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Predictive Value of Hormone Measurements in Maternal and Fetal Complications of Pregnancy

FERNANDO M. REIS, DONATO D'ANTONA, AND FELICE PETRAGLIA

Chair of Obstetrics and Gynecology (F.P.), University of Siena, 53100 Siena, Italy; Department of Obstetrics and Gynecology (D.D.), University of Padua, 35128 Padua, Italy; and Department of Obstetrics and Gynecology (F.M.R.), University of Minas Gerais, Belo Horizonte 30130-100, Brazil

Intrauterine tissues (placenta, amnion, chorion, decidua) express hormones and cytokines that play a decisive role in maternal-fetal physiological interactions. The excessive or deficient release of some placental hormones in association with gestational diseases may reflect an abnormal differentiation of the placenta, an impaired fetal metabolism, or an adaptive response of the feto-placental unit to adverse conditions. This review is focused on the applicability of hormone measurements in the risk assessment, early diagnosis, and management of pregnancies complicated by Down's syndrome, fetal growth restriction, preeclampsia, preterm delivery, and diabetes mellitus. Combined hormonal tests or the combination of hormones and ultrasound may achieve reasonable sensitivity, but research continues to simplify the screening programs without sacrificing their accuracy. Only in a few instances is there sufficient evidence to firmly recommend the routine use of hormone tests to predict maternal and fetal complications, but the judicious use of selected tests may enhance the sensitivity of the risk assessment based solely on clinical and ultrasound examination. (*Endocrine Reviews* 23: 230–257, 2002)

I. Introduction

A PLETHORA OF hormonal changes contribute to the physiological maternal adaptations during human gestation. Fluid balance, blood pressure, digestion, respiration, fuel and mineral metabolism, immune response, and several behavioral functions are reprogrammed during pregnancy and occur under the modulation of hormonal changes, from very early gestation to fetal delivery and beyond (1). These hormonal changes are different in pathological pregnancies and may be monitored for diagnosis or risk prediction of gestational diseases, taking into account both the hormonal levels and the preexisting maternal risk factors.

Intrauterine tissues (placenta, amnion, chorion, decidua) express hormones and cytokines (2) that play a decisive role in maternal-fetal physiological interactions, in the reprogramming of the maternal endocrine system, and in the signaling mechanisms that determine the timing of parturition (3–8). The amount of information regarding placental hormones has dramatically increased over the last few years and has had a great impact on the recognized mechanisms of gestational disorders. At the same time, newly developed assays have become available and have permitted the precise assessment of several placental hormones in the maternal and fetal circulation and in the amniotic fluid. As a natural consequence of these achievements, placental hormones have been investigated as biochemical markers of gestational diseases.

The excessive release of some placental hormones in association with gestational diseases may be part of an adaptive response of the placenta and fetal membranes to adverse environmental conditions, such as hypertension, hypoxia, and infection, or to malformations of the fetus and placenta. The high concentrations of these hormones in maternal peripheral blood, in fetal (cord) blood, and in the amniotic fluid are clinically accessible signs of increased placental hormone synthesis (Fig. 1). Neurohormones and steroids have more endocrine features and are measurable in maternal serum, saliva, or urine, whereas growth factors and cytokines are more often altered in cord serum or amniotic fluid, probably reflecting a fetal/membrane adaptive response. These hormonal changes probably represent a nonspecific functional counterpart of structural changes such as proliferation of the

Abbreviations: AFP, α-Fetoprotein; ANP, atrial natriuretic peptide; DHEA-S, dehydroepiandrosterone sulfate; FGR, fetal growth restriction; GHBP, GH-binding protein; ICAM, intercellular adhesion molecule; IGFBP, IGF-binding protein; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein A; sICAM and sVCAM, soluble forms of ICAM and VCAM; VCAM, vascular cell adhesion molecule.

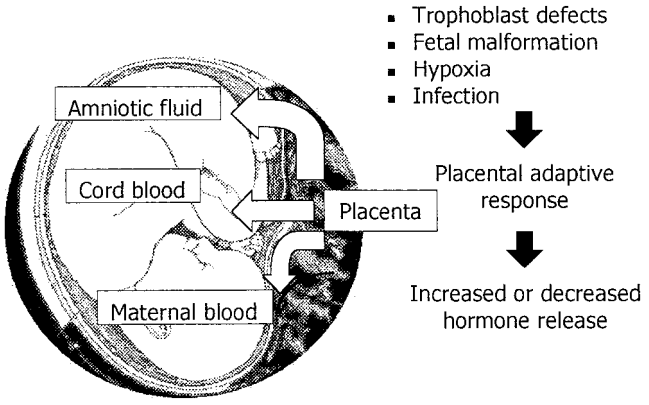

FIG. 1. Adverse environmental conditions, fetal or placental malformation can elicit an adaptive response whose visible side is the increase in placental hormone levels at three major fluid compartments: maternal blood, cord blood, and amniotic fluid.

trophoblast cells and increased prominence of the villous stroma (2).

A potential problem in extrapolating the results of epidemiological studies to clinical practice is that "normal" hormone concentrations may change from one population to another and also from week to week of gestation. This problem is fortunately overcome by the adoption of multiples of the median (MoM) of healthy individuals as a unit of reference to convert hormone concentrations and quantify deviations up or down the normal median. What changes more between studies, however, is the positive predictive value, since the probability that a woman with a positive test will develop a disease depends not only on the accuracy of the test, but also on her preexisting risk factors as well as the expected incidence of the disease in the community to which the woman belongs. Therefore, extreme care should be taken in obstetric counseling to recognize that every screening test has a limited power to predict the absolute risk of an individual.

The aim of the present review is to summarize evidence concerning the applicability of hormone measurements to the risk assessment, early diagnosis, and management of pregnancies complicated by Down's syndrome, fetal growth restriction (FGR), preeclampsia, preterm delivery, and diabetes mellitus. The data discussed here are intended to support clinical decisions regarding the advantages of performing endocrine screening tests on pregnant women, the choice of one or more tests that best combine sensitivity and specificity, and the convenience of using such tests in the first or second trimester to identify women at a higher risk of developing complications of late gestation.

II. Down's Syndrome

Total or partial trisomy of chromosome 21 is responsible for the occurrence of Down's syndrome, which is the most common severe abnormality at birth and has an incidence of 1:700 live births (9). Numerous cytogenetic studies of early and late spontaneous abortions suggest that the incidence of trisomy 21 pregnancies is actually higher, since approximately one forth of the affected pregnancies fails to survive to term (10). The most common cause of trisomy 21 is meiotic error, usually of maternal origin, with paternal meiotic failure being recognized in 4.3% to 35% of the cases (11, 12).

The majority of Down's syndrome babies have low IQ (IQ $<$ 50) and important structural malformations. Gastrointestinal defects are present in 77% of Down's syndrome neonates, cardiac anomalies in 38%, and hematological problems in 11% (13). Consequently, the infant mortality rate is increased up to 24-fold depending on the infant health status, the gestational age at birth, and the residential arrangement (14).

Down's syndrome diagnosis requires invasive testing: 1) chorionic villus sampling in the first trimester, or 2) amniocentesis in the second trimester, or 3) cordocentesis in the second or third trimester. Because invasive tests are associated with a risk of pregnancy loss ranging from 0.5% to 1% (15, 16), noninvasive screening tests have acquired much interest. The current approach combines biochemical markers with maternal age, a variable that is strongly associated with trisomy incidence (17, 18).

A. Endocrinology of Down's syndrome

Down's syndrome is characterized by an alteration in the secretion of placental and fetal proteins and steroids. In 1987, based on the observation of Merkatz and co-workers (19), who reported that aneuploid pregnancies were characterized by low maternal serum α-fetoprotein (AFP) levels, Cuckle *et al.* (20) proposed the screening for Down's syndrome using maternal age in association with AFP. Since then, numerous pregnancy-associated maternal serum markers for fetal trisomy 21 have been evaluated. These include estriol, human CG (hCG), inhibin A, and pregnancy-associated plasma protein A (PAPP-A).

1. AFP. AFP is a glycoprotein produced by the yolk sack and fetal gastrointestinal tract. AFP is a well established screening tool for neural tube defects (21). Elevated maternal serum AFP levels are significantly associated with open spina bifida and anencephaly (22). Maternal serum AFP levels rise steadily until 32 wk of gestation, whereas fetal AFP peaks at 10–13 gestational weeks and then declines progressively until term (22). Amniotic fluid AFP, which reflects the fetal urinary excretion, peaks at 12–14 wk of gestation (23). Maternal serum AFP levels are blunted in Down's syndrome pregnancies at 15–20 wk of gestation (19).

Fetal liver AFP mRNA expression is not significantly different in Down's syndrome and normal pregnancies (24), suggesting that low maternal serum AFP levels in Down's syndrome could result from impaired fetal kidney function or impaired membrane or placental passage (25).

The maternal risk of having a Down's syndrome pregnancy has been calculated from mother's age-related *a priori* risk and maternal serum AFP values normalized to a gestational age-matched control population (20, 26). It has been estimated that screening for Down's syndrome using both maternal age and serum AFP improves the specificity without sacrificing the sensitivity of either marker alone. As a consequence, a greater number of unaffected pregnancies may be spared unnecessary amniocentesis (20).

2. Estriol. Although the placenta is the source of estriol, this hormone may reflect fetal steroidogenesis. The fetal adrenal glands produce dehydroepiandrosterone sulfate (DHEA-S), which is hydroxylated by the fetal liver into 16-α-hydroxy-DHEA-S. The latter is transported to the placenta where it undergoes desulfation by steroid sulfatase and is finally aromatized to estriol (27). In the early phase of pregnancy, fetal adrenal DHEA-S production is independent of fetal ACTH, but in the second trimester ACTH is required for adrenal function. Henceforth, 90% of the estriol production originates from DHEA-S synthesized by the fetal adrenal glands (28). In fact, unlike total estriol, unconjugated estriol is produced almost entirely by the fetal-placental unit and therefore is a more sensitive indicator of fetal health.

Maternal serum unconjugated estriol levels are significantly lower in Down's syndrome pregnancies, ranging from 0.65 (27) to 0.79 MoM (29, 30). Amniotic fluid and placental tissue unconjugated estriol levels are also significantly lower in Down's syndrome. Whereas placental turnover is not affected by trisomy 21, DHEA-S levels in maternal serum, placental tissue, and fetal liver are decreased, suggesting the possibility that the reduction of estriol is linked to diminished fetal DHEA-S synthesis (27).

Unconjugated estriol is a variable independent of maternal age, and therefore it can be used alone or in combination with maternal age for the determination of the relative risk of Down's syndrome. In a routine screening program, maternal serum unconjugated estriol has poor predictive power if used as a single marker, but its inclusion contributes to improving the predictive value of age and AFP (31).

3. hCG. hCG is a glycoprotein composed of two noncovalently linked subunits, α and β, and is produced by syncytiotrophoblast cells of the placenta. hCG has a single β-subunit and an α-subunit also shared by three other glycoprotein hormones: LH, FSH, and TSH. Maternal serum hCG peaks at 8–10 wk and then declines to reach a plateau at 18–20 wk of gestation. Five hCG-related molecules are present in maternal serum: nonnicked hCG, which represents the active hormone; nicked hCG; free α-subunit; free β-subunit; and the nicked free β-subunit (32–34). The free β-subunit can derive from three sources, *i.e.*, direct trophoblast cell production, dissociation of hCG into free α- and free β-subunits, and by macrophage or neutrophil enzymes nicking the hCG molecule (35). The free β-hCG circulating in maternal serum corresponds to only about 0.3–4% of the total hCG (36, 37).

