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Topic 3.3

Prevention of ambiguous genitalia by prenatal treatment with dexamethasone in pregnancies at risk for congenital adrenal hyperplasia*

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Abstract: Congenital adrenal hyperplasia (CAH) refers to a family of monogenic inherited disorders of adrenal steroidogenesis most often caused by a deficiency of the 21-hydroxylase enzyme. In the classic forms of CAH (simple virilizing and salt-wasting), androgen excess causes external genital ambiguity in newborn females and progressive postnatal virilization in males and females. Prenatal treatment of CAH with dexamethasone has been successfully utilized for over a decade. This article reports on 595 pregnancies prenatally diagnosed using amniocentesis or chorionic villus sampling between 1978 and 2002 at the New York Presbyterian Hospital–Weill Medical College of Cornell University. No significant or enduring side effects were noted in the fetuses, indicating that dexamethasone treatment is safe. Prenatally treated newborns did not differ in weight from untreated, unaffected newborns. Based on our experience, prenatal diagnosis and treatment of 21-hydroxylase deficiency is effective in significantly reducing or eliminating virilization in the newborn female. Prevention of genital virilization in female newborns with classic CAH avoids the risk of sex misassignment and diminishes the need for corrective surgery and the resulting psychological impact that may extend into adulthood.

INTRODUCTION

Congenital adrenal hyperplasia is a family of inherited disorders of adrenal steroidogenesis [1]. Each disorder results from a deficiency in one of the five enzymatic steps necessary for normal cortisol synthesis (Fig. 1). Deficiency of the 21-hydroxylase enzyme (21-OHD) accounts for over 90 % of CAH cases. There is a wide range of clinical presentations in classic and nonclassic CAH, from virilization with labial fusion to precocious adrenarche, pubertal or postpubertal virilization, and reduced fertility. A five-stage classification by Prader [2] is used to represent different degrees of virilization (Fig. 2), where on a scale of 1 to 5 (I–V) the genitalia can be scored from slightly virilized (e.g., mildly enlarged clitoris) to indistinguishable from a male. Most classical cases of 21-hydroxylase deficiency are born with Prader IV genitalia.

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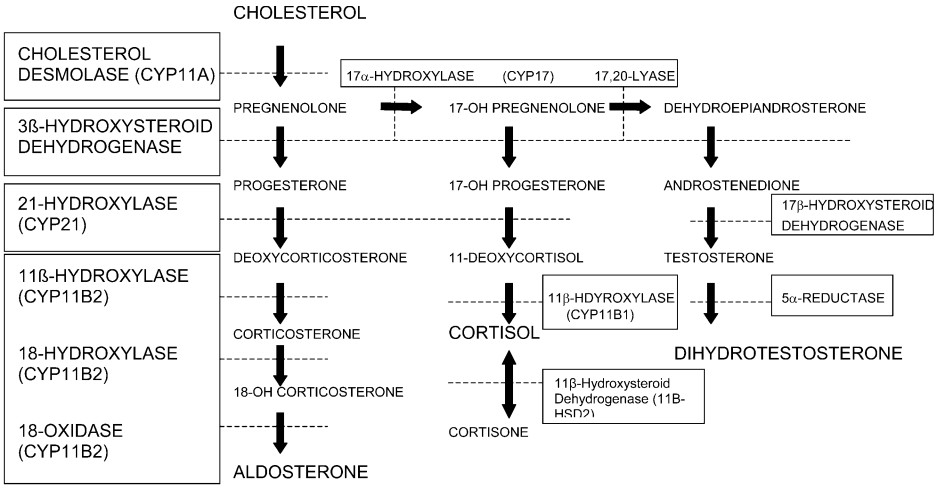
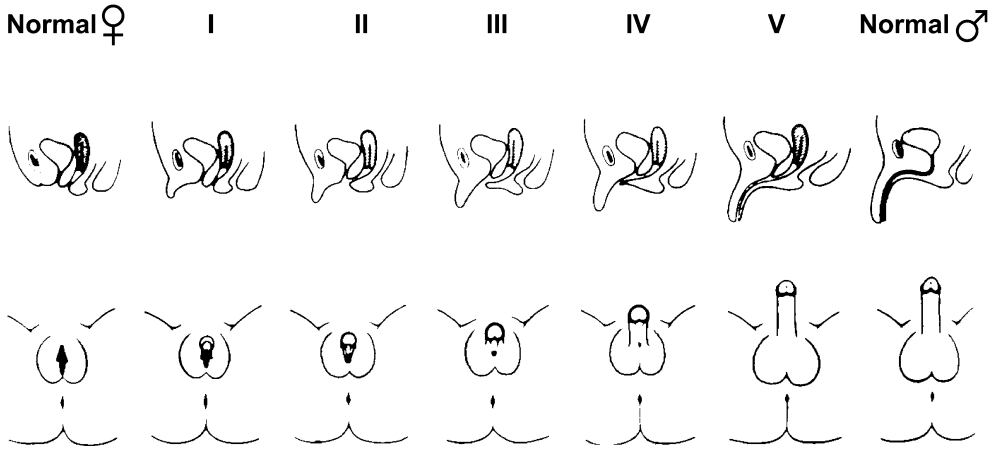


