The obstetric syndromes: Clinical relevance of placental hormones

EXPERT REVIEWS

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Preterm delivery, preeclampsia and intrauterine growth restriction are the major diseases of pregnancy. A key role in their pathogenesis is played by the placenta, which is the source of hormones and other important regulatory molecules providing the metabolic and endocrine homeostasis of the fetal–placental unit. Since obstetric syndromes are characterized by important maternal and neonatal morbidity and mortality worldwide, numerous efforts have been made over the years to prevent and treat them. Due to their complex pathogenesis, however, the therapy is poor and not very effective. Therefore, great emphasis is currently given to the prevention of these diseases through the identification of biochemical and biophysical markers, among which placental factors play a crucial role. The increasing knowledge of the role of placental molecules can indeed lead to the development of new therapeutic and diagnostic tools.

Keywords: abnormal placentation • intrauterine growth restriction • obstetrics syndromes • placental hormones • preterm delivery

The placenta is an endocrine organ capable of producing a wide range of hormones and other regulatory molecules, able to ensure endocrine and metabolic homeostasis in the mother and the fetus during pregnancy [1,2].

When these regulatory mechanisms are impaired, the following obstetrics syndromes may occur: preeclampsia (PE), intrauterine growth restriction (IUGR) and preterm delivery (PTD).

PE is a multisystemic disorder, with unknown etiology, which occurs in approximately 2–8% of pregnancies and is responsible for significant maternal and fetal morbidity/mortality worldwide. The multifactorial pathogenesis includes: failure of trophoblast invasion, oxidative stress, endothelial dysfunction and maternal circulatory impairment. A series of distinct molecular events occur at placentation, and consequently on the rest of gestation [3].

IUGR is the inability of the fetus to achieve its potential growth; it is described as a birth weight below the tenth percentile for gestational age and it is associated with high risk for perinatal morbidity and mortality [4]. It occurs in 2–5% of pregnancies and is often associated with PE.

PTD is one of the major obstetric syndromes, with variable incidence from 7 to 15%, and it causes 35% of all neonatal deaths; it is characterized by a complex etiopathogenesis, where stress and inflammation represent major mechanisms [5].

The obstetric syndromes are responsible for maternal death and perinatal morbidity and mortality [6,7]. In recent times, a common pathogenetic mechanism underlying these obstetric diseases has been hypothesized. Placenta plays a major role in all these syndromes. In fact, defective deep placentation, representing the process of physiologic remodeling of the spiral arteries, was first found in preeclamptic placentas and in cases of IUGR; more recently, this abnormal process has also been related to PTD.

Placentation is a biological process in which fetal and maternal tissues develop into complex structures and uterine vasculature is profoundly remodeled. This physiologic transformation decreases peripheral vascular resistance in the placental bed, maximizing blood flow to the intervillous space. By contrast, failure of physiologic transformation of the myometrial segment of the spiral arteries, probably through ischemia or inflammation, is considered involved in the pathophysiology of the main pregnancy syndromes [7–10].

As the placenta plays a major role in all these diseases, placental factors affect pathogenic mechanisms.

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Considering the impact of these syndromes on social, health and economic levels, numerous efforts have been made over the years to prevent and treat them. Therefore, starting from the studies on pathogenesis, several tools have been tested of potential usefulness for the early diagnosis before symptoms occur and eventually for the treatment of these disorders. Biological fluids such as maternal serum and plasma, saliva, urine, vaginal and amniotic fluid have always been investigated for the detection and measurements of placental molecules. In this way, it may be possible to use these molecules (steroids, neurohormones, peptides and glycoproteins) in order to better understand the pathogenic mechanisms of the main obstetric syndromes. Moreover, in recent decades, biochemical markers have been joined by biophysical markers, encouraged by the continued scientific research in this field [11–13].

Despite the advances in medical research, there are still nonspecific biochemical or biophysical markers that may predict these pregnancy complications, but the frequency of these diseases has not changed. Only the integration of patient's anamnesis with the use of multiple biochemical and biophysical markers may help in identifying patients at risk.

The purpose of this review is to describe the most studied placental hormones in the main pregnancy complications and to identify the possible implications for their use in the clinical management and early detection of these diseases. To be effective, a screening marker needs to be sufficiently sensitive and specific and must provide an adequate positive-predictive value. Today, several promising markers have been described, alone or in combination, that may fulfill these criteria in order to detect early changes responsible for the onset of the pregnancy pathologies and to be used as a therapeutic tool.

Preeclampsia

The major pathogenic mechanism responsible for preeclampsia is represented by abnormal placentation and vascularization, resulting in impaired trophoblast invasion (Figure 1). Abnormal placentation, or defective deep placentation, is characterized by absent or incomplete remodeling of the junctional zone segment of the spiral arteries [14]. In normal pregnancy, a subset of cytotrophoblast cells called 'invasive cytotrophoblasts' migrate through the implantation site and invade tunica media of maternal spiral arteries, replacing its endothelium in a process called 'pseudovascularization' [14]. As a result of these changes, the maternal spiral arteries undergo transformation from small, muscular arterioles to large capacitance and low-resistance vessels, providing an appropriate blood flow to the maternal–fetal interface. In PE, due to genetic, immunologic and other factors not yet well identified, this process is defective. In particular, the physiologic transformation of the spiral arteries in the junctional zone of myometrium is reduced greatly in the central area of the placental bed, which is characterized by a large number of nontransformed myometrial spiral arteries, frequently showing obstructive lesions, such as acute atherosis and thrombosis [15].

Beside the major pathogenic mechanism, other alternative or integrating mechanisms have shown to be involved in the pathogenesis of the disease, such as edema and vascular leakage. It is well known that systemic endothelial cell dysfunction is associated with PE [16,17]: in particular, impaired endothelial barrier function consisting in increased vascular permeability is a characteristic vascular response in the disorder. In fact, vasoconstrictor response, tendency for coagulation and capillary permeability are all enhanced in preeclamptic women; moreover, endothelin and fibronectin (markers of endothelial damage) levels are raised and surface-adhesion molecules are overexpressed on endothelial cells [18,19], confirming the association of disturbed endothelial integrity in the vascular system with this condition.

The diagnostic criteria currently used are represented by the onset of characteristic clinical symptoms (hypertension and proteinuria). In this perspective, the early detection of placental factors causing abnormal placentation may be useful in asymptomatic women.

Prognostic tools

Since abnormal vascularization and placentation occur, the identification of alterations of molecules involved in angiogenic mechanisms and placental dysfunction may result as useful markers for the early diagnosis of the disease.

During the midtrimester, high serum human chorionic gonadotropin (hCG) levels are associated with the risk to develop subsequent PE [20,21].

Low levels of PAPP-A may be a marker for identifying pregnancies at increased risk to develop PE in the second half of pregnancy [22,23]. Therefore, women with low levels of PAPP-A during the first trimester are advised to monitor uterine artery Doppler by ultrasound from 24 weeks of gestation [24].

Low serum levels of PP13, a placental glycoprotein involved in placentation, have been shown in the first trimester of pregnancies that subsequently developed PE with a false-positive rate of 10%. PP13 showed a prediction rate of 80% as a single biochemical marker, and when combined with Doppler ultrasound uterine artery PI, the prediction rate increased to 90% [25]. Furthermore, low first-trimester serum levels of PP13 may be a useful marker for early-onset PE [26].

Since corticotropin-releasing hormone (CRH) and urocortin (Ucn) have vasoactive properties, their increased levels in maternal–fetal circulation in hypertensive disorders suggest a possible role in the adaptive fetoplacental response to adverse conditions [27]. In particular, it was found that increased maternal and fetal serum CRH and Ucn levels are frequent in pregnancies complicated by PE [28] and correlate with Doppler velocimetry patterns [27].

In the past few years, the 'angiogenic imbalance theory' emerged to explain the mechanism leading to PE [29].

