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# Placental Growth Hormones

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**Survival and development of the mammalian conceptus depends on a variety of factors. Fetal growth is controlled by genetic and environmental determinants that may limit the mother's capacity to provide an appropriate environment (e.g., space, nutrients, temperature). Exchanges between the mother and fetus take place within the placenta. Interestingly, despite the diversity of mammalian species in terms of placental structure and hormonal functions, placental size at term always correlates with birth weight, reflecting the essential role of this temporary organ. The placenta is the site of major endocrine activity, including synthesis of a broad range of steroid and peptide hormones, growth factors, cytokines, and other bioactive factors. Some of these are produced exclusively by the placenta, including chorionic gonadotropin, and growth hormone (GH)/prolactin-like hormones. This article focuses on the expression, regulation, and physiologic role of placental GHs in mammalian species. Published data suggest that placental GHs are essential for adapting the maternal metabolism to pregnancy, for normal placental development, and therefore for fetal growth.**

**Key Words:** Placental growth hormone; sheep; syncytiotrophoblast; rhesus monkey; trophoblasts.

## GH Gene and Protein Expression in Placenta

Growth hormone (GH) is important in reproduction, exerting endocrine/paracrine actions. GH and GH receptor (GH-R) gene expression has been demonstrated in mammalian reproductive organs *(1)*. Early studies showed placental GH (PGH)/prolactin (PRL) bioactivity in rodents, ruminants, and primates *(2)*, while more recent reports describe GH mRNA and/or GH protein expression in the rat, sheep, human, and rhesus monkey placenta.

### *Rodents*

Four GH-related proteins have been found in the rat placenta. They were isolated from culture medium of basal zone explants collected on d 15 of pregnancy *(3)* (gestation length of 21 d). Analysis of their N-terminal sequences showed 78% analogy with rat GH precursors. However, the presence of placental GH in rodents has not been confirmed by recent studies. The PRL-related gene family is the most represented in the rat and mouse placenta.

### *Sheep*

In addition to a PRL-related protein—ovine placental lactogen (oPL) or ovine chorionic somatomammotropin (oCS)—the ovine placenta was recently reported to contain GH. GH mRNA is expressed in the placenta between d 27 and d 75 of pregnancy (Fig. 1) (gestation length of 5 mo). Production is highest between d 40 and d 50, when the placental growth rate is maximal *(4)*. GH mRNA expression in the placenta is only one-hundredth of that found in the pituitary, but, on a tissue-weight basis, total GH mRNA content in the whole placenta on d 40–50 is comparable with that in the adult pituitary. Three GH-like transcripts have been identified in the placenta by sequencing studies (*oPGH1*, *oPGH2*, and *oPGH3*). The *oPGH1* sequence is identical to the coding sequence of the pituitary *GH* gene. This transcript encodes a placental protein homologous to pituitary GH. The nucleotide sequences of *oPGH2* and *oPGH3* differ from that of pituitary *GH* gene by four bases. The two transcripts encode the same protein, whose amino acid sequence differs from that of pituitary GH by three amino acids *(5)*. GH transcripts are expressed in the mono- and binucleated trophoblastic cells that constitute the trophectoderm. They are also observed in the syncytium derived from fusion of binucleated trophoblastic cells with the endometrial epithelium *(6)*. GH protein is preferentially located in the syncytium and in the trophectoderm, at the tip and the base of fetal villi, respectively *(5)*. This could result from a maturation gradient of chorionic epithelial cells from the base to the top of the villi *(7)*. The mean hourly rate of oPGH secretion by d 40–45 placental explants is 2.5 ng/g of fresh tissue *(8)*.