Fig. 1 Simplified scheme of adrenal steroidogenesis.



(Courtesy of Andrea Prader. Reprinted from *Helvetica Paediatrica Acta.)*

Fig. 2 Diagrams of different degrees of virilization using the Prader Scale [3].

In the classic form of 21-OHD, prenatal androgen excess causes external genital ambiguity in newborn females (female pseudohermaphroditism) (Fig. 3). Postnatally, males and females exhibit progressive virilization, which may include progressive penile or clitoral enlargement, precocious pubic hair, hirsutism, acne, advanced somatic and epiphyseal development, and central precocious puberty. Reduced fertility and menstrual abnormalities have been observed in women, and testicular adrenal rests in untreated men [3–6]. There are two types of classical steroid 21-OHD, simple virilizing and salt wasting. Three-fourths of classical cases are salt-wasting [1]. To some extent, the symptoms can be arrested or reversed by treatment with glucocorticoids, which suppresses ACTH stimulation of the adrenal cortex. Those patients with aldosterone deficiency require treatment with salt-retaining steroids as well. Analysis of CAH incidence data from almost 6.5 million newborns screened in the general population worldwide has demonstrated an overall incidence of between 1:13 000 and 1:15 000 live births for the classic form of CAH [7–9].

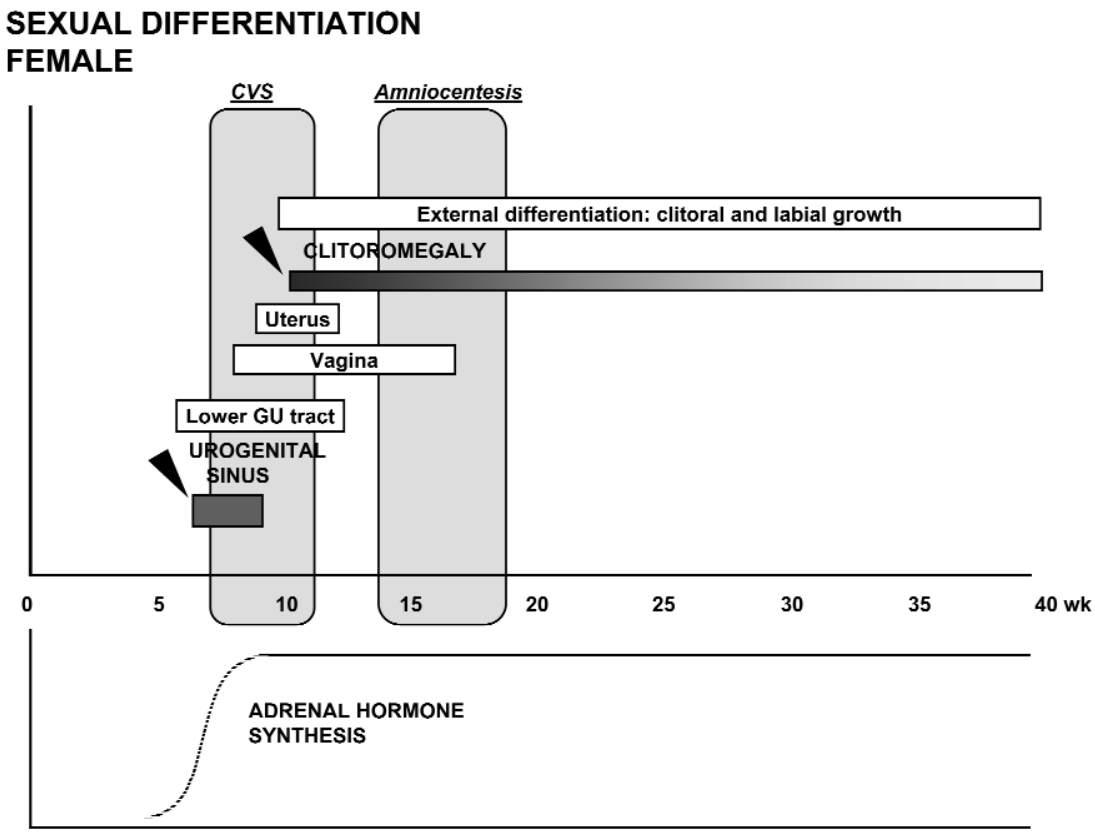


Fig. 3 Timetable of female sexual differentiation.

The most reliable hormonal diagnostic test for 21-OHD has proven to be the ACTH (Cortrosyn, 0.25 mg) stimulation test measuring the serum concentration of 17-hydroxyprogesterone (17-OHP). After intravenous bolus ACTH administration (preferably in the morning due to the diurnal variation of 17-OHP), 17-OHP is measured at 0 and 60 min. A logarithmic nomogram provides hormonal standards for assignment of the 21-OHD type by relating baseline to ACTH-stimulated serum concentrations of 17-OHP [10] (Fig. 4).