Activin A and inhibin A are functionally involved in vascular adaptive mechanisms of pregnancy as well as cytotrophoblast proliferation and differentiation; their placental expression and maternal serum levels are elevated in pregnant women with PE [30]. A recent study on 4764 subjects demonstrated that midtrimester inhibin A concentration of 1.5 multiple of the median (MoM) or greater had a sensitivity of 60% and a false-positive

rate of 16% for the prediction of PE, showing to be the best predictor among other biomarkers studied (maternal serum α-fetoprotein, human chorionic gonadotropin and unconjugated estriol) [31]. Moreover, Akolekar *et al.* showed that maternal plasma inhibin A MoM was significantly higher in the early and late PE pregnancies (1.55 and 1.24 MoM, respectively) compared with the controls (0.98 MoM) [32]. Therefore, inhibin A represents a promising marker to predict the early onset of disease, while at-term high levels have been related to severity of disease. Compared with inhibin A, activin A seems to be a more sensitive marker at 21–25 weeks [33]. The performance of serum activin A and inhibin A as predictive markers is improved when combined with Doppler ultrasound evaluation of uterine artery resistance blood flow at midtrimester [34,35].

Placental *VEGF* mRNA expression is reduced in gestational hypertensive disorders and in the third-trimester placentas of pregnancies with PE compared with normotensive controls [36]. In a recent study, placentas collected from preeclamptic and healthy women delivering by cesarean section showed significantly lower level in PE than in controls [37], confirming abnormality of *VEGF* expression during the disease. Considering the biological function of VEGF, involved in angiogenic processes, its decreased expression may in part explain the structural changes associated with PE placentas, such as deficient growth and differentiation of terminal villi and reduced fetal capillary branching.

Soluble fms-like tyrosine kinase-1 (sFlt-1), a VEGF receptor involved in hypoxic/ischemic response, increases 5 weeks before the onset of PE, without modification in the first trimester [38]. Alterations in sFlt-1 levels correlate with the onset of PE and are higher in women with PE and small for gestational age (SGA) fetuses [39].

PlGF levels, involved in the invasiveness of the extravillous cytotrophoblast [40], are significantly lower in women with early-onset PE compared with controls, pointing out how PlGF significantly improved the ability of systolic blood pressure at the first prenatal visit to predict early-onset PE [41].

The sFlt-1/PlGF ratio has been proposed as a useful tool for the early identification of PE, since a significant increase of the ratio occurs prior to the presentation of clinical symptoms. Significant differences were observed between controls and women with PE, in which circulating sFlt-1 levels are fivefold higher and PlGF levels are fivefold lower than controls. The sFlt-1/PlGF ratio varies during pregnancy, but a cut-off value of 85 offers a sensitivity and a specificity of 82 and 92%, respectively, for the diagnosis of PE during all trimesters of pregnancy, and adding its use in clinical practice could improve the management of the disease and maternal and neonatal outcome [42]. Moreover, the importance of the utility of sFlt-1/PlGF ratio has also been recently shown in identifying the risk for imminent delivery, as in serum of patients with PE/HELLP, its increased concentration is associated with the risk of delivering within 7 days [43]. Similar results were also shown by Molvarec *et al.*, who highlight the decreased PlGF concentration in women with PE associated with IUGR and PTD, focusing on the symbiotic role between the three diseases [44].

Levels of circulating leptin are increased in women with PE, and several studies showed that elevated levels seem to have a prognostic significance for the development of PE before the clinical onset of the disease. An association between higher first-trimester plasma leptin levels and subsequent PE [45], and that the risk of developing PE, is proportional to the increase of serum leptin [46]. Moreover, the observation that leptin rises before the clinical onset of the disease suggests a possible pathophysiological role of this protein. Since it is a potent angiogenic factor, it is responsible for increased apoptosis of trophoblastic cells and of blood supply

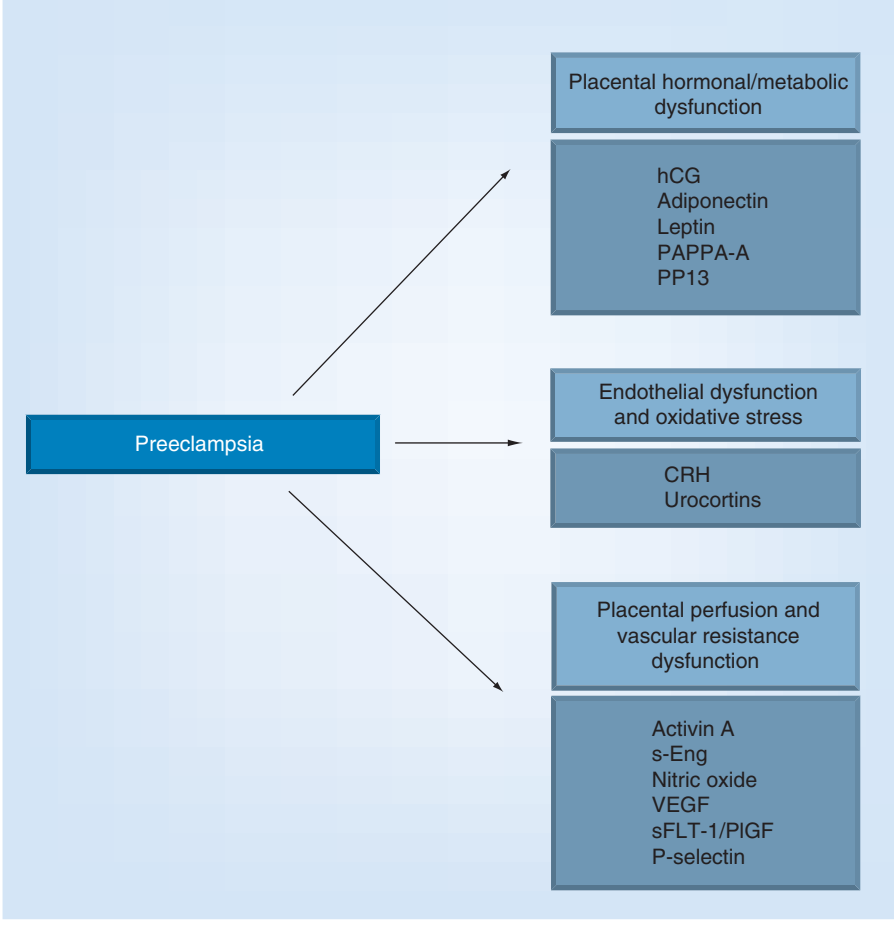

Figure 1. Pathogenesis of preeclampsia and endocrine involvement.
CRH: Corticotropin-releasing hormone; hCG: Human chorionic gonadotropin; sFlt-1: Soluble fms-like tyrosine kinase-1.

to the placenta by neovascularization occurring during PE as a compensatory response to the hypoperfused placenta [46].

As with leptin, adiponectin is also an adipocyte-secreted hormone. It has different roles, as it is recognized as pleiotropic, insulin-sensitizing, anti-inflammatory and anti-atherogenic adipokine. Since low concentrations of adiponectin are associated with obesity, insulin resistance, Type 2 diabetes, hypertension [47] and obesity-related malignancies, a possible role has been hypothesized in the pathogenesis of PE. However, its role has not yet been well defined, as contrasting results have been found [48,49].

Soluble endoglin (S-Eng), a modulator of TGF-β signaling involved in angiogenesis and regulation of the vascular tone, is elevated in PE patients compared with controls, and it is characterized by more elevated levels in case of more severe symptoms, reaching the highest values in case of PE complicated by HELLP syndrome. In pregnancies ending with PE, a pathological increase of S-Eng occurs earlier than in physiologic pregnancies and this distinction becomes considerable 9–11 weeks before the appearance of clinical symptoms. Therefore, a specific prediction cannot be achieved with this analyte alone. For this reason, its potential in combination with PlGF and sFlt-1 for the prediction of PE has been evaluated, with more encouraging results if used together and not as single biomarkers [50].

P-selectin plays a crucial role in inflammatory reactions, as in the recruitment and activation of circulating leucocytes, and in coagulation processes [51] with increased levels in the peripheral blood of PE women [52]. Interestingly, it has recently been shown that alterations in the levels of soluble P-selectin before 20 weeks of gestation antedate the symptoms. Indeed, this early upregulation of soluble P-selectin seems to be a sign of the early but still asymptomatic disturbances of the maternal vascular system [53].