### *Humans*

The human placenta also produces GH-related proteins. The human CS/GH gene family has been extensively studied. It consists of five highly related genes arranged in tandem: pituitary GH (*GH-N*); placental GH (*GH-V*); and the *CS*s *CS-A*, *CS-B*, and *CS-L*. These genes are located in a 47-kb cluster on the long arm of chromosome 17 (q22-q24) *(9–11)*. They are believed to have evolved by gene duplica-

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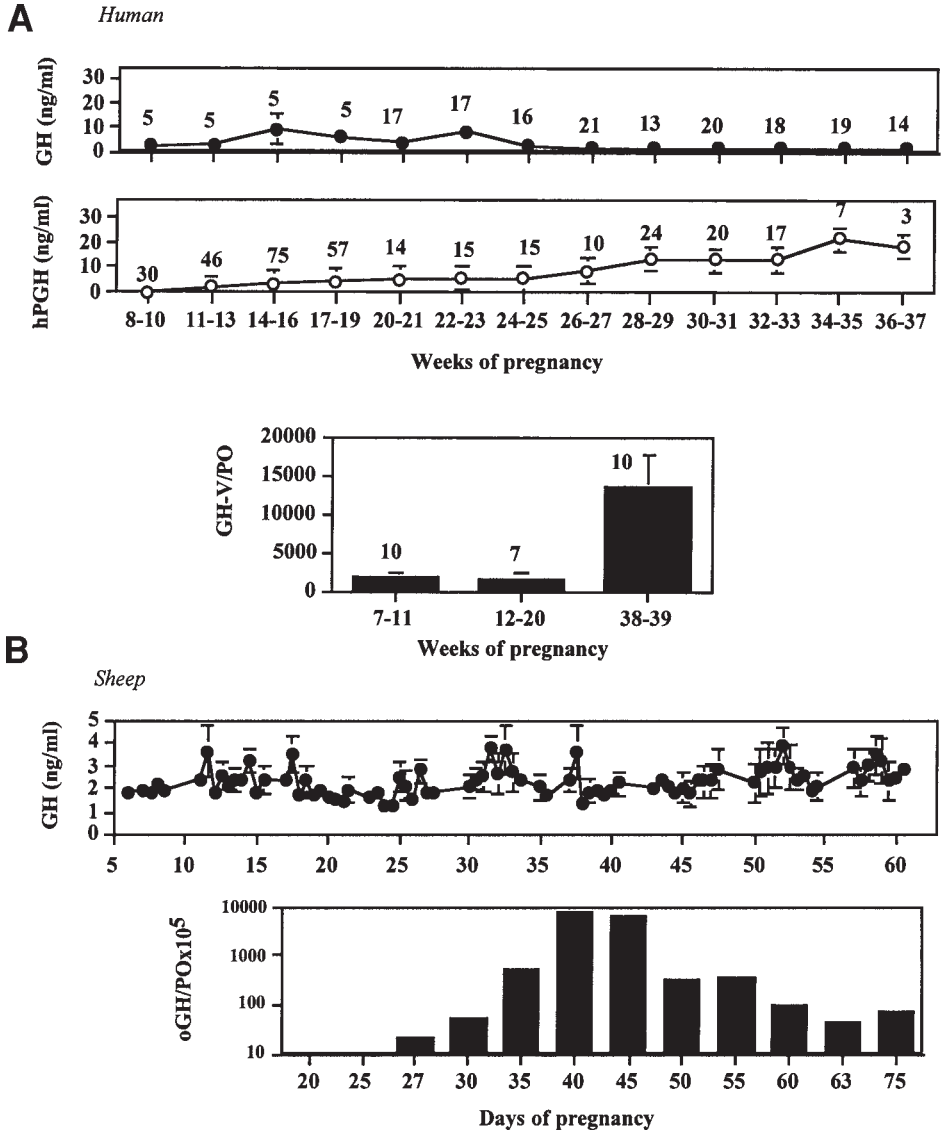


