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Prenatal Diagnosis of P450 Oxidoreductase Deficiency (ORD): A Disorder Causing Low Pregnancy Estriol, Maternal and Fetal Virilization, and the Antley–Bixler Syndrome Phenotype

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We report studies on the second pregnancy of a woman who had previously given birth to a virilized female infant. The cause of the virilization had not been established, but common forms of congenital adrenal hyperplasia (CAH) were excluded. Longitudinal monitoring of the second pregnancy revealed that estriol excretion failed to increase normally, reaching a maximum 0.7 mg/24 hr at the end of pregnancy (normal mean 30 mg/24 hr). The mother showed signs of virilization by the 23rd week of gestation and aromatase deficiency was suspected. However, predicted urinary metabolites for diagnosis of aromatase deficiency (for example, 16α-hydroxyandrosterone) were not increased significantly during the pregnancy. Interestingly, excretion of the androgen metabolite androsterone increased rapidly at the beginning of pregnancy and peaked around the 20th week, suggesting increased production of testosterone and 5αDHT, probably the cause of maternal virilization. Urine steroid analysis by GC/MS showed gradually increasing excretion (9 mg/24 hr) of the normally minor metabolite 5α-pregnane-3β,20α-diol (epiallopregnanediol), an epimer of the dominant progesterone metabolite pregnanediol (5β-pregnane-3α,20α-diol). We believe epiallopregnanediol is largely the maternal urinary excretion product of fetal 5-pregnene-3β,20α-diol, the principal metabolite of pregnenolone, implying a build-up of the latter steroid in the fetal adrenal. These findings suggested that the 'block' in the estriol biosynthetic pathway occurs at an early stage with 17-hydroxylation of pregnenolone being affected. The male baby born of this pregnancy had normal genitalia but showed a urinary steroid profile indicating partial deficiencies of P450c17 and P450c21. However, no mutations in the corresponding *CYP17* and *CYP21* genes were identified. Urinary steroid analysis carried out on his virilized older sibling showed the same pattern of metabolites. Recently, we determined that this disorder is caused by mutations in P450 oxidoreductase (OR), the essential redox partner for CYP17 and CYP21 hydroxylases. The novel metabolic profile has now been seen in many patients, most diagnosed with the skeletal dysplasia Antley–Bixler syndrome. We propose that excessive excretion of epiallopregnanediol together with low estriol may be prenatally diagnostic for OR deficiency (ORD).

KEY WORDS: pregnancy estriol; apparent pregnene hydroxylation deficiency; genital ambiguity; Antley–Bixler syndrome; maternal virilization; luteoma; oxidoreductase deficiency

INTRODUCTION

A focus of our research has been the diagnosis of conditions that result in low estriol synthesis during pregnancy. Follow-up of these cases has become important because of the increasing use of triple-marker-screening for detection of Down syndrome and neural tube defects [Wald and Kennard, 1992]. Unconjugated serum estriol is one of the markers utilized in this test. In the US, more than two million of these tests are performed annually, and a small fraction of these give a low serum estriol value indicating a steroidogenic disorder, likely a result of enzyme inactivity. Disorders causing diminished estriol synthesis can vary in severity from benign to fatal, and can result in multiple anomalies and mental retardation, so establishing the cause is important. We attempt diagnosis of low-estriol conditions by GC/MS analysis of maternal urinary steroids with the rationale that this medium contains metabolites of all steroids that are synthesized in the fetus. Using this technology, we have described the diagnosis of steroid sulfatase deficiency (STS) [Glass et al., 1998], fetal adrenal hypoplasia [Marshall et al., 2003], and 7-dehydrosterol-7-reductase deficiency (DHCR7 deficiency, the Smith–Lemli–Opitz syndrome) [Shackleton et al., 2001].

This study describes the steroid excretion of a pregnant woman who had previously given birth to a virilized female infant. Establishment of the cause of the virilization was not made on that child, but classical forms of congenital adrenal hyperplasia (CAH) were ruled out. When the woman became pregnant again the pregnancy was closely monitored because of the possibility of another baby being born with genital ambiguity. Early in this second pregnancy, it was noted that

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estriol failed to rise appropriately with increasing gestational age and, in addition, by midpregnancy there were indications of maternal virilization. Two conditions are known to result in androgen excess, placental aromatase deficiency [Shozu et al., 1991; Mullis et al., 1997], and pregnancy luteoma [Jenkins et al., 1968; Polansky et al., 1975; Hensleigh and Woodruff, 1978; Schmitt et al., 1990; Illingworth et al., 1992; Warmann et al., 2000; Mazza et al., 2002]. Our studies presented here showed that neither of these conditions existed in this patient and that mutations in P450 oxidoreductase (OR), the redox partner for 17-hydroxylase/17,20-lyase was responsible for the low estriol production [Arlt et al., 2004]. Prior to proving the genetic cause of the disorder we termed it apparent pregnene hydroxylation deficiency (APHD) [Shackleton and Malunowicz, 2003; Shackleton et al., 2004; Adachi et al., 2004a]. APHD is frequently associated with the skeletal dysplasia Antley–Bixler syndrome.