In 1987, Bogart and co-workers (38) reported an elevation of maternal serum hCG levels in Down's syndrome pregnancies, and since then hCG has been introduced in most screening programs (38, 39). In fact, second trimester maternal serum hCG levels are significantly higher in Down's syndrome compared with normal pregnancies, with an average of 2.00 MoM (38, 40, 41). Also free β-hCG levels are augmented in Down's syndrome pregnancies (41–45).

Molecular biology studies have demonstrated that trisomy 21 trophoblasts show a marked increase in β-hCG mRNA and a smaller increase in α-hCG mRNA, suggesting that one of the causes of high hCG levels in maternal serum is the increased hCG production and secretion by the placenta (46). These observations are supported by the relative immaturity of the placenta, which continues to release large amounts of hCG as in the first trimester (47). Furthermore, genetic mappings have shown that neither the genes of the α-subunit nor those of the β-subunit of hCG are located on chromosome 21 but are located on chromosomes 6 and 19, respectively (48, 49), although they can be overexpressed by multiple mechanisms (50).

By the second trimester, maternal serum hCG and free β-hCG are the most sensitive single analytes in screening of Down's syndrome (44, 51, 52). The real advantage of using free β-hCG instead of the intact molecule is still open to debate (53). Several groups of investigators advocate that the free β-subunit hCG can improve the rate of Down's syndrome detection, thanks to a wider separation between the median concentrations in affected and unaffected pregnancies (41, 54, 55). These observations have not been confirmed by other groups who did not report a significant improvement (42, 56); a possible explanation for this discrepancy may lie in some aspect of the storage process of the maternal sera (42).

First trimester maternal serum free β-hCG levels are significantly elevated in Down's syndrome pregnancies (57–59), a fact that may justify moving the screening from the second to the first trimester. A reasonably efficient screening can be performed as early as between 9 and 15 wk of gestation (60). It seems that measuring the free β-subunit improves the performance of the first trimester screening compared with the measurement of total hCG (59, 61, 62), although both measures are highly correlated (60).

4. Inhibin A. Inhibins are glycoproteins that were first isolated from ovarian follicular fluid and named after their ability to inhibit the pituitary secretion of FSH. Inhibins A and B are heterodimers composed by an α-subunit and a βA or βB subunit, respectively, linked by a disulfide bridge (63). Inhibin-related proteins comprise activins, which are homodimers composed by the same β-subunits of the inhibin molecule, and follistatin, a binding protein with affinity for inhibins and activins via the β-subunit. Inhibins and activins are members of the TGFβ superfamily, a group of structurally similar but functionally diverse growth factors (64).

Inhibin α and βA subunits are widely localized in the cyto- and syncytiotrophoblast (65, 66), and the intensity of the hybridization signal for inhibin α and βA subunit mRNA increases throughout pregnancy, peaking in extracts prepared from term placentas (65). Although the decidua (67), membranes (68), and fetus all produce inhibin, the placenta is the major source (7, 69). In consonance with placental expression, maternal serum inhibin A and activin A concentrations increase progressively during gestation, especially in the last trimester (70, 71).

In 1992, a role of immunoreactive inhibin in the screening for Down's syndrome was first suggested by *in vitro* and *in vivo* studies (62, 72), but it was only after the development of a specific assay for inhibin A (73) that it was possible to demonstrate a significant elevation of maternal serum inhibin A levels in the second trimester (74). In fact, many studies have reported values ranging from 1.53 (75) to 2.60 MoM (74) in Down's syndrome pregnancies.

Inhibin pro-α C, the inhibin α-subunit precursor, also reaches higher levels in maternal serum in Down's syndrome pregnancies. These data suggest that the mechanism(s) underlying the elevated inhibin levels observed in Down's syndrome may affect the regulation of both the inhibin α- and βA subunits (75). Interestingly, in affected pregnancies amniotic fluid inhibin A levels are significantly decreased (76), possibly because of a reduced inhibin clearance from the amniotic cavity.

Inhibin A in combination with maternal age, at a fixed 5% false-positive rate, has shown an average detection rate of 42% (42, 77), which is insufficient to support its use as a single marker. However, inhibin A may be effectively introduced in a multiple marker screening for Down's syndrome, as will be discussed further on.

5. PAPP-A. PAPP-A is a 750-kDa glycoprotein produced specifically by the trophoblast. It therefore can be found in pregnant, but not in nonpregnant or male, plasma (78). It is made up of four subunits of which only two are unique to PAPP-A (79).

Maternal serum PAPP-A levels are detectable as early as 8 wk of gestation and then rise throughout pregnancy (80). As first observed in 1991 by Brambati *et al.* (81), maternal serum PAPP-A levels, when measured between 8 and 13 wk of gestation, are significantly reduced in Down's syndrome pregnancies, with an average decrease of 2.5 times (59). Interestingly, maternal serum PAPP-A levels return to normal values between 17 and 19 wk of gestation (82, 83).

The decrease in maternal serum PAPP-A is dissociated from any change in placental synthesis of this protein, since PAPP-A mRNA expression is not significantly decreased in Down's syndrome placentas. Furthermore, the correlation between serum and tissue expression levels of PAPP-A is lost in Down's pregnancies. These observations suggest that the decrease in maternal serum PAPP-A is posttranslational and may be caused by an alteration of the placenta-releasing mechanisms or by a modification of the stability of the secreted protein (84).

PAPP-A is a powerful screening tool for Down's syndrome pregnancies and has the special advantage of achieving satisfactory results as early as the first trimester (85). Compared with other markers alone, PAPP-A provides the highest detection rate for Down's syndrome at early screening (60).

6. Combined hormone measurements for screening of Down's syndrome. The association of maternal age with serum protein levels has been shown to be useful in the screening for Down's syndrome. In fact, this is the most effective approach; it combines maternal serum markers, which should not be correlated, to achieve the highest sensitivity (77).

The use of maternal age of 35 yr or more as the only criterion for performing invasive testing results in identification of only 20% of Down's syndrome pregnancies (18). In the second trimester, the combination of maternal age with AFP elevates the detection rate to approximately 28% (20). The addition of hCG (31) and unconjugated estriol (29) represents a major improvement, as the average detection rate increases to 60% at a fixed false-positive rate of 5% (60, 86–88). This combination (AFP, hCG, and estriol) is called "triple test."

To improve the second-trimester screening sensitivity, many analytes have been proposed and evaluated, *e.g.*, progesterone (89), schwangerschafts-protein 1 (90), and placental alkaline phosphatase (91), but without success, and only the introduction of inhibin A has shown a real advantage.

The use of inhibin A as an additional marker can increase substantially the performance of the triple test (92). This combination has been named "quadruple test." Interestingly, the use of β-hCG, AFP, and inhibin A with the exclusion of unconjugated estriol may improve the sensitivity and reduce the false-positive rate of the triple test (77, 93). The rate of detection of Down's syndrome increases from 53% to 75% with the same specificity when inhibin A is added to maternal age, AFP, and β-hCG (77).

Recent studies have suggested the possibility of moving the maternal serum screening to the first trimester (60, 94). In addition to the clinical advantage of a precocious diagnosis, there is a desire among pregnant women for screening to be conducted earlier than 15 wk of gestation (95). Many possible markers have been evaluated: AFP, estriol, hCG and free β-hCG, CA125, PAPP-A, and glycoprotein of pregnancy (96). Among the possible markers for first-trimester Down's syndrome screening, the combination of maternal age, PAPP-A, and free β-hCG has produced the best results (59, 60). In this double analyte test, PAPP-A is the most informative marker.

In the first-trimester ultrasonography scan, embryo nuchal translucency can be evaluated. Nuchal translucency is a physiological space between the back of the fetal neck and the overlying skin. To date, there have been several publications describing an association of an increased nuchal translucency thickness with fetal aneuploidy (97, 98) and, in particular, with Down's syndrome (99), with a mean detection rate of 72% (100, 101). Interestingly, the mathematical combination of nuchal translucency ultrasound measurement with maternal age, PAPP-A, and free β-hCG has a hypothetical 80% detection rate for a fixed 5% false-positive rate (102); this result is still undergoing validation by appropriate clinical studies.

B. Summary and clinical recommendations

In current practice the most common screening test is the triple test consisting of AFP, estriol, and hCG or free β-hCG, which together with maternal age may achieve a detection rate ranging from 60% to 65% for a false-positive rate of 5%. An alternative triple test substituting inhibin A for estriol has shown evident superiority to the traditional triple-analyte combination, and therefore it is advisable to convert these consistent epidemiological findings into clinical practice. The introduction of inhibin A in routine second-trimester screening for Down's syndrome will require standardization of normal values for different populations to express individual results as MoM and thereby preserve the accuracy attained by the test in controlled studies.

More recently, a passage from second-trimester to first-trimester screening has been proposed. The introduction of nuchal translucency and serum screening based on PAPP-A and free β-hCG seem to be a promising enhancement in the field. This represents an excellent opportunity for early di-

agnosis and should be offered, wherever these resources are available, to pregnant women desiring a screening for Down's syndrome. Until the introduction in clinical practice of the isolation of fetal cells in the maternal circulation for karyotype determination, maternal serum and fetal ultrasound screening are the most effective tools in our hands.

III. Fetal Growth Restriction (FGR)

FGR, also referred to as intrauterine growth restriction/retardation, is a complex condition for which definition has not reached a consensus. From a pathological point of view, FGR is characterized by a disrupted fetal growth and should not be confounded with low birth weight, which encompasses preterm infants with normal development, or even with small-for-gestational-age fetuses, a broad concept that embraces FGR but also normal fetuses with a familial tendency to growth below the population average (103). Traditionally, working definitions of FGR have been birth weight below 2 SDs of the mean (104) or less than the 10th percentile for the same gestational age (105), but these criteria better define small-for-gestational-age infants.

The use of customized fetal growth standards can reduce the rates of false-positive and false-negative diagnoses of FGR (106, 107). There is increasing evidence that customizing fetal growth curves for parental height, weight, parity, ethnic group, and fetal gender improves the distinction between genetically small and growth-restricted fetuses in different populations (108). In addition, customized fetal growth curves are able to detect fetuses born with a normal weight but who fail to reach their full genetic growth potential. These fetuses are also at increased risk of perinatal mortality and cerebral palsy (109).

FGR is a heterogeneous pathology caused by multiple factors of fetal, placental, and maternal origin (110). An abnormal fetal karyotype, *e.g.*, trisomy 21 and 18 (111, 112), or fetal congenital malformations, such as neural tube defects and renal dysplasia, are strongly associated with FGR (110). Multiple gestation is an important cause of FGR, possibly determined by sharing of antenatal maternal nutrients, placental dysfunction, and genetic factors, with an overall incidence of 15–30% (113, 114). Other possible causes of FGR are gross structural placental abnormalities (placental hemangiomas, abnormal cord insertions, bilobate placenta) (115) or abnormal placental localization (*e.g.*, placenta previa) (116). Especially in pregnant women with hypertensive disorders, the incidence of FGR is increased 2- to 3-fold (117, 118), and the severity of the hypertension correlates directly with the presence of FGR. Maternal smoking (119, 120), as well as alcohol consumption (121), maternal malnutrition (122), the mother being underweight, and the presence of a chronic maternal disease (123) or a congenital infection, are associated with various degrees of FGR.

Histopathological studies have demonstrated that FGR placentas present villi with a limited angiogenesis by lower expression of vascular endothelial growth factor (124, 125), linked to placental failure in transferring oxygen and a consequent fall in mean intervillous pO_2 (126). Preeclampsia, which is one of the major contributors to FGR, causes intravascular coagulation, fibrin deposition in the spiral arteries, and, consequently, placental hypoperfusion (127–130). In addition, the apoptotic index is significantly higher in FGR placentas compared with normal pregnancies (131–133).