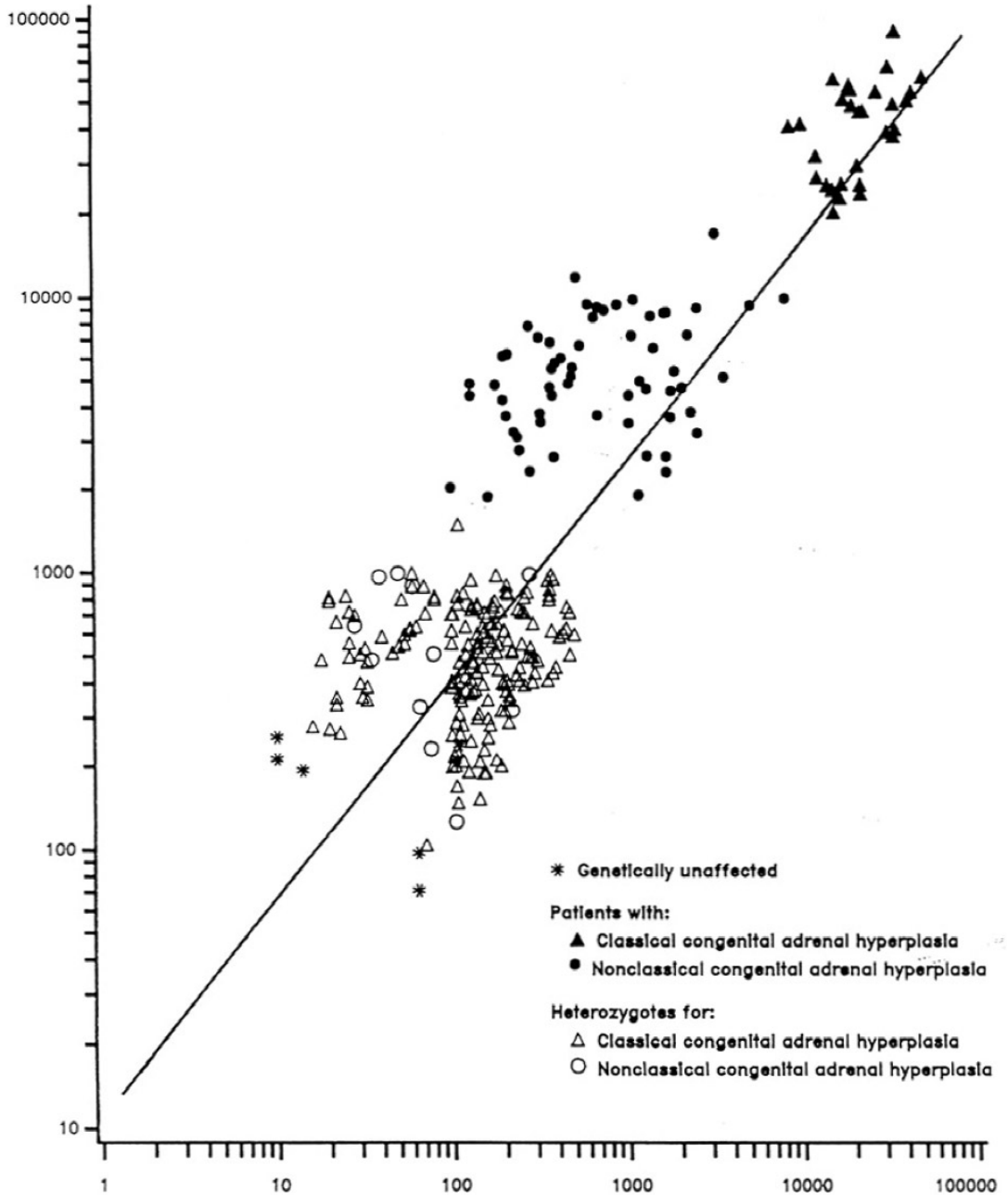


Fig. 4 Nomogram relating baseline to 60′ ACTH-stimulated serum concentrations of 17-hydroxyprogesterone (17-OHP). The scales are logarithmic.

MOLECULAR GENETICS

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is a monogenic autosomal recessive disorder. The gene for adrenal 21-hydroxylase, CYP21, is located about 30 kb from a pseudogene, CYP21P, on chromosome 6p, adjacent to the HLA genes. The high degree of sequence similarity (96–98 %) between CYP21 and CYP21P permits two types of recombination events: (1) unequal cross-

ing-over during meiosis, which results in complete deletions and duplications of CYP21 and the possible transmission of a null allele, and (2) and gene conversion events that transfer deleterious mutations present in the pseudogene to CYP21 [11–13].

Specific mutations may be correlated with a given degree of enzymatic compromise and a clinical form of 21-OHD [14–18]. The genotype for the classical form of CAH is predicted to be a severe mutation on both alleles at the 21-OH locus, with markedly decreased enzymatic activity generally associated with salt wasting. The point mutation A (or C) to G near the end of Intron 2, which is the single most frequent mutation in classic 21-OHD, causes premature splicing of the intron and a shift in the translational reading frame [11,15]. Most patients who are homozygous for this mutation have the salt-wasting form of the disorder [19,20]. Recent studies, however, have demonstrated that there is occasionally a divergence in phenotypes within mutation-identical groups, the reason for which requires further investigation [20,21].

PRENATAL DIAGNOSIS AND TREATMENT

While it was known that CAH-affected fetuses exhibit elevated 17-OHP and Δ^4-androstenedione concentrations in their amniotic fluid, the differentiation of external genitalia and the urogenital sinus begins at approximately the 9^{th} week of gestation. Thus, if prenatal treatment with dexamethasone must begin no later than the 9^{th} week of gestation to effectively prevent virilization of a female fetus, an amniocentesis performed in the second trimester would show