**Fig. 1.** Comparative evolution of GH levels in maternal sera and of pGH mRNA expression, in human (**A**) and sheep (**B**), during pregnancy. (A) Pituitary GH, evaluated in maternal sera with the specific antibody K24, decreased progressively from 20 wk to term (●). pGH, evaluated in maternal sera with the specific antibody E8, increased progressively and replaced the pituitary GH, which became undetectable (○). GH-V mRNA, quantified by real-time reverse transcriptase polymerase chain reaction (RT-PCR) and expressed as a ratio of PO housekeeping gene, was detected in the placenta from wk 7 until term. Its expression increased during the last trimester of pregnancy, reflecting the increase in syncytial mass. Each point represents the mean value ± SEM. The number above each value indicates the number of samples. (B) GH levels, evaluated in maternal sera, were very low and stable during the course of pregnancy (each point represents the mean value ± SEM, $n = 4$). GH mRNA, quantified by real-time RT-PCR and expressed as a ratio of PO housekeeping gene, was detected in the placenta between d 27 and d 75 of pregnancy.

tion and are therefore similar in structure (91–99% sequence identity *[11,12]*; organization: five exons [I–V] separated by four introns [A–D]), transcriptional orientation, and flanking DNA sequences *(13)*. Each gene encodes a mature protein of approx 200 amino acids, preceded by a signal peptide of about 25 amino acids. *GH-N* expression predominates in the pituitary *(14,15)* but is also observed in other cell types *(16)*. In somatotropic cells, efficient *GH-N* expression is dependent on binding of the transcription factor Pit-1 *(17)*. Despite the presence of binding sites for Pit-1 upstream of the GH/CS gene locus *(18–20)* and abundant Pit-1 protein within the pituitary, the *GH-V* gene is not expressed in the pituitary. A member of the nuclear factor-1 family appears to be involved in the repression of the placental *GH/CS* genes in the pituitary *(21)*. Weak GH-V mRNA expression has been described in pituitary adenomatous tissue *(22)*. Contrary to the sheep placenta, *GH-N* is not expressed in the human placenta *(11)*. The remaining four genes (*CS-A*, *CS-B*, *CS-L*,

and *GH-V*) are expressed in the placenta *(11,23)*, as well as in the testis *(24)*. In the placenta, GH-V mRNA accounts for 0.05% of total mRNA, while CS-A and CS-B account for 10–20%, CS-A mRNA being five times more abundant than CS-B mRNA *(25)*. The regulation of *GH-V* gene expression in this organ is poorly documented. Despite Pit-1 transcript and protein expression in the placenta, this transcription factor does not seem to regulate *GH-V* expression *(26,27)*. Similarly, in lymphocytes, where GH-V and GH-N mRNA are coexpressed, Pit-1 is not involved in *GH-V* gene regulation *(28)*.

The *GH-V* gene expressed in the placenta yields an 800-nucleotide mRNA coding for a 22-kDa protein, hereafter referred to as placental GH (hPGH) *(29)*. In addition to *GH-V* cDNA, a distinct cDNA designated *GH-V2* has been described *(30)*. *GH-V2* represents more than 30% of total *GH-V* transcripts *(30)*. GH-V2 mRNA (1250 nucleotides long instead of 800) is predicted to encode a protein with a membrane-spanning region *(30)*. The translated protein, however, has not yet been detected. Two additional GH transcripts have been reported in the human placenta *(31,32)*.

Frankenne and colleagues *(33,34)* identified hPGH as the *GH-V* gene product. It is a 191 amino acid protein differing from pituitary GH by 13 amino acids. It has a more basic isoelectric point than pituitary GH and possesses an N-glycosylation site at position 140–142 *(33)*.

*GH-V* is predominantly expressed in the placenta (Fig. 1) *(22,30)*. *In situ* hybridization (ISH) with a *GH-V*-specific probe showed that the syncytiotrophoblast is the *GH-V* production site *(35)*. hPGH has also been localized by immunohistochemistry in the syncytiotrophoblast *(36)*. Using the well-established in vitro model of villous cytotrophoblast differentiation into syncytiotrophoblast *(37,38)*, we confirmed by real-time quantitative RT-PCR that *GH-V* transcript levels increase during syncytiotrophoblast formation *(39,40)*. Using cultured villous trophoblast cells, agents that stimulate or inhibit villous cytotrophoblast differentiation were found to modulate *GH-V* expression. hPGH secretion increases after cyclic adenosine monophosphate (cAMP) treatment *(41)*, and *GH-V* transcript levels increase after retinoid and peroxisome proliferator-activated receptor (PPAR)-ligand treatment *(42)*; both of these treatments stimulate villous trophoblast differentiation. Conversely, overexpression of copper-zinc superoxide dismutase, which inhibits villous trophoblast fusion and differentiation, decreases *GH-V* expression *(40)*. *GH-V* expression, like *CS* expression, appears to be a good marker of syncytiotrophoblast formation.

