Placental Steroid Hormone Synthesis: Unique Features and Unanswered Questions[1]

Jerome F. Strauss III,[2] Federico Martinez, and Marianthi Kiriakidou

Center for Research on Women's Health and Reproduction, Department of Obstetrics and Gynecology
University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT

Despite the amazing diversity of placental architecture across species, a number of common elements can be found, including the ability of all placentae to synthesize and metabolize steroid hormones; the assignment of steroidogenic activities to specific trophoblast phenotypes; the use of novel mechanisms to control expression of steroidogenic enzyme genes, which differ from those employed in the adrenal cortex and gonads; and interactions with the maternal and fetal compartments encompassing supply of steroid hormone precursors as well as regulatory influences of maternal ovarian and pituitary hormones and fetal adrenal cortical steroids.

INTRODUCTION

The placenta performs several important functions during pregnancy: it physically anchors the fetus to the uterus; it transports nutrients from the maternal circulation to the fetus; it excretes fetal metabolites into the maternal compartment; it has an immunomodulatory role in the maternal acceptance of the fetal semi-allograft; and it produces hormones that regulate maternal and fetal organs.

This review will focus on one aspect of placental function, the elaboration of steroid hormones. We have two goals: 1) to illustrate how the placenta utilizes novel mechanisms to accomplish the task of steroid hormone synthesis and 2) to point out interesting and as yet poorly understood aspects of placental steroidogenesis. While several excellent reviews of placental steroidogenesis have been published in recent years [1–6], none of these articles has approached the topic with these perspectives.

THE DIVERSITY OF PLACENTAL MORPHOLOGY AND FUNCTION

The structure of the placenta varies remarkably across species, and anatomists have extensively categorized the various types of maternal-fetal interfaces. The placentae of some orders such as carnivores, lagomorphs, and rodents are generally similar. However, in insectivores and primates there is a broad range of morphologies, prompting Leiser and Kaufmann [7] to reflect that this gives " . . . the impression that several animals have acquired their specific placental types only by chance."

The placenta is composed of several different trophoblast cell phenotypes that have specialized functions (i.e., transport/exchange, endocrine duties, etc.). In some species, functions are combined in one phenotype, whereas in others they are performed by different cell phenotypes. For example, the human syncytiotrophoblast, which comes into direct contact with maternal blood, is both a transporting epithelium and an endocrine cell, positioned to take up precursors from maternal plasma and secrete hormones into the maternal compartment. In the mouse and rat, the labyrinthine zone syncytial trophoblast cells, which contact maternal blood, carry out transport functions, whereas the endocrine activities appear to be restricted to the giant trophoblast cells and the spongiotrophoblast.

There is a spectrum of placental endocrine activities across species (Table 1). In some mammals, the placenta eclipses the pituitary in the maintenance of ovarian function (e.g., mouse and rat). In the human and some infrahuman primates and in the sheep, horse, cat, and guinea pig, the placenta acquires the ability to substitute for the ovaries in the maintenance of gestation at various times during pregnancy. It should be noted that even though the placentae of other species cannot substitute for ovarian function, all placentae critically studied to date express steroidogenic enzymes [7–13]. Therefore, the ability to elaborate or metabolize steroid hormones is one common feature of trophoblast cells despite the marked differences in placental morphologies. However, the extent to which the placenta is capable of producing steroid hormones and the repertoire of steroidogenic enzymes expressed varies. In the human, rhesus monkey, baboon, and horse, the placenta does not express 17α-hydroxylase [1, 2, 10]. Placental estrogen synthesis in these species depends upon a source of androgen precursor from the fetus; the fetal adrenal glands in the case of primates, the gonadal interstitial cells in the case of the horse. In contrast, the trophoblast cells of the rat, pig, sheep and cow express 17α-hydroxylase [4, 8, 14–17] and are able to synthesize androgens and in some species estrogens.

The striking diversity in placental structure and endocrine function represents a veritable feast for the comparative biologist but an inevitable source of frustration for scholars who seek unifying concepts in reproductive biology. Clearly, caution must be exercised in extrapolating

TABLE 1. Dependence of pregnancy on maternal ovarian and pituitary secretions in various species.

