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THE HUMAN PLACENTA: AN ATYPICAL ENDOCRINE ORGAN

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RESUM

La placenta humana es caracteritza per la intensitat i especificitat de les seves funcions endocrines. La hormones de la placenta són necessàries per a l'establiment i manteniment de l'embaràs, per a l'adaptació a aquest de l'organisme femení, per al creixement del fetus així i per al desenvolupament dels mecanismes implicats en el part. El teixit endocrí de la placenta és el sinciciotrofoblast, que cobreix les vellositats coriòniques o estructura principal d'intercanvi. La utilització de cultius primaris de citotrofoblasts ha proporcionat molta informació sobre els mecanismes implicats en la formació del sinciciotrofoblast per fusió cèl·lula-cèl·lula. Immers en la sang materna, el sinciciotrofoblast secreta la major part de les seves hormones polipeptídiques a la circulació materna. Entre d'altres, la gonadotrofina coriònica (hCG) fa una funció essencial en el manteniment del cos luti i està directament implicada en la diferenciació del trofoblast. L'hormona de creixement (GH) placentària està també secretada contínuament pel sinciciotrofoblast i substitueix la GH hipofisària durant l'embaràs. Mitjançant la captura del colesterol a partir de les lipoproteïnes maternes, el sinciciotrofoblast sintetitza una gran quantitat de progesterona necessària per a l'estabilitat de l'úter. El sinciciotrofoblast, en no tenir l'enzim citocrom P450 17 α-hidroxilasa/17-20-liasa, utilitza els andrògens adrenals materns i fetals per a sintetizar estròg ens. Com a conclusió, és important esmentar que en l'observació de qualsevol anomalia hormonal durant l'embaràs hauràn de tenir-se en compte aquestes dades i, en particular, les característiques enzimàtiques de la placenta.

Paraules clau: hormones de l'embaràs, trofoblast humà, sinciciotrofoblast, hCG, esteroidogènesi.

SUMMARY

The human placenta is characterized by the intensity and the specificity of its endocrine functions. Placental hormones are required for the establishment and maintenance of pregnancy, the adaptation of the maternal organism to pregnancy, fetal growth and well being, and the development of the mechanisms involved in parturition. The endocrine tissue of the placenta

is the syncytiotrophoblast, which covers the chorionic villi, the main structure of exchange. Primary cultures of villous cytotrophoblasts have provided insight into the mechanisms involved in syncytiotrophoblast formation by cell-cell fusion. Bathing in maternal blood, the syncytiotrophoblast secretes the majority of its polypeptide hormones into maternal circulation. Among those, hCG (human chorionic gonadotropin) plays an essential role in the maintenance of the corpus luteum and is directly implicated in trophoblastic differentiation. The placental GH (growth hormone) secreted continuously by the syncytiotrophoblast replaces the maternal pituitary GH during pregnancy. Capturing the cholesterol from the maternal lipoproteins, the syncytiotrophoblast synthesizes large amounts of progesterone essential for uterine quiescence. Deprived of cytochrome P450 17αhydroxylase/17-20lyase, it uses the maternal and fetal adrenal androgens to synthesize estrogens. The observation of any maternal hormonal anomaly during pregnancy must take into account these data and, in particular, the enzymatic characteristics of the placenta.

Keywords: pregnancy hormones, human trophoblast, syncytiotrophoblast, hCG, steroidogenesis.

The human placenta is a villous placenta; the structural and functional unit of the human placenta is the chorionic villous, which becomes apparent in its definitive structure as early as day 21 of pregnancy (Loke and King, 1993; Bernischke and Kaufmann, 2000) (see figure 1).

