Klotz Communications: Evolution of hormones during pregnancy

Evolution of steroids during pregnancy: Maternal, placental and fetal synthesis

Évolution des stéroïdes pendant la grossesse : origine maternelle, origine placentaire, origine fœtale ?

Yves Morel*, Florence Roucher, Ingrid Plotton, Claire Goursaud, Véronique Tardy, Delphine Mallet

Service d’hormonologie, endocrinologie moléculaire et maladies rares, CPBE, groupement hospitalier Lyon-Est, 69677 Lyon-Bron, France

Abstract

Progesterone, estrogens, androgens and glucocorticoids are involved in pregnancy from implantation to parturition. Their biosynthesis and their metabolism result from complex pathways involving the fetus, the placenta and the mother. The absence of expression of some steroidogenic enzymes as CYP17 in placenta and in adrenal fetal zone and the better determination of the onset and variation of others especially HSD3B2 during the pregnancy explain the production of the steroid hormones. Moreover the consequences of some disorders of steroidogenesis (especially aromatase, POR, CYP11A1 and 21-hydroxylase deficiencies) in fetus and mother during the pregnancy have permit to elucidate these complex pathways. This better knowledge of steroid hormones production associated with their dosages in maternal plasma/urine or amniotic fluid using new specific assays as LC-MS MS could facilitate the follow-up of normal and pathological pregnancies. Moreover, these advances should be a basis to evaluate the impact of multiple pathologies of the pregnancy and pharmacologic and xenobiotic consequences on their metabolism. © 2016 Elsevier Masson SAS. All rights reserved.

Keywords: Aromatase/deficiency; Androgens/metabolism; CYP11A1/deficiency; Estriol/metabolism; Estrogens/metabolism; Fetus/*enzymology/metabolism; Glucocorticoids/metabolism; Gonadal steroid; Hormones/biosynthesis/*metabolism; Humans; Placenta/*enzymology/metabolism; Pregnancy; Progesterone/metabolism

Résumé

Progesterone, estrogènes, androgènes et glucocorticoides interviennent lors de la grossesse de la nidation à l’accouchement. Leur biosynthèse et leur métabolisme impliquent de voies complexes dues à l’interaction entre le fœtus, le placenta et la mère. L’absence d’expression de quelques enzymes de la stéroïdogenèse comme CYP17 dans le placenta et la zone fœtale des surrenales et la meilleure détermination du début et des variations d’expression d’autres en particulier HSD3B2 lors de la grossesse expliquent la production de ces stéroïdes. De plus, les conséquences de quelques anomalies de leur biosynthèse (en particulier les déficits en aromatase, POR, CYP11A1 et 21-hydroxylase) chez le fœtus et la mère lors de la grossesse a permis d’élucider ces voies métaboliques complexes. Cette meilleure connaissance de la production des hormones stéroïdes associée à leurs dosages dans le plasma et les urines de la mère ou le liquide amniotique, en utilisant des méthodes plus spécifiques comme la LC-MS/MS, peut faciliter la surveillance des grossesses normales et pathologiques. De plus, ces avancées devraient être une base pour évaluer le retentissement des nombreuses pathologies de la grossesse et les conséquences de médicaments et xénobiotiques sur leur métabolisme. © 2016 Elsevier Masson SAS. Tous droits réservés.

Mots clés : Aromatase/déficit ; Androgènes/métabolisme ; CYP11A1/déficit ; Oestriol/métabolisme ; Oestrogènes/métabolisme ; Fœtus/*enzymology/métabolisme ; Glucocorticoides/métabolisme ; Gonadal steroid ; Hormones/biosynthèse/*métabolisme ; Homme ; Placenta/*enzymology/métabolisme ; Grossesse ; Progesterone/métabolisme

* Corresponding author.
E-mail address: yves.morel@chu-lyon.fr (Y. Morel).

http://dx.doi.org/10.1016/j.ando.2016.04.023
0003-4266/© 2016 Elsevier Masson SAS. All rights reserved.
hormones are prevented from entering the fetal compartment. The placenta functions as a hypothalamic-pituitary-end organ-like entity with stimulatory and inhibitory feedback mechanisms to dynamically regulate factors that affect fetal growth and development under a variety of conditions. The fetus and the placenta produce and secrete steroids and peptides into the maternal circulation as well as stimulate maternal hormone production [1,2]. Progesterone, estrogens, androgens and glucocorticoids are involved in pregnancy from implantation to parturition. They are synthesized and metabolized in complex pathways involving the fetus, the placenta and the mother. This review is focusing on the origin and the metabolism of steroids during the pregnancy except their modifications occurring during the preterm labor and delivery.