It is well known that in normal pregnancies, nitric oxide production is elevated and this increase seems to be implicated in vasodilatation; however, in pregnancies complicated by PE, its production is decreased. Recently, the evidence that nitric oxide products are decreased in maternal serum of patients at risk for PE between 22 and 26 weeks of pregnancy has suggested a possible role to predict the development of preeclampsia [54].

Recent evidence has demonstrated an important role of the immune system in the etiology of PE [55]. In this context, an activation of the complement system has been widely demonstrated. Recently, elevated amounts of complement activation markers in the systemic circulation during the third trimester of pregnancy and, furthermore, in PE patients have been shown, with an excessive activation of the terminal pathway in case of fetal growth restriction [56]. More elevated serum concentrations of heat-shock protein 70, considered a potent activator of the classical pathway of the human complement system, are present in PE patients and its elevated levels are associated with proinflammatory changes in circulating cytokine profile, suggesting that it may contribute to the development of the excessive systemic inflammatory response characteristic of the maternal syndrome of the disease [57]. Another evidence of the activation of the immune system is demonstrated by the inflammatory environment which characterized PE: an increase of proinflammatory cytokines, such as IL-6 and TNF-α, of chemokines, as IL-8, IP-10 and MCP-10, and of adhesion molecules, such as ICAM-1 and VCAM-1, has been highlighted in PE, suggesting the potential role of these molecules in endothelial dysfunction [58]. More relevant data with regard to prevention of this important disease are those relating to markers of pathology, already present midtrimester, such as factor B-derived Bb activation fragment from the alternative complement pathway, which may be used as a marker of the complement activation. At 20 weeks of pregnancy, its concentration is more elevated in women with subsequent PE as compared with uneventful pregnancies, suggesting that it may be an early biomarker of the pathogenic events that start in early pregnancy and then lead to PE [59]. Furthermore, elevated serum levels of activation fragment C3a in midtrimester are an indicator of adverse pregnancy outcome, as demonstrated by its elevation in women with subsequent PE before 20 weeks of pregnancy [60].

Regarding the prevention of PE, the use of metabolomics seems to be promising. This method is able to identify all the metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. Recently, different studies have demonstrated a profound change in the first-trimester metabolite profile in women with subsequent early-onset PE, supporting the hypothesis that this new science may be critical for the prevention of the disease in the future [61,62].

In summary, several placental factors have been investigated as potential biomarkers for early diagnosis/prediction of PE. Among them, no single biomarker emerged as a risk predictor of PE, and the complexity of the pathogenic mechanisms may in part explain the impossibility to rely on a single predictor. However, although there are currently no arms in our possession to prevent this important disease of pregnancy, there are excellent perspectives for future early diagnosis of PE.

Intrauterine growth restriction

Despite the fact that the etiology of IUGR is rather complex and characterized by numerous factors, the key point of this disease is represented by the abnormalities in placental structure and function (**Figure 2**).

The process of implantation and placentation requires the production of several growth factors, cell-adhesion molecules, extracellular matrix proteins, hormones and transcription factors, which often exhibit altered expression within the placenta of IUGR pregnancies.

The decrease of villous number, diameter and surface area, as well as the decrease in arterial number, lumen size and branching are aspects which characterize the placental structure in IUGR gestations [63–66]. If during normal pregnancy, extravillous trophoblast cells migrate and invade the spiral artery vessel walls within the decidua and myometrium, in case of PE and/or IUGR, this invasion is restricted to the decidual portion of the spiral arteries, with consequent failure of these arteries to become low-resistance vessels. This leads to an increased fetoplacental vascular resistance and consequently to decreased blood flow and decreased availability of nutrients and oxygen to the fetus.

A number of factors have been associated with extravillous trophoblast invasion, including angiogenic growth factors and their respective receptors. While it remains unresolved as to the exact mechanism causing deficient trophoblast invasion, angiogenic growth factors are logical candidates as regulatory molecules in placental development and function [67].

As angiogenesis and vascular transformation are important in normal placental development, IUGR is thought to result from impaired trophoblast invasion of the maternal spiral arteries in early pregnancy, leading to reduced uteroplacental perfusion and placental hypoxia [68–70]. It has been hypothesized that placental hypoxia may stimulate the release of factors by the placenta which cause widespread maternal endothelial cell damage and derangement of placental angiogenesis. What causes the deficient trophoblast invasion remains unknown. In this context, IUGR is currently diagnosed by ultrasound measurements (abdominal circumference and estimated fetal weight <tenth centile) and confirmed postnatally by birth weight below the tenth percentile corrected for gestational age [71]. When IUGR is caused by placental insufficiency, the diagnosis is improved including oligohydramnios and/or pathologic umbilical and uterine artery pulsatility indices at Doppler examination [72].

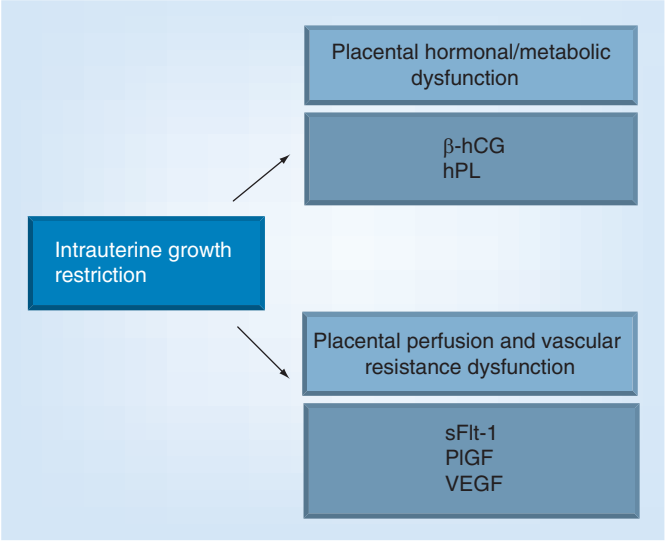

Figure 2. Pathogenesis of intrauterine growth restriction and endocrine involvement.
hCG: Human chorionic gonadotropin; hPL: Human placental lactogen; sFlt-1: Soluble fms-like tyrosine kinase-1.

Prognostic tools

There are strong indications that angiogenic growth factors related to VEGF family may be implicated, especially PlGF and its soluble receptor sFlt-1, altered in maternal and fetal serum from pregnancies complicated by IUGR. In IUGR pregnancies, sFlt-1 is increased, and PlGF is decreased, in both maternal and umbilical vein serum. Levels of VEGF are higher and levels of sFlt-1 are lower in serum from the umbilical vein and umbilical artery compared with maternal serum. These findings suggest an imbalance of angiogenic and antiangiogenic factors in IUGR, with formation of an antiangiogenic state in maternal blood altering the terminal differentiation of placental capillary loops, and placenta appears to play a central role in the release of these factors [73–75].

Furthermore, in pregnancies complicated by SGA or IUGR, altered levels of placental factors, such as glycoproteins, involving in growth of the fetoplacenta unit and in trophoblast proliferation were found.

Moreover, low levels of free β-hCG in the first trimester have been associated to with growth restriction. For example, Ong *et al.* found that first-trimester maternal β-hCG circulating levels below tenth centile of the reference range were associated with subsequent development of IUGR [76]. Similarly, Kirkegaard *et al.* demonstrated a strong association between free β-hCG levels below 0.3 MoM and SGA fetuses [77]. Finally, in a group of 3728 pregnancies affected by one or more of the pregnancy complications of low birth weight, IUGR, PTD and stillbirth, first-trimester free β-hCG levels below 0.5 MoM showed relative risks of 2.3 for developing IUGR [78]. It is still necessary to specify that there are conflicting opinions on the role of free β-hCG in PE and IUGR [11].