After nidation, the trophoblast differentiates into two forms: the villous and the extravillous trophoblast. In the villous phenotype, the cytotrophoblastic cells of the floating villi (in the intervillous space) remain attached to the villous basement membrane, forming a monolayer of epithelial cells. These cells proliferate and differentiate by fusion to form a syncytiotrophoblast that covers the entire surface of the villus (see figure 1). This membrane fusion process is complex and involves different factors. The so-called "phosphatidylserine flip" (Adler *et al.*, 1995) associated with caspase 8 activity (the caspase initiator) (Huppertz *et al.*, 2001) has been implicated. The requirement of an endogenous retroviral envelope protein (Blond *et al.*, 2000; Mi *et al.*, 2000; Frendo et al. 2003*a*), connexin 43 (Frendo *et al.*, 2003*b*; Cronier *et al.*, 2003) and cadherin 11 (Getsios and MacCalman, 2003), has been demonstrated. In the extra-villous phenotype, the cytotrophoblastic cells of the anchoring villi, in contact with the uterine wall, proliferate, detaching from the basement membrane and aggregating into multilayered columns of non-polarized cells that rapidly invade the uterine wall. This trophoblastic invasion is confined to the endometrium, the first third of the myometrium, and the associated spiral arterioles. This invasion process is associated with the complete remodeling of the spiral artery wall, leading to the disappearance of the muscle layer and the replacement of endothelial cells by trophoblasts (endovascular trophoblasts) (Pijnenborg et al., 1981). This trophoblastic endovascular invasion is of major importance to the feto-placental physiology: intra-arterial plugs of endovascular trophoblasts prevent, until the twelfth week of gestation, the access of maternal blood to the intervillous space and, therefore, protect the conceptus from excessively high oxygen levels during this very critical stage of development (Burton *et al.*, 1999; Hustin *et al.*, 1990; Hustin and Schapps, 1987). In addition, an abnormally deficient arterial remodeling is involved in pre-eclampsia, a disorder that is specific to human pregnancy and manifests itself during the second trimester of pregnancy, with maternal hypertension and proteinuria (see for review Sibai *et al.*, 2005).

The syncytiotrophoblast is multifunctional, but its primary functions are absorption, exchanges, and hormonal production. The syncytiotrophoblast is strongly polarized and secretes the majority of its polypeptide hormones into maternal circulation (Linnemann *et al.,* 2000). The syncytiotrophoblast, which has the same chromosomal pattern as the fetus, is a female or male endocrine factory. The syncytiotrophoblastic mass appears to be more important in female placentas. This could explain the slightly higher hormone levels of syncytiotrophoblastic origin found in maternal circulation in the event of a female fetus (Chellakooty *et al.*, 2004). It should, however, be noted that the differences in the hCG levels in maternal serum, according to fetal sex, are not sufficient to interfere with the screening of fetal trisomy 21 by maternal serum markers. Studies of primary cultures have provided insight into human villous trophoblastic differentiation. Isolated villous cytotrophoblasts from early and term placentas adhered to plastic dishes, aggregated and fused together to form a non-proliferative, multi-nucleated syncytiotrophoblast producing specific hormones (Kliman *et al.*, 1986; Alsat *et al.*, 1991; Tarrade *et al.*, 2001*a*). This model can be used for studies of cell-cell fusion, the regulation of hormone production, and trophoblastic differentiation . It has also been used to explore the genetic control of villous trophoblastic development, using a subtractive cDNA library (Morrish *et al.*, 1996) and microarray technology (Aronow *et al.*, 2001; Handwerger and Aronow 2003) and by proteomic analysis (Hoang *et al.*, 2001). It has also made it possible to identify a certain number of genes, such as those involved in coding for the retinoid receptors and the activators of the peroxisomes (PPARγ), which are directly implicated in the formation and the differentiation of the syncytiotrophoblast (Handwerger *et al.,* 2003; Tarrade *et al.,* 2001*b*, *c*)