Recent studies have shown *GH-V* and hPGH expression in invasive extravillous cytotrophoblasts *(43)*, which were already known to produce human placental lactogen (hPL), the product of *CS* genes *(44,45)*. GH-V mRNA expression has also been reported in human trophoblastic neoplasms, samples of complete hydatidiform moles, and choriocarcinoma cells *(46,47)*. BeWo and JAR choriocarcinoma cells express *GH/CS* family genes but not JEG-3 *(46,48)*. *GH-V* gene expression is reported only in BeWo cells *(46,48)*, but GH secretion was evaluated using a polyclonal antibody that crossreacts with hPGH and hPL. We cultured JEG-3, JAR, BeWo, and BeWo b30 cells in the presence or absence of cAMP *(49)* and measured hPGH production over 48 h with a specific radioimmunoassay *(50)*. Only BeWo b30 cells produced hPGH, and cAMP treatment did not increase this production (unpublished data).

### *Nonhuman Primates: Rhesus Monkey*

The rhesus placenta also expresses several members of the GH/CS family, which is at least as complex as the human counterpart. Four genes are expressed in the placenta: three highly homologous *CS* genes (*mCS1, mCS2, mCS3*) and a *GH-V* gene (*mGH-V*). The four genes show strong homology (90%) with the human placental CS/GH mRNAs *(51)*. As in humans, the rhesus pituitary does not express any of these placental transcripts, and the placenta does not express pituitary GH transcripts. CS/GH mRNAs are expressed early in gestation, being detectable in the blastocyst and, from d 18, in the placenta (gestation length of 180 d) *(51)*. During the first trimester, mCS1 mRNA is strongly expressed, whereas the other three mRNAs are all weakly expressed. During the second and third trimesters, mGH-V mRNA levels increase to reach those of mCS1 mRNA, and the two mRNAs represent 75% or more of all *CS/GH* transcripts in the placenta. *mGH-V* is more highly expressed in monkeys than in humans, accounting for 37% of all placental transcripts *(51)*. As in humans, *CS/GH* genes are expressed at the trophoblast level. Cultured trophoblasts differentiated into a syncytium express the four genes, but *mCS1* and *mGH-V* are expressed two to six times more strongly than *mCS2* and *mCS3 (52)*. Precise localization of CS/GH-V mRNA by ISH is lacking in this species. Placental *CS/GH* gene regulation has been extensively studied at the molecular level in monkeys. As in humans, CS/GH mRNA expression in cultured trophoblasts is increased by cAMP treatment *(52)*. This activation is specific for placental cells and is developmentally regulated; it is observed only in second- and third-trimester trophoblastic cells *(53)*. The monkey *GH-V* gene transcriptional start site contains binding sites for Sp1 and Pit1/GHF-1. As in humans, Pit-1 is expressed in the rhesus monkey placenta, and its expression is increased by cAMP; however, it does not seem to have a functional role in *mGH-V* gene transcription *(54)*. On the other hand, two Sp1/Sp3 sites, and additional elements directly adjacent to them, are involved in the cAMP responsiveness of the *mGH-V* gene *(55)*.

The four placental *CS/GH* genes code for four 217 amino acid proteins with predicted molecular weights 22–25 kDa *(51)*. The deduced amino acid sequence codes for a protein with 87% homology and 77% identity with hPGH. Placental mGH is predicted to have a basic isoelectric point, like hPGH, but lacks an N-glycosylation site *(51)*.