Species	Duration of pregnancy (days)	Duration of nonpregnant luteal phase (days)	Day of pregnancy when hypophysectomy without effect (days)	Day of pregnancy when ovariectomy without effect (days)
Man	260–270	12–14	?	40
Monkey (M. mulatta)	168	12–14	29	21
Sheep	147–150	16–18	50	55
Guinea pig	60	16	3	28
Rat	22	10–12	12	Term
Mouse	20–21	10–12	11	Term
Cat	63	30–60	Term?	50
Horse	330–340	20–21	?	150–200
Cow*	280–290	28–20	?	Term
Dog*	61	61	Term?	Term?
Pig*	115	16–18	Term	Term
Rabbit*	31	12	Term	Term
Goat*	150	16–18	Term	Term

*These results do not mean that these species are solely dependent on pituitary and ovarian function. It is established that some are partially dependent on support from placental sources which are inadequate when acting alone. (Reprinted from Johnson M, Everitt B. Essential Reproduction. Blackwell Scientific Publications Oxford, 3 ed, 1988; pp 257 with permission).

findings regarding placental function from one species to another.

A UNIQUE ENDOCRINE ORGAN

It has been common for scholars to approach the study of the placenta with the anticipation that parsimony in nature would demand that mechanisms used in the control of placental endocrine functions would mimic those used postnatally in the hypothalamus, pituitary, adrenals, ovary, and testis. Indeed, the existing literature on placental endocrinology is replete with suggested parallels between the placenta and the adult endocrine system. These are undoubtedly misguided notions. The placenta is an ephemeral organ that is discarded after its roles in the maintenance of pregnancy and the fetus are accomplished. The forces that drive evolution of the placenta are different from those that drive evolution of the fetus and adult organism. Thus, the placenta could use unique transporters to acquire nutrients and unique receptors to respond to maternal or fetal agents; it could use protein mimics or unique modes of regulation of gene expression to accomplish its tasks. Given these considerations, it should not be a surprise that certain "placental-specific" gene products are among the most rapidly evolving proteins known. The sequences of ovine and bovine placental lactogens are remarkably different, suggesting a very rapid rate of evolution [18].

THE IMPORTANCE OF PLACENTAL STEROIDOGENESIS

In species in which pregnancy continues after removal of the ovaries, it is evident that the placenta can assume a major role in the production of the sex steroid hormones required for maintenance of the reproductive tract. In the human, the placenta at term produces about 300 mg of progesterone per day, which is 10 times the quantity of progesterone secreted by the midluteal phase CL.

The ability of placental steroidogenesis to sustain human pregnancy was demonstrated in studies in which pregnant women underwent luteectomy at the time of tubal sterilization before therapeutic pregnancy termination at various times during the first trimester [19, 20]. Removal of the CL before 8 wk of gestation resulted in a substantial number of pregnancy losses, whereas removal of the CL after this time did not produce abortion.

Yoshimi et al. [21] examined levels of progesterone and 17α-hydroxyprogesterone in women undergoing ovulation induction therapy. Because the human placenta does not express cytochrome $P450_{17\alpha}$, whereas the CL does, the plasma levels of 17α-hydroxyprogesterone were taken to reflect CL secretion, while plasma progesterone concentrations were taken to represent the contributions of both the CL and placenta. This study suggested a functional life span of the CL of 10 wk and the emergence of quantitatively significant placental secretion by the eighth week of pregnancy.

Analysis of progesterone levels in women with ovarian failure, who participated in an assisted reproductive technology program of oocyte donation, revealed that placental steroidogenesis, determined as increases in peripheral blood progesterone and estradiol levels over the background levels achieved with a constant replacement regimen of progesterone and estradiol, is quantitatively significant by the fifth week of pregnancy [22].