PLACENTAL POLYPEPTIDE HORMONES

The syncytiotrophoblast secretes many polypeptide hormones. They are primarily: hCG (human chorionic gonadotropin) (see for review: Jameson and Hollenberg, 1993), hPL (human placental lactogen), or hCS (human somatomammotropic hormone) and placental GH (growth hormone) (see for review: Alsat *et al.,* 1997; Lacroix *et al.,* 2002). The glycoprotein hormone hCG is the key hormone of human pregnancy. It behaves like a super-agonist of LH, allowing the transformation of cyclic ovary corpus luteum in gravidic corpus luteum, ensuring the maintenance of ovarian progesterone secretion during the first 6 weeks of pregnancy (Jameson and Hollenberg, 1993; Srisuparp *et al.*, 2001; Maston and Ruvolo, 2002). After six weeks of pregnancy, the steroidogenic activity of the feto-placental unit compensates for the maternal ovarian functions. Thus, an ovariectomy after 6 weeks of pregnancy has no effect on a pregnancy's outcome. HCG is made up of two subunits, an alpha sub-unit and a beta subunit. The alpha subunit is the same in the other glycoproteic hormones (FSH, LH, and TSH). The alpha subunit is made up of 92 amino acids with two N-glycosylation sites. It is encoded by only one gene on the chromosome 6q21.1-23. The beta subunit is made up of 145 amino acids with two sites of N-glycosylation and 4 sites of O-glycosylation. It is encoded by a whole set of genes, six beta genes, a CG beta pseudogene and an LH beta gene on the chromosome 19q13.3. These CG beta genes evolve by duplication from the LH beta gene and are controlled differently on the level of their promoter. Compared to LH, the 31 additional amino acids in the C-terminal position of hCG and hCG's very high glycosylation levels allow its intracellular trafficking towards the apical membrane of the syncytiotrophoblast, its secretion directly into maternal circulation, and its prolonged half-

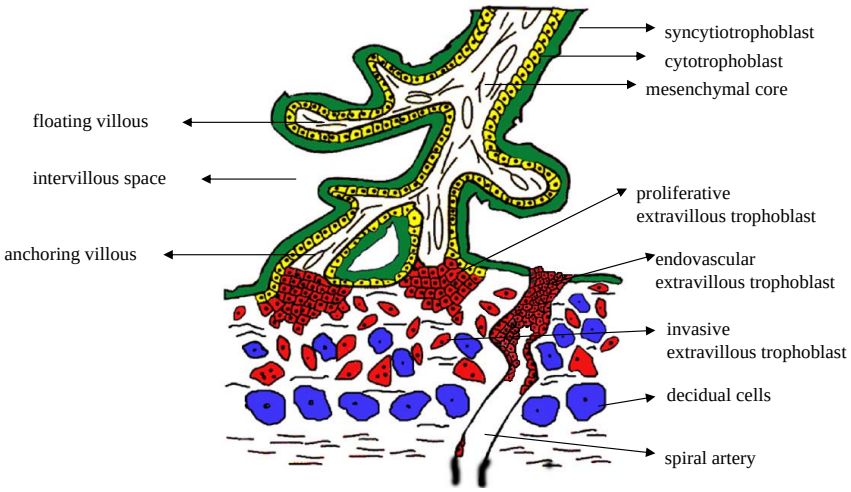

FIGURE 1. Scheme of human chorionic villi.

life. (Jablonka-Shariff *et al.,* 2002). By an autocrine and paracrine mechanism, hCG plays an essential role in trophoblastic differentiation and stimulates *in vitro* the differentiation of the cytotrophoblasts in the syncytiotrophoblast (Shi *et al.,* 1993; Yang *et al.,* 2003). Recently hCG was characterized as a new angiogenic factor involved in the establishment and the development of the human placenta (Zygmunt *et al.,* 2002). The human trophoblast expresses two types of hCG receptor: a truncated 50 kD isoform and an 80 kD full-length isoform (Licht *et al.*, 1993). The truncated isoform is expressed during the first trimester of pregnancy, and the full-length isoform is expressed in the highly-differentiated term trophoblast. Other hCG receptors have been identified in non-gonadal tissue and shown to be involved in the normal course of pregnancy (Licht *et al.*, 2001; Rao, 2001). To our knowledge, no informative mutations affecting hCG genes have been described, suggesting that hCG is absolutely required for the initiation of pregnancy. The secretion of hCG by the trophoblast appears very early; it begins as soon as the 7th day after fecundation at the time of implantation. The concentrations of maternal hCG increase gradually, reaching a maximum peak by about the tenth week, and then decrease very clearly during the 3rd month to remain practically stationary until childbirth. The reasons for this maternal plasmatic peak of hCG during the first trimester of pregnancy remain to be discussed. The following hypotheses have been suggested: 1, at this stage of pregnancy the presence of the truncated form of the receptor would block the autocrine regulation of hCG synthesis; 2, the synthesis of hCG by the trophoblast varies during the pregnancy and is higher during the first trimester; and, 3, hCG would be controlled by an autocrine/paracrine mechanism, by GnRH, produced by the cytotrophoblasts that are present in greater numbers during the first trimester (see for review: Malassiné *et al.,* 2003). Several recent studies have demonstrated the importance of the glycosylation state of hCG, which varies according to the stage of the pregnancy (Diaz-Cueto *et al.*, 1996). Choriocarcinoma cells (Eliot *et al.*, 1997) and trophoblast cells displaying chromosome 21 trisomy (Frendo *et al.*, 2004) produce abnormally glycosylated forms of hCG with low biological activity. It should