Among women who delivered a IUGR baby, human placental lactogen (hPL) remained at low levels throughout gestation. hPL, a polypeptide with a somatotropic effect, correlates with placental weight and fetal size. Evaluating women at risk for IUGR around 18 weeks of pregnancy, the prevalence of IUGR associated with lower hPL levels is 35% [79]. The physiologic increase in maternal serum hPL with advancing gestation does not occur in IUGR fetuses, and may be related to the unsuccessful fetal growth [80].

In summary, IUGR is burdened with significant neonatal morbidity and its diagnosis is still only made through ultrasound. Since its diagnosis is often delayed and therapeutic support available is scarce, further studies investigating new potential biomarkers for IUGR diagnosis/prediction are necessary. Indeed, given its parallelism and its coexistence with PE, in a good percentage of cases, the identification of early markers of PE could help to detect fetuses at risk of IUGR.

Preterm delivery

PTD is a syndrome characterized by a complex pathogenesis (Figure 3). The most common lesions found in the placenta of patients with spontaneous preterm parturition are those of acute inflammation (acute chorioamnionitis and funisitis). Vascular lesions are the second most common histologic appearance in the placenta of these patients. Placentas of patients with preterm labor and intact membranes show a greater degree of failure of transformation of the spiral arteries in the myometrial and decidual segments compared with women with term labor. However, this aspect is even more relevant in patients with PE. Several placental vascular lesions have been described in patients with preterm premature rupture of membranes (PROM), as failure of physiologic transformation of the decidual segment of the spiral arteries, thrombosis and atherosis. Thus, patients who deliver preterm may be classified in two major categories: patients with acute inflammatory lesions and patients who deliver preterm, but at a later gestational age, in whom the principal lesions are vascular.

Maternal vascular lesions, leading to uteroplacental ischemia, may be responsible for a preterm labor. Although this process is still largely unclear, there are some assumptions. For example, thrombin is suggested to play an important role in the activation of the common pathway of parturition, in particular when uteroplacental ischemia is severe enough to cause decidual necrosis and hemorrhage. Deficient angiogenesis, thrombosis, and/or insufficient physiologic transformation of the spiral arteries can cause ischemia of the placenta and the uterus [3,4].

Uteroplacental ischemia has recently been implicated as a mechanism of disease in preterm PROM, as well as in preterm parturition with intact membranes. Failure of physiologic transformation, decidual thrombosis and other vascular lesions have been identified in a substantial portion of these patients, as result of the failure of physiologic transformation in the myometrial segments of the spiral arteries. Herein, it is possible to add preterm PROM to the group of obstetric syndromes where abnormal placentation has been documented [10].

As an adaptive mechanism to these pathological changes, placenta produces and releases several factors, in the maternal circulation, that can be used as diagnostic or predictive markers of PTD and some of them as therapeutic tools.

Diagnostic tools

Although the diagnosis of PTD is mainly clinical, currently the use of biochemical and biophysical tools is of fundamental support in the routine practice. In symptomatic and asymptomatic patients at risk of PTD, vaginal digital examination for assessing the cervix is subjected to large variation among examiners [81], and therefore, the measurement of cervical length (CL) by transvaginal ultrasonography represents a more reliable tool in detecting patients at risk of PTD [82]. In the highest risk women, a CL of 25 mm has a positive-predictive value of 70% at 14–18 weeks and of 40% at 18–22 weeks for PTD <35 weeks of pregnancy [83]. In addition, fetal fibronectin (fFN) testing has shown to be a promising predictor of PTD. A positive fFN test in vaginal fluids is the most accurate investigation in predicting spontaneous preterm birth (PTB) within 7–10 days among women with symptoms of threatened PTD and improves the predictive value of cervical ultrasonography to identify patients at risk [84]. A fFN positive test represents a powerful marker of PTD when measured at or after 22–24 weeks of gestation in asymptomatic high-risk women [85].

In summary, with regard to the complex etiopathogenesis of PTD syndrome, the combination of biochemical markers, such as fFN, and biophysical parameters, primarily transvaginal CL, may help to better diagnose this condition and provide a valuable tool for the identification of high-risk patients.

Prognostic tools

Although the presence of changes in the placental vascular bed represents the latest findings in order to better understand the mechanisms that lead to PTD, this complex syndrome has been understood over the years by analyzing the changes of both steroid and stress placental hormones.

Among steroids, the association between estriol and PTD has long been investigated. The salivary estriol profile is similar between women with term deliveries and PTDs, showing a characteristically increase throughout the second half of pregnancy, increasing approximately 3 weeks before parturition. The primary difference between women who gave birth at term and those who had spontaneous PTD was in the timing of the estriol elevation [86]. Salivary estriol levels were higher in patients delivered preterm than in women delivered at term. This difference appeared as early as at 24–26 weeks of gestation and continued until 34–36 weeks [87]. In symptomatic women, salivary estriol is associated with an increased risk of delivery within 2 weeks, but its clinical utility as a routine screening test to predict preterm labor in asymptomatic women has not been established [88]. Recently, due to increased knowledge of the hormonal changes underlying both term deliveries and PTD, the assessment of the estriol/estradiol ratio may be a useful marker to predict PTD. In fact, the increase of estriol production is related to the timing of the onset of labor, however, a pivotal event of parturition is represented by a change in the ratio of the estradiol and estriol as labor approaches, leading to a more than tenfold excess of estriol. Since

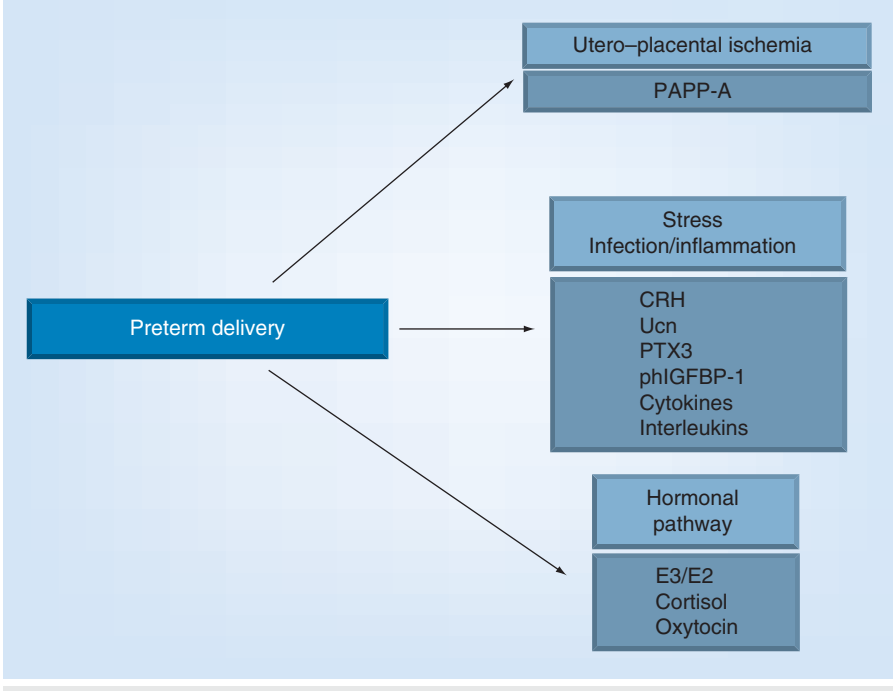

Figure 3. Pathogenesis of preterm delivery and endocrine involvement.
CRH: Corticotropin-releasing hormone; phIGFBP-1: IGF binding protein nonphosphorylated isoform; Ucn: Urocortin.

estriol/estradiol ratio increased in the month before delivery, creating an estrogenic environment at the onset of labor, the estriol increase and the altered estriol/estradiol ratios may be clinically useful in predicting PTD [89].

It has been known for long that the hypothalamic–pituitary–adrenal (HPA) axis hormones play a significant role in the mechanisms of response to the stress of delivery. Placental CRH is thought to play a crucial role in the regulation of fetal maturation and the timing of delivery, and it has also been implicated in the control of fetoplacental bloodflow.