The studies summarized above establish that the placenta can sustain human pregnancy in the absence of ovarian function at some point in the first trimester. What of the converse? Could human pregnancy be maintained in the absence of placental steroidogenesis without replacement therapy? This question is much more difficult to answer, particularly regarding the role of placental progesterone secretion. In fact, the concept that placental progesterone production is essential is supported by negative data. There are no known cases of cytochrome P450 cholesterol side-chain

cleavage enzyme (P450$_{scc}$) deficiency in man. Absence of functional P450$_{scc}$ would prevent placental progesterone synthesis as well as fetal adrenal and gonadal steroidogenesis. In contrast, homozygous mutations in the P450$_{scc}$ gene have been discovered in the rabbit, a species in which the ovaries maintain pregnancy [23]. There are also no known individuals homozygous for mutations in the type I 3β-hydroxysteroid dehydrogenase gene expressed by trophoblast cells [24]. Such mutations would presumably compromise placental progesterone production.

In contrast to the absence of informative mutations affecting genes involved in placental progesterone synthesis, there are two examples of genetic disease that demonstrate that placental estrogen synthesis, at least at high levels, is not apparently required for maintenance of human pregnancy. Human gestation goes to term when the placenta and fetus lack sulfatase, an enzyme required for the hydrolysis of dehydroepiandrosterone sulfate generated by the fetal adrenal cortex, which is the primary precursor of estrogens during the third trimester [1, 2, 25, 26]. Moreover, pregnancies reach term with severe fetal and placental aromatase deficiency [27]. Although pregnancy is maintained in the face of low placental estrogen synthesis, the changes in the reproductive tract that precede parturition, particularly ripening of the cervix, do not occur, revealing a significant role for placental estrogens in the preparation for birth. Moreover, in the case of aromatase deficiency, both the mother and fetus are virilized as a consequence of diminished aromatization of androgens.

UNIQUE FEATURES OF PLACENTAL STEROIDOGENESIS

Regulation of Placental P450$_{scc}$ Expression: Trophoblast-Specific Elements in Steroidogenic Enzyme Genes

Rcho-1 cells, a line established from a transplantable rat choriocarcinoma, can be manipulated to differentiate into giant cells that are the rat placental trophoblast cells that produce steroid hormones [28]. As Rcho-1 cells differentiate into giant cells in vitro, they increase their secretion of progesterone. In addition, exposure of the Rcho-1 cells to a cAMP analog stimulates progesterone production [29]. Are the mechanisms underlying the differentiation-dependent up-regulation of progesterone secretion in these trophoblast cells and their response to cAMP similar to those underlying the granulosa cell response to the LH-induced cAMP increase at ovulation that induces luteinization?

The increase in steroidogenic activity of the Rcho-1 cells is due to an increase in transcription of the P450$_{scc}$ gene, just like the increase in P450$_{scc}$ gene expression in granulosa cells as they luteinize [29]. However, this increase in gene transcription does not involve the same *cis* elements in the P450$_{scc}$ promoter that govern P450$_{scc}$ gene transcription in the ovary [29]. The promoter sequences needed for the dif-
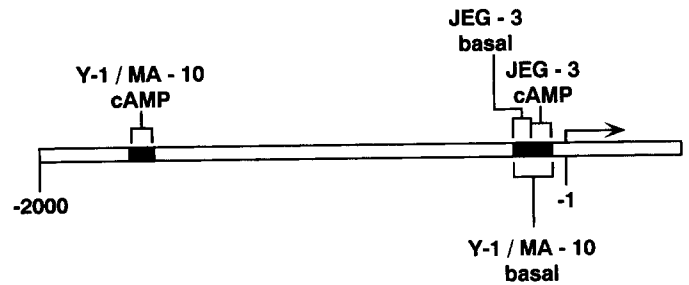

FIG. 1. Map of *cis* elements in human P450$_{scc}$ promoter that regulate expression of the gene in trophoblast (JEG-3 choriocarcinoma cells), gonadal (MA-10 Leydig tumor cells), and adrenocortical cells (Y-1 adrenal cortical tumor cells). *Cis* elements responsible for basal and cAMP-regulated expression are indicated on the basis of data presented in references [34–36]. Bent arrow denotes transcription start site.

ferentiation-induced expression of P450$_{scc}$ lie upstream of those that are implicated in gonadotropin regulation of the gene in ovarian cells. Moreover, cAMP does not stimulate the accumulation of P450$_{scc}$ mRNA or stimulate P450$_{scc}$ promoter activity in Rcho-1 cells [29]. Thus, the cAMP stimulation of progesterone secretion does not involve increased P450$_{scc}$ mRNA expression.