In this communication we suggest prenatal diagnostic criteria for OR deficiency (ORD) and present hypotheses as to the origin of the maternal and fetal virilization.

SUBJECTS AND METHODS

Patients Studied

The patients described were born to parents of German origin. The first pregnancy of the 31-year-old woman resulted in the birth of a girl (46, XX) with fused labia majora and moderate clitoral enlargement. Since CAH due to 21- and 11β-hydroxylase defects are well known causes of this disorder, these conditions were suspected, but gene analysis failed to confirm the presence of either disorder. Fractionated plasma steroids were analyzed by the method of Sippell et al. [1980] and apart from mildly elevated progesterone, all steroids were within normal range. Gas chromatographic analysis of urinary steroids (Prof. J.Homoki, Ulm) showed elevated excretion of corticosterone metabolites. Virilization did not progress in the infant after birth suggesting a transient, possibly maternal cause. Maternal virilization was not noted during this pregnancy and luteoma of pregnancy was not suspected. The patient had craniofacial and skeletal abnormalities consistent with Antley–Bixler syndrome.

The second pregnancy, and the subject of this article, resulted in the delivery of a healthy boy (46, XY). By the 23rd week of pregnancy the mother was showing signs of virilization such as acne and increased body hair. Ultrasound indicated that the fetus was male and had no apparent abnormalities. Urine samples were collected at the 7th, 20th, 23rd, 33rd, and 38th weeks of gestation. A control (nonpregnancy) sample was collected 10 weeks after birth of the child. The infant had an elevated 17-hydroxyprogesterone as determined by newborn screening, but this returned to normal by 10 weeks after birth. Urine samples from the male child and his female sibling were analyzed.

Molecular studies have recently demonstrated that the infants were compound heterozygous for Y178D and C566Y mutations in P450 OR [Arlt et al., 2004].

Urinary Steroid Analysis

Urinary steroids were analyzed by a GC/MS selected-ion-monitoring (SIM) quantitative method [Shackleton, 1993; Caulfield et al., 2002]. The final analytical samples are the methyloxime-trimethylsilyl (MO-TMS) derivatives of steroids released from conjugation. While multiple steroids were quantified by SIM, excretions of only select ones are reported here. For the maternal samples, these include the androgen metabolites androsterone and etiocholanolone, their 16α-hydroxy metabolites, estriol, pregnanediol (PD), 17α-hydroxypregnanolone (17HP and its 5α-epimer), and pregnanetriol (PT). The urine sample derivatives were also analyzed by repetitive scanning, a technique that, unlike SIM, identifies all steroids excreted. A striking feature apparent in the scanned data was the presence of a prominent steroid metabolite eluting early in the chromatogram before androsterone. Exhaustive studies on this component showed it to be (17E)-5α-pregn-17(20)-en-3β-ol (epiallopregnenol). We established that this steroid is formed from epiallopregnanediol-20-sulfate (5α-pregnane-3β,20α-diol-20-sulfate) by desulfation and dehydration during the derivitization process (Fig. 1). Epiallopregnanediol-20-sulfate arises from the enzymatic hydrolysis (*Helix pomatia* β-glucuronidase and sulfatase) of urinary epiallopregnanediol-3,20-disulfate. Sulfate esters of the 20-hydroxyl of steroids are resistant to enzymatic hydrolysis so are still present at the time of derivatization. We believe excreted epiallopregnanediol has two origins, the disulfate conjugate probably being of fetal origin while a glucuronidated or 3-monosulfate form is likely a both a fetal metabolite and a placental progesterone metabolite. Epiallopregnanediol proved to be the key steroid defining the condition described here, so quantitation was required. We separately measured the excretions of the two forms; epiallopregnanediol (from the 3-glucuronide) and the epiallopregnenol artifact formed from epiallopregnanediol disulfate.