CRH and Ucns play an important role in pathogenesis PTD, as shown in the past by *in vitro* and *in vivo* studies on placental explants. Many lines of evidence support the involvement of placental CRH in the mechanisms controlling the onset of labor, both at term or preterm. The evolution of maternal serum CRH concentrations parallels the CRH curve of normal pregnancy but the levels are higher when labor occurs preterm [90]. CRH is involved in pathogenetic mechanisms leading to PTD in which inflammatory and infective pathways represent central events [91]. Maternal plasma CRH levels at midgestation are higher in women who subsequently have spontaneous PTD than in pregnant women delivering at term [92]; moreover, women affected by threatened preterm labor present higher plasma CRH levels when delivering within 24 h than when delivering later [92]. Measurement of maternal serum CRH would not satisfy the requisites of a screening test for PTD in a low-risk population, although it should be considered a potential marker to be used in populations with a higher risk [93]; however, its clinical relevance has still to be confirmed.

Also maternal plasma Ucn levels are increased in PTD [94]. Ucn directly enhances myometrial contractility by augmenting myometrial contractile response to prostaglandins and activating the signaling pathways that regulate myometrial contractility [95]. In women with threatened preterm labor, maternal plasma Ucn levels are increased in patients who experience preterm labor before 34 weeks of pregnancy, and among women laboring prematurely are higher in those delivering within 7 days, compared with those delivering later [94]; therefore this measurement provides a promising new biochemical marker which may add significant prognostic information for predicting PTD among women at risk.

Changes of other placental molecules, such as glycoproteins, were investigated in association with PTD, showing a correlation which has proven to be less viable than that obtained with other hormones; in particular, lower levels of PAPP-A in maternal serum between 10 and 13 weeks of pregnancy are associated with a higher risk of PTD [96], while elevated maternal serum hCG levels in the second trimester are associated with an increased risk for PTD in low-risk pregnancies [97,98], also demonstrating an increased risk with higher levels of hCG [97]. Moreover, hCG concentrations in cervicovaginal secretions between 24 and 36 weeks of gestation, at the cut-off value of 50 mUI/ml, can predict PTD before 34 weeks with a sensitivity of 50% and a specificity of 87% [99].

Maternal plasma PTX3 concentration is significantly elevated during labor at term and in the presence of spontaneous preterm labor or preterm PROM, suggesting that the increased PTX3 concentration is part of the physiologic or pathologic activation of the proinflammatory response in the maternal circulation during the process of labor, both at term or preterm [100].

While there is no significant association between maternal IGF and PTD, there is much literature about the association between IGF binding protein nonphosphorylated isoform (phIGFBP-1) and PTD [101]. A positive cervical phIGFBP-1 conferred a significantly increased risk of delivery before 34 weeks in women with a cervix ≤30 mm, and a significantly increased risk of delivering within 7 days in the subgroup of women with a CL of 20–30 mm [102] with high negative-predictive value (88%) and a low positive-predictive value (24%) [101]. Since the most common cause of PTD is intrauterine infection, it has been hypothesized that the reduction of IGF may be caused by increased production of inflammatory mediators [103].

Concerning the unquestionable importance of infection/inflammatory processes in the pathogenesis of PTD, throughout the years, several cytokines and interleukins have been investigated as possible biomarkers for PTD.

In particular, IL-2, -6, -8, and -10, TNF-α, granulocyte colony-stimulating factor, stromal cell-derived factor-1α, IFN-γ, matrix metalloproteinase-8, secretory leukocyte proteinase inhibitor, soluble VCAM-1, soluble ICAM-1, C-reactive protein, ferritin and alkaline phosphatase have been more or less correlated with the PTD [104,105].

Among these, IL-6 demonstrates the greatest predictive value, since in asymptomatic women at 22–25 weeks its concentration in cervical fluid is significantly in case of subsequent PTD and in symptomatic women, affected by threatened PTD, it demonstrates ability to predict delivery within 2 and 7 days with a sensitivity and specificity of 60 and 77%, respectively, for predicting delivery within 2 days, and 62 and 80%, respectively, for predicting delivery within 7 days [106]. Furthermore, also in amniotic fluid, its concentration in asymptomatic women undergoing mid-trimester amniocentesis is higher in women with subsequent spontaneous PTD within 32 weeks of gestation, than those who delivered at term, showing an association of IL-6 levels in amniotic fluid and early PTD [107].

In summary, it is well known that the early identification of women at risk of giving birth prematurely would allow to activate possible preventive measures to reduce the impact of this condition. Since spontaneous PTD is frequently preceded by threatened PTD, characterized by the presence of uterine contractility in absence of cervical changes at the obstetric examination, the identification of those experiencing PTD after diagnosis of threatened PTD would allow the optimization of obstetric management in symptomatic women. Biomarkers identified so far lack enough accuracy to represent single predictors for PTD; studies conducted to identify them are heterogeneous as far as population to which address them as predictive biomarkers. Therefore, the combination of them with other predictors, such as ultrasound parameters (CL, funneling) together with a better identification of risk factors, would improve the estimation of the risk of PTD both in symptomatic and asymptomatic women, and allow an

optimization of the clinical practice, by addressing only those at high risk to therapeutic or preventive measures.

Therapeutic tools

Interestingly, the central role that placental hormones play in the pathogenesis of PTD is also reflected in the therapeutic appearance of the disease.

The use of 17-α-hydroxy-progesterone caproate supplementation has been approved during pregnancy to reduce the risk of recurrent PTD in women with a history of at least one prior spontaneous PTD. In addition to the weekly intramuscular administration in women with a history of previous PTD, there is also evidence that in cases of cervical shortening (<15 mm) diagnosed by transvaginal ultrasound prior to 24 weeks women may also benefit of 17-α-hydroxy-progesterone caproate administration [108].

Progesterone treatment is also recommended in singleton pregnancies of women with no prior PTD and CL <20 mm at 24 weeks; by vaginal progesterone is associated with reduction in PTD and perinatal morbidity and mortality [109].

Another treatment largely used in women with PTD is the antenatal administration of synthetic glucocorticoids for accelerating fetal lung maturation and decreasing neonatal mortality and morbidity in infants born before 34 weeks of gestation [110]. The administration of antenatal corticosteroids in women at risk of imminent PTD is associated with decreased neonatal morbidity and mortality [111], improving fetal lung maturation by promoting surfactant synthesis, increasing lung compliance, reducing vascular permeability and generating a greater response to postnatal surfactant treatment. The maximal effectiveness in preventing neonatal complications of prematurity is when delivery occurs within 2–7 days after administration [112], and a single course of antenatal corticosteroids should be considered routine for all preterm deliveries [113].

Although the short-term outcomes and benefits of exposure to single and repeated doses of synthetic glucocorticoids is reassuring, there is reason for caution in extrapolating these findings for the long term based on the reported adverse long-term and permanent changes on the HPA axis [110]. Exposure to excess levels of endogenous and exogenous glucocorticoids can lead to permanent modification of HPA function and stress-related behavior, causing dysregulation of the fetal and neonatal HPA axis and life-long consequences, such as adaptation to stress, cognition, behavior and the cardiovascular and immune responses [114].

Conclusion

After an overview of the literature on the etiopathogenetic mechanisms taking part in the development of the main obstetric syndromes, PE, IUGR and PTD, we have shown how placental dysfunction plays a common and central role in all these diseases. Several placental factors have been studied as potential diagnostic and predictive biochemical markers, although none of them can be considered as a single biomarker to early identify either women affected by or those at risk for the development of the syndromes. In such a complex scenery, we therefore show how a greater knowledge and awareness of the etiologic and pathogenetic mechanisms may be useful for the identification of novel biomarkers or at least for the strategic combination of them with other tools (ultrasound parameters, risk factors) to improve prediction and prevention of these diseases, still burdened by a significant rate of maternal and fetal morbidity.

Expert commentary & five-year view

PE, IUGR and PTD are considered the major obstetric syndromes, complicated by significant maternal and fetal morbidity and mortality. Despite new diagnostic techniques and new therapies, their incidence in recent years has not diminished.

The understanding of the pathogenetic mechanisms involved in these diseases may offer new tools for the future. In particular, the altered placental vascularization associated to the altered production of placental factors suggests a possible use as diagnostic or predictive markers. The fibronectin test and phIGFBP-1 are currently used as diagnostic testing. A possible prognostic role is supposed for sFLT-1/PlGF for PE and for CRH and Ucns for PTD.