Progesterone secretion by bovine and ovine placentome cells is not affected by agents that raise cAMP levels or cAMP analogs but is increased by agents that activate the protein kinase C pathway (cattle) or arachidonate metabolites (sheep and goats) [30–33].

Cyclic AMP increases P450$_{scc}$ gene transcription in human trophoblast cells [34–36]. However, the *cis* elements controlling this response are different from those in the adrenal and gonads. Figure 1 presents a map of the *cis* elements that govern basal and cAMP-stimulated expression of the human P450$_{scc}$ gene in cells of the trophoblast lineage (JEG-3 human choriocarcinoma cells) and adrenal cortex (Y-1 murine adrenal tumor cells), and in gonadal cells (MA-10 Leydig tumor cells). Note that the cAMP-responsive elements are widely separated and that the elements controlling basal expression, though closely clustered, are different.

Collectively these observations indicate 1) that placental P450$_{scc}$ gene expression is controlled by different transcriptional mechanisms than in the adrenal cortex and gonads and 2) that cAMP may not have a prominent role in regulating placental steroidogenesis in some species or that its actions on placental steroidogenesis involve different pathways than in the adrenal cortex and gonads.

"Trophoblast-Specific" Trans Factors Governing Expression of Steroidogenic Enzymes

The findings reviewed above suggest that expression of the P450$_{scc}$ gene in trophoblast cells is controlled by trans acting factors that are different from those in the adrenal cortex and gonads. Indeed, several well-characterized transcription factors involved in adrenal cortical and gonadal

expression of steroidogenic enzymes are not apparently involved in placental steroidogenesis. Steroidogenic factor-1 (SF-1), also called adrenal-4-binding protein (Ad4BP), is a 58-kDa orphan nuclear receptor that plays a key role in the control of steroidogenesis in the adrenals and gonads [37–39]. The promoters of cytochrome P450 enzymes involved in steroidogenesis, including P450$_{scc}$ and aromatase, and the type II 3β-hydroxysteroid dehydrogenase gene (but not the type I enzyme gene), have sequences that bind SF-1. These sequences may also be involved in cAMP stimulation of transcription of these genes since experiments suggest that phosphorylation of this orphan nuclear receptor by protein kinase A is required for its effects on transcription. It is noteworthy that the deletion of the SF-1 gene in the mouse by homologous recombination prevents development of the adrenals and gonads [40].

Human trophoblast cells (Sugawara and Strauss, unpublished results) and Rcho-1 cells [29] express negligible levels of SF-1. Moreover, placental development and function appears to be normal in the SF-1 "knock-out" mouse [40]. The relative absence of SF-1 expression in the trophoblast suggests that this transcription factor does not play a role in placental gene expression. This distinguishes the placenta from the gonads and adrenal cortex. Dax-1 is another orphan nuclear receptor/transcription factor regulating adrenal cortex and gonadal function that is not expressed in the placenta [41]. Dax-1 binds to retinoic acid response elements but is not activated by retinoids. Mutations in the Dax-1 gene cause X-linked congenital adrenal hypoplasia and hypogonadotropic hypogonadism with associated deficiencies in adrenocortical and gonadal steroidogenesis.

Are there "placenta-specific" transcription factors regulating steroidogenic enzyme expression? Hum et al. identified sequences in the P450$_{scc}$ promoter that determined basal (bp −131 to −155) and cAMP-stimulated expression [36]. They also identified a 55-kDa nuclear protein that bound (bp −89 to −108) within this sequence by gel shift analysis and Southwestern blotting. This 55-kDa DNA binding protein was not found in adrenal cortex cell nuclear extracts but was detectable in extracts of some nonsteroidogenic cells (e.g., T84 human colon carcinoma cells). These findings support the idea of trophoblast regulatory factors that act on cis elements that are distinct from those that control P450$_{scc}$ gene expression in gonads and adrenal cortex. Whether these trans factors are indeed "placenta-specific" remains to be determined.

