Review Article

Pituitary-Adrenal Axis During Human Development

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Abstract. Investigation of early human fetal tissue has helped us elucidate the onset of the activation of the pituitary-adrenal axis during human development. Adrenal steroidogenesis and ACTH secretion from the pituitary starts at 7–8 weeks postconception, providing the rationale for prenatal treatment using dexamethasone offered to fetuses at risk of 21-hydroxylase deficiency (21-OHD). Fluctuation of 3beta-hydroxysteroid dehydrogenase (HSD3B2) in human fetal adrenal has several significant meanings. Its activity during early gestation is essential for inhibiting androgen production in the adrenal and safeguarding normal female sexual development. The enzyme may be reduced during mid-gestation in order to maintain pregnancy and to prevent preterm labor. Its reappearance in late gestation is crucial for fetal maturation and parturition at term. Late-onset circulation failure observed in extremely low birth weight newborns may be associated with the paucity of HSD3B2 in their adrenals. In fetuses with 21-OHD, a proportion of increased 17alpha-hydroxyprogesterone may be converted to dihydrotestosterone through the backdoor pathway and contribute to the virilization of female fetuses.

Key words: human fetus, 3beta-hydroxysteroid dehydrogenase, sexual differentiation, fetal adrenal, prenatal treatment

Introduction

Since the first report by David et al. in 1984 (1), prenatal treatment has been given to approximately one thousand fetuses at risk of 21-hydroxylase deficiency (21-OHD) worldwide by administration of dexamethasone (DEX) via their mothers in the first trimester (2–4).

This treatment is aimed at the restoration of negative feedback from glucocorticoid in the pituitary-adrenal (PA) axis of affected fetuses with insufficient cortisol biosynthesis due to impaired steroidogenic enzyme activity and is based upon the hypothesis that the PA axis and its negative feedback from adrenal-derived cortisol is already functioning even in the fetuses in the first trimester, just as in newborns or in adults.

However, direct evidence of the PA axis during the first trimester has been hampered by restricted access to human fetal materials during this period. The available data about the onset of human fetal pituitary and adrenal function has been inconclusive (5–7).

In this review article, a summary of the current knowledge of the PA axis of human fetuses is presented, with a focus on recent findings (8) and unresolved issues.
Development of the Human Fetal Adrenal and Onset of Its Steroidogenic Function

The human adrenal cortex is distinct from the bipotential gonad at 33 days postconception (dpc) (9, 10). By 41 dpc, vessels penetrate the embryonic adrenal (8). Mitotic activity of the fetal adrenal cortex is limited to the outer definitive zone (11), and proliferated cells migrate inward to form the fetal zone (12).

The expression profile of steroidogenic enzymes in human fetal adrenal was well described in a report by Narasaka et al. (5). However, they only examined fetal tissues after 14 weeks postconception (wpc), but not the tissues of the first trimester, the critical period for the differentiation of male or female phenotype. There have been several reports about the expression of type 2 3beta-hydroxysteroid dehydrogenase (HSD3B2) (5–7) in human fetal adrenal. However, the results of many studies were inconclusive about the expression of the enzyme before 24 wpc (5, 6), and none of them examined fetal adrenal glands before 10 wpc.

Nevertheless, in cord blood from human fetuses between 10 and 20 weeks of gestation, the concentration of cortisol was greater in the umbilical artery than in the umbilical vein (13), indicating that the fetal adrenal already produces the steroid during this period.

As an explanation for this inconclusive evidence, the current hypothesis is that fetal adrenal may use the abundant amounts of progesterone existing in the placenta as a potential source and convert it to cortisol (14). This is confirmed in human fetal adrenal after 16 wpc (15). However, as human cytochrome P450 17-hydroxylase (CYP17) rarely converts 17alpha-hydroxyprogesterone (17-OHP) to androstenedione (16) (Fig. 1), this hypothesis cannot solely explain the mechanism of the virilization of the genitalia observed in female fetuses with 21-OHD.