1. Progesterone

Progesterone is requiring for the maintenance of pregnancy by reducing myometrial contractility. Its maternal plasma concentration increase progressively during the pregnancy to reach a high concentration (in order of 130 ng/ml) in the third trimester (Fig. 1). Its production approximates 250 mg per day.

Following rupture of follicle, capillaries and fibroblasts from the theca proliferate and penetrate the basal lamina. The mural granulosa cells undergo morphologic changes referred as luteinization and secrete progesterone. After implantation, the hCG secreted by trophoblast appear in maternal circulation 9 days after LH peak of the conception cycle and rescue corpus luteum function that otherwise would regress. Hence, this progressive increase of maternal plasma progesterone and estrogens reflects the activity of the corpus luteum of pregnancy. It progressively involutes. The luteal-placental shift occurs between the 6th and 8th week of gestation. Human placental synthesis of progesterone requires two steps. The first step is the conversion of cholesterol to pregnenolone by cytochrome P450scc (CYP11A1) in mitochondria in a reaction requiring electrons delivered via adrenodoxin reductase and adrenodoxin. As the biosynthesis of cholesterol in placenta is very limited, the cholesterol comes essentially from LDL-cholesterol of the mother, but also from the fetus. Some authors suggest that pregnenolone sulfate originating in the adrenal fetal zone should bypass this step and is a key progesterone precursor [3]. Nevertheless, the progesterone levels and miscarriage do not differ in anencephalic fetuses [4,5] and during the pregnancy of mothers having an affected CAH fetus treated by dexamethasone [6]. The delivery of cholesterol to placental CYP11A1 is not dependent of StAR protein, but to MLN64, a placental constitutive protein containing a C-terminal START domain homologous to StAR protein and a number of other lipid transfer protein [7,8].

The second step is the conversion of Pregnenolone to progesterone by type 1 3β-hydroxysteroid dehydrogenase (HSD3B1). This HSD3B1 enzyme is bound to the membrane, but its intracellular localization remains unclear (see reviews [9,10]). Enzymatic activity has been located in microsomes but also in mitochondrial fractions [11,12]. Using immunogold labeling, 3β-HSD immunoreactivity is found in mitochondria and endoplasmic reticulum [13,14]. The 10-fold lower Km values of HSD3B1 for both pregnenolone and DHEA than that of HSD3B2 expressed in adrenals and gonads may facilitate the processing of low concentrations of pregnenolone in the placenta. Based on these data, it seems likely that HSD3B1 located in the inner mitochondrial membrane convert pregnenolone to progesterone and HSD3B1 located in the endoplasmic reticulum convert DHEA to androstenedione (Fig. 2).

The identification of the molecular defects of the first step of the steroidogenesis should confirm the essential role of progesterone to maintain pregnancy until term by suppressing uterine contractility. The majority of molecular defect is due to StAR
mutation. Patients homozygous for null StAR mutations have no miscarriage. All steroids in amniotic fluid of an affected fetus homozygous for a null mutation were low except pregnenolone and progesterone [15]. These reports confirm that the biosynthesis of progesterone in placenta is StAR independent as described above. In contrast, when the patients carry homozygous or compound heterozygous for CYP11A1, a residual activity of CYP11A1 is observed and sufficient to synthesise placental progesterone to drive a term pregnancy [16–26] (Table 1). Hence, patients with CYP11A1 null mutations are rare, born before term and their mothers have histories of early miscarriages [16,19]. Nevertheless, two patients homozygous for p.R232X [19,27] are born at term suggesting that a longer survival of maternal corpus luteum may provide enough progesterone to maintain pregnancy until the term.