The Human Trophoblast Does Not Express Steroidogenic Acute Regulatory Protein

The transfer of cholesterol from the outer to the inner mitochondrial membrane, where cytochrome P450$_{scc}$ resides, is acutely stimulated by tropic hormones acting through the intermediacy of cAMP in the adrenal cortex and gonads [42].

This translocation of cholesterol, which accounts for the rapid increase in steroidogenesis in response to tropic stimulation, is apparently mediated by a newly discovered protein called steroidogenic acute regulatory protein (StAR) [43]. StAR is derived from a precursor containing an N-terminus mitochondrial targeting sequence that is cleaved upon entry into the mitochondria to yield a 30-kDa mature protein. Mutations in the StAR gene cause the severest form of congenital adrenal hyperplasia, lipoid congenital adrenal hyperplasia, in which the synthesis of all gonadal and adrenal steroids is impaired and cholesterol accumulates in the cytoplasm of the steroidogenic cells because of its inability to be efficiently transferred to the inner mitochondrial membrane [44]. The fact that pregnancies with a fetus affected with congenital lipoid adrenal hyperplasia go to term indicates that StAR is not involved in placental steroidogenesis. Indeed, the StAR gene is highly expressed in the human adrenal cortex, testis, and ovary but not in placenta, isolated trophoblast cells, or choriocarcinoma cells [45]. These observations indicate that StAR does not function in the human placenta and that other factors must be responsible for the movement of cholesterol to the trophoblast P450$_{scc}$ system.

The Trophoblast 3β-Hydroxysteroid Dehydrogenase Is Different from That Expressed in the Gonads and Adrenal Cortex

There are several enzymes encoded by different genes that catalyze the 3β-hydroxysteroid dehydrogenase reaction [46]. The type II enzyme predominates in gonads and adrenals. It is not expressed in the placenta. Another enzyme that differs from the type II enzyme in 12 amino acids, the type I enzyme, is expressed in trophoblast and skin. The type I enzyme has about a 10-fold lower K_m for pregnenolone compared to the type II enzyme, which would make it more efficient in metabolizing low levels of pregnenolone into progesterone. Mutations that inactivate the type II enzyme and cause severe congenital adrenal hyperplasia do not affect placental progesterone production [46, 47]. As mentioned previously, as yet, no mutations have been identified in the type I gene.

The Placental Aromatase Gene Is Transcribed from Unique Promoters

There is one aromatase gene, which encodes the enzyme that converts androgens into estrogens [48, 49]. The gene is quite large, at least 70 kb in size. The aromatase gene gives rise to several different transcripts, which display a tissue-specific pattern due to the use of alternative promoters. The promoter driving transcription of the primary placental transcript is upstream of exon I.1 and is 40 kb in front of the start site of translation. It is different from the promoter driving transcription of the aromatase gene in the ovary, which

is immediately proximal to the exon containing the translation start site. The use of alternative promoters in the placenta predicts the involvement of different trans acting factors from those controlling gonadal aromatase expression. Whether the placental trans factors important in controlling trophoblast P450$_{scc}$ expression are the same as those involved in placental aromatase transcription is not yet known.

It is noteworthy that the use of tissue-specific aromatase promoters is not restricted to the human. These have also been identified in the cow, pig, and horse, species in which the placenta also synthesizes estrogens [50, 51]. However, the regulation of the placental aromatase promoters seems to differ across species since the bovine placental aromatase promoter is not active in JEG-3 human choriocarcinoma cells whereas the corresponding human placental promoter is.

INTERACTIONS WITH THE MOTHER AND FETUS

The placenta is positioned to utilize steroid precursors contributed by both the mother and the fetus and to be influenced by hormones of maternal and fetal origin (Fig. 2). Indeed, there is good evidence indicating that the placenta engages in a dynamic steroid-mediated dialogue with both the maternal pituitary and ovary and the fetal adrenal cortex.