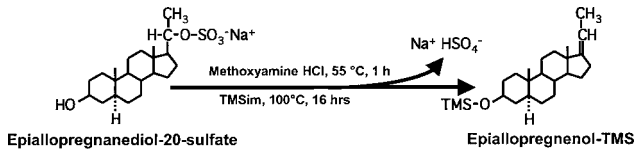


Fig. 1. The formation of epiallopregnenol artifact from epiallopregnanediol-20-sulfate during trimethylsilylation.

For the infant urine samples the analytes measured were three cortisol metabolites (tetrahydrocortisone, THE; tetrahydrocortisol, THF, and 5α-tetrahydrocortisol, 5α THF), four corticosterone metabolites (tetrahydro-11-dehydrocorticosterone, THA; tetrahydrocorticosterone, THB, and their 5α-reduced epimers), Other steroids quantified were 17HP, PT, pregnanetriolone (PTONE), PD, 5-pregnene-3β,20α-diol (5PD), androsterone (An), and etiocholanolone (Et).

RESULTS AND DISCUSSION

Steroid Excretion of the two Infants

Pregnancy estriol is almost exclusively a product of feto-placental steroid synthesis. The primary synthesis occurring in the fetal adrenal and liver, with terminal steps carried out by the placenta. Thus, the importance of defining the steroid biosynthetic ability of an infant born of a low-estriol pregnancy is a vital aid in interpreting maternal urinary steroid data.

The excretion of steroids by the two infants was determined on more than one occasion, and the results for the male and female siblings at age 1 year and 5 months, and 3 years and 6 months, respectively, are given in Table I. The steroid values obtained for normal 2–5-year-old children are also given (mean and range). Notable in the urinary steroid data are elevated excretions of the pregnenolone metabolite pregnenediol (5-pregnene-3β,20α-diol), PD (the progesterone metabolite) as well as the diagnostic analytes for 21-hydroxylase deficiency (PT, 17HP, and PTONE) and 17-hydroxylase deficiency (THA, 5αTHA, THB, and 5αTHB). The excretion of three of the major cortisol metabolites (THE, THF, and 5αTHF) was normal.

The elevated production of the "precursor" steroid metabolites is most easily recognized by relating their quantitative excretion to the excretion of major metabolites of cortisol [Shackleton et al., 2003], a form of "precursor/product" ratio. These values are also reported in the table.

TABLE I. Steroid Excretion of Infants (μg/24 hr)

	Infant 2	Infant 1	Normal 2–5 years (n = 9)	
	XY	XX	XX + XY	
Karyotype				
Age	17 months	3 years 6 months	Mean	Range
Steroid excretion				
Androsterone (An)	13	25	19	2–38
Etiocholanolone (Et)	6.3	17	17	2–38
Pregnanediol (PD)	221	1,274	23	6–28
Pregnanetriol (PT)	183	993	30	7–39
17-hydroxypregnanolone (17HP)	83	352	9	2–19
Pregnanetriolone (PTONE)	71	392	6	2–12
Pregnenediol (5PD)	340	964	74	2–20
THA	136	606	37	18–100
5α-THA	158	410	43	12–76
THB	33	110	19	7–34
5α-THB	444	944	122	18–199
THE	522	1,841	602	163–1,094
THF	60	172	239	102–430
5α-THF	215	286	354	111–557
Diagnostic ratios				
(An + Et)/(PT + 17HP)	0.073	0.031	0.900	0.6–1.0
Bs/Fs	0.967	0.900	0.184	0.11–0.45
17HP + PT/Fs	0.334	0.585	0.033	0.006–0.08
PTONE/Fs	0.089	0.171	0.005	0.002–0.02
PD/Fs	0.277	0.554	0.019	0.006–0.04
5PD/Fs	0.427	0.419	0.013	0.004–0.03

Bs, cumulative excretion of four corticosterone metabolites; Fs, cumulative excretion of three cortisol metabolites.

The steroid excretions and the diagnostic ratio results were distinctive for a condition we termed APHD [Shackleton and Malunowicz, 2003; Shackleton et al., 2003]. While this disorder exhibits attenuated 21- and 17-hydroxylation (particularly 17,20-lyase activity), we now know this is secondary to ORD [Arlt et al., 2004].

Maternal Steroid Excretion

Quantitative excretions of selected steroids by the mother are listed in Table II. Table II and Figure 2A report the PD and estriol excretions in comparison to those of normal pregnancies. While PD was normal, estriol excretion failed to increase significantly as pregnancy proceeded, suggesting a deficiency within the biosynthetic pathway. If estriol production is low (in the absence of fetal adrenal hypoplasia), a prominent replacement should be present in maternal urine, the identity of which is dependent on the particular biosynthetic block.