2. Estrogens

Estrogens are formed mainly in the placenta using fetal and maternal androgens and diffuse to the maternal and fetal compartment. The production rate increases continuously during pregnancy and reaches 100–120 mg/24 h [28]. The synthesis of estriol (E3), which constitutes 60–70% of the total estrogens, begins at 8 weeks of gestation when the fetal adrenal and liver are functional. The plasma maternal mean values increases from 0.07 to 16 ng/ml at the end of the pregnancy [29] (Fig. 1).

The fetal-placental unit becomes the primary source of estrogen production during pregnancy making ovarian steroidogenesis unnecessary. The fetal liver and placenta play both a major role. First the large amounts of DHEA and DHEA sulfate (DHEA-S) produced by the human fetal adrenal are 16α-hydroxylated by CYP3A7 in the liver [30] and then metabolized sequentially by placental sulfatase, 3β-hydroxysteroid dehydrogenase of type I (HSD3B1), 17-hydroxysteroid oxdoreductase (HSD17B1) and aromatase (CYP19A1) to yield estriol, the principal placental estrogen (Fig. 2) [28]. DHEA could produce estradiol by the same pathway. As the liver lacks HSD3B and CYP17A1 and has abundant aromatase (CYP19A1), only fetal testosterone could be the substrat of the synthesis of estrogens by fetal liver. As the placenta lack P450c17α-hydroxylase/17,20 lyase (CYP17A1) [31], all placental estrogen synthesis depends on uptake of circulating C19 steroids. The majority of estrogens come from the placenta by the umbilical vein to human fetal liver and are either sulfated through the SULT enzymes [32] or inactivated by glucuronidation. Moreover, the human fetal liver secretes very high level of alpha-fetoprotein and SHBG, which acts to bind estrogen in the circulation and limit levels of bioactive estrogen [33]. Hence the fetal liver protects estrogenic exposure of the fetus.

In summary, the origin of plasma estrogens during pregnancy depends essentially of the fetal adrenal. The fetal ovary is quiescent in terms of steroid production. Nevertheless some intracrine and paracrine ovarian secretion should occur because the human fetal ovary expresses the machinery to produce and detect multiple steroid signaling pathways, including estrogenic signaling, with the oocyte acting as a key component [34]. The part of maternal origin is unclear. Maternal estrone and estradiol can cross the placenta readily, whereas maternal DHEA, DHEA-S and testosterone are converted to E1 and E2 by placental enzymes [5]. The physiologic role of estrogens during the pregnancy remains unclear except at the end of pregnancy although the preterm labour and delivery of fetus with aromatase deficiency appear normal.

3. Androgens

During pregnancy, testosterone biosynthesis and his conversion to DHT by SRD5A2 is essential for male sexual development. The biosynthesis of testosterone begins around
Table 1
Outcome of pregnancy in patients with CYP11A1 deficiency.