In our experiments using human fetal tissues of ages ranging from 33 dpc to 14 wpc (8), steroid

![Fig. 1 Steroidogenic enzymes in human fetal adrenal. CYP17 rarely converts 17-OHP to androstenedione.](image-url)
acute regulatory protein (StAR), P450 side-chain cleaving enzyme (CYP11A), cytochrome P450 17-hydroxylase (CYP17), steroid 21-hydroxylase (CYP21), and 11beta-hydroxylase (CYP11B1)/aldosterone synthase (CYP11B2) were detected after 50–52 dpc within the inner fetal zone by immunohistochemistry. HSD3B2, the enzyme that used to be doubted to exist in fetal adrenal of early gestation, was also detected at 50-52 dpc within the outer definitive zone. However, it showed transient expression peaking at 8–9 wpc, decreasing thereafter, and disappearing by 14 wpc (8).

It is known that steroidogenic enzymes such as CYP11A, CYP17, CYP21, and CYP11B are transcriptionally regulated by ACTH (17). Steroidogenic factor 1 also takes part in regulating these steroidogenic enzymes (18). In contrast, as HSD3B2 shows a different distribution and transient presence, it may be regulated by some unique mechanisms.

Recently, HSD3B2 was determined to be regulated by the orphan nuclear receptor named nerve growth factor-induced clone B (NGFI-B) during late gestation (19). Our experiments have revealed remarkably similar expression profiles of NGFI-B and HSD3B2 even in the first trimester (8). Both of them were absent by 14 wpc (8), irrespective of constant secretion of ACTH from the pituitary, indicating that NGFI-B may regulate HSD3B2, even in the first trimester, and that this regulation may be independent of ACTH. However, the question still remains of what determines the expression profile of NGFI-B.

We found that human fetal adrenal expressed all the steroidogenic enzymes needed for production of cortisol or testosterone by 50–52 dpc and melanocortin 2 receptor (MC2R) by 8 wpc, and also that these steroids are secreted from the cultured fetal adrenal after 8 wpc and are increased by the stimulation of ACTH (8).

Development of the Human Fetal Pituitary and Onset of Its Function

In an attempt to prevent virilization of female fetuses with 21-OHD, prenatal treatment has been offered to mothers who have previously given birth to a child with the disease, by administration of DEX in the first trimester (1–4). To prevent the virilization effectively, it is recommended that DEX be administered at 6–7 wpc (20, 21).

The pituitary is shaped from the fusion of Rathke’s pouch derived from oral ectoderm and infundibulum derived from diencephalons. However, the fusion does not take place before 6 wpc (22) and it is not clear when the fetal pituitary begins its regulatory function. Although it is confirmed that the fetal pituitary regulates adrenal steroidogenesis by secreting ACTH after 12 wpc (14), the evidence of its function in early gestation has been hampered by restricted access to human fetal material. Although immunoreactivity of ACTH in fetal pituitary at 7 wpc has been reported (23), it has never been correlated to adrenal function. Therefore, the function of the fetal PA axis at this stage has been hypothetical.

We found that LIM homeobox gene 3 (LHX3), which is known to be specifically expressed in pituitary of fetal mice (24), is already expressed in human fetal pituitary as early as 41 dpc, but that ACTH is still absent at the stage (data not shown). However, cytoplasmic expression of ACTH and nuclear expression of glucocorticoid receptor (GR) were detected by 50–52 dpc, and the distribution of their positive cells was overlapping (8).

We also found that ACTH was already being secreted from a cultured fetal pituitary after 8 wpc and that the secretion was suppressed by the addition of DEX (8).
Fetal PA Axis in Early Gestation and the Adequacy of Prenatal Treatment

As mentioned above, the human fetal adrenal commences expression of steroidogenic enzymes around 7 wpc and secretes cortisol by 8 wpc, and the secretion is increased by the stimulation of ACTH. The fetal pituitary also begins expression of ACTH and GR around 7 wpc and secretes ACTH by 8 wpc, and the secretion is suppressed by the addition of DEX. This evidence suggests that the human fetal PA axis is already functioning at 7–8 wpc and provides the rationale for prenatal treatment offered to fetuses at risk of 21-OHD in early gestation.

