely, T halves, which are induced by stress by angiogenin that has not yet been fully characterized in relation to preeclampsia. 122

ECVs are an important component of the STB stress response so they need to be well-characterized preeclampsia as do their cargoes of miRNAs. 123

Senescence

Cellular senescence is a state of permanent cell cycle arrest. The cell cannot reenter the cell cycle in response to external stimuli, such as growth factors, and resists apoptotic stimuli. 124 Senescent cells continue to be metabolically active. They maintain a low-grade inflammatory response (typically secretion of interleukin [IL-6 and IL-8], with what is called the senescence-associated secretory phenotype [SASP], which underlies chronic inflammatory conditions of old age. 16 Senescent tissues have to “make do” with a population of dysfunctional cells that are damaged but retain some useful functions. The lay perception of senescence is of a weakened state. In fact, senescent cells are stable and persistent and can have important barrier functions in, for example, neoplastic tissues. 125

Premature senescence occurs with a long-lasting stress response to external stimuli, which wear the system out faster. Replicative senescence occurs because each cell has its “cell by date” determined by telomeres, which are caps that stabilize the ends of individual chromosome. The caps are progressively eroded during cell division. 126 The degree of shortening depends on how many divisions the cell has completed. 126 Increased shortening exposes chromosomal ends to damage or end-to-end fusion with other telomeres either of which can activate a DNA damage response. Telomeres are sensitive to oxidative stress, which accelerates their dysfunction. This is because they are rich in guanine nucleotides, which are unusually vulnerable to oxidative stress. 127

STB is formed by two processes—fusion and senescence. Cell fusion occurs in other contexts as a stress response, for example, to infection from the measles virus. 146 The human trophoblast depends on a retroviral gene ERVWE1 encoding
Syncytiotrophoblast fusion itself triggers cell cycle arrest, conventionally deemed a feature of senescence. Several markers of senescence (p53, p21, p16, and senescence-associated beta galactosidase [SA-βGal]) have been documented in normal STB of which p16, p21, and SA-βGal progressively increase with maturity, although they remain stable in villous cytotrophoblast, except for SA-βGal, which declines. The endpoint is that the placenta is coated in a resilient and relatively stable cellular barrier and that it is senescent that appears to be essential for its formation and functions. Increased production of inflammatory cytokines is certainly a feature of the preeclamptic placenta, but whether they are produced specifically by the third-trimester STB itself and could be classified as SASP is unclear. In other contexts, long-standing cellular senescence is associated with senescence-associated heterochromatin foci (SAHF), which are clusters of cells with characteristic heterochromatin nuclei. Presumably, the equivalent structures in STB are the syncytial knots, but further characterization is needed. The generation of SAHF depends on a senescence pathway mediated by 2 tumor suppressor proteins, retinoblastoma protein (Rb) and p16. As stated above, p16 is expressed in STB. However, there is only indirect evidence for that of Rb.

If the STB must be senescent to perform its functions, further stress that comes with aging, with the associated damage to the nuclei, which provokes a DNA damage response and impaired organellar functions, such as that of the mitochondria, may contribute to a decline in STB function. These aspects are being better characterized now and together constitute the phenotype of what we call the “twilight placenta.”

The “Twilight Placenta”: What is it, What are its Clinical Consequences, and how is it Diagnosed?

We previously reviewed the evidence that term preeclampsia results from a late decline in placental function. The evolution of this decline has now been clearly documented in samples taken from normal pregnancies at 37 weeks of gestation to postterm pregnancy. Progression from term to postterm pregnancy is associated with larger changes in the STB in terms of lipid and DNA or RNA oxidation and the distribution and size of autophagosomes. The most extreme changes, the phenotype of the twilight placenta, were seen in samples from cases with unexplained stillbirths. The patterns could be replicated in placental explants stressed by culture in a serum-free medium.

A similar pattern was also clearly shown in another study using different markers, including the tumor suppressor protein p21, lipofuscin (formed from oxidized lipids and protein), 8-hydroxy-2’-deoxyguanosine (a marker for DNA and RNA oxidation), and activation of the DNA damage response. That this decline is caused by “senescence” is informative but incomplete. It is clear that senescence progresses in STB, but in terms of cell cycle arrest, STB is always senescent from the moment it forms. Thus, when “markers” of senescence increase toward term, as they do, what does this signify and what causes the added stress? It has been suggested that the stress markers may be “donated” by cytotrophoblast, during fusion. As cytotrophoblast does replicate and must have its own senescence program, this may be reflected in the markers detected in STB. Term is also characterized by increasing hypoxia. This may be caused by, and is certainly associated with, physical constraints on placental growth, which is restricted by the size of the uterus, by increasing the compression of the chorionic villi, and by limits on uteroplacental perfusion. We have previously called this restricted placental capacity. These limits would create the stresses that we have highlighted and cause cytotrophoblast senescence.