FGR has an incidence of 3–10% in developed countries and 6–30% in the developing world (134–137). The relationship between decreasing birth weight percentiles and increasing fetal morbidity and mortality has been demonstrated by several authors (138–141). Specifically, FGR is strongly associated with neonatal death, necrotizing enterocolitis, and respiratory distress syndrome and has a less strict association with intraventricular hemorrhage (142). Epidemiological studies suggest that FGR is a significant risk factor for the subsequent development of chronic hypertension, ischemic heart disease, diabetes, and obstructive lung disease in adult life (143, 144).

Because of the relevant incidence of FGR and its fetal-neonatal consequences, it is of primary importance to use screening tools to predict this heterogeneous pathology. In addition to ultrasound protocols, maternal serum screening is under extensive investigation, and new markers emerge continuously, as no single method thus far has proved completely satisfactory (145).

A. Endocrinology of FGR

Because FGR is a multifactorial heterogeneous pathology, it is difficult to find a common endocrine pattern. For a long period in the history of obstetrics, maternal serum and urinary estriol and placental lactogen (PL) levels have been tools for the monitoring of fetal welfare (146, 147) and fetal growth (148–150), and a reduction of their levels was associated with FGR. Infusion of DHEA-S with subsequent determination of plasma E2 and estetrol concentrations was proposed to assess placental and fetal function in the 1970s (151). After a period of ostracism, when most centers abandoned endocrinological testing in favor of various ultrasound approaches, there has been a resurgence of enthusiasm for hormone markers of fetal well-being since hCG was shown to predict FGR (152, 153). More recently, attention has been focused on the possible correlation between fetal growth and the placental production of GH, GH-binding protein (GHBP), IGFs, and leptin.

1. hCG. In the first trimester, women with serum hCG levels below the 10th percentile have an increased risk of subsequent FGR (154). The low hCG levels probably reflect a derangement of trophoblast function that will culminate in placental insufficiency and FGR.

Although the association between high second-trimester hCG values and FGR has been widely investigated, it is a matter of controversy. Several authors have reported a significant increase of second-trimester maternal serum hCG levels in patients who developed FGR, ranging from 1.21 to 2.5 MoM (152, 153, 155), and an approximately 2- to 3-fold increase in the risk of FGR has been observed among individuals with unexplained elevated hCG levels in the second trimester (153, 156). Also free β-hCG has been associated with poor pregnancy outcome and FGR (157). More recently, high second-trimester maternal urine β-core hCG fragment

levels have been reported in association with FGR (158). The β-core fragment is the metabolite of hCG that accumulates in maternal urine and might be a useful marker of trophoblast activity (159). However, FGR is closely associated with preeclampsia, and the latter is a strong confounding factor because the pathological features of preeclampsia include an excessive placental secretion of hCG. A study designed to solve this confusion has actually demonstrated that hCG levels in the second trimester were not elevated in women who carried growth-retarded fetuses but did not develop preeclampsia (160).

In the third trimester, maternal serum hCG levels are significantly higher in FGR pregnancies associated with pathological umbilical artery flow velocimetry, underlying vascular placental insufficiency, whereas FGR pregnancies with normal Doppler parameters show normal hCG levels (161).

2. Human PL (hPL). hPL is a polypeptide of 191 amino acids that has structural and functional homology with PRL and GH. The levels of hPL in the maternal circulation are very low in early pregnancy and increase progressively, showing some correlation with placental weight (8). hPL production and expression are localized in the placenta, but the actions of the hormone affect both fetus (with a weak somatotropic effect) and mother (altering the lipid and carbohydrate metabolism and thereby increasing the availability of energy substrate to the fetus) (162). Maternal serum hPL levels reflect placental biosynthesis and are positively correlated with the size of the fetus (163), suggesting the possibility of using this hormonal marker for the screening of FGR.

Serum hPL was proposed in the 1970s as a screening test to detect and monitor pregnancies at risk, particularly those with FGR, since declining levels of hPL would be an early sign of impaired placental function and chronic fetal distress (147). Indeed, low maternal serum hPL levels have been associated with FGR (149, 150, 164, 165). A major limitation of maternal hPL measurement in the screening of FRG is the fact that its screening efficiency begins at 30–35 wk of gestation (145, 150), with low if any predictive value in the second trimester (145, 148) or in the first trimester (166).

3. Estrogens. Among the estrogens, estriol seems to have the most relevant role in FGR screening. From the mid-1960s to the 1970s, maternal estriol has been used to assess fetal welfare with serial urine and serum measurements between 30 and 42 wk of gestation (146). While mothers carrying growth-restricted fetuses have serum estriol levels reduced to approximately half the normal levels (167), maternal E2 does not change meaningfully (148).

Decreased second-trimester unconjugated estriol levels (below 0.75 MoM) are significantly associated with FGR (168, 169). Furthermore, maternal urine estriol below the 10^{th} percentile is found in about 30% of FGR pregnancies (170).

Low estriol levels could be associated with either a placental or a fetal pathological condition, alone or in combination (168). In fact, infants with intrauterine growth restriction/retardation have been reported to have disturbed adrenocortical function (171, 172) and, at birth, umbilical vein DHEA-S and estriol are significantly reduced (167). Placental DHEA-S conversion could be impaired by vascular pathology (173) and/or by reduced blood flow (174).

4. GH and IGFs. During pregnancy, maternal pituitary GH is progressively replaced by placental GH (175, 176), a variant GH molecule produced by the syncytiotrophoblast (177). Its biological activity is modulated by GHBP (178, 179), the pregnancy levels of which are similar to those in the nonpregnant state (180). Placental GH is thought to play a role in the development and function of the placenta and to have an indirect influence on fetal growth, since no significant amounts of this hormone enter the fetal circulation (176, 181).

Maternal serum and placental GH levels are lower in FGR pregnancies (182), with an average reduction of 50% in the third trimester, when the circulating GH is produced almost entirely by the placenta (182, 183). Interestingly, free GH levels are further reduced by increased concentrations of GHBP (182). The low level of placental GH may be due not only to placental size, but also to the reduced production of GH by the placenta. The population of cells expressing placental GH is reduced in FGR pregnancies, although the relative levels of GH mRNA in the single nuclei are not different (184). These cells are functionally normal, but their numerical reduction suggests an impairment of the functional organization of the placenta.

IGFs are a family of pro-insulin-like polypeptides that stimulate cell division and differentiation. IGF-I and IGF-II mRNAs are present in many fetal tissues from 9 wk of gestation (185), and the proteins are detectable in the fetal circulation from 15 wk (186). In postnatal life, most of the growth-promoting effect of GH is mediated by IGF-I (187). However, the importance of IGF-I and IGF-II to fetal growth has been demonstrated in gene knockout mice, in which disruption of either the IGF-I (188) or IGF-II (189) gene determines severe FGR, suggesting a prominent role of IGFs in fetal growth. More recently, Woods *et al*. (190) reported the first observation of a case of a 15-yr-old boy with a homozygous partial IGF-I gene deletion, resulting in extreme growth failure beginning *in utero* and continuing after birth.

Both maternal and fetal IGF-I influence placental metabolism and thereby regulate the availability of substrates required for fetal growth (191). Maternal and fetal IGFs appear to be independently regulated since there is no correlation between maternal serum and cord serum IGF-I levels. Therefore, maternal IGF-I may influence fetal growth only through its effect on placental function (192).

Low maternal IGF-I levels have been observed in last trimester pregnancies complicated by FGR and by an abnormal pattern of umbilical artery blood flow (193–196), but a consistent relationship between maternal IGF-I levels and fetal birth weight has not been established. Interestingly, this hormonal pattern is associated with a significant increase in IGF-binding protein 1 (IGFBP-1) but not IGFBP-3 levels (197). The measurement of IGF-I and IGFBP-1 in cord blood obtained by cordocentesis during the third trimester appears to increase the predictive value of ultrasound in estimating the weight of fetuses affected by growth retardation. Infant birth weight is positively correlated with cord blood IGF-I concentrations and is inversely correlated with IGFBP-1 (198).

Low maternal IGF-I levels may be a consequence of abnormal placentation or inappropriate placental hormone stimulation, such as hPL or GH, of the maternal IGF system (Fig. 2). An increase in IGFBP-1 may result from maternal

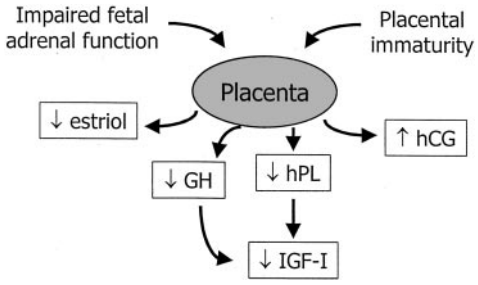

FIG. 2. Hormone changes associated with FGR.

carbohydrate metabolic changes as well as from alterations in placental or decidual function (197), but the finding of low IGFBP-1 levels in association with preeclampsia (199) suggests that changes in this protein do not simply reflect placental dysfunction but also involve regulatory mechanisms specific to each pathological process.

5. Leptin. Leptin is an important modulator of food intake and energy balance, the major source of which is the adipose tissue (200, 201). Thus, serum leptin levels are positively associated with the percentage of body fat or body mass index, suggesting that obese individuals have a decreased sensitivity to the appetite modulator effect of the hormone (202, 203). Moreover, leptin has been linked to the adaptive response to fasting as it affects GH secretion, thyroid and adrenal function, and several components of the reproductive hormonal axis (204).

Leptin has been isolated from placental tissue (205, 206), which may explain the significant increase of serum leptin levels during pregnancy (207). The rise in leptin levels during pregnancy is far beyond the expected increase due to pregnancy-induced gain of body adiposity (208). The postpartum period is characterized by a rapid drop of leptin concentrations, consistent with the placental source withdrawal (209). Most of the leptin produced by the placenta is released into the maternal circulation, but significant amounts also enter the fetal circulation (210).

Leptin is detectable in umbilical cord serum as early as 18 wk of gestation, and its levels rise considerably after the 34th wk, when the fetus starts to accumulate most of the adipose tissue (211, 212). Umbilical cord serum leptin levels are positively correlated with fetal birth weight (213–215) but do not show any significant association with maternal or obstetric factors (215, 216). Nor do fetal leptin levels assessed by cordocentesis reveal differences between normal and growth-restricted fetuses, at least before 34 wk (215, 217). No significant correlation exists between maternal and fetal leptin levels (214, 215) and, most important, maternal leptin concentrations do not predict fetal birth weight (213, 214, 218). Therefore, leptin does not seem to be a suitable antenatal marker of FGR.

B. Summary and clinical recommendations

FGR is a multifactorial heterogeneous pathology (110) and therefore it is impossible to elaborate a single guideline for its detection. In clinical practice, an early detection of fetuses at risk for, or already affected by, growth restriction is of fundamental importance to establish an intensive program of fetal surveillance the goal of which is to define the best timing for parturition (219).

Even though ultrasound remains the standard technique for detection of FGR and subsequent fetal surveillance, the addition of biochemical markers such as cord blood IGF-I can enhance the precision of ultrasound-based weight gain estimates (198). In addition, maternal serum biochemical markers may have a place in estimating the risk of FGR. Second-trimester maternal serum hCG levels of 2.0 MoM or more are in fact associated with a 2- to 3-fold increased risk of FGR. Less useful markers are maternal serum hPL, estrogens, and leptin, with little or no diagnostic value. GH and IGFs are promising markers but their importance in clinical practice is still limited.

IV. Preeclampsia

Preeclampsia is a well defined clinical entity affecting pregnant women, the etiology of which remains uncertain. Some authors have suggested that it may not be a single disease, but a syndrome of many possible origins (220). Severe and/or early-onset preeclampsia is an important cause of fetal and maternal morbidity and mortality.