It is recommended that DEX be administered at 6–7 wpc to prevent virilization (20, 21). However, it is only after 10 wpc that prenatal diagnosis through the analysis of DNA from a chorionic villous biopsy is available. Therefore, to perform effective treatment to only 1 affected female fetus with 21-OHD, theoretically 7 fetuses have to receive needless treatment for 3-4 weeks until the diagnosis is confirmed, and the adequacy of this treatment has been controversial (20, 21). Since there are concerns that antenatal exposure to glucocorticoids may be related to reduced birth weight (25) and an increased risk of cardiovascular and metabolic disorders in later life (26, 27), this treatment needs further assessment.

Transient Appearance of HSD3B2 and Normal Female Sexual Development

Integrating our data (8) with those of others (5–7), HSD3B2 is considered to appear in fetal adrenal around 7 wpc, disappear by 14 wpc, and reappear around 24 wpc (Fig. 2). This unique fluctuation of HSD3B2, which is not observed with other steroidogenic enzymes, suggests novel functioning of the early adrenal.

Human fetal adrenals biosynthesize and secrete androgens such as androstenedione and testosterone by 8 wpc, and they can be increased by the stimulation of ACTH (8). Nevertheless, as the activity of the placental aromatase is still absent during this period (28), androgens derived from the fetal adrenal cannot be converted to estrogens. Consequently, normal female differentiation of genitalia is vulnerable to androgens derived from the adrenal and is easily virilized.

| 5 wpc | 10 wpc | 15 wpc | 20 wpc | 25 wpc |
|-------|--------|--------|--------|--------|
| Mesiano et al (1993) | | 17 | 24 | |
| Parker et al (1995) | 11 | 15 | | 24 | 41 |
| Narasaka et al (2001) | | 14 | | 23 | 41 |
| Our results (2006) | 6 | 7 | 11 | 14 |
| Integration of the data above | 7 | | | 24 |

Fig. 2 Expression profiles of HSD3B2 in human fetal adrenal. The bold lines and the dotted lines indicate observed presence and absence of the enzyme, respectively, in each report.
The transient appearance of HSD3B2 in fetal adrenal around 7 wpc may inhibit adrenal androgen biosynthesis and safeguard normal female sexual differentiation, first by competing with CYP17 for precursors such as pregnenolone and 17-hydroxypregnenolone (29), and second by increasing cortisol biosynthesis and inhibiting ACTH production from pituitary corticotroph via its negative feedback (Fig. 3).

In support of this model, Mendonca et al. found phenotypes of female pseudohermaphroditism characterized by enlarged clitoris, labial fusion, and a urogenital sinus in a case with homozygous inactivating mutation of the GR gene (30). The patient showed elevated ACTH and androgen secretion. Although the patient also carried one disrupted allele of the CYP21 gene, this would be insufficient by itself to generate an intersex phenotype because 21-OHD is inherited in an autosomal recessive manner. Therefore, the GR mutation was proposed as the major cause of genital ambiguity in this case.

The phenotype of this case is evidence that disrupted negative feedback caused by GR inactivation can solely cause female pseudohermaphroditism by increasing ACTH and androgen secretion. Therefore, activating negative feedback on the pituitary by the transient appearance of HSD3B2 and cortisol production in the adrenal of a normal early fetus may play a crucial role in female sexual development.

**Fluctuation of HSD3B2 and Maintenance of Pregnancy**

HSD3B2 appears in fetal adrenal during early gestation and decreases during mid-gestation (Fig. 2). This leads to the reduction of cortisol biosynthesis.

It is known that an increase in fetal plasma cortisol is essential for parturition at term (31). Inversely, cortisol deficiency is correlated to prolongation of pregnancy seen in fetuses with 21-OHD (32). Therefore, suppressed cortisol secretion from fetal adrenal during mid-gestation may prevent preterm labor.