We propose the hypothesis that the twilight phase immediately precedes STB failure when its renewal by fusion with cytotrophoblast is no longer enough. There is circumstantial evidence that we have cited, however, direct evidence needs to be sought. The clinical correlates are clear: increasing preeclampsia, late-onset FGR, and “unexplained” stillbirth are all well-documented consequences in postterm pregnancies.

How many pregnancies end with a twilight placenta is not known. Much clinical research is based on comparisons between normal and abnormal. However, at term, what is a normal placenta? At best, it is a declining placenta delivered before maternal preeclampsia occurs. Today’s normal placenta may be tomorrow’s preeclamptic placenta.

Exceptional Causes of Syncytiotrophoblast Stress and Preeclampsia

So far, we have considered malplacenta and senescence as the two main causes of STB stress and preeclampsia. However, there are other unusual causes, including infection with parvovirus and immune attack by antiphospholipid autoantibodies. The former may be associated with the mirror syndrome, comprising edema that affects both the mother and fetus. In addition, the mother may develop preeclampsia, which regresses after recovery from the viral infection. In such cases, the STB demonstrates apoptosis, while maternal sFlt-1 and PI GF, biomarkers of STB stress, increase and decrease, respectively, as they do in preeclampsia of other causes. Antiphospholipid autoantibodies may be directed against cardiolipin located in the mitochondria or annexin of which the latter is a protective layer for STB. They are mostly associated with early pregnancy loss, but associations with preeclampsia have been documented. Anticardiolipin antibodies bind to purified placental mitochondria and activate the ROS production. Hence, there is a possible mechanism to explain their adverse effects on pregnancy outcomes, but so far, it has not been possible to invoke participation of STB directly. It is likely that there are other unrecognized causes of STB stress.

Syncytiotrophoblast Stress, Prediction, Diagnosis, and Perinatal Outcome

Stress causes STB to activate a new and wide-ranging transcriptional program of
protein synthesis through the UPR. Proteins that promote homeostasis are prioritized, and the production of others are restricted (“negative” stress response proteins). Some enter the maternal circulation and contribute to the preeclampsia syndrome. They are the “biomarkers” of the condition. Some increase and some decline. Of special interest are those that are exclusively produced by STB or at least with a minimal contribution from maternal systems. A comprehensive list is not appropriate, but sFlt-1, activin A and inhibin A, corticotropin-releasing hormone, soluble endoglin, and plasminogen activator-1 are some examples. Of these, the first is used in clinical practice often coupled with measures of PlGF. In trophoblast, various stressors, including tumor necrosis factor alpha (TNFα), angiotensin II, or hypoxia, induce sFlt-1. This depends on a stress sensor GADD45a, which responds to DNA damage within cells, including STB. PIGF declines with the stress of preeclampsia and is an example of a negative stress factor. Its measurement in maternal blood may be complicated by the partition between free and bound PlGFs. However, researchers agree that its production by trophoblast is reduced with hypoxia.

Maternal circulating levels of sFlt-1 and PIGF are therefore biomarkers of STB stress when they are increased and decreased, respectively. The time courses of their circulating measures in normal pregnancies are clear. From about 30 to 32 weeks’ gestation, something causes STB stress, which progressively increases toward term (Figure 6). Remember that these are pregnancies with normal outcomes but display relatively universal and consistent changes ascribable to STB stress. This is consistent with the concept of the limited lifespan of the placenta. At term, its function declines, leading to increasing rates of preeclampsia, late-onset FGR, and unexplained term stillbirths.

In summary, a blood-borne biomarker that is a stress product of STB reflects the health of the STB. sFlt-1 and PIGF are the first to be brought into practice. However, there are many more that await investigation. Of these, STB microvesicles are promising. They carry many signals, which can be delivered in a targeted way. They are not easy to study in blood samples, but the techniques are rapidly improving. If they fulfill the promise of enabling peripheral biopsy of STB, we speculate that it could become possible to determine the “biological age” of a placenta rather than its gestational age. Some placentas at 40 weeks’ gestation may have a potential life expectancy of 3 to 4 more weeks. Others at 38 weeks’ gestation may be in the final stages of STB failure.