Initially we suspected aromatase deficiency was the cause of low estriol because of the fetal and maternal virilization observed [Shozu et al., 1991; Mullis et al., 1997]. If the patient had P450 aro deficiency, metabolites of 16α-hydroxyandrostenedione, the steroid utilized by the aromatase enzyme, would be expected to be in excess. Excretion of the predicted metabolites of 16α-hydroxyandrostenedione (16α-hydroxyandrosterone and 16α-hydroxyetiocholanolone) only slightly increased during this pregnancy, arguing against deficient aromatase as a cause of low estriol. If attenuated fetal hepatic 16α-hydroxylation was contributory to low estriol then 16-deoxy estrogen excretion should be elevated. There was no significant increase in estrone or estradiol excretion during the pregnancy.

However, significant amounts of an abnormal component eluting before androsterone was found in the GC/MS profile. This was identified as epiallopregnenol, which was determined to be a methodological artifact formed from epiallopregnanediol disulfate. We suspected that this was the primary estriol substitute. Epiallopegnanediol disulfate is not a metabolite of placental progesterone. Figure 2B illustrates the excretion of epiallopregnenol and total epiallopregnanediol (representing epiallopregnanediol 3-monosulfate or glucuronide and epiallopregnanediol disulfate). While the excretion of both forms increased during the course of the pregnancy, only the elevated excretion of epiallopregnenol seemed to be exclusive to this pregnancy, a significant excretion of monoconjugated epiallopregnanediol being found in normal pregnancies. We report for normal pregnancies the excretion of epiallopregnenol and total epiallopregnanediol at the 19th week of gestation (Table II), a period when we often attempt prenatal diagnosis of low-estriol disorders by steroid analysis.

The most likely fetal precursor of epiallopregnanediol disulfate was pregnenediol disulfate (5-pregnene-3β,20α-diol disulfate), the sulfate ester of the 20α-reduced metabolite of pregnenolone. A high production of fetal pregnenolone would be indicative of attenuated 17- and 21-hydroxylation, reinforcing our diagnosis previously based on the steroid profile of the affected infants. For pregnenediol disulfate to be converted to the epiallopregnanediol disulfate we find in maternal urine, reduction of the Δ^5 double bond to yield a 5α hydrogen is required, but how or where this occurs is yet unknown. One possibility is that placental sulfatase first removes the 3β-hydroxy sulfate of pregnenediol disulfate (the 20-sulfate being resistant to cleavage), and the resulting pregnenediol 20-sulfate is converted to 20α-dihydroprogesterone-20-sulfate by 3βHSD. When it passes to the maternal compartment, the latter compound is probably reduced "3β,5α" prior to re-sulfation of the 3β-hydroxyl and urinary excretion of the di-conjugate. Total epiallopregnanediol in urine is probably not solely a fetal product; monoconjugated epiallopregnanediol is likely to be at least in part a minor metabolite of placental progesterone.

TABLE II. Excretion of Selected Steroids Throughout Gestation (μg/24 hr)

Gestational age (weeks)	Concentration							Ratio	Concentration		Ratio
	PD	Estriol	16-OH-An	16-OH-Et	Epiallo pregnenol	EpialloPD	An	Et	An Et	17HP	3α,5α17HP	17HP3α5α 17HP
7	12,825	24 (80)[a]	119	59	179	413	7,068	5,134	1.38	1,670	185	0.11
20	17,256	220 (4,500)	1,118	306	922 (48; 2-143)[c]	1,000 (272; 69-826)[c]	7,684	1,781	4.31	1,805	561	0.31
23	22,181	304 (7,500)	1,647	440	1,930	1,171	10,727	2,339	4.59	2,493	776	0.31
33	41,954	743 (20,000)	1,138	454	3,747	1,995	5,424	1,231	4.41	2,196	609	0.28
38	35,113	733 (31,000)	1,509	540	5,593	4,108	5,593	1,178	4.75	2,814	938	0.33
Control[b]	1,309	10	74.1	41.8	40	44	4,167	4,137	1.01	180	16	0.09

PD, pregnanediol; 16-OH-An, 16-hydroxyandrosterone; 16-OH-Et, 16-hydroxyetiocholanolone; EpialloPD, 5α-pregane-3β,20α-diol; An, androsterone; Et, etiocholanolone; 17HP, 5β-pregnane-3α,17α-diol-20-one; 3α,5α17HP, 5α-pregnane-3α,17α-diol-20-one.

[a]Normal values for estriol excretion in brackets. From Brown [1960]

[b]Control. This sample of urine was collected 10 weeks after birth of the children.

[c]Mean and range for normal pregnancies (n = 13) at 19 weeks.