Placental Metabolism of Maternal Steroid Precursors

The human placenta utilizes maternal lipoprotein-carried cholesterol as a major substrate for progesterone synthesis (reviewed in [52]). This conclusion is based on studies in which the conversion of labeled plasma cholesterol into progestin metabolites was determined. The low levels of low-density lipoproteins (LDL) found in maternal plasma in hypobetalipoproteinemia are associated with plasma progesterone concentrations that are about one half those found in normal pregnancy, an observation consistent with a key role for maternal plasma apo B-containing lipoproteins in supporting placental progesterone synthesis. Trophoblast cells express a number of receptors that recognize lipoproteins, including three members of the LDL receptor family of receptors: the LDL receptor, which binds VLDL and LDL [52]; the VLDL receptor [53], which binds very low-density lipoproteins (VLDL) and chylomicron remnants; and the α_2-macroglobulin receptor/LDL receptor-related protein, which binds apo E-enriched VLDL [54]. These receptors provide mechanisms for uptake of maternal lipoproteins. Moreover, trophoblast cells produce apo E, a ligand for all three of these receptors. This creates a mechanism for secretion-recapture whereby the trophoblast cells release into the intervillous space a ligand that can associate with lipoprotein particles and enhance their affinity for the receptors expressed on the trophoblast plasma membranes [55]. The expression of LDL receptors on the baboon trophoblast appears to be dependent
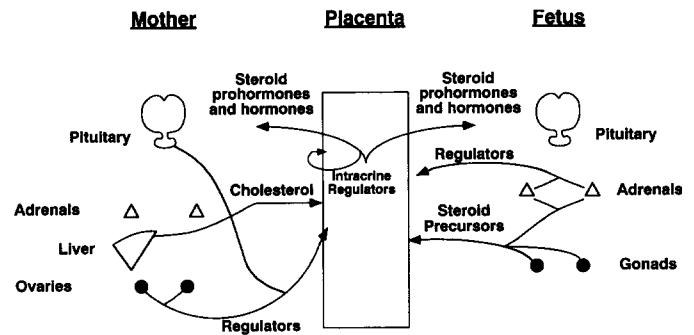

FIG. 2. Placental interactions with maternal and fetal compartments. Maternal substrates (cholesterol) are utilized for steroid synthesis. In addition, maternal and fetal prohormones are converted into active steroids in placenta (e.g., human fetal adrenal cortex dehydroepiandrosterone [DHEA]-SO$_4$ is converted into estrogens in placenta). Conversely, in some species, placental prohormones are converted into active steroids in maternal and fetal compartments (e.g., placental-derived androsteroidione is aromatized in rat ovary). Steroid-mediated regulatory loops encompassing maternal ovaries and pituitary and fetal adrenal cortex exist.

upon estrogen, and antagonism of estrogen action results in reduced LDL receptor expression and diminished placental progesterone production [56].

Maternal Ovarian and Pituitary Hormones Can Affect Placental Steroidogenesis: Evidence for a Dialogue between the Ovary and the Pituitary and Placenta

In the rat, estrogen, synthesized by the ovaries, suppresses placental expression of 17α-hydroxylase [15]. Since the rat placenta elaborates androgens that are potential precursors for ovarian aromatization [57, 58], a dialogue between the placenta and ovary may take place in this species. Estrogens not only regulate 17α-hydroxylase expression, they control placental mass [59]. The rat placenta hypertrophies in response to ovariectomy, and this hypertrophy is blocked by exogenous estrogen. These findings support the notion of an ovarian-placental interaction.

Pituitary hormones may also affect rat placental function [17]. Durkee et al. [17] suggested that placental 17α-hydroxylase is suppressed by LH on the basis of the inverse relationship between LH levels in pregnant rats and levels of enzyme in the placenta. They also found that administration of hCG to pregnant rats suppresses 17α-hydroxylase expression. This action of LH seems to be directly upon the placental cells. Together, these findings demonstrate that placental function can be influenced by a dialogue between the ovaries and pituitary gland, providing a framework for compensatory interactions.

Fetal Hormones Regulate Placental Steroidogenesis

Steroid hormones derived from the fetus influence placental function by providing precursors for steroidogenesis as in the case of estrogen biosynthesis in primates and the horse. The fetal adrenal cortex (primates) or gonadal interstitial cells (horse) secrete androgens that are aromatized in

the placenta [1, 2, 5, 6]. The estrogen produced from fetal adrenal androgens in the baboon has an impact on placental function since blockade of estrogen action reduces placental progesterone production [56, 60].