The combination of both biochemical and biophysical markers is a developing method for the prevention of the major obstetric syndromes.

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Key issues

- Placental disorders and impaired placentation are common mechanisms for the obstetric syndromes.
- Preeclampsia and intrauterine growth restriction are characterized by the dysfunction of placental perfusion, of endothelium and of placental hormones and metabolism.
- Preterm labor is a syndrome resulting from infection/inflammation, stress and altered vascularization in placental–fetal unit.
- Placental factors may be used as diagnostic or prognostic markers for the obstetric syndromes, in combination with biophysical markers.
- A possible therapeutic implication may be hypothesized.

References

1 De Bonis M, Torricelli M, Severi FM, Luisi S, De Leo V, Petraglia F. Neuroendocrine aspects of placenta and pregnancy. *Gynecol. Endocrinol.* 28(Suppl. 1), 22–26 (2012).

2 Feldt-Rasmussen U, Mathiesen ER. Endocrine disorders in pregnancy: physiological and hormonal aspects of pregnancy. *Best Pract. Res. Clin. Endocrinol. Metab.* 25(6), 875–884 (2011).

3 Cetin I, Huppertz B, Burton G *et al.* Pregenesys preeclampsia markers consensus meeting: what do we require from markers, risk assessment and model systems to tailor preventive strategies? *Placenta* 32(Suppl.), S4–S16 (2011).

4 American College of Obstetrician and Gynecologists. Intrauterine growth restriction. *ACOG Practice Bulletin N.* 12 (2000).

5 Romero R, Espinoza J, Kusanovic JP *et al.* The preterm parturition syndrome. *BJOG* 113(Suppl. 3), 17–42 (2006).

6 Brosens I, Pijnenborg R, Vercruysse L, Romero R. The 'Great Obstetrical Syndromes' are associated with disorders of deep placentation. *Am. J. Obstet. Gynecol.* 204(3), 193–201 (2011).

7 Romero R, Kusanovic JP, Chaiworapongsa T, Hassan SS. Placental bed disorders in preterm labor, preterm PROM, spontaneous abortion and abruptio placentae. *Best Pract. Res. Clin. Obstet. Gynaecol.* 25(3), 313–327 (2011).

8 Khong Y, Brosens I. Defective deep placentation. *Best Pract. Res. Clin. Obstet. Gynaecol.* 25(3), 301–311 (2011).

9 Pijnenborg R, Vercruysse L, Brosens I. Deep placentation. *Best Pract. Res. Clin. Obstet. Gynaecol.* 25(3), 273–285 (2011).

10 Kim YM, Chaiworapongsa T, Gomez R *et al.* Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. *Am. J. Obstet. Gynecol.* 187(5), 1137–1142 (2002).

11 Reis FM, D'Antona D, Petraglia F. Predictive value of hormone measurements in maternal and fetal complications of pregnancy. *Endocr. Rev.* 23(2), 230–257 (2002).

12 Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr. Rev.* 27(2), 141–169 (2006).

13 Torricelli M, Voltolini C, De Bonis M *et al.* The identification of high risk pregnancy: a new challenge in obstetrics. *J. Matern. Fetal. Neonatal. Med.* 25(Suppl. 1), 2–5 (2012).

14 Brosens JJ, Pijnenborg R, Brosens IA. The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am. J. Obstet. Gynecol.* 187(5), 1416–1423 (2002).

15 Hering L, Herse F, Verlohren S *et al.* Trophoblasts reduce the vascular smooth muscle cell proatherogenic response. *Hypertension* 51(2), 554–559 (2008).

16 Haller H, Hempel A, Homuth V *et al.* Endothelial-cell permeability and protein kinase C in preeclampsia. *Lancet* 351(9107), 945–949 (1998).

17 Wang Y, Lewis DF, Alexander JS, Granger DN. Endothelial barrier function in preeclampsia. *Front. Biosci.* 12, 2412–2424 (2007).

18 Perry KG Jr, Martin JN Jr. Abnormal hemostasis and coagulopathy in preeclampsia and eclampsia. *Clin. Obstet. Gynecol.* 35(2), 338–350 (1992).

19 Haller H, Ziegler EM, Homuth V *et al.* Endothelial adhesion molecules and leukocyte integrins in preeclamptic patients. *Hypertension* 29(1 Pt 2), 291–296 (1997).

20 Kalinderis M, Papanikolaou A, Kalinderi K *et al.* Elevated serum levels of interleukin-6, interleukin-1β and human chorionic gonadotropin in preeclampsia. *Am. J. Reprod. Immunol.* 66(6), 468–475 (2011).

21 Bahado-Singh RO, Oz U, Isozaki T *et al.* Midtrimester urine human chorionic gonadotropin β-subunit core fragment levels and the subsequent development of preeclampsia. *Am. J. Obstet. Gynecol.* 179(3 Pt 1), 738–741 (1998).

22 Dugoff L, Hobbins JC, Malone FD *et al.* First-trimester maternal serum PAPP-A and free-β subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *Am. J. Obstet. Gynecol.* 191(4), 1446–1451 (2004).

23 Odibo AO, Zhong Y, Goetzinger KR *et al.* First-trimester placental protein 13, PAPP-A, uterine artery Doppler and maternal characteristics in the prediction of preeclampsia. *Placenta* 32(8), 598–602 (2011).

24 Spencer K, Cowans NJ, Nicolaides KH. Low levels of maternal serum PAPP-A in the first trimester and the risk of preeclampsia. *Prenat. Diagn.* 28(1), 7–10 (2008).

25 Nicolaides KH, Bindra R, Turan OM *et al.* A novel approach to first-trimester screening for early preeclampsia combining serum PP-13 and Doppler ultrasound. *Ultrasound Obstet. Gynecol.* 27(1), 13–17 (2006).

26 Romero R, Kusanovic JP, Than NG *et al.* First-trimester maternal serum PP13 in the risk assessment for preeclampsia. *Am. J. Obstet. Gynecol.* 199(2), 122.e1–122.e11 (2008).

27 Florio P, Torricelli M, De Falco G *et al.* High maternal and fetal plasma urocortin levels in pregnancies complicated by hypertension. *J. Hypertens.* 24(9), 1831–1840 (2006).

28 Giles WB, McLean M, Davies JJ, Smith R. Abnormal umbilical artery Doppler waveforms and cord blood corticotropin-releasing hormone. *Obstet. Gynecol.* 87(1), 107–111 (1996).

29 Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. *Annu. Rev. Med.* 59, 61–78 (2008).

30 Florio P, Ciarmela P, Luisi S *et al.* Preeclampsia with fetal growth restriction: placental and serum activin A and inhibin A levels. *Gynecol. Endocrinol.* 16(5), 365–372 (2002).

31 Ree PH, Hahn WB, Chang SW *et al.* Early detection of preeclampsia using inhibin a and other second-trimester serum markers. *Fetal. Diagn. Ther.* 29(4), 280–286 (2011).

32 Akolekar R, Minekawa R, Veduta A, Romero XC, Nicolaides KH. Maternal plasma inhibin A at 11–13 weeks of gestation in hypertensive disorders of pregnancy. *Prenat. Diagn.* 29, 753–760 (2009).

33 Muttukrishna S, North RA, Morris J *et al.* Serum inhibin A and activin A are elevated prior to the onset of preeclampsia. *Hum. Reprod.* 15(7), 1640–1645 (2000).

34 Spencer K, Yu CK, Savvidou M, Papageorghiou AT, Nicolaides KH. Prediction of preeclampsia by uterine artery Doppler ultrasonography and maternal serum pregnancy-associated plasma protein-A, free β-human chorionic gonadotropin, activin A and inhibin A at 22 + 0 to 24 + 6 weeks' gestation. *Ultrasound Obstet. Gynecol.* 27(6), 658–663 (2006).