Two features of the maternal urinary profile indicated an increased relative production of 5α-reduced compounds, a significant finding in view of the pregnancy being hyperandrogenic. There was a marked increased excretion of androsterone at mid-pregnancy (Fig. 2C) and in addition, the ratio of androsterone to etiocholanolone (its 5β epimer) was significantly elevated compared to nonpregnancy values. This was not solely due to increased production of androsterone but also a decreased etiocholanolone excretion relative to nonpregnancy values. In one normal pregnancy, we were able to study there was no increased excretion of androsterone relative to etiocholanolone, the ratio of the two remaining constant (Fig. 2C).

Finally, a precursor of androsterone, 17α-hydroxyallopregnanolone (5α-pregnane-3α,17α-diol-20-one), had increased excretion during gestation, particularly relative to its more prominent 5β-epimer (Fig. 2D). In the normal pregnancy studied, the 5α/5β ratios of these 17HP were low and constant.

The Origin of low Estriol

The biochemical results from the mother and infants were appropriate to a diagnosis of APHD [Shackleton and Malunowicz, 2003; Shackleton et al., 2003], the low-estriol primarily resulting from attenuated 17,20-lyase activity preventing the conversion of 17α-hydroxypregnenolone (and/or sulfate ester) to DHEA (and/or sulfate ester) in the fetal adrenal (Fig. 3A). DHEA sulfate is the first C_{19} steroid on the biosynthetic pathway leading to estriol.

After completion of the steroid investigations molecular studies confirmed that the disorder in these patients was a result of disabling mutations in OR, the essential redox partner for 17-hydroxylase/17,20-lyase [Arlt et al., 2004].

While attenuated side-chain cleavage was the primary cause of low-estriol, reduced activity of fetal hepatic 16α-hydroxylase and placental aromatase, which also utilise OR, are likely contributory factors.

Recently, another pregnancy has been reported where estriol was undetectable at triple marker screening and, following birth, it was noted that the baby had ABS phenotype [Cragun et al., 2004].

The Cause of Virilization

The finding of elevated excretion of the primary androgen metabolite androsterone in early gestation, peaking at midterm was strong evidence of increased androgen production. A condition that has repeatedly been reported to cause hyperandrogenemia is luteoma of pregnancy [Jenkins et al., 1968; Polansky et al., 1975; Hensleigh and Woodruff, 1978; Schmitt et al., 1990; Illingworth et al., 1992; Warmann et al., 2000; Mazza et al., 2002]. One study in particular [Begue et al., 1977] reports elevated urinary androgen metabolite excretion in a pregnancy with luteoma, and in similar fashion to our case, a disproportionate production of androsterone (a 5α steroid) relative to etiocholanolone (a 5β steroid). This latter observation probably indicates increased production of the active androgen 5α-DHT. In our case, the excretion of androsterone doubled while etiocholanolone halved compared to the nonpregnant state. In a report of Antley–Bixler syndrome, associated with a clinical presentation similar or identical to ours, pregnancy luteoma was found [Warmann et al., 2000; Roth et al., 2000]. However, no luteoma was detected in our patient. Attenuated fetal hepatic 16α-hydroxylation (in the pathway leading to 16α-hydroxy DHEA sulfate) secondary to ORD could also contribute to increased placental androstenedione and maternal excretion of the metabolite androsterone.

Whatever the androgen source, it is necessary to rationalize the fetal virilization seen in the disorder. Placental