Another example of a regulatory role for fetal-derived steroids is in the induction of placental 17α-hydroxylase and aromatase activity in the sheep placenta at term by fetal adrenal glucocorticoids [16, 61]. The induction of 17α-hydroxylase is envisioned to lead to the catabolism of progesterone as well as the production of androgen precursors for aromatization. The communication between the fetal adrenal cortex and placenta is not unidirectional. Estrogens may restrain fetal adrenal cortical androgen production and thus modulate estrogen precursor supply through a negative feedback mechanism [62].

Is the Trophoblast Cell Steroidogenic Machinery Compartmentalized?

The interactions of the placenta with the maternal and fetal compartments raise the question of whether the steroidogenic machinery is organized within the trophoblast cell to facilitate utilization of precursors derived from one compartment or another and to facilitate ready release of steroid products into a compartment.

The syncytiotrophoblast of the human, baboon, and macaque placentae are highly polarized cells, providing a unique opportunity to explore the organization of the steroidogenic machinery. The apical surfaces of these cells face the maternal circulation; their basal surfaces rest on a basement membrane that overlies fetal capillaries.

Although a comprehensive ultrastructural study of the topography of steroidogenic enzymes in the placenta has not been reported, available information suggests that certain enzymes could be localized to specific domains [63, 64]. Immunocytochemistry at the ultrastructural level has localized aromatase and 17β-hydroxysteroid dehydrogenase to syncytiotrophoblast endoplasmic reticulum [65]. Interestingly, aromatase was found associated with the microvilli on the apical surface [63]. Sulfatase, the enzyme that generates the aromatase substrate, was also associated with endoplasmic reticulum and plasma membrane [64]. The co-localization of these two enzymes near the apical portion of the syncytiotrophoblast positions them to generate estrogen for secretion into the maternal compartment. This localization of aromatase assists the placental "biochemical barrier" to entry of maternal androgens into the fetus.

UNSOLVED MYSTERIES OF PLACENTAL STEROIDOGENESIS

The unique aspects of placental steroidogenesis described above raise a number of interesting questions for which there are presently no clear answers.

Ontogeny of Placental Steroidogenesis and the Lineage of Placental Steroidogenic Cells

When do trophoblast cells gain steroidogenic competence? A number of recent reports have described changes in the levels of steroidogenic enzyme proteins and their respective mRNAs in the placentae of laboratory animals and domestic animals [8–10, 14, 17]. These studies have clearly demonstrated that genes encoding the steroidogenic machinery are expressed relatively early in gestation. Although some of these studies have included in situ hybridization and immunohistochemical analyses, it is notable that in some instances the cells expressing these enzymes and their location in the placenta have not been defined by these methods (vide infra). This is unfortunate since unexpected observations have been made in the course of such work, including the discovery that maternal decidual cells in the basalis and capsularis of the rodent express $P450_{scc}$ in the periimplantation period and early pregnancy [9, 66]. These findings in mouse and rat, which are consistent with biochemical studies on steroid release from endometria of other species, raise the interesting possibility of maternal-placental interplay in the intrauterine production of progestins.

Relatively little is known about the ontogeny of steroidogenic machinery in the primate placenta beyond the clinical studies reviewed previously that mark the time that placental steroidogenesis in the human becomes quantitatively significant. We know that the genes encoding the subunits of chorionic gonadotropin are expressed by trophoblast cells of the preimplantation blastocyst. When are the components of the cholesterol side-chain cleavage system first expressed?

The question of ontogeny of the steroidogenic machinery goes beyond the descriptive mapping of the temporal and spatial patterns of gene expression. It leads directly to the question of the differentiation of the steroidogenic cell phenotype in the placenta. Available information, although far from comprehensive, indicates that only specific trophoblast cell phenotypes express steroidogenic enzymes (e.g., the syncytiotrophoblast of the primate placenta or the trophoblast giant cells of the rodent placenta). Parenthetically, we should remind readers that the trophoblast cell types responsible for steroidogenesis have not been firmly established in species that have been well-studied. Some authors have concluded that the binucleate cells of the ruminant placenta are steroidogenic [67, 68], whereas others have found that steroidogenic enzymes like $P450_{scc}$ are expressed in mononucleate cells, not binucleate cells [69]! What genes determine the pathway of differentiation of endocrine trophoblast cells? Do intrinsic signals or factors derived from the maternal compartment control expression of the genes coding for steroidogenic enzymes? Do environmental factors influence the ontogeny of enzyme expression?