35 Florio P, Reis FM, Pezzani I, Luisi S, Severi FM, Petraglia F. The addition of activin A and inhibin A measurement to uterine artery Doppler velocimetry to improve the early prediction of preeclampsia. *Ultrasound Obstet. Gynecol.* 21(2), 165–169 (2003).

36 Andraweera PH, Dekker GA, Laurence JA, Roberts CT. Placental expression of VEGF family mRNA in adverse pregnancy outcomes. *Placenta* 33(6), 467–472 (2012).

37 Cooper JC, Sharkey AM, Charnock-Jones DS, Palmer CR, Smith SK. VEGF mRNA levels in placentae from pregnancies complicated by preeclampsia. *Br. J. Obstet. Gynaecol.* 103(12), 1191–1196 (1996).

38 Smith GC, Crossley JA, Aitken DA *et al*. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. *Obstet. Gynecol.* 109(6), 1316–1324 (2007).

39 Levine RJ, Maynard SE, Qian C *et al*. Circulating angiogenic factors and the risk of preeclampsia. *N. Engl. J. Med.* 350(7), 672–683 (2004).

40 Lacroix MC, Guibourdenche J, Fournier T *et al*. Stimulation of human trophoblast invasion by placental growth hormone. *Endocrinology* 146(5), 2434–2444 (2005).

41 Vandenberghe G, Mensink I, Twisk JW, Blankenstein MA, Heijboer AC, van Vugt JM. First trimester screening for intrauterine growth restriction and early-onset preeclampsia. *Prenat. Diagn.* 31(10), 955–961 (2011).

42 Verlohren S, Herraiz I, Lapaire O *et al*. Risk stratification of hypertensive pregnancy disorders. *Eur. Ob. Gyn.* 7, 14–17 (2012).

43 Verlohren S, Herraiz I, Lapaire O *et al*. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am. J. Obstet. Gynecol.* 206(1), 58.e1–58.e8 (2012).

44 Molvarec A, Szarka A, Walentin S, Szucs E, Nagy B, Rigó J Jr. Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with preeclampsia. *Hypertens. Res.* 33(9), 892–898 (2010).

45 Ning Y, Williams MA, Muy-Rivera M, Leisenring WM, Luthy DA. Relationship of maternal plasma leptin and risk of preeclampsia: a prospective study. *J. Matern. Fetal. Neonatal. Med.* 15(3), 186–192 (2004).

46 Miehle K, Stepan H, Fasshauer M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and preeclampsia. *Clin. Endocrinol. (Oxf.)* 76(1), 2–11 (2012).

47 Tan KC, Xu A, Chow WS *et al*. Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. *J. Clin. Endocrinol. Metab.* 89(2), 765–769 (2004).

48 D'Anna R, Baviera G, Corrado F, Giordano D, Di Benedetto A, Jasonni VM. Plasma adiponectin concentration in early pregnancy and subsequent risk of hypertensive disorders. *Obstet. Gynecol.* 106(2), 340–344 (2005).

49 Dalamaga M, Srinivas SK, Elovitz MA, Chamberland J, Mantzoros CS. Serum adiponectin and leptin in relation to risk for preeclampsia: results from a large case-control study. *Metab. Clin. Exp.* 60(11), 1539–1544 (2011).

50 Grill S, Rusterholz C, Zanetti-Dällenbach R *et al*. Potential markers of preeclampsia – a review. *Reprod. Biol. Endocrinol.* 7, 70 (2009).

51 Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J. Thromb. Haemost.* 3(8), 1590–1596 (2005).

52 Chaiworapongsa T, Romero R, Yoshimatsu J *et al*. Soluble adhesion molecule profile in normal pregnancy and preeclampsia. *J. Matern. Fetal. Neonatal. Med.* 12(1), 19–27 (2002).

53 Banzola I, Farina A, Concu M *et al*. Performance of a panel of maternal serum markers in predicting preeclampsia at 11–15 weeks' gestation. *Prenat. Diagn.* 27(11), 1005–1010 (2007).

54 Shaker OG, Shehata H. Early prediction of preeclampsia in high-risk women. *J. Womens. Health (Larchmt.)* 20(4), 539–544 (2011).

55 Rusterholz C, Hahn S, Holzgreve W. Role of placentally produced inflammatory and regulatory cytokines in pregnancy and the etiology of preeclampsia. *Semin. Immunopathol.* 29(2), 151–162 (2007).

56 Derzsy Z, Prohászka Z, Rigó J Jr, Füst G, Molvarec A. Activation of the complement system in normal pregnancy and preeclampsia. *Mol. Immunol.* 47(7–8), 1500–1506 (2010).

57 Molvarec A, Szarka A, Walentin S *et al*. Serum heat shock protein 70 levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in women with preeclampsia. *Clin. Chim. Acta* 412(21–22), 1957–1962 (2011).

58 Szarka A, Rigó J Jr, Lázár L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol.* 11, 59 (2010).

59 Lynch AM, Murphy JR, Byers T *et al*. Alternative complement pathway activation fragment Bb in early pregnancy as a predictor of preeclampsia. *Am. J. Obstet. Gynecol.* 198, 385.e1–e9 (2008).

60 Lynch AM, Gibbs RS, Murphy JR, Giclas PC, Salmon JE, Holers VM. Early elevations of the complement activation fragment C3a and adverse pregnancy outcomes. *Obstet. Gynecol.* 117(1), 75–83 (2011).

61 Bahado-Singh RO, Akolekar R, Mandal R *et al*. Metabolomics and first-trimester prediction of early-onset preeclampsia. *J. Matern. Fetal. Neonatal. Med.* 25(10), 1840–1847 (2012).

62 Kenny LC, Broadhurst DI, Dunn W *et al*.; Screening for Pregnancy Endpoints Consortium. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. *Hypertension* 56(4), 741–749 (2010).

63 Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. *Br. J. Obstet. Gynaecol.* 92(1), 31–38 (1985).

64 Krebs C, Macara LM, Leiser R, Bowman AW, Greer IA, Kingdom JC. Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree. *Am. J. Obstet. Gynecol.* 175(6), 1534–1542 (1996).

65 Macara L, Kingdom JC, Kaufmann P *et al*. Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. *Placenta* 17(1), 37–48 (1996).

66 Salafia CM. Placental pathology of fetal growth restriction. *Clin. Obstet. Gynecol.* 40(4), 740–749 (1997).

67 Regnault TRH, Galanb HL, Parkera TA *et al*. Placental development in IUGR pregnancies. *Placenta* 23, 119–129 (2002).

68 Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe preeclamptic pregnancies. *Br. J. Obstet. Gynaecol.* 101(8), 669–674 (1994).

69 Redline RW, Patterson P. Preeclampsia is associated with an excess of proliferative immature intermediate trophoblast. *Hum. Pathol.* 26(6), 594–600 (1995).

70 Robertson WB, Brosens I, Dixon G. Uteroplacental vascular pathology. *Eur. J.*

Obstet. Gynecol. Reprod. Biol. 5(1–2), 47–65 (1975).

71 Gardosi J, Mongelli M, Wilcox M, Chang A. An adjustable fetal weight standard. *Ultrasound Obstet. Gynecol.* 6(3), 168–174 (1995).

72 Ferrazzi E, Bulfamante G, Mezzopane R, Barbera A, Ghidini A, Pardi G. Uterine Doppler velocimetry and placental hypoxic-ischemic lesion in pregnancies with fetal intrauterine growth restriction. *Placenta* 20(5-6), 389–394 (1999).

73 Wallner W, Sengenberger R, Strick R *et al*. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin. Sci.* 112(1), 51–57 (2007).

74 Ahmed A, Dunk C, Ahmad S, Khaliq A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen – a review. *Placenta* 21(Suppl. A), S16–S24 (2000).

75 Nevo O, Many A, Xu J *et al*. Placental expression of soluble fms-like tyrosine kinase 1 is increased in singletons and twin pregnancies with intrauterine growth restriction. *J. Clin. Endocrinol. Metab.* 93(1), 285–292 (2008).

76 Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free β human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* 107(10), 1265–1270 (2000).