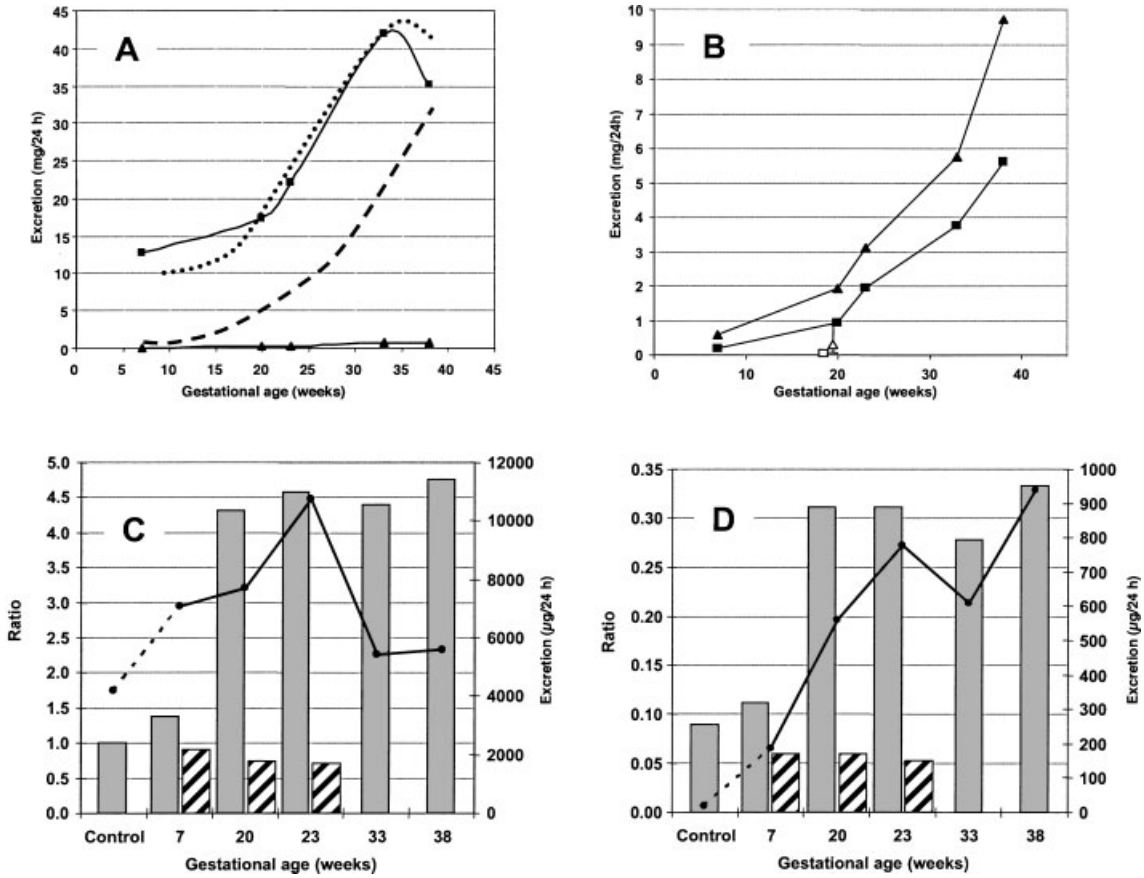
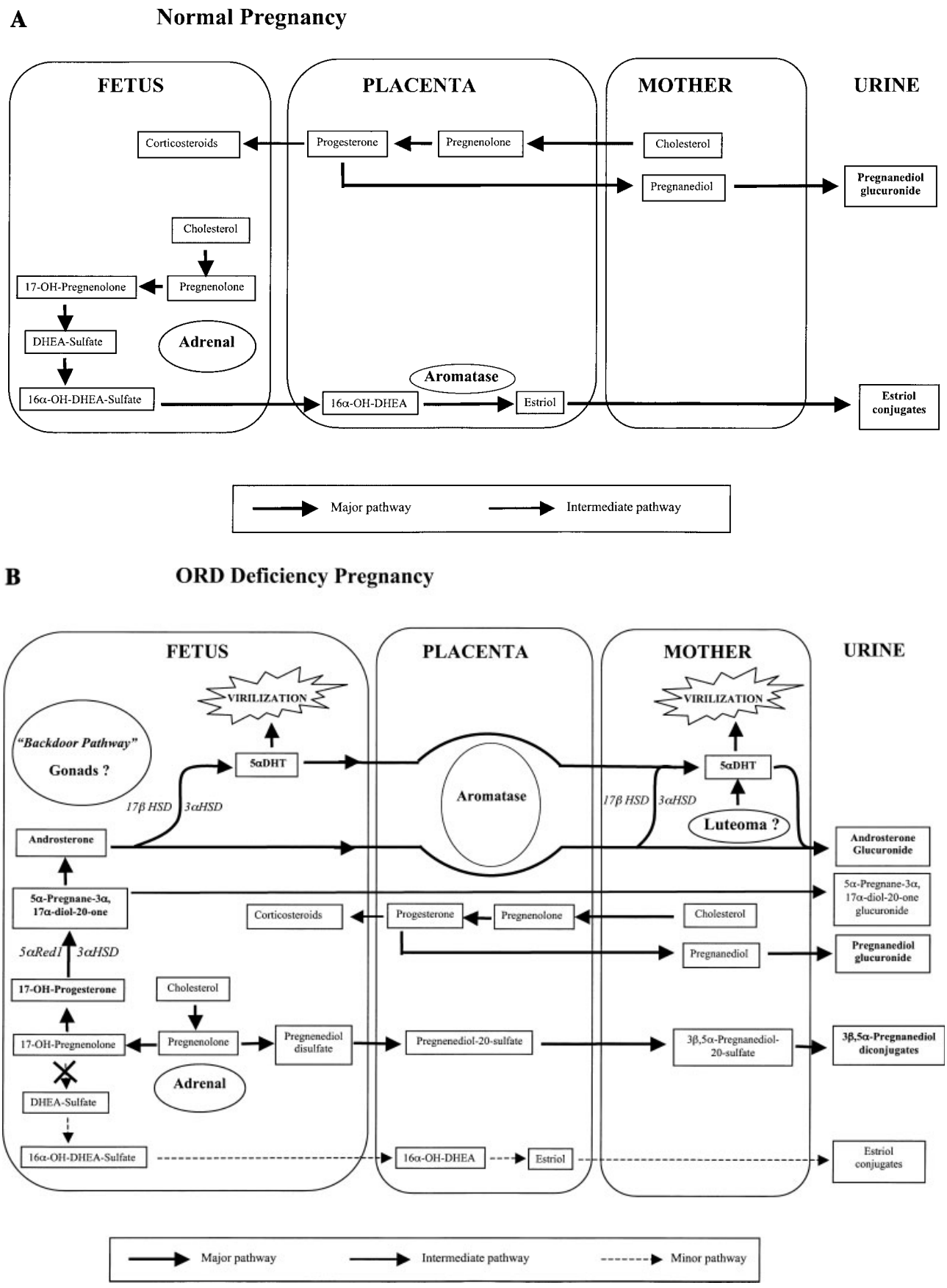