There has been recent progress in the identification of factors that affect the development of certain placental cell types. Gene knock-out studies in the mouse have suggested important roles for the basic-loop-helix-loop transcription factor, Mash-2 [70], in the development of the spongiotrophoblast and hepatocyte growth factor/scatter factor [71], a growth factor produced by mesenchymal cells that acts on epithelial cells, in the development of the labyrinthine zone of the placenta. Both knock-outs result in embryonic lethality, due to deficient placental development. However, in both cases the trophoblast giant cell lineage, and presumably their steroidogenic functions, appear grossly to be normal.

One of the unmet needs of investigators working on the problem of trophoblast lineage specification is a toti-potent trophoblast stem cell system. At present, only cell culture systems with restricted differentiation patterns like the Rcho-1 cells exist. This represents an important area for future research.

How Is Cholesterol Transferred Within the Trophoblast Mitochondria to P450$_{scc}$?

The absence of expression of StAR in the human placenta raises the question of how cholesterol is moved to the cholesterol side-chain cleavage system. There are several possible explanations for the ability of the placenta to produce progesterone in the absence of StAR.

1) StAR or a StAR-like protein is not required. Some cholesterol is able to move from the outer to inner membranes in cells that do not express StAR. Thus, COS cells transfected with P450$_{scc}$ and adrenodoxin produce pregnenolone, but at rates that are 4- to 20-fold less than those achieved when StAR is coexpressed with cholesterol side-chain cleavage enzyme [44].

A human CL that weighs about 1 g and expresses StAR produces 25 mg of progesterone/day. A human placenta that weighs 500 g (100 g being trophoblast) produces 300 mg of progesterone/day or 3 mg of progesterone/g of trophoblast [26]. Hence, the mass of the placenta could compensate for the absence of facilitators to move cholesterol to the inner mitochondrial membrane.

2) There is another yet to be identified StAR expressed in the placenta. There appears to be only one StAR structural gene and a pseudogene. Genomic Southern blotting at low stringency has not suggested the existence of related gene sequences (Sugawara and Strauss, unpublished results).

3) Other mechanisms such as the mitochondrial peripheral benzodiazepine receptor facilitate sterol delivery to the placental P450$_{scc}$. This remains a possibility since other putative factors involved in intramitochondrial cholesterol translocation like the peripheral benzodiazepine receptor system are expressed in the placenta [72, 73]. However, definitive evidence for the involvement of this protein and its associated proteins in placental steroidogenesis remains to be provided.

4) The syncytiotrophoblast mitochondria differs from the mitochondria of adrenal cortical and gonadal cells. Although several investigators have studied steroid synthesis by human placental mitochondria, establishing that the steroidogenic machinery is associated with these organelles, the location of P450$_{scc}$ in these organelles and the structural relationships of the inner and outer mitochondrial membranes has not been described. This leaves open the possibility that placental mitochondrial structure differs from that of the adrenal cortex and gonads.

It is of interest to note that the mitochondria of the syncytiotrophoblast have a distinctly different morphology from those of the cytotrophoblasts, which give rise to the syncytiotrophoblast by a process of cell fusion. The cytotrophoblasts do not express steroidogenic enzymes at high levels, and their mitochondria are large, with an electron lucent matrix (Martinez and Strauss, unpublished result). The syncytiotrophoblast mitochondria are smaller with a more electron-dense matrix. The change in surface-to-volume ratio of the mitochondria could lead to facilitated movement of cholesterol to the inner membranes. How the changes in mitochondrial structure noted above are brought about during the process of trophoblast differentiation and whether they are indeed significant for mitochondrial steroidogenic function are questions that have not been explored.

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