Preeclampsia has been traditionally defined as persistent blood pressure elevation, edema, and proteinuria newly diagnosed in pregnancy. Whereas edema is no longer recommended as a diagnostic marker because it is a common and nonspecific finding, proteinuria and hypertension remain the cornerstone of the diagnosis and management. According to a recent classification proposed by the National High Blood Pressure Education Program, the minimal criteria for diagnosis of preeclampsia are proteinuria, defined as 300 mg or more of urinary protein excretion per 24 h, and hypertension, defined as blood pressure of 140/90 mm Hg or higher and first diagnosed after 20 wk of gestation (221).

A reliable early marker of preeclampsia would permit identification of patients who might benefit from prophylaxis, when it becomes available. Until now, preventive interventions, such as calcium dietary supplementation (222), the use of aspirin (223), and the supplementation of n-3 fatty acids such as fish oil (224), have shown a limited efficacy. The use of low molecular weight heparin, the supplementation of vitamin C and E with a beneficial effect on oxidative stress (225), and the use of atenolol (226) have produced more promising results, but their role in clinical practice is still under investigation, and strict surveillance remains the chief strategy to prevent complications. Even in this present context, predicting the risk of preeclampsia is important so that selected patients may be submitted to more intensive antenatal care.

A huge number of tests have been proposed to predict preeclampsia, ranging from asking the simple question of how the patient is feeling (227) and standard methods of antenatal care such as blood pressure measurement and proteinuria by dipstick, to blood and urine biochemical tests, infusion of vasoconstrictor substances, hematological markers, and ultrasonographic evaluation. In the past decade an increasing number of reports have established a link between the risk of preeclampsia and uterine artery Doppler alter-

ations (228–230). The increased vascular resistance is responsible for the typical modifications in the Doppler wave forms and velocimetry of the uterine arteries (231). Between 20 and 24 wk of gestation, the presence of an early diastolic notch and a mean resistance index of 0.65 or greater for the unilateral notch and 0.55 or greater for the bilateral notch has an approximately 65% sensitivity and a false-positive rate of 11% (230). A systematic quantitative review showed that uterine artery Doppler flow velocity has limited diagnostic accuracy in predicting preeclampsia (232).

Because none of the current methods combines accuracy, reproducibility, and simplicity to become a universal predictive marker of preeclampsia (233, 234), there continues to be a compelling demand for new markers, and placental hormones have been investigated with this purpose.

A. Endocrinology of preeclampsia

Recent observations suggest that preeclampsia originates from an abnormally shallow endovascular cytotrophoblast invasion in the spiral arteries, characterized by an increased apoptotic index of the cytotrophoblast, an exaggerated inflammatory response, an endothelial cell activation, and a relative placental ischemia (235–238). In normal pregnancy, the low oxygen tension in the first trimester prevents the trophoblast from differentiating toward an invasive phenotype, and this mechanism is mediated by TGFβ3 (239, 240). The physiological increase in oxygen tension between 10 and 12 wk of gestation determines a decrease of TGFβ3 and, hence, allows the trophoblast to differentiate into a more invasive type. In the preeclamptic placenta, TGFβ3 levels remain high, and the trophoblast is arrested at an immature state while its invasiveness is reduced (241). Another important feature of preeclampsia is that the invasive trophoblasts reach the vicinity of the spiral arteries but fail to penetrate them, precluding the conversion of the spiral arteries into low-resistance channels and inducing the placenta to secrete hypertensive substances (220).

Since trophoblastic abnormalities play a central role in the development of preeclampsia and precede the appearance of clinical signs and symptoms, it is not surprising that some placental hormones change in the maternal circulation, indicating the derangement of placental function (Fig. 3). The levels of several placental hormones are elevated in maternal serum long before overt preeclampsia is diagnosed, and

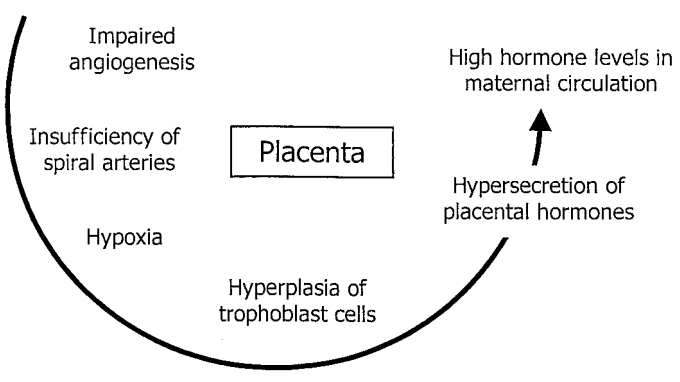

FIG. 3. Putative mechanisms involved in the increased placental hormone levels associated with preeclampsia.

these may be considered preclinical manifestations of the earlier stages of the disease. Therefore, such hormones have been proposed as early predictive markers of preeclampsia.

1. hCG. Patients with overt preeclampsia in the third trimester have increased maternal serum hCG levels. There is general agreement that the placenta remains the main source of hCG in patients with preeclampsia, but whether the cause of the high circulating levels of the hormone is placental overproduction is still debated. Some advocate that hCG secretion may be increased as a consequence of abnormal placental invasion or placental immaturity (156). It may also be linked to the trophoblast response to hypoxia with the development of a hypersecretory state (242). Compared with normal pregnancies, the placentas of patients with unexplained elevated maternal hCG levels in the second trimester tend to be larger and to have an increased density of hCG-positive trophoblasts along with an increased intensity of hCG immunostaining within the placental villi (243). However, this is not corroborated by a small sample study that found equivalent expression of β-hCG mRNA in normal and preeclamptic placental tissues (244).

On average, maternal hCG levels are already increased in the second trimester in pregnancies that subsequently develop preeclampsia (155, 245). The measurement of hCG levels during the second trimester for Down's syndrome screening has already been incorporated into clinical practice at many antenatal clinics worldwide. The availability of thousands of records of midtrimester hCG levels for women attending screening programs and their respective outcomes have permitted the investigation of whether the finding of elevated hCG concentrations in maternal serum is predictive of preeclampsia.

There are accumulating data from studies that evaluated whether a single elevated hCG value (usually above 2.0 MoM) between 14 and 24 wk of gestation is predictive of preeclampsia (34, 155, 159, 246–252). The results of these studies are convergent in suggesting that women with elevated hCG levels in the second trimester are at increased risk for preeclampsia, but there is divergence regarding the accuracy of this test and, by consequence, its predictive value (Table 1). Many reasons contribute to the disagreement between studies. The sensitivity and specificity of the test may change according to the method of assay, the clinical and epidemiological background of the subjects, the gestational age at which samples were collected, and the cutoff chosen to distinguish high from normal hCG levels.

Nevertheless, a consistent observation throughout these studies is that no more than one third of the future cases of preeclampsia can be predicted by a second-trimester screening using hCG alone, if one assumes that the false-positive rate must remain within 10%. Lowering the cutoff point from 2.0 MoM to 1.0 MoM would obviously increase the test sensitivity, but the high false-positive rate would render the test useless as an indicator for prophylactic intervention (155, 253). Since preeclampsia has a relatively low prevalence in most populations, any new screening test should have a high specificity and positive predictive value to identify only high-risk women and avoid the emotional and social costs imposed by false-positive results.

TABLE 1. Performance of maternal serum hCG levels at midtrimester in predicting preeclampsia in low-risk populations

Study	Subjects (n)	Cutoff point	Gestation (wk)	hCG type	Pretest probability[a] (%)	Posttest probability (95% CI)[b]	Sensitivity (95% CI)
Muller *et al.*, 1996 (155)	5,776	>2.0 MoM	15–18	Total	0.6	1.9% (0.8–3.0)	32.4% (16.7–48.1)
Önderoğlu and Kabukçu, 1997 (246)	562	>2.0 MoM	15–20	Total	2.7	8.6% (2.5–14.7)	46.7% (21.4–71.9)
Ashour *et al.*, 1997 (247)	6,138	>2.0 MoM	15–22	β	3.2	5.2% (3.5–6.9)	17.5% (12.2–22.8)
Vaillant *et al.*, 1996 (248)	434	>2.0 MoM	14–20	β	3.7	15.1% (6.9–23.3)	68.8% (46.1–91.5)
Luckas *et al.*, 1998 (249)	430	>2.0 MoM	15–18	Free-β	4.4	8.5% (0.5–16.4)	21.0% (2.7–39.3)
Pouta *et al.*, 1998 (250)	637	>2.0 MoM	15–19	Free-β	4.7	5.9% (1.3–10.5)	20.0% (5.7–34.3)
Morssink *et al.*, 1997 (251)	2,008	>2.5 MoM	15–20	Total	2.0	4.6% (0.3–8.8)	10.0% (0.7–19.3)

[a] Prevalence in the whole study population.
[b] Positive predictive value. CI, Confidence interval.

Another aspect that has been considered is whether hCG screening would have a different outcome in predicting preeclampsia in nulliparous and multiparous women. A study including only nulliparous women has concluded that hCG measurement and Doppler ultrasound of the uterine arteries had similar predictive value, with many operational advantages for the former (248). However, a much larger (n = 6,138) and well controlled study that evaluated nulliparous and multiparous women separately reached different conclusions (247). High maternal serum hCG levels at midtrimester were associated with development of preeclampsia among multiparous, but not among nulliparous, women. The association was stronger for severe preeclampsia (247). A population-based cohort of nulliparous women in Finland (250) revealed that the probability of developing preeclampsia after a positive test was very similar to the overall prevalence of the disease, indicating that second-trimester hCG screening adds little information to the risk prediction of preeclampsia among nulliparous women (Table 1).

An alternative to serum hCG testing is measuring the urinary excretion of the hCG β-subunit core fragment, which is the end product of hCG metabolism. There appears to be an increased risk of preeclampsia in association with elevated urine β-core fragment concentrations (34, 159). However, the test has poor screening efficiency and does not add sensitivity to the measurement of serum hCG.

Table 2 summarizes data pooled from six studies evaluating the value of maternal serum hCG measurement at the second trimester to predict preeclampsia. All studies adopted the 2.0 MoM cutoff point to define individuals with high hCG levels, which was considered a positive result in the screening test. This cutoff conferred a specificity of near 90%. The test proved to be poorly sensitive, since only 23.7% of the women who ultimately developed preeclampsia had high second-trimester hCG levels. The relative risk of 2.54 reflects the positive predictive value, which was approximately twice the overall prevalence of the disease. The test performed better when applied to populations with a prevalence of preeclampsia higher than 3.5%, achieving a positive predictive value of 9.5% (Table 2).

Something that emerges from all these studies is the high negative predictive value of midtrimester maternal serum hCG (Table 2). Values lower than 2.0 MoM (which are detected in 80–90% of all primigravidas) indicate a very low (<4%) probability of developing preeclampsia, which at first glance may sound useful to predict a good outcome after a negative test. This finding merits cautious interpretation because all studies have been performed on populations whose prevalence of preeclampsia was lower than 5%, and it is probable that in selected populations with a higher prevalence or with specific risk factors the probability of preeclampsia would increase even among subjects with a normal hCG screening test.

TABLE 2. Analysis of pooled data from six studies evaluating the accuracy of maternal serum hCG measurements at second trimester for prediction of preeclampsia

Total number of subjects	13,977
Cases of preeclampsia	308
Sensitivity (95% CI)	23.7 (19.0–28.4)%
Specificity (95% CI)	89.4 (88.9–89.9)%
Likelihood ratio for positive result (95% CI)	2.24 (1.82–2.75)
Likelihood ratio for negative result (95% CI)	1.17 (1.10–1.25)
Relative risk (95% CI)	2.54 (1.97–3.29)
Positive predictive value (95% CI)	
Studies with lower prevalence[a]	4.0 (2.9–5.0)%
Studies with higher prevalence[b]	9.5 (5.6–13.3)%
Negative predictive value (95% CI)	
Studies with lower prevalence[a]	98.3 (98.1–98.5)%
Studies with higher prevalence[b]	96.6 (95.6–97.6)%

All studies used 2.0 MoM as cut-off point (155, 246–250). CI, Confidence interval.
[a] Prevalence of preeclampsia < 3.5% (155, 246, 247).
[b] Prevalence of preeclampsia > 3.5% (248–250).