suppressed 17-OHP levels and be unreliable for diagnosis. The later use of human leukocyte antigen (HLA) genetic linkage marker analysis was also unsuccessful because recombination or haplotype sharing lead to many diagnostic errors. The method currently used is direct DNA analysis of the 21-OH gene (CYP21) with molecular genetic techniques [22–24]. Chorionic villus sampling (CVS) can also be used to obtain fetal tissue for prenatal diagnosis by molecular genetic analysis at 9–11 weeks gestation.

An algorithm was first published in 1990 for the prenatal diagnosis of 21-OHD congenital adrenal hyperplasia using direct molecular analysis of the 21-OH locus and dexamethasone treatment [25] (Fig. 5). Unlike hydrocortisone, dexamethasone crosses the placenta. Because maternally administered dexamethasone is not bound by corticosteroid-binding globulin, (also termed transcortin), and dexamethasone is not converted by 11B-hydroxysteroid dehydrogenase to cortisone it undergoes efficient placental transfer to the fetus.

Since the first report of prenatal therapy by David and Forest in 1984 [26], there have been several large studies of prenatal treatment [24,27,28]. When the proper protocol is followed, these studies have proved extremely successful in preventing virilization in females affected with classic CAH. Of the 595 pregnancies prenatally diagnosed using amniocentesis or chorionic villus sampling between 1978 and 2002 at the New York Presbyterian Hospital–Weill Medical College of Cornell University, 281 were prenatally treated for CAH, owing to a risk for 21-hydroxylase deficiency.

The prevention of genital virilization in female newborns with classic CAH has significant implications. For the prenatally treated child, there is the benefit of unambiguous genitalia, including the diminished need for future vaginoplasty and the resulting psychological impact of genital surgery. Prenatal treatment also avoids a potential male sex assignment to virilized female newborns and has been suggested to prevent gender ambiguity sometimes seen in CAH females, attributed to the high levels of androgens exposed to the brain during differentiation [29].