## Biologic Properties of PGHs

### *Humans*

Humans are the only species for which we have information on the biologic properties of placental GH. hPGH has high somatogenic activity and low lactogenic activity. It binds to GH-R with similar affinity to pituitary GH *(56–58)*. However, hPGH has considerably lower affinity than pituitary GH for lactogenic receptors *(58,59)*. hPGH is equipotent to pituitary GH as a ligand for circulating GH-binding protein *(60)* and therefore circulates in the maternal circulation in both free and bound form *(61)*. The somatogenic activity of hPGH is illustrated by a 40–90% increase in size in transgenic mice bearing the *GH-V* gene as compared with controls *(62)*. This increase is similar to that seen in transgenic mice containing the *GH-N* gene. hPGH induces body weight gain in hypophysectomized rats, to a similar extent to pituitary GH *(63)*. In vitro, hPGH binds to intact fat cells and produces insulin-like and lipolytic responses in rat adipose tissue, similarly to biosynthetic pituitary GH *(64)*. However, the mitogenic response of lactogen-inducible $Nb_2$ cells to hPGH is significantly lower than the response to pituitary GH *(63,65)*.

### *Sheep*

The placental transcript *oPGH1* encodes a protein homologous to pituitary GH. This placental GH probably has the same capacity as pituitary GH to bind GH-R, inducing receptor homodimerization and signal transduction. *oPGH2* and *oPGH3* transcripts code for the same protein, whose amino acid sequence differs from that of pituitary GH by three amino acids. One of these three amino acids is located in a loop structure involved in GH-R binding. The same amino acid variation, at the same position, is observed in oPL *(5)*. Consequently, the protein encoded by *oPGH2*/*oPGH3* is presumed to bind GH-R, leading to the formation of either a homodimer, or a heterodimer composed of one GH-R molecule and one PRL-receptor molecule, as reported for oPL *(66)*.

## PGH Secretion into Maternal and Fetal Compartments

### *Humans*

The existence of a monoclonal antibody specific for hPGH (E8; IRMA, Biocode SA, Lieges, Belgium) allows pituitary GH and hPGH to be distinguished in maternal serum. A cross-sectional study of GH concentrations in pregnant women reveals that early in pregnancy (up to 15 wk), pituitary GH is the main form present in the maternal circulation, displaying a highly pulsatile 24-h profile *(67,68)*. From 10–20 wk to term, hPGH gradually replaces pituitary GH, which becomes undetectable (Fig. 1) *(68–70)*. In contrast to pituitary GH, the 24-h serum concentration profile of hPGH is basically nonpulsatile *(71)*. hPGH is not released in the fetal compartment; it is not detected in fetal blood *(69)*. Interestingly, hPGH levels in maternal serum *(72)*, like those of human chorionic gonadotropin (hCG) *(73,74)* and hPL *(75)*, are significantly higher in pregnancies with a female fetus *(76)*, suggesting an increase in syncytiotrophoblast mass or in trophoblast hormonal production. Placental removal after Cesarean section in late pregnancy leads to a rapid fall (within 1 h) in the serum hPGH concentration *(67)*, confirming both the relatively short half-life of the hormone and its placental origin. A drastic decrease in hPGH levels is observed at the onset of labor *(68)* and is likely related to changes in uteroplacental blood flow and to release of placental proteases. Consequently, any studies on hPGH in maternal circulation must include samples taken before the onset of labor in order to avoid artifacts and large variation due to the initiation of labor.

### *Sheep*

The antibody used to evaluate GH concentrations in sheep circulation during gestation does not discriminate between pituitary GH and oPGHs. Nevertheless, maternal circulating GH levels during early pregnancy are very stable and low (Fig. 1; 3 ng/mL). They do not rise significantly between d 30 and 50, when the oPGH is maximally expressed in the placenta. During this period, mean placental oPGH content ranges between 100 and 1000 ng. This strongly suggests that in sheep, contrary to humans, oPGH release in the maternal circulation is very low or nonexistent. On the other hand, GH is detected in the umbilical vein and artery as early as d 35. This GH is not of fetal origin as GH mRNA was not detected in the fetal pituitary before d 50. It is not of maternal origin either, because GH does not cross the sheep placenta (unpublished data) and GH concentrations are twice those found in the maternal serum at the same stage of pregnancy. These results support the hypothesis that a portion of oPGHs is released into the fetal compartment via the umbilical cord *(77)*.