2. Inhibin A and activin A. Maternal serum activin A and inhibin A levels are substantially increased in the presence of hypertensive disorders (254–258). Although this might happen only because of hemoconcentration or decreased urinary clearance, activin A levels begin to rise modestly but significantly before the onset of hematological or renal manifestations of clinical disease (256, 259, 260). The most probable mechanism for the high activin A and inhibin A concentrations in patients with preeclampsia is increased placental production (261). Because activin A is involved in the control of trophoblast cell differentiation in the first trimester (262), an aberrant expression of this protein would possibly affect placental invasiveness resembling the pathogenesis of preeclampsia, but this hypothesis remains speculative. The physiological correlation between activin A levels and gestational age is lost in women with established preeclampsia in the third trimester (254), suggesting that the exaggerated activin A production is more likely to represent a placental response to the hostile environment than a primary overproduction of the protein.

An early study carried out to evaluate activin A in hypertensive disorders of pregnancy revealed that maternal serum activin A concentration was markedly high in patients with preeclampsia, while patients with chronic hypertension or non-proteinuric pregnancy-induced hypertension had activin A concentrations in the normal range. At that time it was suggested that activin A might be a diagnostic and prognostic marker of preeclampsia in hypertensive pregnancies (256). A cohort of 10 patients with chronic hypertension was followed with repeated activin A determinations, and those subjects who developed superimposed preeclampsia presented with an increase of activin A secretion before the onset of clinical signs of the disease (256). Muttukrishna *et al.* (258) confirmed and expanded this observation, showing that inhibin A, activin A, and the inhibin precursor pro-αC were significantly higher in preeclampsia than in normal pregnancies. Going further, Silver *et al.* (255) confirmed that inhibin A and activin A levels were higher in women with preeclampsia and observed that before 34 wk of gestation there was a more pronounced difference in the average levels of both analytes between normal and complicated pregnancies, with almost complete separation of the ranges of values found in each group. These findings prompted new studies, the goal of which was to determine how early inhibin A and activin A levels begin to increase in women who will eventually develop preeclampsia, and how accurate the measurement of these analytes would be in predicting preeclampsia.

While small-sample studies failed to detect any precocious elevation (263, 264), larger studies have indicated that inhibin A is elevated several weeks before the onset of clinical signs of preeclampsia (245, 259, 260, 265–267) (Table 3). A retrospective comparison of 30 women who developed preeclampsia and 30 normotensive controls showed higher second-trimester inhibin A levels in patients than in controls, and the elevation in inhibin A was more pronounced among women with preeclampsia delivering at preterm (259). An extensive analysis of unselected women who had inhibin A measured between 15 and 19 wk of gestation showed that women with an inhibin A concentration exceeding 2.0 MoM were more likely to develop preeclampsia, to be delivered of a small-for-gestational-age infant, and to have a stillbirth or neonatal death (266).

This association is corroborated by a nested case-control study showing that inhibin A levels were significantly elevated in women who later developed preeclampsia (245). Interestingly, inhibin A levels tended to be higher when the onset of preeclampsia occurred within a shorter interval after collection of the second-trimester screening sample. These observations suggested that second-trimester inhibin A would be more effective in predicting early-onset than later-onset disease (245).

The longitudinal evaluation of serial blood samples collected from 8–13 wk to term showed that mean inhibin A (and also activin A) concentrations were markedly elevated as early as at 15–19 wk in the group of women who subsequently developed preeclampsia (260). Another important finding of this study was that the earlier the disease onset, the earlier the beginning of elevation in inhibin A and activin A levels. Thus, inhibin A was particularly sensitive in predicting the occurrence of preeclampsia before 34 wk, when the impact of the disease on maternal-fetal outcome is worse (260).

Compared with inhibin A, activin A seems to be a more sensitive marker at 21–25 wk (260). This may possibly occur because activin A is produced also by circulating inflammatory cells activated by the systemic inflammatory response that is part of the syndrome of preeclampsia (260). When both proteins are measured at 15–19 wk, however, inhibin A appears to be more sensitive than activin A in predicting cases of early-onset preeclampsia culminating with delivery before 34 wk (260).

Altogether, the studies evaluating second-trimester inhibin A and activin A measurements to predict preeclampsia suggest that these markers have limited sensitivity and low positive predictive value when applied to low-risk populations (Table 3). However, inhibin A is still more accurate than hCG and other routine markers in predicting preeclampsia (268). Future studies should evaluate its applicability to high-risk populations as well as whether repeated testing would improve its sensitivity. Since inhibin A begins to be incorporated into the screening of Down's syndrome (74–76), it seems useful to consider it together with other clinical and biochemical parameters in the risk assessment of preeclampsia.

3. Vasoactive and natriuretic peptide systems. Direct or indirect measurements of the activity of the renin-angiotensin system have been proposed to predict preeclampsia, based on the observation that patients with hypertensive disorders of pregnancy show a decreased activity of this vasoactive system together with an increased response to the pressor effect of angiotensin II. A study of patients with severe preeclampsia has shown that plasma renin activity and aldosterone are decreased in these patients compared with gestational age-

TABLE 3. Performance of maternal serum inhibin A levels at midtrimester in predicting preeclampsia in low-risk populations

Study	Subjects (n)	Cutoff point	Gestational age (wk)	Pretest probability (%)	Posttest probability (95% CI)	Sensitivity (95% CI)
Aquilina *et al.*, 1999 (266)						
All women	640	>2.0 MoM	15–19	5.5	23.6% (13.8–33.4)	48.6% (32.0–65.2)
Nulliparous	313	>2.0 MoM	15–19	6.7	31.3% (15.2–47.3)	47.6% (26.2–68.9)
Lambet-Messerlian *et al.*, 2000 (245)[a]	359	>1.9 MoM	15–21	5.0[b]	15.9%[c]	18% (9–28)
Muttukrishna *et al.*, 2000 (260)[a]	297	95th centile	15–19	4.8[b]	14.5%[c]	23% (19–28)
	291	90th centile	21–25	4.8[b]	10.6%[c]	27% (21–32)

[a] Nested case-control studies.
[b] In the whole cohort where cases and controls were selected.
[c] Indirect estimate using the pretest odds and likelihood ratio (383).

matched normotensive controls (269). Accordingly, the maternal plasma concentrations of active renin, angiotensin I, angiotensin II, and aldosterone, and the activity of angiotensin-converting enzyme, are lower in patients with preeclampsia than in normotensive women in the third trimester (270). The placenta does not seem to be involved in this adaptive process, at least in patients with moderate preeclampsia in whom the activity of the placental renin-angiotensin system at term is similar to that in normal pregnancy (271).

The first attempt to evaluate the renin-angiotensin system in pregnant women to unmask early changes was the angiotensin sensitivity test, an invasive and complicated procedure proposed in the 1970s (272). The test consists of determining the minimum amount of angiotensin II infused per kilogram of body weight per minute that causes a rise in diastolic blood pressure of 20 mm Hg, and a test is generally considered positive when this amount of angiotensin II is less than 10 ng/kg (273). Due to its complex technical requirements and low sensitivity, this test has not proved to be an effective tool in the screening of patients at risk of preeclampsia (274).

A simpler and noninvasive method to assess the activity of the renin-angiotensin system is the determination of angiotensin II receptors in representative and accessible target cells, the circulating platelets. Platelet angiotensin II binding has been measured during the second trimester in women who later developed preeclampsia, and no difference was observed in relation to a matched group of pregnant women who remained normotensive (275). Even repeated tests applied to a large cohort failed to identify women who ultimately developed hypertensive complications of pregnancy (276).

Kallikreins are proteases with indirect vasomotor effects mediated by kinins and by the renin-angiotensin system. Millar *et al*. (277) developed a predictive test based on the ratio between inactive urinary kallikrein and urinary creatinine concentrations at 16–20 wk of gestation. On average, the ratio between inactive kallikrein and creatinine was 5-fold lower in women who developed preeclampsia. The method did not achieve high sensitivity and specificity due to a considerable dispersion and overlapping of values in patients who remained normotensive and those who developed nonproteinuric hypertension or preeclampsia. It may, however, add some prognostic information since the probability of developing pregnancy-induced hypertension or preeclampsia was approximately 3 times higher among subjects with a positive test, defined by a kallikrein-creatinine ratio below the best discriminating threshold, than in the whole study population.

Another family of vasoactive peptides that may be involved in the adaptive response to hypertensive disorders of pregnancy is atrial natriuretic peptide (ANP) and its related peptides. In the third trimester, the plasma levels of the N-terminal peptide of pro-ANP are higher in preeclamptic women than in healthy pregnant controls and also higher in women with severe preeclampsia than in women with mild preeclampsia (278). Furthermore, the N-terminal peptide of pro-ANP is particularly elevated in the subgroup of hypertensive pregnancies with abnormal Doppler velocimetry (278). Despite these encouraging observations in late gestation, an attempt to measure the N-terminal peptide of pro-ANP in second-trimester serum samples failed to detect any difference between women who later developed preeclampsia or gestational hypertension and those who remained normotensive (250). Hence, this peptide does not work well as a predictive marker for second-trimester screening of women at risk for preeclampsia. ANP is synthesized by the human placenta, but there is no evidence that the elevated circulating levels reflect placental production (279). This may explain why ANP is not altered in the preclinical stage of preeclampsia. With the development of new assays, other ANP-related peptides may become future candidate markers of preeclampsia.

Potent vasoconstrictor substances have been identified in human placenta, some of which are more abundantly expressed in preeclampsia. For example, endothelin-1 mRNA and immunoreactive protein were found to be increased in preeclamptic placentas (280, 281). Although maternal plasma endothelin-1 levels have been found to be elevated in the first trimester of pregnancies complicated by preeclampsia (282), a cohort study comprising all trimesters of gestation did not confirm such an association (283). Recently, a neuropeptide classified as tachykinin and named "neurokinin B" was identified in human placental tissue (284). Women with pregnancy-induced hypertension and preeclampsia showed high plasma concentrations of this peptide, which was able to cause hypertension in pregnant rats. It is possible that vasoconstrictor peptides such as endothelin-1 and neurokinin B represent the link between placental vascular malformation and blood pressure elevation in the pathogenesis of preeclampsia. Although these substances are interesting therapeutic targets, current evidence does not support their use as diagnostic or predictive tools.

4. Progesterone and other neurosteroids. The placenta is a source of progesterone and its derivatives, 5α-pregnane-3α-ol-20-one (allopregnanolone) and 5α-dihydroprogesterone, and its precursor, pregnenolone sulfate (285). These hormones are neurosteroids (steroid hormones synthesized by the nervous system) that may contribute to the neurochemical and behavioral changes of pregnancy and puerperium. It has been hypothesized that the neuroendocrine effects of progesterone explain, in part, some neural/behavioral symptoms of preeclampsia (227).

The measurement of maternal serum progesterone levels did not result into a powerful index of placental function and neither progesterone nor 5α-dihydroprogesterone levels identified women at risk of developing pregnancy-induced hypertension (286, 287). In further support of these observations, a recent longitudinal study showed no change in progesterone levels associated with hypertensive disorders of pregnancy (288). A multiple marker cohort study involving more than 1,000 nulliparous women found that progesterone levels assayed between 25 and 34 wk of gestation were higher in women who eventually presented with preeclampsia, but the accuracy of progesterone as a predictive marker was low in the third trimester and negligible in the second trimester (233).