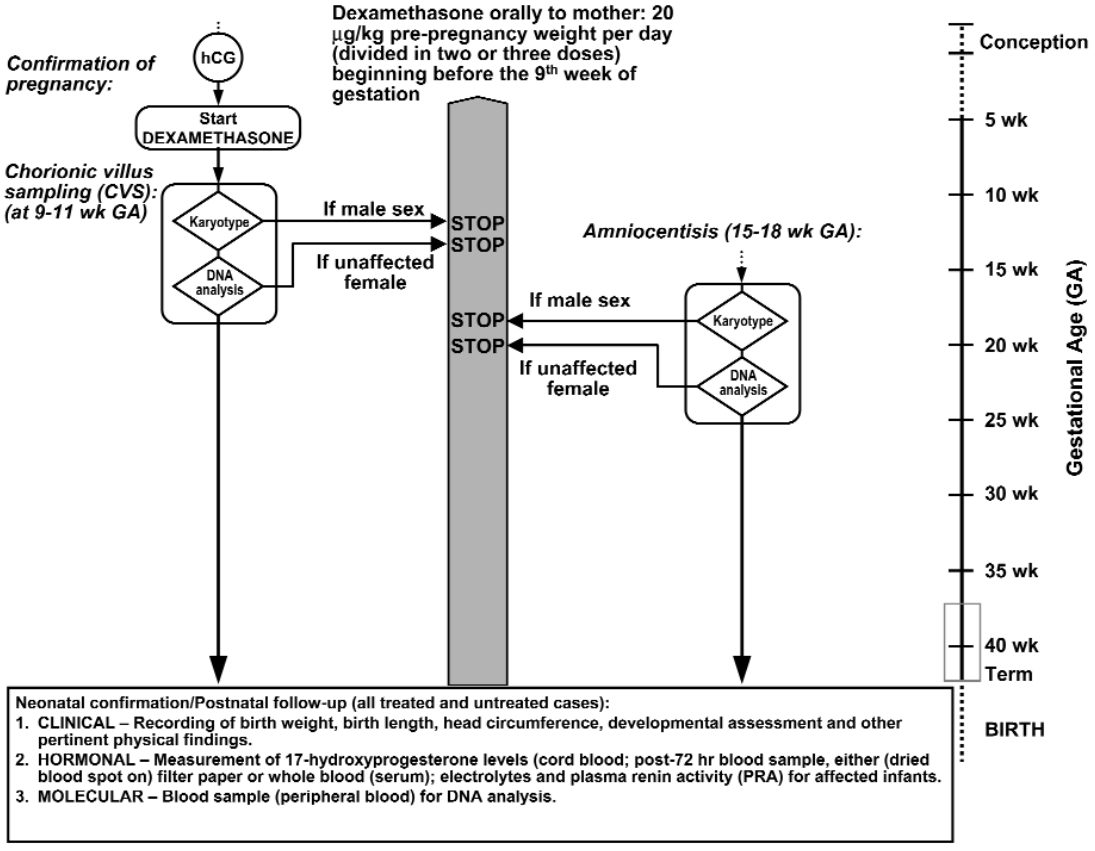


Fig. 5 Algorithm depicting prenatal management of pregnancy in families at risk for a fetus affected with 21-OHD [25].

THE PROTOCOL

Dexamethasone (20 µg/kg/day in 3 divided doses) was administered to the pregnant mother before the 9^{th} week of gestation, blind to the affected status of the fetus, to suppress excess adrenal androgen secretion and prevent virilization should the fetus be an affected female (Fig. 6). Diagnosis by DNA analysis requires chorionic villus sampling in the 9^{th} to 11^{th} week gestation or sampling of amniotic fluid cells obtained by amniocentesis in the second trimester. The fetal DNA is used for specific amplification of the CYP21 gene utilizing polymerase chain reaction (PCR) [30]. If the fetus is determined to be an unaffected female upon DNA analysis or a male upon karyotype analysis, treatment is discontinued. Otherwise, treatment is continued to term.