be noted that no CG beta genes have been found in mice (Maston and Ruvolo, 2002).

It has been known for many years that the syncytiotrophoblast secretes very large amounts of hCS or hPL into the maternal compartment. This hormone is also found in fetal blood, though in much smaller amounts than in maternal blood. The increase in the secretion of hPL during pregnancy follows the evolution of the placental mass, and more particularly, the syncytiotrophoblastic mass, which is the site of its synthesis. Its real physiological role remains to be elucidated. Indeed, normal pregnancies have been described in the absence of hPL secretion. In the last few years several studies have underlined the important role of a growth hormone specifically produced by the placenta, i.e., the placental growth hormone (review Alsat *et al.,* 1997; Lacroix *et al.,* 2002). This hormone, a product of the GH-V gene, is expressed specifically in the syncytiotrophoblast and differs from the pituitary growth hormone by 13 amino acids. It gradually replaces the pituitary growth hormone in maternal circulation and becomes undetectable during the second trimester of pregnancy. Secreted continuously by the placenta, it seems to control the synthesis of maternal IGF-1. Indeed, maternal IGF-1 levels are in correlation with placental GH levels. Moreover, in pathology, during pregnancies of acromegalic women, maternal IGF-1 increases gradually, thus, following the profile of placental GH, in spite of very high stable levels of pituitary GH. The secretion of placental GH, but not of hPL or hCG, is inhibited *in vitro* by glucose in explants and in trophoblastic cells. *In vivo*, in the event of gestational diabetes, the levels of placental GH decrease in maternal blood during an oral glucose tolerance test. This suggests a metabolic role for placental growth hormone, secreted specifically in the maternal compartment and not detected in fetal circulation. In the event of a fall in maternal glycemia, placental GH, which is then secreted abundantly by the placenta, will maintain an energetic flux to the fetus. Recently, we demonstrated that this hormone is also involved in the complex mechanisms, which regulate trophoblastic invasion into the maternal uterine wall. Indeed, placental GH, secreted by the invasive trophoblast, increases trophoblastic invasion by an autocrine/paracrine mechanism (Lacroix *et al.,* 2005).

The syncytiotrophoblast also secretes other peptide hormones with maternal levels that increase gradually throughout pregnancy, thus, reflecting the progressive increase in the syncytiotrophoblastic mass. Such is the case with inhibin A and activin A (Debiève *et al.,* 2000). These two hormones, members of the TGFβF super-family, are dimeric hormones, whose exact role during pregnancy remains to be elucidated. Only *in vitro* studies on cultures of trophoblastic cells underline their modulating role on trophoblastic hormonal secretion. Recently, the synthesis of leptin, produced by fat cells, was also localized in the syncytiotrophoblast, and its circulating levels are high in maternal circulation. Its role during pregnancy is unknown. High levels of maternal circulating leptin are observed, in the event of maternal diabetes or of pre-eclampsia (see for review: Sagawa *et al.,* 2002).

STEROID HORMONES

As early as the 6th week of pregnancy, the human placenta is the site of an important production of steroid hormones, which are mainly progesterone and estrogens: estriol, estradiol and estrone. At term, the daily placental production of progesterone is about 300 mg (see for reviews: Albrecht and Pepe, 1990; Payne and Hayles, 2004)..