Allopregnanolone seems more promising as a predictive

marker of preeclampsia. Maternal serum allopregnanolone levels increase throughout gestation and are higher in patients with chronic hypertension than in healthy women (288). Within this high-risk group consisting of chronically hypertensive women, those who will develop preeclampsia have higher allopregnanolone levels as early as the first trimester compared with those who will not (288). The mechanism of such change is still unknown since extraplacental sources may be involved. The time course of allopregnanolone production in low-risk pregnancies ending in preeclampsia, as well as the feasibility and accuracy of this neurosteroid as a predictive marker, should be investigated.

5. Adhesion molecules. Adhesion molecules are receptors that modulate inflammatory responses by permitting leukocyte adhesion to the inflammatory site. Some adhesion molecules are considered to be important for leukocyte extravasation in the placental bed during trophoblast invasion. Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are largely expressed by the vascular endothelium of human decidua (289).

Since the soluble forms of ICAM-1 (sICAM-1) and VACM-1 (sVCAM-1) are markers of arterial wall inflammation and endothelial dysfunction, it would not be surprising to find high circulating levels of both molecules in preeclamptic patients. This is actually valid for sVCAM-1 (290, 291), but probably not for sICAM-1, whose levels have been reported to be unchanged (290, 292) or slightly decreased (291) in preeclamptic women. In addition, preeclampsia does not change the placental expression of these or other adhesion molecules (293), leaving unclear the mechanism of the increased sVCAM-1 levels. The possibility of using sVCAM-1 for prediction of preeclampsia is discouraged by the lack of alteration in its serum levels in the second trimester (290).

6. Corticotropin-releasing hormone. Corticotropin-releasing hormone (CRH) is a neuropeptide that stimulates ACTH release from both the anterior pituitary gland and the placenta (294). It is produced by the placenta and released into maternal and fetal circulation at increasing rates during gestation with maximum maternal serum concentrations occurring around labor and delivery (4, 295).

Increased maternal serum CRH levels are a frequent feature of pregnancies complicated by preeclampsia. Increased placental synthesis of CRH is evident at both the mRNA and protein levels (296, 297). Umbilical cord plasma concentrations of CRH are higher in preeclampsia than in normotensive pregnancies, and concentrations are higher in venous than in arterial cord blood, indicating the secretion of CRH from the placenta into the fetal circulation (4). The intimate mechanisms leading to excessive placental production of CRH in preeclampsia are not known.

Relatively high CRH concentrations may be detectable as early as the second trimester in women who subsequently develop preeclampsia (298). However, in a selected sample of women at high risk of developing pregnancy-induced hypertension, we observed that a consistent elevation of maternal CRH levels did not occur before the onset of manifested disease (nonproteinuric hypertension or preeclampsia) (299).

7. Leptin. Elevated maternal leptin levels have been described in women with preeclampsia in the third trimester (300–302) but not at delivery (303). The rise in total leptin represents an increase of free leptin levels, as the bound fraction is paradoxically decreased (302). The most probable mechanism of leptin increase in preeclampsia is increased placental production (304), and this explains why preeclampsia subverts the physiological relationship between adiposity and leptin levels in pregnant women (305). A longitudinal study showed increased leptin levels beginning at 20 gestational weeks in women prone to developing preeclampsia, suggesting that leptin might be an early marker of the disease (301). This potential clinical application should be addressed in future research.

8. Combined hormonal tests to predict the risk of preeclampsia. An attempt has been made to extract the best information from midtrimester screening tests to identify, within a low-risk population, those women at risk of developing severe preeclampsia. Several complex models including clinical and biochemical markers have been constructed and tested by multivariate logistic regression to fit a prediction model with the highest sensitivity and as few variables as possible (306). Some clinical variables, such as maternal age, race, and body mass index, or second-trimester hCG and AFP concentrations, were not predictive at all, probably because the study endpoint was severe preeclampsia, and the patients with mild to moderate forms of the disease were considered nonaffected. The best model included nulliparity, a history of preeclampsia, elevated screening mean arterial pressure, and low second-trimester estriol concentration, but it achieved only modest sensitivity and specificity rates (306).

The HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome is a rare life-threatening condition associated with severe preeclampsia. A retrospective study of more than 10,000 pregnancies showed a remarkable increase of the risk of HELLP syndrome among women with unexplained elevated hCG (relative risk = 12), AFP (relative risk = 11), or both analytes (relative risk = 47) at midtrimester screening (307). Nonetheless, the positive predictive value ranges from 1.2% (only hCG $>$ 2.5 MoM) to 6.3% (both hCG and AFP $>$ 2.5 MoM), which is much higher than the expected incidence of the syndrome (0.3%) but does not seem to justify any intervention or particular surveillance in patients with an altered screening test.

The usefulness of CRH as an early predictor of preeclampsia has been tested alone and in combination with AFP. In a cohort of 1,021 low-risk Chinese women sampled for CRH and AFP in the second trimester, receiver operating characteristic curves for prediction of preeclampsia have indicated a weak performance of both analytes either alone or combined (298). The inclusion of AFP improved the sensitivity of CRH alone but did not change the low positive predictive value of the test (298).

It has been suggested that inhibin A can be more sensitive than total hCG in predicting preeclampsia and that the addition of hCG does not improve the sensitivity of inhibin A alone (268). This putative superiority of inhibin A over hCG has not been confirmed by another study of similar design (245), but there is agreement that using the double test does

not add much sensitivity to the single makers because they probably reflect a common pathophysiological phenomenon leading to the placental overexpression of both hormones.

B. Summary and clinical recommendations

The use of hormone markers to predict the risk of preeclampsia may serve more than one objective. First, to be used in a screening program covering all pregnant women indiscriminately, any test should combine a high sensitivity, to justify the costs, with a high positive predictive value, to avoid unnecessary interventions. None of the tests reviewed here fulfill these requirements. However, unexplained elevated mid-trimester hCG, AFP, inhibin A, or activin A (above 2.0 MoM) suggests that the woman carries a higher risk of preeclampsia than otherwise expected, but the absolute risk may be modest if she does not accumulate other risk factors. Other putative markers such as serum allopregnanolone and leptin and urinary kallikrein should await further validation.

A second objective may be to identify among high-risk pregnant women, *i.e.*, patients with chronic hypertension or a history of preeclampsia, those with a very low probability of presenting preeclampsia in their present gestation. In this case a hormonal test that achieves optimal negative predictive value may be used to reduce anxiety in the patient and physician and to prevent unnecessary hospitalization. This will require more sensitive tests than those currently available. Meanwhile, the possible utility of second-trimester markers such as hCG, AFP, inhibin A, and activin A to predict the risk of preeclampsia remains to be investigated in high-risk populations.

In any case, placental hormone markers do not predict future disease. They denounce the early placental changes that are part of the evolving disease and only predict the imminent installation of the preeclamptic syndrome. This paradigm explains why tests are better predictors when preeclampsia supervenes shortly, and why screening in the first trimester is unlikely to work as well as in the second and third trimesters. While the earliest placental changes associated with preeclampsia cannot be detected with sufficient accuracy, the diagnosis of preeclampsia still relies on regular blood pressure measurement and urine analysis for proteinuria, which are late but unequivocal markers.

V. Preterm Delivery

Preterm delivery is defined as delivery occurring before 37 completed gestational weeks. It complicates nearly 10% of all births and accounts for approximately 70% of all neonatal deaths throughout the world (308, 309). Preterm birth remains a leading cause of neonatal morbidity and mortality in places as distant (geographically and economically) as the United States (310), Southern Brazil (311), and sub-Saharan Africa (308). Its enormous impact on public health has not been reversed by constant improvements in obstetric and neonatal care (311).

The persistence of a high proportion of preterm births nowadays may be related to the lack of more effective therapies based on the primary causes of preterm labor and on the mechanisms leading to impending preterm birth. Approximately one third of the cases of preterm labor are associated with intrauterine infection, which is a preventable cause, as screening and treatment of lower genital tract infection has been proven to reduce the incidence of preterm delivery (5, 312). However, many cases are still labeled as idiopathic, and therefore a rational prophylaxis cannot be instituted. Another limitation of the prevention of preterm birth is the poor predictive value of clinical risk assessment to identify the women requiring close surveillance and prophylactic interventions.

To ameliorate the limitation of clinical scores, many complementary tests have been proposed, including evaluation of cervical length by ultrasound and detection of biochemical markers of fetal membrane lesion, among which fetal fibronectin gained the most widespread use (5). Fetal fibronectin is a basement membrane protein produced by fetal and placental tissues, the presence of which in the cervicovaginal fluid in the transition from second to third trimester probably indicates disruption of the chorion-decidua interface (313). A recent meta-analysis concluded that fetal fibronectin accurately predicts preterm delivery among patients with symptoms of preterm labor (314). The finding of elevated vaginal fetal fibronectin levels in asymptomatic women screened at about 24 wk implies an extremely increased risk of spontaneous preterm birth before 28 wk (315). However, the appearance of fetal fibronectin in the vagina is likely to occur late in the course of intrauterine infection, making it questionable if any intervention will be effective to prevent preterm birth (313).

A. Endocrinology of preterm labor

Labor at term is initiated by a physiological change in the pattern of myometrial activity that switches from irregular contractures to regular contractions (5). It appears that labor begins when the uterus is released from an inhibitory control that makes it quiescent throughout pregnancy and continues with active uterine stimulation by an integrated parturition cascade (5). Uterine quiescence is maintained by substances mostly of placental origin such as progesterone, prostacyclin, relaxin, nitric oxide, PTH-related peptide, and putatively CRH and hPL. The cascade of parturition is activated by endocrine (maternal and fetal) as well as local factors from the uteroplacental unit. Once activated, parturition is stimulated by oxytocin and stimulatory PGs (316).

Preterm labor is a syndrome that may be initiated by many causes, representing either a breakdown in the mechanisms responsible for maintaining uterine quiescence or an overwhelming of the normal parturition cascade (5). A number of hormones and cytokines have been found to be altered in pregnancies prone to end at preterm and have been considered potential markers of the syndrome, even though many of these markers lack a proven role in the mechanisms of labor (Table 4). The rationale of these tests is variable, as some are designed to detect precocious elevations of hormones that indeed rise as parturition approaches in physiological conditions, *e.g.*, CRH and estriol, while others, *e.g.*, hCG and hPL, are interpreted as markers of placental dysfunction and/or fetal involvement. In addition, proinflammatory cytokines have been chosen as potential markers because they

TABLE 4. Possible markers of increased risk of preterm labor and/or impending preterm birth in several gestational compartments

Compartment	Maternal serum	Maternal saliva	Vaginal fluid	Amniotic fluid	Cord serum
Placental hormones					
CRH	+				
hCG	+		+		
hPL	+				
Unconjugated estriol	+	+			
Fetal and membrane proteins					
AFP	+				
Fetal fibronectin			+		
Interleukins					
IL-6	+			+	+
IL-8	+			+	
IL-2 receptor	+				
Other cytokines					
Granulocyte-colony + stimulating factor	+				
TNF-α				+	

signal the immune and inflammatory responses accompanying intrauterine infection (Fig. 4).

1. Cytokines. Among several routes through which bacteria can migrate to invade the pregnant uterus, the most common is passage from the vagina through the cervix. Ideally, infection should be eradicated from the vagina before the bacteria ascend to the uterus, but this strategy is often unfeasible. There are chronic infectious processes that begin during early gestation or even before conception and culminate in preterm labor in the third trimester. Although there is no sufficient evidence that treatment with antibiotics can reverse the risk of preterm labor after the uterus has been colonized by bacteria, detection of asymptomatic cases is an important goal in obstetric care (312). With this aim, a rapid and safe approach may be quantification of various proinflammatory cytokines in maternal blood and genital tract fluids.