### *Rhesus Monkey*

No data are available on maternal or fetal GH concentrations in the rhesus monkey.

## Physiologic Role of PGHs

### *Humans*

In humans, hPGH exerts its biologic effects on the mother and the placenta. In vivo and in vitro experimental models (see Biologic Properties of PGHs) clearly show that hPGH has somatotrophic activity and can alter the maternal metabolism. In the maternal liver and other organs, hPGH strongly stimulates gluconeogenesis, lipolysis, and anabolism, thereby increasing nutrient availability for the fetoplacental unit. hPGH is considered a key regulator of maternal insulin-like growth factor-1 (IGF-1). Cross-sectional studies of a large number of normal and defective pregnancies have revealed that IGF-1 values in maternal plasma correlate with corre-

sponding hPGH values but not with CS values, regardless of the type of complication and gestational age *(61,68,78)*. In women with acromegaly, despite high levels of pituitary GH and high IGF-1 concentrations, maternal IGF-1 levels increase gradually during pregnancy, following the pattern of hPGH secretion *(79)*. The recent description of a gradual elevation of IGF-1 levels in the circulation of a pregnant woman with Pit-1 deficiency supports the hypothesis that hPGH is the prime regulator of maternal serum IGF-1 during pregnancy *(27)*. Through its nonpulsatile secretion into the maternal circulation and its metabolic effect on IGF-1, hPGH is considered a major mediator of the insulin resistance of pregnancy. Recent data obtained with transgenic mice that overexpress the *GH-V* gene corroborate this hypothesis; these animals display fasting and postprandial hyperinsulinemia, and minimal glucose lowering in response to insulin injection *(80)*.

The presence of GH-Rs in the villous trophoblast *(81–83)* and hPGH expression in the extravillous trophoblast *(43)* also suggest an autocrine and/or paracrine role of this hormone in placental development and function. This potential impact of hPGH on placental development, metabolism, and substrate supply might indirectly control fetal growth. hPGH does not appear to be directly involved in fetal growth, however, because it is not detected in the fetal circulation.

### *Sheep*

Contrary to humans, oPGHs are probably not involved in regulating the maternal metabolism, because they are secreted for only 1 mo and probably do not enter the maternal compartment. GH-R is detected in the uterus and placenta during the oPGH secretion period. Furthermore, GH-R mRNA appears in the fetal liver simultaneously with GH mRNA expression in the placenta *(77)*. The involvement of oPGH in GH-R regulation at the fetoplacental level, and of placental metabolic factors such as placental leptin *(84)* or glucose transporter *(85)*, remains to be investigated. After d 50 of pregnancy, fetal GH may take over the role of placental oPGH.

## Regulation of pGH Secretion

### *Humans*

The regulation of hPGH secretion is very different from the regulation of pituitary GH secretion. GH-releasing hormone (GHRH) mRNA and peptide are detected in the human placenta *(86)*, but GHRH does not modulate hPGH production in vivo *(87)* or in vitro *(88)*. In vitro, hPGH secretion is inhibited by glucose in term placental explants and in cultured trophoblast cells *(89)*. In vivo, hPGH concentrations fall during an oral glucose tolerance test in women with gestational diabetes *(34,90)*, while no change in hPL, leptin, or hCG secretion is observed. Bjorklund et al. *(91)* reported a mean 27% increase in hPGH during a hyperinsulinemic hypoglycemic clamp in pregnant women with insulin-dependent diabetes mellitus. These results suggest that the syncytiotrophoblast, which is in direct contact with the maternal blood and expresses the major glucose transporter Glut1, responds rapidly to variations in maternal blood glucose levels by modifying hPGH secretion *(92)*.

Preliminary results obtained recently with cultured trophoblast cells suggest that nitric oxide may be an important regulator of hPGH secretion (unpublished data).