Fig. 2. Steroid interrelationships during pregnancy. **A**: shows the pregnanediol (PD, solid squares) and estriol (solid triangles) excretion of the mother with ORD affected fetus in comparison to mean normal values reported by Brown [1960] for estriol (dashed line) and James [1972] for PD (dotted line). This clearly illustrates the deficient estriol synthesis in the affected pregnancy. **B**: illustrates the increasing excretions of epiallopregnenol (the artifact from epiallopregnanediol disulfate, solid squares) and total epiallopregnanediol (solid triangles). The mean values (and range) for normal pregnancies at 19 weeks are also shown (open symbols, values given in Table II). **C**: shows the elevation in androsterone excretion during gestation (solid circles and lines), and also the increased androsterone/etiocholanolone ratio (grey histograms) Ratio values for one normal pregnancy obtained on the 10th, 20th, and 23rd weeks of gestation are also given (hatched histograms). **D**: shows the increased excretion of the purported androsterone precursor 5α-pregnane-3α,17α-diol-20-one (3α,5α17HP) and the 3α,5α17HP/17HP ratio. Ratio values for the normal pregnancy are also given (hatched histograms). The control values for (C, D) were obtained from a urine sample collected 10 weeks after birth.

aromatase is considered the gatekeeper responsible for detoxifying fetal and maternal androgens thereby preventing maternal or fetal virilization. While aromatase, per se, was not defective in the fetuses studied, three hydroxylations requiring six electrons are needed for removal of the 19-methyl group and aromatization, so this transformation is also likely to be sensitive to inactivating mutations in OR, a known electron donor for P450 aromatase. Thus, attenuated aromatization may result in active androgen crossing the placenta in both directions. However, there is evidence that an alternate biosynthetic route to androgens is active in fetal life which does not involve the production of androgens that can be aromatized (Fig. 3B). Auchus et al. [Gupta et al., 2003] have recently shown that human P450c17 has a much greater substrate affinity for 17-hydroxyallopregnanolone (5α-pregnane-3α,17α-diol-20-one) than 17-hydroxypregnenolone or 17-hydroxyprogesterone. 17-Hydroxyallopregnanolone is produced in excess in the ORD fetus by sequential action of 5α-reductase 1 and 3α-hydroxysteroid dehydrogenase (3αHSD) acting on 17α-hydroxyprogesterone. It is readily converted to androsterone, which in turn could be converted by 3αHSD (through a "backdoor" pathway) to 5αDHT, a steroid that would cross the placenta bypassing aromatase. In contrast, as previously described in relation to estriol synthesis, the conventional "frontdoor" pathway to androgens involving conversion of 17α-hydroxypregnenolone to DHEA is curtailed by ORD. Activity of the backdoor pathway is transient and during the first months of life gonadal expression of the 5α-reductase 1 will be replaced by 5α-reductase 2, which is not able to convert 17-hydroxyprogesterone to 17α-hydroxyallopregnanolone. As a consequence, backdoor androgen synthesis ceases, compatible with a phenotype of high androgens during fetal life and low or normal androgens after birth as observed in patients with APHD, that is, ORD [Shackleton et al., 2003]. This backdoor pathway may also have an important pathophysiological role in the prenatal virilization of children with classic, isolated 21-hydroxylase deficiency.