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutations</th>
<th>Activity</th>
<th>Gestation</th>
<th>Maternal Story</th>
<th>Karyotype</th>
<th>External genitalia</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L14I1W/V415E</td>
<td>38.5%/0%</td>
<td>Term</td>
<td>None</td>
<td>46,XY</td>
<td>Female</td>
<td>[16]</td>
</tr>
<tr>
<td>2</td>
<td>R360W/R405X</td>
<td>30–38%/0%</td>
<td>Term</td>
<td>Threatened miscarriage, treated with progesterone</td>
<td>46,XY</td>
<td>Male</td>
<td>[17]</td>
</tr>
<tr>
<td>3a</td>
<td>R451W+/+</td>
<td>30%</td>
<td>?</td>
<td>?</td>
<td>46,XY</td>
<td>Female</td>
<td>[17]</td>
</tr>
<tr>
<td>3b</td>
<td>R451W+/+</td>
<td>30%</td>
<td>Term</td>
<td>?</td>
<td>46,XY</td>
<td>Male</td>
<td>[26]</td>
</tr>
<tr>
<td>4</td>
<td>R451W+/+</td>
<td>30%</td>
<td>Term (40 weeks)</td>
<td>Consanguineous</td>
<td>46,XY</td>
<td>Male</td>
<td>Personal data</td>
</tr>
<tr>
<td>5–12 (9 patients)</td>
<td>R451W+/+</td>
<td>30%</td>
<td>Term</td>
<td>None</td>
<td>46,XY (n=4)</td>
<td>46,XX (n=5)</td>
<td>[26]</td>
</tr>
<tr>
<td>13</td>
<td>A359V+/+</td>
<td>11.70%</td>
<td>Term (40 weeks)</td>
<td>Consanguineous, 2 miscarriages</td>
<td>46,XY</td>
<td>Female</td>
<td>[21]</td>
</tr>
<tr>
<td>14</td>
<td>A269V+/+</td>
<td>11.00%</td>
<td>Term</td>
<td>Consanguineous</td>
<td>46,XY</td>
<td>Male</td>
<td>Cryptorchidism</td>
</tr>
<tr>
<td>15a</td>
<td>c.835delA/A269V</td>
<td>0%/11%</td>
<td>Term</td>
<td>?</td>
<td>46,XY</td>
<td>Male</td>
<td>[22]</td>
</tr>
<tr>
<td>15b</td>
<td>c.835delA/A269V</td>
<td>0%/11%</td>
<td>Term</td>
<td>?</td>
<td>46,XY</td>
<td>Female</td>
<td>[21]</td>
</tr>
<tr>
<td>16</td>
<td>R353W/A189V</td>
<td>8%/0%*</td>
<td>Term (41 weeks)</td>
<td>None</td>
<td>46,XX</td>
<td>Female</td>
<td>[23]</td>
</tr>
<tr>
<td>17</td>
<td>L222P++</td>
<td>7%</td>
<td>Term</td>
<td>Consanguineous, twin died at 2.5 y</td>
<td>46,XY</td>
<td>Male</td>
<td>Hypospadias, cryptorchidism</td>
</tr>
<tr>
<td>18a</td>
<td>R232X/F215S</td>
<td>0%/3.5%</td>
<td>Term</td>
<td>Consanguineous</td>
<td>46,XY</td>
<td>Female</td>
<td>Small penis</td>
</tr>
<tr>
<td>18b</td>
<td>R232X/F215S</td>
<td>0%/3.5%</td>
<td>Term</td>
<td>Consanguineous</td>
<td>46,XY</td>
<td>Female</td>
<td>ND</td>
</tr>
<tr>
<td>18c</td>
<td>R232X/F215S</td>
<td>0%/3.5%</td>
<td>Term</td>
<td>Consanguineous</td>
<td>46,XY</td>
<td>Male</td>
<td>[19]</td>
</tr>
<tr>
<td>18d</td>
<td>R232X/F215S</td>
<td>0%/3.5%</td>
<td>Term</td>
<td>Consanguineous</td>
<td>46,XX</td>
<td>Female</td>
<td>[19]</td>
</tr>
<tr>
<td>19</td>
<td>271insGD+/−</td>
<td>0%/?</td>
<td>Term</td>
<td>None</td>
<td>46,XY</td>
<td>Female with clitoromegaly</td>
<td>[25]</td>
</tr>
<tr>
<td>20</td>
<td>G138R/L170V/?X30</td>
<td>70%/?</td>
<td>36 weeks</td>
<td>None</td>
<td>46,XX</td>
<td>Female</td>
<td>[20]</td>
</tr>
<tr>
<td>21</td>
<td>c.835delA/TVS3(2-3)insT</td>
<td>0%/0%*</td>
<td>39 weeks</td>
<td>2 miscarriages, low estriol</td>
<td>46,XY</td>
<td>Female</td>
<td>[16]</td>
</tr>
<tr>
<td>22</td>
<td>c.835delA/++</td>
<td>0%</td>
<td>31 weeks</td>
<td>2 miscarriages, low estriol</td>
<td>46,XY</td>
<td>Female</td>
<td>[27]</td>
</tr>
<tr>
<td>23</td>
<td>R232X+/+</td>
<td>0%</td>
<td>Term</td>
<td>Consanguineous, 2 miscarriages</td>
<td>46,XX</td>
<td>Female</td>
<td>[19]</td>
</tr>
<tr>
<td>24</td>
<td>R232X+/+</td>
<td>0%/0%</td>
<td>Term</td>
<td>Consanguineous, 2 miscarriages</td>
<td>46,XY</td>
<td>Female</td>
<td>[19]</td>
</tr>
<tr>
<td>25</td>
<td>R120X++</td>
<td>0%</td>
<td>37 weeks</td>
<td>1 miscarriage</td>
<td>46,XX</td>
<td>Female</td>
<td>[19]</td>
</tr>
</tbody>
</table>