Competing Odds
As STB stress increases with advancing gestational age so does the likelihood of an adverse outcome (preeclampsia, late-onset FGR, stillbirth). If delivery never occurred, all pregnancies would presumably be eventually affected. However, they do not, because delivery supervenes. In most instances, it does so in good time, and the outcome is normal. In effect, there is a race between 2 biological processes: spontaneous labor or decompensated STB stress. Current theories of what triggers spontaneous labor concern senescence in the amniochorion. Senescent amniochorionic cells activate an inflammatory SASP, which in time stimulates myometrial contractility and labor. Hence at the end of pregnancy, there are 2 clocks counting down to competing outcomes. This can be expressed in statistical terms as competing odds. In this study of nearly 66,000 pregnancies, the mean time for getting preeclampsia was estimated to be 55 weeks. Hence, only a small proportion (2.7%) succumbed. Post-maturity pregnancy beyond 41 to 42 weeks is now rare with modern antenatal care, and induction at an earlier gestational age is commonplace if
hypertension begins to develop (Figure 7). This practice developed over the last 40 years has prevented an unknown but not insubstantial number of cases of preeclampsia.

The Preeclampsia Syndrome—more than a Single Disorder?

One of the clear messages that comes from the delineation of the pathophysiology of early- and late-onset preeclampsia as using different pathways to arrive at the common endpoint, STB stress (Figure 8), is that there may be more than “one kind” of preeclampsia. Are there subsets of preeclampsia other than early and late onset? This likelihood is supported by several lines of evidence.

The clinical presentation of preeclampsia is remarkably diverse. The condition may evolve relatively slowly or may progress explosively to severe illness in 24 hours. Organ involvement is also quite variable. In some women, hepatic and coagulation changes predominate with hemolysis, elevated liver enzymes, and low platelet count; in others, cortical blindness and cardiac or CNS findings are of major impact.

The long-range implications of preeclampsia are quite different in subsets of women. With preeclampsia delivering at term in only 1 pregnancy, the risk of later life CVD is about doubled. However, having had preeclampsia in more than 1 pregnancy results in a 3.5-fold risk, and the women with preeclampsia before 34 weeks’ gestation have almost a 10-fold risk. Laboratory findings are also inconsistent with considerable overlap between women defined as preeclamptic and those who have normal outcomes. This is more of a problem at term when normality is being replaced by the problems of the aging placenta.

Differences in laboratory findings also include measurement of factors considered as important parts of the pathophysiological pathways to preeclampsia, such as inflammation, oxidative stress, and angiogenic imbalance. In a study from our group of 50 women with preeclampsia followed up longitudinally through pregnancy, PlGF was minimally different from concentrations in normal pregnant women in about half of the women, whereas the other half had PlGF concentrations below the fifth centile of normal pregnancy throughout gestation. This may have been an index of malplacentation, which would explain the higher incidence of preterm preeclampsia in this group.

Although not as convincing, further critical evidence is the nearly universal observation that attempts at preventive treatment for preeclampsia follow a common pathway. Early, small studies are successful, whereas larger studies stimulated by these successes are not effective. An obvious explanation is, of course, publication bias with small successful studies being reported, whereas small unsuccessful studies are not. Although this is a likely explanation, an alternative is the possibility that small studies succeed because of a
Early-onset disease is characterized by a long first stage and more severe placental and fetal sequelae. Late-onset disease has a shorter first stage and less severe sequelae if delivery supervenes normally. They both cause syncytiotrophoblast stress, associated with the specific stresses as listed. Early-onset preeclampsia is based predominantly on spiral artery dysfunction that causes focal oxidative stress in the relevant territory of the artery. Late-onset disease is more diffuse and affects syncytial health in a less focused way. Adapted from Redman and Redman and Staff.10,30

With this consideration, is it possible that preterm birth, growth restriction (without maternal findings), and stillbirth, which share many placental pathologic and epidemiologic findings with preeclampsia may also share pathophysiological pathways but with a different clinical phenotype?

In summary, identifying different variants of preeclampsia could have a major impact on the prevention and prediction of the syndrome. These advances might also be shared with other disorders not considered part of the preeclampsia syndrome.

**Identifying Subtypes of the Preeclampsia Syndrome**

To begin to search for subtypes, it would require that we determine how such subtypes arise. Although there is a common endpoint, STB stress, in the pathogenic pathway to preeclampsia, there is heterogeneity in its antecedents: in possible additional routes to STB stress, in the multiple uncharted signals that stressed STB sends to the mother, and in the range of responses by different mothers (Figure 9).

Maternal factors contribute to this heterogeneity of preeclampsia. Chronic hypertension, obesity, nutrition, environmental toxins, genetic predisposition, and other factors known and unknown contribute to an increased risk of preeclampsia and influence pathophysiological responses. The question to be answered ultimately resolves into the question of the mechanisms of the interaction between maternal factors and the STB stress (Figure 10). Are these effects secondary to increasing propensity to STB stress, increased maternal response to this insult, or perhaps both are at play for some of these factors?