Progesterone essentially acts on the uterus and is required for myometrium quiescence.

During the first six weeks of pregnancy, the production of progesterone is primarily carried out by the gravidic ovary cor-

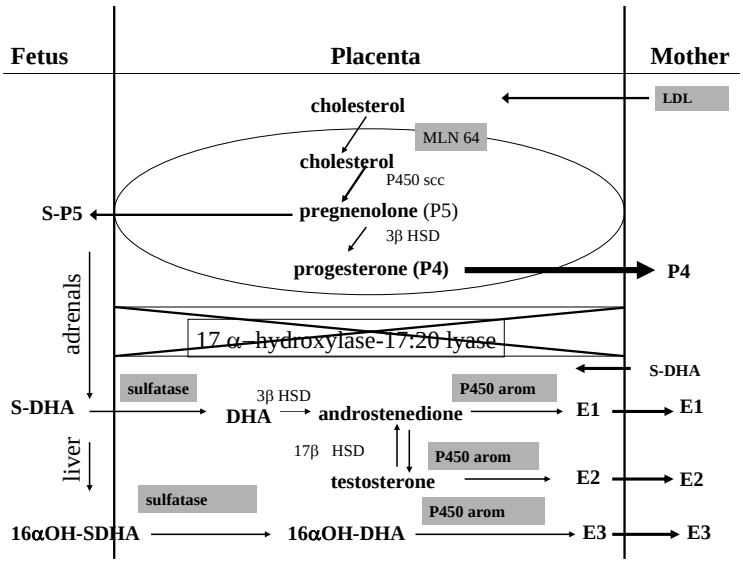

FIGURE 2. Scheme of placental steroidogenesis. E1: estrone; E2: estradiol; E3: estriol; P5: pregnenolone; P4: progesterone; DHA: dehydroepiandrosterone; MLN64: metastatic lymph node 64.

pus luteum. It is thus associated with a secretion of 17αhydroxy progesterone, produced exclusively by the ovary at this stage of pregnancy. Placental progesterone production gradually takes over with the appearance within the syncytiotrophoblast of the various enzymatic systems necessary for its synthesis. The precursor to progesterone is cholesterol. The *de novo* synthesis of cholesterol from acetyl CoA is not significant in the placenta. The maternal origin of placental cholesterol has been demonstrated; the trophoblast captures cholesterol carried by maternal plasmatic lipoproteins (Wadsack *et al.*, 2003). Indeed, second and third trimesters of pregnancy are characterized by high levels of maternal circulating total cholesterol, triglycerides, low-density lipoproteins (LDL cholesterol), and high-density lipoproteins (HDL cholesterol). The syncytiotrophoblast's apical microvillus membrane, in direct contact with maternal blood, presents specific receptors for these lipoproteins (LDL receptor, VLDL receptor, B1 scavenger receptor). It has been shown that the syncytiotrophoblast captures and metabolizes LDL by specific receptor-mediated endocytosis. Indeed, as early as the 6th week of pregnancy, LDL receptors are present on the syncytiotrophoblast's microvillus membrane. After internalization, LDL is degraded in the lysosomes to release cholesterol, which will be used for steroidogenesis and cellular membrane synthesis. The direct involvement of LDL receptors in progesterone synthesis is confirmed by the observation that *in vitro* estrogens stimulate LDL receptor expression, inducing an increase in progesterone synthesis (Albrecht and Pepe, 1990). However, this pathway is not sufficient to explain the very high placental requirement in cholesterol. Recent studies point to the complementary role of other ways of accomplishing cholesterol uptake. For example, another manner could include the selective cholesterol uptake pathway, which involves the scavenger receptor B1 (SR-B1), localized in syncytiotrophoblastic microvilli. The SR-B1 binds HDL, LDL, and VLDL, as well as mod-