* Deduced from In vitro minigene study, a percentage of normal splicing could not be excluded.
9th WG in Leydig cells and indicates the end of the testis formation [35]. The “classic” biosynthetic pathway from cholesterol to testosterone via DHEA and Δ4-androstenedione in the testis and the subsequent conversion of testosterone to DHT in genital skin is well established. However, recently found mutations in AKR1C2/4 genes in undervirilized 46,XY individuals have established a role for a novel alternative backdoor pathway for fetal testicular DHT synthesis. In this pathway, which has been first elucidated for the tammar wallaby pouch young, 17-hydroxyprogesterone is converted directly to DHT by 5α-reductase without going through the androgens of the classic pathway. Nevertheless the role of this backdoor pathway via the predominant classic one remains to be elucidated (see review [36]).

The disorders of testosterone biosynthesis cause 46,XY DSD but fetuses develop normally, reach term gestation except some cases of CYP11A1 mutations and undergo normal parturition and delivery (see the extensive review [37]). In contrast, disorders of steroid biosynthesis producing fetal androgen excess, aromatase deficiency and some rare luteoma have be useful to a better understanding of the pathophysiology of steroid biosynthesis and metabolism during the pregnancy. Fetal androgen excess due to CYP21A2 and CYP11B1 deficiencies did not virilize the mother because placental aromatase transform androgens to estrogens (Fig. 2). Moreover, the pregnant mother is protected against hyperandrogenism by a high level of SHBG and a high level of progesterone.

Some rare pregnancy luteoma are responsible for virilization of both the fetus and the mother suggesting that in these cases the placenta could not protect totally the fetus against the huge maternal testosterone production [38,39]. The aromatase defect due to CYP19A1 or POR deficiencies remains the best example of pathophysiology to explain the role of placenta to protect maternal and fetus from androgen excess. In these defects, high placental production of testosterone due to conversion of DHEA and DHEA-S produced by adrenal fetal zone cause virilization of external genitalia of female fetus and her mother. Maternal testosterone is elevated and estriol very low in the 2nd and 3rd trimesters of pregnancy (Fig. 2). At birth after delivery, all steroids return to normal values [40,41].

4. Glucocorticoids

The human adrenal cortex was first detected in human embryos by hematoxylin and eosin staining at 33 days post-conception (dpc) with distinction between the definitive and fetal zones possible at 52 dpc. Vascular development was apparent within the adrenal gland at 41 dpc. StAR, CYP11A1, CYP17 and CYP21 were expressed centrally within the fetal zone at 50 dpc and all later time points during the first trimester [42]. Within the outer definitive zone, StAR, CYP11A1, CYP11B1, CYP11B2 immunoreactivity also was weaker visible, but detected by RT-PCR [43]. Early studies having only detected HSD3B2 late in the second trimester were not compatible to early cortisol biosynthesis [44,45]. Although ACTH by immunohistochemistry has been detected at eight week of gestation in anterior pituitary [46], some authors have been suspicious about the presence of negative feedback upon ACTH secretion and the efficiency and the mechanism of dexamethasone treatment giving to pregnant mother to prevent genital virilization of external genitalia of female CAH fetus [47]. Goto et al’s report predicts cortisol biosynthesis in the first trimester, but in a narrow window (8–12 WG) due to the decline of HSD3B2 in the definitive zone [43]. This window of early cortisol biosynthesis corresponds to the critical period of normal androgen-mediated male sexual differentiation and safeguard female fetus susceptible to virilization before the protective appearance of placental aromatase [37,43,48]. The unique 46,XX DSD due homozygous frameshift mutation for glucocorticoid receptor gene supports this hypothesis [49]. Moreover, the critical date of 8.0 WG to begin dexamethasone treatment to prevent virilization of female CAH fetus deduced from accurate datation of pregnancy is agreed with the beginning of the window of early cortisol biosynthesis [50]. Nevertheless, the secretion of ACTH is maintained during the pregnancy and some cortisol biosynthesis should occur because the diagnosis of 21-hydroxylase could be easily done between 12 and 22 WG by the increase of 17OH-progesterone in amniotic fluid [51,52]. Moreover, immunoreactivity for HSD3B2 is present again in some cells of adrenal definitive zone from 12 to 18 WG, then in all almost cells from 19 WG [53].