There are 2 ways to search for different disease pathways. The first is the traditional hypothesis testing driven by questions generated by clinical observation. Why are early- and late-onset preeclampsia so different in clinical features of long-range morbidity epidemiologic outcomes? We present the evidence for different pathways to STB stress.164 Does obesity result in a different subtype of preeclampsia? Is the same disorder
associated with undernutrition in low-resource settings? Do women with preeclampsia with markedly different biomarkers (eg, PlGF) pursue a different pathway to preeclampsia? Are women in whom aspirin is effective different from women in whom it is not? These questions generate testable hypotheses.

The second approach is discovery science. The availability of powerful analytical approaches permits investigators to perform nondirected studies of analytes assayed by state-of-the-art techniques (proteomics, metabolomics, genomics, etc.). From these approaches, systems analyze group findings into clusters, indicating potential pathways to disease, once again generating testable hypotheses. An especially powerful strategy, again taking advantage of currently available analytical power, is the attempt to combine different analytes from different “omics” strategies with linked demographic and clinical findings.

**Clinical Implications**

The problem of late-onset preeclampsia has received minimal attention not only in terms of the pathophysiology but also in the prediction and prevention of preeclampsia. Thus, prediction has been largely considered unimportant because delivery is considered a “safe” option. Interestingly, this approach has even been extended to normal pregnancy, in which the increase in stillbirths after 39 weeks’ gestation has stimulated studies demonstrating the safety of delivering nulliparous women who are at low risk at 39 weeks’ gestation leading to the adoption of this approach in several settings. These attitudes are perhaps relevant to high-resource settings, but for low-resource settings, in which late-onset preeclampsia remains a substantial maternal and infant risk, such management is not consistent with available resources. In these settings, identifying women at risk and marshaling resources to deliver women at risk would be a major advance. Even in high-resource settings, delivery near term but before an increase in maternal and infant morbidity is desirable. Furthermore, because the biomarkers marking the genesis of preeclampsia are signs of placental senescence and stress, they are likely signaling the premature expression of normal placental stress accompanying the biological termination of pregnancy. Carefully ovulation dated pregnancies indicate that pregnancy duration is 268±9 days (282±9 days by menstrual dating) with a range of 37 days. Thus, arbitrary delivery at 39 weeks’ gestation results in 80% of pregnancies being delivered before their biologically determined endpoint. The ability to date the biologically programmed end of pregnancy rather than making decisions based on variable temporal endpoints has obvious advantages.

We have advanced the hypotheses that there are different pathways to the convergence point of the pathophysiology.
Four ways in which maternal factors influence the expression of SBT stress. A, Placentation is influenced by maternal and paternal (fetal) immunologic interactions. There are also maternal-specific determinants, including decidual preparation and preconditioning of vessels perfusing the placental bed by previous pregnancies. B, There are other recognized and not yet identified maternal factors that contribute to SBT stress. C, Maternal factors influence the rate of placental senescence or SBT stress. D, The response to the SBT stress signals, both qualitatively and quantitatively, will be different in different women.

SBT, syncytiotrophoblast.


of preeclampsia, STB stress, and different maternal responses to the resulting placental response. If this is the case, there will never be a 1-way strategy to prevent all cases of preeclampsia as we first suggested in 1999. This prediction will be at best extraordinarily complex and at worst highly inaccurate. However, the recognition of different subtypes of preeclampsia with clinical-demographic and analytical markers would allow directed assessment of risks and specific treatments. With this knowledge, we will be able to predict, and hopefully prevent, preeclampsia (perhaps even other pregnancy-specific disorders not currently considered part of the preeclampsia syndrome).

**Laboratory Research**

This discussion does not apply to the scope and content of research of placentation and its defects. Laboratory research into the health and dysfunction of STB in the third trimester of pregnancy has special facets that need to be recognized. Analysis of a minced whole placental tissue is too imprecise. However, confocal or electron microscopy with immunocytochemistry can be exceptionally informative in terms of discriminating between the STB and cytotrophoblast in close proximity. Conclusions from experiments with trophoblast cell lines, which can be induced to syncytialize, will always need to be validated on primary tissue. Ex vivo preparations of primary trophoblast cells are expensive and limited because they can only be used in 1 set of experiment. Careful culture of placent al explants are easier and more robust. With either method, the tissue may be altered in variable ways by stresses induced by labor, so that mode of delivery should be controlled. Research on delivered placentas is confounded by the need to match for gestational age and normality. As discussed, the concept of normality at term is, to an extent, spurious. Hence, 1 of the top priorities is to determine the biological age of a pregnancy in terms of measures of STB stress, which can be used to grade the “normality” of placentas delivered to apparently normal women at term. This may be feasible by analysis of circulating STB-derived microvesicles. Their cargoes are complex and differ if there is preeclampsia. This issue merits detailed investigation in relation to the time course of the end-stage placenta. Large quantities of pure STB microvesicles can be prepared by dual perfusion of single placental lobes to allow their properties in vitro to identify the features of highest interest. These can be used to distinguish preeclampsia from normal or to grade biological age and hence distinguish “twilight” vesicles from those that are biologically younger.