ified lipoproteins. In this case, the cholesterol esters are captured by the syncytiotrophoblast without the internalization and degradation of apoproteins (Wadsack *et al.*, 2003). Free cholesterol is transported by the Sterol Carrier Protein-2 (SCP2) to the external membrane of the mitochondria and then on to the internal membrane by way of the MLN64 (metastatic lymph node 64). MLN64 presents a final domain similar to the stAR(steroidogenic acute regulatory protein), a protein expressed in the other steroidogenic tissues (corpus luteum, adrenals) (Strauss III *et al.*, 2000; Tuckey *et al.*, 2001). Within the internal membrane of the mitochondria, P-450 cytochrome scc (cholesterol side chain cleavage) allows the conversion of cholesterol into pregnenolone. This reaction requires electrons provided by mitochondrial adrenodoxin (ADX) and adrenodoxin reductase (AdRed) activities. Only one gene (P-450XIA) coding for P-450scc, localized on chromosome 15, is present in the placenta, as early as the 10th week of pregnancy. Pregnenolone is then converted into progesterone by the 3βhydroxysteroid-deshydrogenase/isomerase (3β HSD), also localized in the mitochondria. Several genes, coding for different 3β HSD isoforms, are described. In the placenta, only the type 1 gene is expressed. The coordinated action of two transcription factors determines the specific expression of the 3βHSD1 in the human placenta (Peng *et al.*, 2004). Those two transcription factors are TEF-5 (transcription enhancer Factor V), which is strongly expressed in the human placenta, and a GATA-like protein.

For estrogen synthesis, in contrast to other steroidogenic organs, the placenta does not express cytochrome P450 17α - hydroxylase/17:20 lyase and, therefore, cannot convert pregnenolone and progesterone into androgens. Thus, the production of placental estrogens is the tributary of a precursor androgen, sulphate of dehydroepiandrosterone (S-DHA), produced by the maternal and fetal adrenal glands. Estrogen synthesis, originating from the fetal adrenal glands, increases progressively during pregnancy. At term, fetal adrenal glands are involved in 40% of the production of estrone and estradiol and in 90% of the production of estriol. S-DHA diffuses from fetal blood to the syncytiotrophoblast and is hydrolyzed by a sterol steroid sulfatase; DHA is then metabolized in androstendione by a 3β HSD. Androstendione is transformed into testosterone by a 17 β-hydroxysteroid dehydrogenase (17 βHSD), encoded by the HSD17B1 gene, localized on chromosome 17 (17q11-q21). C19 androsterone and testosterone are then aromatized in C18 estrogens (estrone and estradiol, respectively) by the P450 cytochrome aromatase, encoded by the CYP19 gene, only present in the syncytiotrophoblast. Fetal adrenal S-DHA can also undergo 16α-hydroxylation in the fetal liver, leading to the formation of 16α-hydroxy S-DHA, the precursor to estriol androgen. Thus, 90% of placental estriol arises from the activities of the fetal adrenal glands and the liver. This cooperation between the placenta and the fetus has led to the concept of a feto-placental unit (see figure 2).

If numerous factors, such as hCG, cAMP, and prostaglandins, have been described to modulate *in vitro* placental estrogen synthesis, the quality of the fetal adrenal glands' development remains the essential factor.

If progesterone is absolutely required for the well being of pregnancy, the role of estrogens still remains uncertain. Estrogens induce progesterone receptors' synthesis in the myometrium and stimulate the incorporation of lipoproteins and CYP11A1 expression, therefore, modulating progesterone synthesis. In addition, *in vitro* E2 stimulates the formation of the syncytiotrophoblast (Malassiné and Cronier, 2002). However, as will be detailed further, genetic deficiencies in placental sulfatase or aromatase, leading to very low levels of maternal circulating estrogens, are associated with normal pregnancy.