As mentioned above, the human fetal glucocorticoids are needed at about 8 to 12 WG, but it is not clear that there are needed after. Hence, a newborn presenting on the 1st day of life a severe glucocorticoid resistance due to a homozygous frameshift mutation of glucocorticoid receptor is born at term, with a normal pulmonary function and have no abnormal fetal development suggesting that glucocorticoid action is not requiring for normal human fetal development [54].

5. What steroids to measure during pregnancy?

During several past decades, immunoassays have been the best method to measure steroids during pregnancy [28,55]. Nevertheless, samples had to be extracted and separated by chromatography to improve the sensitivity and the specificity because of risk of cross-reactivity between these steroids and their multiple placental metabolites. In practice, these steroids are measured in maternal plasma and urine and in amniotic fluid, rarely umbilical cord plasma [56]. Recently, GC-MS [57] and LC-MS/MS [58,59] methods become highly reliable and sensitive methods with the advantage over immunoassay to measure multiple steroids at once.

Maternal estriol is only present during pregnancy and a good marker for fetal adrenal and adrenocorticotropic functions, but only in the second and third trimester. Hence, the synthesis begins after 8 WG due to the DHEA-S synthesis by fetal adrenal (fig. 1). Moreover, except in low levels, the efficiency of maternal plasma E3 for routine non-invasive diagnosis is better if two samples are at least spaced over one month because of large inter-individual variations. Low maternal E3 is useful in prenatal diagnosis of aromatase and POR deficiencies [60–62], X-linked steroid sulfatase deficiency [63,64], Smith-Lemli-Opitz [65], adrenal insufficiency [66], defects of the first step of
the steroidogenesis [16], isolated familial adrenocorticotropic (TPIT) deficiency [67], isolated hypopituitarism [68] or associated with septo-optic dysplasia [69]. E3 is also used in maternal triple-marker screening. Moreover, E3 is very useful to evaluate the efficiency of a dexamethasone treatment of female CAH fetus on fetal adrenal [50,51].

Measurement of steroids in amniotic fluids (AF) has been essentially used in the three past decades for prenatal diagnosis of 21-hydroxylase deficiency [55,70]. With the recent advances of ultrasound technology (US) and the widespread use of amniocentesis, prenatal diagnosis of DSD appears more common especially if a mismatch between karyotype and external genitalia detected by US occurs. Recently accurate and specific determination of normal values of steroids by LC-MS/MS has been reported and should be useful to evaluate DSD during this prenata-

6. Conclusion

During these recent years, the elucidation of disorders of the biosynthesis of steroid hormones and the progress in the measurement in different compartments of the fetal-placenta unit and the pregnant mother have largely contributed to a better understanding of the biosynthesis and metabolism of steroid hormones. Further studies should elucidate the regulation of these biosyntheses to explain the complex metabolome. These advances should be a basis to evaluate the impact of multiple metabolites, and inhibition by product steroids. J Steroid Biochem 1988;31:785–93.

Nevertheless non-invasive approach in the maternal urine has been proposed by some authors [60,71].

Disclosure of interest

The authors declare that they have no competing interest.

References