77 Kirkegaard I, Henriksen TB, Uldbjerg N. Early fetal growth, PAPP-A and free β-hCG in relation to risk of delivering a small-for-gestational age infant. *Ultrasound Obstet. Gynecol.* 37(3), 341–347 (2011).

78 Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. *Prenat. Diagn.* 20(8), 652–656 (2000).

79 Gardner MO, Goldenberg RL, Cliver SP, Boots LR, Hoffman HJ. Maternal serum concentrations of human placental lactogen, estradiol and pregnancy specific β 1-glycoprotein and fetal growth retardation. *Acta Obstet. Gynecol. Scand. Suppl.* 165, 56–58 (1997).

80 Bersinger NA, Ødegård RA. Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies. *Acta Obstet. Gynecol. Scand.* 83(1), 37–45 (2004).

81 Holcom WL Jr, Smeltzer JS. Cervical effacement: variation in belief among clinicians. *Obstet. Gynecol.* 78, 43–45 (1991).

82 Sonek JD, Iams JD, Blumenfeld M, Johnson F, Landon M, Gabbe S. Measurement of cervical length in pregnancy: comparison between vaginal ultrasonography and digital examination. *Obstet. Gynecol.* 76(2), 172–175 (1990).

83 Mella MT, Berghella V. Prediction of preterm birth: cervical sonography. *Semin. Perinatol.* 33(5), 317–324 (2009).

84 Honest H, Forbes CA, Durée KH *et al*. Screening to prevent spontaneous preterm birth: systematic reviews of accuracy and effectiveness literature with economic modelling. *Health Technol. Assess.* 13(43), 1–627 (2009).

85 Berghella V, Hayes E, Visintine J, Baxter JK. Fetal fibronectin testing for reducing the risk of preterm birth. *Cochrane Database Syst. Rev.* 4, CD006843 (2008).

86 Goodwin TM. A role for estriol in human labor, term and preterm. *Am. J. Obstet. Gynecol.* 180(1 Pt 3), S208–S213 (1999).

87 McGregor JA, Jackson GM, Lachelin GC *et al*. Salivary estriol as risk assessment for preterm labor: a prospective trial. *Am. J. Obstet. Gynecol.* 173(4), 1337–1342 (1995).

88 Heine RP, McGregor JA, Goodwin TM *et al*. Serial salivary estriol to detect an increased risk of preterm birth. *Obstet. Gynecol.* 96(4), 490–497 (2000).

89 Smith R, Smith JI, Shen X *et al*. Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. *J. Clin. Endocrinol. Metab.* 94(6), 2066–2074 (2009).

90 Lockwood CJ. The diagnosis of preterm labor and the prediction of preterm delivery. *Clin. Obstet. Gynecol.* 38(4), 675–687 (1995).

91 Petraglia F, Aguzzoli L, Florio P *et al*. Maternal plasma and placental immunoreactive corticotrophin-releasing factor concentrations in infection-associated term and pre-term delivery. *Placenta* 16(2), 157–164 (1995).

92 Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. *Am. J. Obstet. Gynecol.* 180(1 Pt 3), S257–S263 (1999).

93 McLean M, Bisits A, Davies J *et al*. Predicting risk of preterm delivery by second-trimester measurement of maternal plasma corticotropin-releasing hormone and α-fetoprotein concentrations. *Am. J. Obstet. Gynecol.* 181(1), 207–215 (1999).

94 Florio P, Linton EA, Torricelli M *et al*. Prediction of preterm delivery based on maternal plasma urocortin. *J. Clin. Endocrinol. Metab.* 92(12), 4734–4737 (2007).

95 Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. *Endocr. Rev.* 31(6), 783–816 (2010).

96 Spencer K, Cowans NJ, Molina F, Kagan KO, Nicolaides KH. First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of preterm or early preterm delivery. *Ultrasound Obstet. Gynecol.* 31(2), 147–152 (2008).

97 Lieppman RE, Williams MA, Cheng EY *et al*. An association between elevated levels of human chorionic gonadotropin in the midtrimester and adverse pregnancy outcome. *Am. J. Obstet. Gynecol.* 168(6 Pt 1), 1852–1856; discussion 1856 (1993).

98 Jelliffe-Pawlowski LL, Baer RJ, Currier RJ. Second trimester serum predictors of preterm birth in a population-based sample of low-risk pregnancies. *Prenat. Diagn.* 30(8), 727–733 (2010).

99 Bernstein PS, Stern R, Lin N *et al*. β-human chorionic gonadotropin in cervicovaginal secretions as a predictor of preterm delivery. *Am. J. Obstet. Gynecol.* 179(4), 870–873 (1998).

100 Cruciani L, Romero R, Vaisbuch E *et al*. Pentraxin 3 in maternal circulation: an association with preterm labor and preterm PROM, but not with intra-amniotic infection/inflammation. *J. Matern. Fetal. Neonatal. Med.* 23(10), 1097–1105 (2010).

101 Cooper S, Lange I, Wood S, Tang S, Miller L, Ross S. Diagnostic accuracy of rapid phIGFBP-I assay for predicting preterm labor in symptomatic patients. *J. Perinatol.* 32(6), 460–465 (2012).

102 Danti L, Prefumo F, Lojacono A, Corini S, Testori A, Frusca T. The combination of short cervical length and phIGFBP-1 in the prediction of preterm delivery in symptomatic women. *J. Matern. Fetal. Neonatal. Med.* 24(10), 1262–1266 (2011).

103 Demendi C, Börzsönyi B, Nagy ZB, Rigó J Jr, Pajor A, Joó JG. Gene expression patterns of insulin-like growth factor 1, 2 (IGF-1, IGF-2) and insulin-like growth factor binding protein 3 (IGFBP-3) in human placenta from preterm deliveries: influence of additional factors. *Eur. J.*

Obstet. Gynecol. Reprod. Biol. 160(1), 40–44 (2012).

104 Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. *BJOG* 118(9), 1042–1054 (2011).

105 Goldenberg RL, Iams JD, Mercer BM *et al.*; Maternal-Fetal Medicine Units Network. The Preterm Prediction Study: toward a multiple-marker test for spontaneous preterm birth. *Am. J. Obstet. Gynecol.* 185(3), 643–651 (2001).

106 Coleman MA, Keelan JA, McCowan LM, Townend KM, Mitchell MD. Predicting preterm delivery: comparison of cervicovaginal interleukin (IL)-1β, IL-6 and IL-8 with fetal fibronectin and cervical dilatation. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 95(2), 154–158 (2001).

107 Gervasi MT, Romero R, Bracalente G *et al.* Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-γ-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. *J. Perinat. Med.* 40(4), 329–343 (2012).

108 Norwitz ER, Caughey AB. Progesterone supplementation and the prevention of preterm birth. *Rev. Obstet. Gynecol.* 4(2), 60–72 (2011).

109 Society for Maternal-Fetal Medicine Publications Committee, with assistance of Vincenzo Berghella. Progesterone and preterm birth prevention: translating clinical trials data into clinical practice. *Am. J. Obstet. Gynecol.* 206, 376–386 (2012).

110 Waffarn F, Davis EP. Effects of antenatal corticosteroids on the hypothalamic-pituitary-adrenocortical axis of the fetus and newborn: experimental findings and clinical considerations. *Am. J. Obstet. Gynecol.* 207(6), 446–454 (2012).

111 Berkman ND, Thorp JM Jr, Hartmann KE *et al*. Management of preterm labor. Evidence Report/Technology Assessment No. 18 (Prepared by Research Triangle Institute under Contract No. 290-97-0011). AHRQ Publication No. 01-E021. Agency for Healthcare Research and Quality (Level III), Rockville, MD, USA (2000).

112 Crowley P. Prophylactic corticosteroids for preterm birth. *Cochrane Database Syst. Rev.* 18, CD000065 (2007).

113 Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst. Rev.* 3, CD004454 (2006).

114 Kapoor A, Dunn E, Kostaki A, Andrews MH, Matthews SG. Fetal programming of hypothalamo–pituitary–adrenal function: prenatal stress and glucocorticoids. *J. Physiol. (Lond.)* 572(Pt 1), 31–44 (2006).