Granulocyte colony-stimulating factor is a cytokine produced by monocytes that has been shown to increase in the maternal circulation after preterm rupture of membranes. High plasma granulocyte colony-stimulating factor concentrations at 24–28 wk of gestation are associated with an increased risk of spontaneous preterm labor and delivery (317). It may be deduced from the reported data that elevated concentrations of maternal plasma granulocyte colony-stimulating factor at 24 wk are present in half the cases of birth before 32 wk, but its potential clinical utility as a predictive test remains to be determined.

The value of the determination of cervicovaginal secretion of IL-8 at 28 wk in predicting birth before 37 wk was investigated in twin pregnancies (318). IL-8 values higher than 1.75 ng/g mucus were associated with an increased risk of preterm delivery (relative risk = 2.2). The test identified nearly 80% of the women destined to give birth preterm, but there were many false-positive tests, which resulted in a poor predictive value of positive results.

A quantitative assessment of TNFα in cervicovaginal secretion was performed in a cohort of healthy women evaluated during routine prenatal visits before 36 wk of gestation (319). The median concentrations of TNFα were similar in the women who ultimately delivered at preterm and in the remaining women whose pregnancy proceeded to term. In another group of patients with threatened preterm labor,

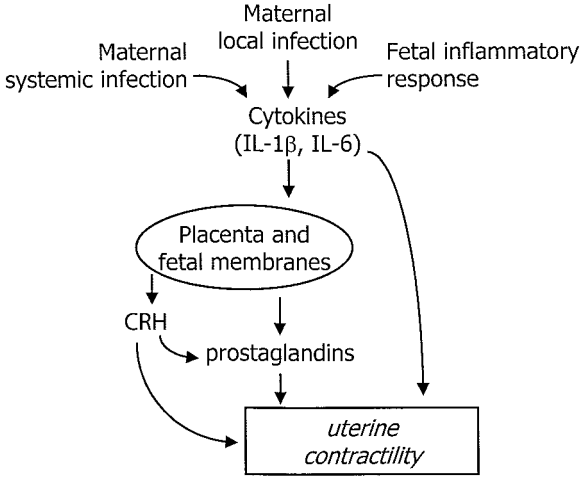

FIG. 4. Placental endocrine and paracrine response to infection in women with preterm labor.

higher levels of TNFα in the cervicovaginal secretion were predictive of preterm delivery (319).

For reasons still unclear, the concentrations of some cytokines may be particularly increased in patients destined to give birth soon after the onset of labor compared with those who will respond to tocolysis and deliver near term. The IL-2-soluble receptor, being a marker of activated immune cells in peripheral blood, may help to identify patients at greater risk of delivery within 48 h from the onset of preterm labor (320).

Maternal serum IL-6 concentration has been evaluated as a potential marker of imminent delivery in women with threatened preterm labor without clinical signs of infection, all of whom submitted to a standard tocolytic treatment (321). The probability of giving birth within 5 d of hospitalization may be as high as 95% among patients with serum IL-6 levels higher than 6 pg/ml, and only 15% among those with IL-6 levels lower than this cutoff point (321). This information may be important in selecting patients who would benefit from immediate administration of corticosteroids to accelerate fetal lung maturation. Successful tocolysis, defined as at least 2 wk elapsed from treatment to delivery, may

also be predicted from relatively low IL-6 and IL-8 concentrations in the amniotic fluid (322).

A simpler way to assess IL-6 production by intrauterine tissues is to use cervical and/or vaginal secretion. A prospective cohort study has shown that the levels of IL-6 in the cervical secretion are increased in patients delivering at preterm (323). However, another study concluded that IL-6 concentration in cervicovaginal secretion does not predict preterm labor in asymptomatic women or preterm delivery in patients hospitalized with preterm labor (319).

Fetuses with preterm premature rupture of the membranes and a systemic inflammatory response syndrome, indicated by elevated cord blood IL-6 levels, are at higher risk for impending spontaneous preterm delivery than those without the syndrome (324). The current concept is that the fetus takes active part in the mechanism of preterm labor induced by intrauterine infection (312). Hence, a fetal inflammatory response denoted by IL-6 predicts that the fetus is likely to activate defense mechanisms, such as increased cortisol production by the adrenals (312), leading to an accelerated organ maturation and predisposing to premature delivery.

2. CRH. Many lines of evidence support the involvement of placental CRH in the mechanisms controlling the onset of labor, either term or preterm (3, 4). Elevated CRH levels are present in pregnancies complicated by preterm labor without infection (325). The evolution of maternal serum CRH concentrations parallels the CRH curve of normal pregnancy but the level is displaced upward (326). This discrimination is detectable before any clinical manifestation of uterine contractility, a fact that prompted the design of controlled studies focusing on the value of second-trimester CRH level in predicting preterm birth.

In a case-control study, mean maternal plasma CRH levels at 18–20 wk of gestation were found to be 3-fold higher in the group of women who subsequently had spontaneous preterm delivery compared with matched controls (327). There was a clear separation of CRH concentrations between case and control groups with no overlapping, but the small number of cases prevented additional inference about the predictive value of CRH as an early screening test.

In spite of the average elevation of maternal CRH levels as early as 18–20 wk in pregnancies that will eventually end before term (327, 328), the attempts to validate second-trimester CRH as a predictive test for preterm labor have been less encouraging. An extensive cross-sectional study of asymptomatic women revealed a poor discrimination between pregnancies ending at term and preterm on the basis of second-trimester maternal serum CRH concentrations (329). A large cohort study performed in Hong Kong followed more than 1,000 low-risk women starting at 15–20 wk of gestation (328). CRH levels were higher in the group who delivered before 34 wk but not in the whole preterm group, and its positive predictive value was only 3.6% for an outcome whose prevalence in the study population was 1.1%. It became clear that the measurement of maternal serum CRH would not satisfy the requisites of a screening test for preterm delivery in a low-risk population, although it should be considered a potential marker to be used in populations with a higher prevalence of the problem.

This perspective was confirmed by a large prospective study carried out in Australia (330). The value of midtrimester CRH measurement to predict the risk of preterm birth (either spontaneous or planned) was evaluated alone and in combination with AFP and a clinical risk factor score. CRH alone had a positive predictive value of 27%, a 4-fold increase compared with the 7.3% prevalence of preterm birth in the study population. The combination of CRH with AFP and a risk factor score enhanced the sensitivity of all single markers without losing specificity (330). Although these results cannot be freely extrapolated to different populations due to the heterogeneity of the syndrome, they suggest that CRH testing can be usefully added to a risk assessment program to improve the sensitivity of clinical scores. Because CRH is particularly elevated in idiopathic (not associated with infection) cases of preterm labor (330), a prerequisite for its implementation is that preventive interventions be developed to target this group of patients.

There is no doubt that placental CRH plays a role in the control of human parturition, but its precise role is still a matter of debate. The finding of elevated midtrimester CRH levels in women who will have preterm delivery does not necessarily represent an early release of the cascade of parturition, since many women with relatively high midtrimester CRH levels still proceed to term. The precocious elevation of plasma CRH levels could be an epiphenomenon rather than a trigger for the mechanisms leading to preterm labor. An unsolved paradox emerges from biochemical evidence for a myometrial relaxing effect of CRH before term, favoring uterine quiescence, in contrast to its indirect uterotonic effect *in vitro* and its increased bioavailability at term (3, 316). This apparently dual role of CRH might explain its limited predictive power for preterm labor.

3. Growth factors. Angiogenin is a potent inducer of neovascularization released by tissues in which there is ischemia and chronic inflammation, two phenomena frequently observed in placentas of preterm deliveries. Amniotic fluid angiogenin concentrations have been measured retrospectively in amniotic fluid samples obtained during the second trimester at routine amniocentesis for genetic screening and were already elevated in pregnancies ending before term (331). The association between high second-trimester amniotic fluid angiogenin levels and spontaneous preterm delivery remained significant after correction for maternal race, FGR, and other potential confounders (331), suggesting that this marker correlates with the early placental dysfunction that is present in at least part of the cases of preterm labor. Angiogenin measurement after amniocentesis in selected patients might be considered a potential prognostic marker of preterm labor, but its predictive value has not yet been established.

Maternal activin A levels are increased in women experiencing preterm labor. Furthermore, activin A levels are higher in women who deliver preterm compared with those with preterm labor who respond to tocolysis (332). Activin A production by intrauterine tissues is stimulated by hypoxia (333) and by inflammation mediators (334) but, intriguingly,

its tissue concentration *in vivo* does not seem to be higher in women who experience preterm than in those with term labor (335). The potential use of activin A to predict preterm labor has yet to be determined. A small study (13 cases and 81 controls) of women admitted to hospital with preterm labor suggests that activin A has low predictive value for preterm delivery in this group of patients (336).

4. Estriol, hCG, and hPL. The usefulness of plasma estriol to predict preterm labor has been evaluated in twin gestations. The concentrations of maternal plasma estriol at 31–34 wk were, on average, higher in women destined to initiate spontaneous labor before 37 wk, but they were not distinctive enough to predict this outcome (337).

To overcome the inaccuracy of plasma estriol determinations, due to the fact that most of the steroid circulates in a protein-bound state, estriol assays have been developed using saliva as matrix. Saliva estriol levels reflect the concentrations of free, unconjugated estriol in plasma and therefore correspond to the biologically active fraction of the circulating hormone. Salivary assays have the advantages that samples can be collected frequently by a noninvasive and comfortable procedure, stability is preserved during sample transport, and there is a good reproducibility of the estriol estimations (338, 339).

Estriol concentrations in maternal saliva have been correlated with the occurrence of preterm labor. In a pioneer case series of 13 women who delivered at preterm after spontaneous labor with intact membranes, 12 had a salivary estriol-to-progesterone ratio higher than the 95th percentile of uneventful pregnancies in the last 1–4 d before delivery (338). This increase in the estriol-to-progesterone ratio was due to high estriol concentrations, the putative mechanism of which is an increased fetal adrenal activity, possibly triggered by stressful stimuli (338, 340).

A prospective study of weekly estriol measurements, including both low-risk and high-risk subjects, revealed that salivary estriol levels were higher in the group of patients delivered of preterm infants than in the group of women delivered at term. This difference appeared as early as at 24–26 wk of gestation and continued until 34–36 wk (340). A large triple-blind multicenter study discovered that a single salivary estriol value higher than 2.1 ng/ml between 21 and 25 wk in asymptomatic women is associated with an increased risk of preterm birth, and in this regard the estriol measurement achieves greater sensitivity than a standard clinical test (Creasy scoring) (339). A potential contribution of salivary estriol measurement comes from negative tests in high-risk populations. Among patients labeled as "high-risk" by the Creasy score, two consecutive weekly estriol tests in the normal range suggest that the patient is likely to proceed to term, either without threatened preterm labor or by responding to tocolysis (339). In symptomatic women, salivary estriol is associated with an increased risk of delivery within 2 wk, but its predictive power is modest (341).

Salivary estriol may be more accurate than clinical risk assessment in predicting either preterm labor or preterm birth (339, 340), but its possible clinical utility as a routine screening test to predict preterm labor in asymptomatic women has not been established. Specifically, it remains to be determined whether women with elevated salivary estriol levels have a significantly higher probability of undergoing preterm labor compared with their pre-test probability, given by demographic and clinical variables.

Elevated second-trimester serum β-hCG levels predict an increased risk of perinatal death (153, 342), low birth weight, small-for-gestational-age infants, preterm premature rupture of membranes, and preterm birth (152, 156, 246), albeit not of early preterm delivery (before 34 wk) (343). Cervical and vaginal secretions may also be assayed for hCG in patients with increased risk of preterm labor. A single β-hCG value higher than 50 mIU/ml obtained between 24 and 28 wk of gestation in cervicovaginal secretion predicts a 2-fold increased risk of giving birth before 34 wk (344).

hPL has been validated in the past as a nonspecific marker of placental dysfunction and fetal distress. It has been considered predictive of fetal deterioration in pregnancies complicated by severe hypertension (147) but has not proved helpful in predicting spontaneous preterm labor.