OTHER FACTORS

In the last few years, the production of neuropeptides has been highlighted in the placenta, an organ deprived of innervation. These neuropeptides are similar to those found in the hypothalamo-pituitary system or in the digestive tract (TRH, GnRH, CRH, somatostatin, grhelin...). In trophoblastic cultured cells *in vitro*, they modulate the placental hormonal secretion by autocrine or paracrine mechanisms (Petraglia *et al.*, 1996). It must be pointed out that, during pregnancy, the placenta and the fetal membranes produce large amounts of CRH (Corticotropin Releasing Hormone). Placental CRH progressively increases during pregnancy, due to an increase in its gene expression. It was thus proposed that CRH, interacting with estrogens, fetal adrenal steroids and prostaglandins, establishes a stimulative autocrine loop, which could initiate parturition (Mc Lean and Smif, 2001). Lastly, the placenta is the site of expression of many growth factors implicated in its development, such as IGFs and cytokines (Fowden, 2003). Some placental factors implied in angiogenesis could be early markers for pre-eclampsia. Thus, an increase in the levels of the soluble VEGF receptor (vascular endothelial growth Factor) and a reduction in the levels of PLGF (placental growth Factor) are observed in maternal circulation during the first trimester of pregnancy, before any of the clinical signs of pre-eclampsia have presented (Lewine *et al.*, 2004; Tadani *et al.*, 2004).

HORMONAL PATHOLOGIES OF PREGNANCY

The peak of hCG in maternal circulation is associated with a decrease in maternal TSH levels. Indeed, hCG binds to TSH receptors and stimulates thyroid cells. This thyreostimulating effect of hCG can lead to the appearance of a maternal goiter, observed more frequently in twin pregnancies, where hCG levels are higher. Recently, several studies stress the importance of the glycosylation state of hCG, which varies according to the stage of the pregnancy. The glycosylation of hCG is modified in choriocarcinomas and in feto-placental trisomy 21. The increased levels of hCG observed in maternal circulation, in the case of feto-placental trisomy 21, are used in clinical practice as a maternal serum marker in prenatal screening. It was recently demonstrated that this hCG increase is related to an abnormal glycosylation of this hormone, modifying its biological activity and its clearance in the materno-placental compartment (Frendo *et al.*, 2004). It is now well established that the levels of placental GH and IGF-1 are lowered in cases of intra-uterine growth retardation (Mirlesse *et al.*, 1993). The placental sterol sulfatase deficit leads to a very important reduction in the secretion of the estrogens. This enzymatic deficit is linked to the X chromosome, affecting the male fetus and is associated with ichthyosis (Bedin *et al.*, 1987). Another genetic abnormality, associated with very low levels of maternal estrogens, is the deficit in CYP19 (P450 aromatase). In this case, maternal and fetal virilisation is observed, due to the weak aromatization of androgens (Shozu *et al.*, 1991). Finally, the family deficit of β lipoproteins is one of the rare conditions of abnormal placental steroidogenesis and is related to an insufficient uptake of cholesterol in the syncytiotrophoblast. Apparently, it is associated with normal pregnancies. The specificity of human placental steroidogenesis was recently illustrated by the fact that the protein implied in the translocation of cholesterol to the internal mitochondrial membrane (STAR) was not expressed in the trophoblast, but rather, in the fetal adrenals (Tuckey *et al.*, 2004). Thus, a null mutation of this gene, which is associated with a potentially lethal fetal adrenal lipoid hyperplasia, does not modify the normal course of pregnancy. However, the absence of testosterone

production by Leydig cells is associated with a female phenotype at birth (Hasegawa *et al.*, 2000).

In addition, maternal or fetal cortisol can be deactivated by the placenta into cortisone. This regulates the quantity of active glucocorticoids available to the fetus. The syncytiotrophoblast expresses the 11β-hydroxysteroid deshydrogenase (type 2) throughout pregnancy. Any defect in syncytiotrophoblastic development or function decreases this enzyme, therefore, inducing a fetal hypercortisolism (Seckle *et al.*, 2000).Furthermore, an intra-uterine growth retardation is associated with a null mutation or a reduction in the expression of the 11β-hydroxysteroid deshydrogenase (Dave-Sharma *et al.*, 1998). This trophoblastic enzymatic activity has therapeutic implications. In the prenatal treatment of 21α hydroxylase deficiency in a female fetus, the maternal treatment must be unmetabolized dexamethasone, instead of hydrocortisone, which is transformed into cortisone by the trophoblast.

CONCLUSION

This article is dedicated to José Sáez who trained Danièle Evain-Brion in Endocrinology and allowed her to discover the fascinating interaction between clinical and fundamental research. José Sáez was an exceptional master and a great scientist with whom it was always a great pleasure to discuss any research topic. José Sáez was a friend.

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