ORD and ABS

Recent studies [Arlt et al., 2004; Fluck et al., 2004; Adachi et al., 2004b] have shown that APHD is a result of ORD. APHD is a disorder with varying severity, sometimes only noticeable through the observation of genital ambiguity, but it can also be associated with the more severe skeletal and craniofacial disorder ABS. How deficient OR results in ABS is still


A
Normal Pregnancy
FETUS
PLACENTA
MOTHER
URINE
Corticosteroids
Progesterone
Pregnenolone
Cholesterol
Pregnanediol
Pregnanediol glucuronide
Cholesterol
17-OH-Pregnenolone
Pregnenolone
DHEA-Sulfate
Adrenal
Aromatase
16α-OH-DHEA-Sulfate
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URINE
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VIRILIZATION
"Backdoor Pathway"
Gonads ?
5αDHT
5αDHT
17β HSD
3αHSD
Aromatase
17β HSD
3αHSD
Luteoma ?
Androsterone
Androsterone Glucuronide
5α-Pregnane-3α, 17α-diol-20-one
5α-Pregnane-3α, 17α-diol-20-one glucuronide
Corticosteroids
Progesterone
Pregnenolone
Cholesterol
Pregnanediol
Pregnanediol glucuronide
5αRed1
3αHSD
17-OH-Progesterone
Cholesterol
17-OH-Pregnenolone
Pregnenolone
Pregnenediol disulfate
Pregnenediol-20-sulfate
3β,5α-Pregnanediol-20-sulfate
3β,5α-Pregnanediol diconjugates
Adrenal
DHEA-Sulfate
16α-OH-DHEA-Sulfate
16α-OH-DHEA
Estriol
Estriol conjugates
Major pathway
Intermediate pathway
Minor pathway

Fig. 3.

unknown, but Kelley et al. [2002] provide evidence that ABS may be a result of a defect in cholesterol synthesis at the stage of lanosterol 14α-demethylation, an attractive hypothesis in light of the increasing appreciation of the relationship of defective cholesterol synthesis to malformation syndromes [Porter, 2002]. While *CYP51*, the gene coding the enzyme responsible for 14α-demethylation has not been shown to be mutated in ABS, demethylation involves hydroxylation requiring the redox partner OR. Thus, both disordered steroidogenesis and sterologenesis can be a consequence of the same genetic fault.

SUMMARY

We present here data supporting reduced estriol production caused by attenuated 17,20-lyase activity secondary to inactivating mutations in OR. Reduced fetal DHEA sulfate synthesis results in deficient synthesis of the primary precursor of estriol, 16α-hydroxy-DHEA sulfate. Attenuated 17-hydroxylase/17,20-lyase activity (secondary to ORD) results in pregnenolone being the major fetal adrenal steroid product. Pregnenolone in turn is probably finally disposed of in maternal urine as a disulfate of epiallopregnanediol. Maternal androsterone excretion is high, as is the excretion of a potential precursor 17-hydroxyallopregnanolone, indicating a hyperandrogenic condition. We propose that androsterone and its product 5αDHT are produced prenatally by a novel pathway. These androgens can freely cross the placenta without being aromatized so could be responsible for the maternal and fetal virilization observed in our cases.

ORD must be added to the disorders considered when a low-estriol is found at triple-marker-screening. We predict epiallopregnanediol (particularly the epiallopregnenol artifact) will become the analyte of choice for prenatal diagnosis of APHD (including ABS phenotypes) caused by ORD.

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Fig. 3. Altered steroid synthesis and metabolism in the affected pregnancy. This figure offers a simplified rendering of steroid synthesis and metabolism in a normal pregnancy and one with an ORD affected fetus. A single arrow between steroids may represent a multiple step conversion. In a normal pregnancy (**A**) two urinary steroids dominate, PD the metabolite of placental progesterone and estriol, the metabolite of fetal DHEA sulfate. Not illustrated are the necessary syntheses of fetal androgens for normal male development. In affected ORD pregnancies (**B**) estriol synthesis is attenuated because of a 17,20-lyase block secondary to the ORD deficiency. We propose that a major estriol "replacement" in these pregnancies is epiallopregnanediol, a maternal metabolite of fetal pregnenolone. A pregnancy luteoma has been suggested as the cause of hyperandrogenism in some affected pregnancies, but in our patient this was not evident, and we suggest the existence of a fetal "backdoor" pathway to 5αDHT, an androgen resistant to aromatase.

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