B. Summary and clinical recommendations

The use of predictive markers of preterm labor and delivery has two complementary goals. The first is the early identification of women at greater risk of preterm labor as a prerequisite for planning preventive strategies, if not to delay birth, at least to improve perinatal outcome. The ideal marker should not only be accurate but also be present as early as necessary to permit effective prophylaxis. Such an ideal test is difficult to attain due to the heterogeneity of the preterm labor syndrome and probably also because the pathological process leading to preterm labor often develops late in the course of pregnancy, making it hard to predict before its installation. A second objective is the prediction of preterm birth after the onset of spontaneous preterm labor, which can help in the clinical decision to optimize preventive and therapeutic measures against the adverse consequences of prematurity.

Of the several endocrine and nonendocrine biochemical markers reviewed here, only the combination of CRH and AFP shows moderate accuracy in early prediction of the risk of preterm labor (Table 5). In our view, there is no sufficient evidence supporting the use of endocrine markers in the first trimester or early in the second trimester to predict the risk of preterm labor.

In the late second trimester and early third trimester, salivary estriol and vaginal fetal fibronectin are powerful predictive markers of preterm labor. Nevertheless, convincing evidence is still lacking to support their adoption for the routine screening of low-risk, asymptomatic women.

The prediction of impending preterm birth among patients with symptoms of preterm labor is feasible with current biochemical tests. One of the best serum markers is IL-6, which definitely adds valuable information to the prognosis of patients admitted to the hospital with preterm labor and thereby to the management of these patients according to the expectation of imminent or postponed delivery. While IL-6 achieves the highest predictive values for positive results, fetal fibronectin shows the highest predictive value for neg-

TABLE 5. Usefulness of second-trimester maternal serum markers of pregnancy outcome

Marker	Down's syndrome	FGR	Preeclampsia	Preterm birth	References
Single analytes					
AFP	4.0–5.6				(20)
hCG	6.9	6.2	2.2		(44,153)
Free β-hCG	7.5				(44)
uE_3	5.0				(31)
Inhibin A	8.4		3.6		(245,260)
Activin A			5.9		(260)
CRH			1.9	1.5–4.8	(329,330)
Combined analytes					
hCG + AFP + uE_3	3.6–4.0				(42,93)
Free β-hCG + AFP	10.6				(77)
Free β-hCG + AFP + inhibin A	15.0				(77)
CRH + AFP			2.6	7.4	(298,330)
Inhibin A + hCG			4.6		(245)

Data are expressed as likelihood ratio for positive tests, defined as the ratio between the proportion of positive results among affected individuals (*i.e.,* sensitivity) and the proportion of positive tests among normal controls (*i.e.,* false positive rate) (383). Likelihood ratios of more than 10 are regarded as definitely useful, 5 to 10 are regarded as moderately useful, 2 to 5 as slightly useful, and less than 2 as not at all useful (384).

ative results. Combining both tests will theoretically optimize the prognostic information.

VI. Diabetes Mellitus

Diabetes mellitus is a common complication of pregnancy and is responsible for an augmented risk of adverse maternal and fetal outcomes. Gestational diabetes, defined as glucose intolerance newly diagnosed during pregnancy, can be diagnosed by simple screening tests such as fasting plasma glucose (345) and abbreviated glucose challenge test (346). This screening has been adopted universally for many years, but there is considerable controversy about whether the recognition and management of gestational diabetes is beneficial to maternal-fetal outcome (347).

It would be desirable that the individual's susceptibility to gestational diabetes be recognized before the onset of glucose intolerance, but this remains a difficult task (348, 349). Debate continues over whether gestational diabetes represents a pregnancy-induced state of glucose intolerance or only the exacerbation of a preexisting insulin resistance that would become evident in the future regardless of pregnancy. There are many similarities between gestational diabetes and the syndrome of insulin resistance in nonpregnant subjects (350, 351), but attempts to predict the development of gestational diabetes using indexes of glucose intolerance such as glycosylated hemoglobin or fructosamine have been fruitless (349). Nonspecific markers of placental and fetal endocrine function such as hCG, hPL, and estriol have also failed to distinguish diabetic from normal pregnancies (352, 353).

As a consequence of enhanced insulin production by the fetal pancreas after excessive transplacental glucose load, infants of diabetic mothers may be large for gestational age and even macrosomic. Macrosomia is defined as infants weighing more than 4,000 g (354). Macrosomic infants are more susceptible to birth trauma and operative delivery (355–357). In addition, large-for-gestational-age infants are at increased risk for neonatal hypoglycemia due to residual insulin excess. Thus, early identification of fetal excessive growth/macrosomia is important to prevent obstetric trauma and to better plan neonatal assistance (354).

The current standard method for antenatal diagnosis of macrosomia is ultrasonography, but the use of serial ultrasound scans to assess fetal growth is an expensive, time-consuming method, the accuracy of which remains unsatisfactory (358–360). In this context, biochemical markers of fetal overgrowth would be useful tools, particularly if they were as sensitive and specific as ultrasound biometry. Placental hormones might be candidates to such markers because several endocrine and metabolic functions of the placenta are altered in diabetic pregnancies (361–365).

A. Endocrinology of maternal diabetes and fetal macrosomia

1. Somatotropic hormones. Pronounced fetal macrosomia may occur even with adequate maternal blood glucose control (355, 366). The severe fetal hyperinsulinemia in this case may be imputed to some factor other than excessive glucose load, possibly hPL, which induces proliferation and enhanced function of pancreatic β-cells (366). In concert with this hypothesis, maternal serum hPL concentrations measured in the third trimester are higher in diabetic pregnancies complicated by fetal macrosomia (355).

IGFs and IGFBPs of both placental and fetal origin may account for the fetal macrosomia associated with diabetes. This is supported by studies in knockout mice demonstrating a role of increased IGF-II gene expression in the mechanism of fetal overgrowth (367) and by the finding of high circulating IGF levels in macrosomic infants (368–371).

Similarly to normal pregnant women, diabetic women have increasing serum concentrations of IGF-I and IGF-II with gestation, reaching a plateau by the third trimester. The levels of both IGFs as well as IGFBP-1 to -3 are comparable in normal and diabetic maternal circulation, whereas IGF-I, IGF-II, and IGFBP-3 concentrations are higher in the cord blood of neonates born to diabetic mothers (369, 371). It has

been suggested that IGF-I is increased in cord serum of macrosomic fetuses regardless of maternal diabetes, whereas IGF-II is increased in fetuses of diabetic mothers regardless of macrosomia (368, 370). However, this has not been a consistent finding in different studies (369, 371, 372).

The mechanism of IGF increase in fetuses of diabetic mothers is still controversial. Increased placental production is suggested by the more intense expression of IGF-II mRNA in term placentas from diabetic pregnancies (373), but this finding has not been consistently confirmed (370).

2. Leptin. Cord blood leptin levels are augmented in large-for-gestational-age infants and are directly correlated with the infant's birth weight and body mass index (374–376). Interestingly, leptin levels are higher in asymmetric than in symmetric large-for-gestational-age infants (377). Although the fetal adipose tissue is a potential source of leptin, an increased placental production seems to contribute to the fetal hyperleptinemia in diabetic pregnancies (361).

Maternal leptin levels appear to be increased in diabetic women (378), but this only reflects the usually increased body mass index of these women, as revealed by multiple regression analysis (379). Indeed, pregnant women with glucose intolerance have relatively lower leptin levels than their healthy counterparts after adjustment for body mass index (379). Functional hypoleptinemia in diabetic pregnancy is further suggested by the finding of increased concentrations of soluble leptin receptors that compete with membrane receptors for hormone binding (208).

Of broader interest, however, is the possibility of using maternal leptin levels in the screening of fetal macrosomia. Several studies have addressed this possibility, and they are quite convergent in concluding that maternal leptin levels do not correlate with fetal leptin levels and do not predict birth weight (216, 218).

3. Other placental hormones. Basic fibroblast growth factor is a potent mitogen and angiogenic factor produced by various fetal and adult tissues but detectable in peripheral circulation only in very specific circumstances, including pregnancy. Basic fibroblast growth factor levels in cord serum and amniotic fluid from both pregestational diabetic patients and those with gestational diabetes are much greater than in normal subjects (380). The levels in maternal serum tend to be higher in diabetic than in normal pregnancies and correlate positively with birth weight.

Maternal serum α-hCG levels were measured longitudinally in well controlled insulin-dependent diabetic patients and matched nondiabetic controls (381). Maternal serum α-hCG levels were lower in diabetic women but did not correlate with fetal macrosomia.

B. Summary and clinical recommendations

There is accumulating evidence for the participation of placental IGFs, IGFBPs, and leptin in the mechanisms leading to fetal macrosomia associated with maternal diabetes mellitus. Notwithstanding the link between these endocrine markers and fetal macrosomia, their lack of significant diagnostic power is disappointing. While new putative markers are being investigated, the prenatal diagnosis of fetal macrosomia still relies on ultrasound biometry.

VII. Conclusions

The availability of noninvasive, cost-effective, reliable methods of risk estimation and early diagnosis is an essential requisite for a rational selection of the target population for preventive interventions against gestational diseases and their sequelae. Biochemical assays in maternal biological fluids are the most convenient laboratory tests for this scope and, among them, placental hormones may be useful markers due to their abundance in the maternal circulation and their close relationship with the pathophysiological background of almost every complication of pregnancy. In the near future, the use of microarray technology will offer an extraordinary opportunity to determine clusters of genes that may be up-regulated or down-regulated in maternal and fetal organs affected by particular diseases (382), and this will permit new markers to be discovered at an amazingly fast pace.

Before adopting one or more hormone markers to predict the risk of gestational diseases, we must be aware of the power and limitations of the tests to extract the best information they can provide. It should be kept in mind, however, that not only the test must be accurate, giving substantially more true than false results, but also the probability of having (or not having) a disease after a positive or negative test has to differ materially from the expected prevalence of that disease; otherwise the test will be useless. The judgment of which test is useful and which is not may differ depending on whether we are considering to use it in selected individuals or in a whole population screening.

The hormonal tests proposed to evaluate the risk and to predict the outcome of gestational diseases may bring relevant information to the clinician provided they are interpreted carefully. As summarized in Table 5, of several maternal serum markers measured in the second trimester, only the triple test proposed for Down's syndrome screening has a satisfactory detection rate combined with high specificity, but tests with modest-to-moderate performance can also be judiciously introduced into clinical practice. At present, there remains an open question about who should benefit from hormone measurements to predict preeclampsia, FGR, or preterm labor, but there is more certainty about which tests perform better and when they should be applied.

Complex, multifactorial diseases may require more than one marker to be accurately predicted or diagnosed. Therefore, it is not surprising that combined hormonal tests or the combination of hormones and ultrasound may achieve better sensitivity than single markers in predicting gestational diseases, but ideally we should be able to simplify the screening programs including as few items as possible to obtain maximal screening efficiency at a lower running cost. In any case, these data indicate a great future area of development for clinical implications of placental hormones and signaling factors: human placenta as a source of signals, which gives information about the fetal-maternal unit and, in particular, reflects the genetic, vascular, infective, or metabolic disturbances that may occur during gestation.

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Address all correspondence and requests for reprints to: Felice Petraglia, M.D., Chair of Obstetrics and Gynecology, University of Siena Policlinico Le Scotte, Viale Bracci, 53100 Siena, Italy. E-mail: petraglia@unisi.it

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