As reduced activity of HSD3B2 during mid-gestation hinders pregnenolone and 17-hydroxypregnenolone from entering the delta 4 pathway, these substrates are apt to be converted by CYP17 and the production of dehydroepiandrosterone sulfate (DHEA-S) increases exponentially (33).

Abundant DHEA-S produced in the fetal adrenal during mid-gestation is carried to the placenta and converted to estrogens such as estradiol and estriol by placental aromatase. Thus, maternal plasma concentration of estrogens increases abruptly during mid-gestation (34). These estrogens have been used as markers of fetal well-being and their function has not been clarified.
completely. However, the series of reactions during mid-gestation, starting with the fall of HSD3B2 activity in fetal adrenal and resulting in increased production of fetal DHEAS and maternal estrogens, may play a significant role in maintaining pregnancy.

The absence of HSD3B2 in fetal adrenal during mid-gestation may not completely disconnect the PA axis, as ACTH still regulates transcription of other adrenal steroidogenic enzymes including CYP17, CYP21, and CYP11B, all of which are essential for conversion of placenta-derived progesterone to cortisol, and the produced cortisol may inhibit ACTH production from the pituitary.

**Association with Glucocorticoid-Responsive Hypotension in Extremely Low Birth Weight Newborns**

Since the report by Helbock (35), extremely low birth weight (<1000 g) infants (ELBWI) suffering from late-onset circulation failure characterized by glucocorticoid-responsive hypotension, hyponatremia, and hypouria have been observed at various institutions. This may be equivalent to what Ng et al. call transient adrenocortical insufficiency of prematurity in very low birth weight (< 1500 g) infants (36). These babies may have adrenal insufficiency and fail to produce adequate amounts of cortisol, concordant with their stress.

Advancement in neonatology has enabled us to rescue preterm newborns even at 22 weeks of gestation, which is equivalent to 20 wpc. However, HSD3B2 is not supposed to reappear in the adrenal of newborns of this age (Fig. 2). If they were in the uterus, fetuses of this age could use progesterone derived from the placenta and biosynthesize cortisol (15). However, as preterm newborns are dissected from the placenta and deprived of the progesterone supply, cortisol production in their adrenals may be hampered and this may result in adrenal insufficiency. The question of why they do not suffer from adrenal insufficiency until around 1 week of age remains unanswered. The reason may be associated with the fact that plasma concentration of some steroid precursors increases for some period after delivery in preterm newborns, while they fall soon after delivery in term infants (37). A slower steroid clearance in immature liver or kidneys of ELBWI may be one reason for the late-onset symptoms of glucocorticoid insufficiency.

**The Importance of the Backdoor Pathway in Fetal Adrenal**

The backdoor pathway, which converts 17-OHP to dihydrotestosterone (DHT) not via a testosterone-dependent route, but via substrates such as 5alpha-pregnane-3alpha, 17alpha-diol-20-one and androsterone, has recently been demonstrated in marsupials (38, 39) and rodents (40).

As urine steroid profile analysis in patients with cytochrome P450 oxidoreductase deficiency shows increases in the substrates derived from the backdoor pathway, the presence of the backdoor pathway is also suggested in humans (41). As 17-OHP is abnormally increased in adrenals of fetuses with 21-OHD, a proportion of the substrate may be converted to DHT through the backdoor pathway, and it may contribute to the virilization of external genitalia observed in patients with 21-OHD. Further studies are needed to confirm this hypothesis.

**Conclusions**

In conclusion, the evidence of the early onset of the PA axis provides the rationale for prenatal treatment using DEX offered to fetuses at risk of 21-OHD. Fluctuation of HSD3B2 in human fetal adrenal may be crucial for sex differentiation, maintenance of pregnancy, and parturition at term.

Further studies are needed to elucidate the etiology of late-onset circulation failure observed in ELBWI and the role of the backdoor pathway in the virilization of fetuses with 21-OHD.
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