Finally, STB cannot be considered in isolation of its supporting cytotrophoblast. In terms of all the issues discussed in this paper, detailed analysis of cytotrophoblast condition and stress is urgently needed.

**Clinical Research**

Innovative discovery strategies supported by hypothesis-guided research now have the capacity to unravel the complexity of abnormal pregnancies. Use of these tools to decipher the pathophysiologies of preeclampsia should be a major priority of future studies. All the authors have been frustrated by being involved in clinical trials to prevent preeclampsia, which have had minimal or no success. These trials were guided by conventional wisdom of the time. However, in retrospect, we can see that they also shared the major conceptual shortcoming of considering preeclampsia to be a single disorder, which could be solved by a single “magic bullet.” We recommend that further large clinical trials should await the recognition of subsets that provide specific targets for the test intervention and are tested exclusively in that subgroup.
Big Data
For successful understanding of pre-eclampsia, we must understand the syndrome and the pathophysiology of its subtypes. This mandates a new emphasis on collaborative research where sharing is the norm and a major factor in the study design, data collection, and biops specimen accrual. Although powerful analytical tools are now available, their effective use demands large amounts of carefully collected and harmonized data and biological materials. This is crucial for the big data approach to understanding any disease. However, sharing of data and biological materials has not been a priority in preeclampsia research.

In fact, for many years, academic departments, funders, and publishers have discouraged investigators from being part of large studies in which they are not a primary investigator. New efforts to change this institutional mindset to facilitate data sharing by standardizing sample collection, data fields, and outcome recording have begun.

Conclusions
Preeclampsia is not just a placental disorder but specifically one of STB stress. STB stress may be caused by maternal malperfusion secondary to malplacentation, in early pregnancy, or to late pregnancy factors related to placental growth and compression, which cause malperfusion and hypoxia. Malplacentation is not a stage in the progression of all preeclampsia or is preeclampsia the only outcome of malplacentation. It is a separate condition and, in addition to being a powerful risk factor for early-onset preeclampsia, predisposes to FGR without preeclampsia or some cases of preterm birth. STB stress, secondary to damage caused by antiphospholipid autoantibodies or parvovirus, is an atypical cause of preeclampsia. There are likely other routes to STB stress that are currently not recognized.

The role of senescence in late pregnancy is complex. STB is always semi-senescent because it cannot replicate or can it undergo apoptosis as it is characterized in mononuclear cells. This stable state may be perturbed by superimposed stresses, which stimulate autophagy, shedding of multinuclear fragments, breaks in the STB, or necrosis. STB stress can explain many aspects of the placental pathology of preeclampsia and its downstream consequences, such as syncytiot knots and perivillous fibrin deposition. It could also account for some cases of villitis of unknown etiology.

The decline in health, at and after term, which we call the “twilight placenta,” affects all pregnancies but with different time courses. The degree to which villous cytotrophoblast undergoes senescence is not known. It could be the final determinant of STB failure, when it ceased to be able to renew the overlying STB.

The best biomarkers of preeclampsia are generated by STB stress. They indicate that on average, STB stress begins at 30 to 32 weeks’ gestation in normal pregnancies. The outcome of term pregnancy depends on competing risks as to which comes first: spontaneous (or induced) delivery or preeclampsia with or without STB failure (unexplained stillbirth). STB-derived microvesicles are complex signals to the mother, which are stimulated by stress. As circulating biomarkers of syncytiot health, they have the potential to determine biological rather than gestational age, to monitor the twilight placenta at the end of pregnancy.

Maternal risk factors modulate the generation, evolution, and consequences of STB stress. They are not the cause of preeclampsia, because the risk factors persist, whereas the syndrome remits.

The several pathways leading to STB stress, the multiple insults generated by STB stress, and the variable responses to these insults, all modified by maternal factors, mandate a new approach to studying and understanding preeclampsia. It is a complex disorder with multiple targets for prevention that will not be appropriate in all women. The identification of these subsets requires coordinated and collaborative research efforts.

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