### *Sheep*

Some aspects of oPGH regulation are similar to those in humans. We investigate oPGH regulation by using perifused cotyledon explants. We have previously detected GHRH production by the ovine placenta *(93)*, but oPGHs are not regulated by this releasing factor. As in humans, oPGH production is deceased within 4 h of a glucose challenge; the mechanism is posttranscriptional, since *oPGH* transcript expression is not affected *(8)*.

## hPGH in Complications of Pregnancy

hPGH levels in the maternal circulation are normal in cases of fetal anencephalia, supporting the notion that the regulation of hPGH secretion is independent of the fetal pituitary axis *(94)*.

The course and outcome of pregnancy is normal in women in whom the *CS-A-B-GHV* gene locus is fully deleted and therefore have no circulating hPL. However, unexpected placental expression of *GH-N*, at a low level, can be observed in these women *(34)*. This points to a compensatory mechanism that allows the placenta to secrete a hormone with the same metabolic somatogenic effect. Recently, two reports of children with a 45-kb gene deletion within *GH/CS* gene cluster, encompassing the *GH-N*, *GH-V*, *CS-A*, and *CS-L* genes, have also been published *(95,96)*. One of these reports provides few data on related pregnancies and fails to state length at birth, an essential datum *(95)*. The other report states that the four affected newborns had short bodies *(96)*. These cases show that fetal viability was not affected by the lack of placental hPGH synthesis, although this was a potential cause of the intrauterine growth defect. Indeed, fetal growth during late pregnancy is essentially dependent on the maternal substrate supply *(97)*, and hPGH is one placental hormone that allows the maternal metabolism to adapt to this strongly catabolic phase. Maternal IGF levels and both total and free hPGH levels are low in intrauterine growth retardation (IUGR) *(61,68,94)*. Likewise, the mean number of placental cells per unit area expressing GH-V mRNA, as evaluated by ISH, is significantly lower in IUGR (normal: $12.8 \pm 0.9$ cells/unit area; IUGR: $4.9 \pm 2.4$ cells/unit area; $p < 0.004$). These data suggest that, in IUGR, the decreased levels of hPGH in the maternal circulation do not result exclusively from the reduced size of the placenta, but also from abnormal placental development and/or abnormal regulation of hPGH synthesis *(98)*.

We recently observed abnormal placental development in fetal Down's syndrome (trisomy 21) *(39,99)*. The placental aneuploidy is associated with decreased syncytiotrophoblast formation in vitro, which is related to genetic overexpression of copper-zinc superoxide dismutase *(40)* and, thus, to a reduced syncytiotrophoblast mass. A significant reduction in maternal circulating hPL levels reflects the decrease in hormone production by the syncytiotrophoblast. However, we observed a slight increase (1.2 × the median) in circulating hPGH in a large cohort ($n = 105$) of fetal trisomy 21–affected pregnancies (unpublished finding). This is in keeping with preliminary results from Moghadam et al. *(100)* and suggests that the hPGH metabolic clearance rate is modified in this setting, possibly owing to a posttranscriptional modification of hPGH.

Other recent studies have pointed to a modification of *GH-V* expression in diabetic pregnancies. In particular, Hu et al. *(101)* observed a higher GH-V/CS-L mRNA ratio than in normal term placentas. McIntyre et al. *(61)* found a strong correlation between hPGH and glucose levels at 28–30 wk of gestation and postulated that hPGH levels in diabetic pregnancies tend to increase glycemia in the long term.

## Conclusion

The existence of placental GH synthesis is clearly established in primates and sheep. In humans, at midgestation, the placenta partially takes control of the maternal metabolism, as hPGH gradually takes over from pituitary GH. This does not seem to be the case in sheep. opGH is probably involved at precise developmental stages of the fetoplacental unit, activating key growth factors or inducing the expression of other factors indirectly involved in fetoplacental growth. In humans, the presence of GH-Rs in villous and extravillous trophoblasts poses intriguing questions on the possible paracrine or autocrine roles of GH in implantation and placental development.

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