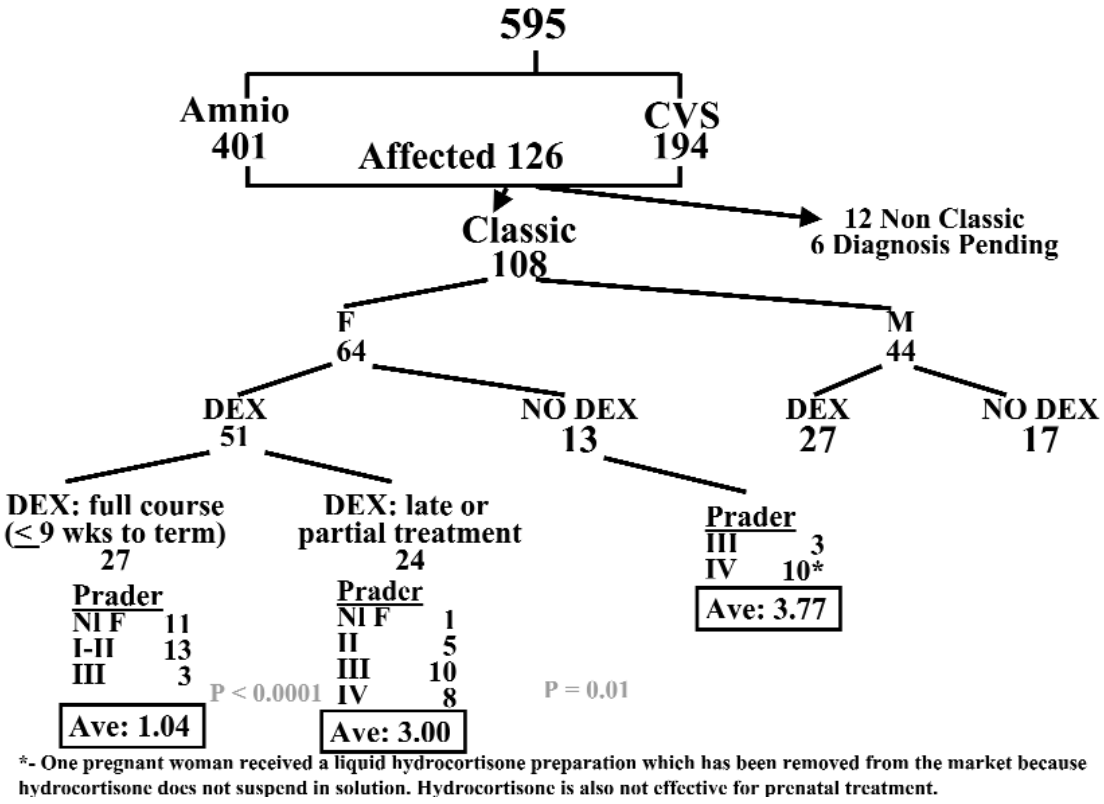


Fig. 6 Diagram depicting prenatal dexamethasone treatment outcome by Prader scores in fully and partially treated affected newborns. Partial treatment includes initiation of dex >9 weeks gestation, noncompliance, inadequate dosage for body weight. Dex = dexamethasone; Amnio = amniocentesis; CVS = chorionic villus sampling; F = female; M = male; Nl = normal.

RESULTS

Of 595 pregnancies evaluated, 126 fetuses were found to be affected with 21-OHD, of whom 108 were classical cases. Of the classical cases, 64 were female, 51 of whom were treated prenatally with dexamethasone. Dexamethasone administered at or before 9 weeks of gestation (27 affected female fetuses) was effective in reducing virilization. Of these 27, 11 fetuses were born with entirely normal female genitalia, while 13 were significantly less virilized (Prader stages 1–2) than those untreated (Fig. 7). Sixteen affected females treated with dexamethasone full-term had untreated affected female sibs. In all 16 cases, the external genitalia of the treated females were less virilized than the genitalia of the untreated sibs. Most of the newborn females whose genitalia were rated Prader stages 3–4 who had been treated prenatally with dexamethasone, started treatment late, were under-treated by referring physician, or were noncompliant.

Overall for affected females, the average Prader score for those treated prenatally at or before 9 weeks gestation was 1.04 while those with no prenatal treatment had an average Prader score of 3.77 ($P << 0.003$). The external genitalia of affected females prenatally treated at or before 9 weeks were less virilized than the partially treated affected females (mean Prader score of 3.00; $P < 0.0001$). The Prader scores of partial dexamethasone treatment and no treatment in affected females (mean Prader score 3.77) are also statistically significant ($P = 0.01$).

No significant or enduring side effects were noted in newborns and children who were prenatally treated. Fetal wastage did not differ statistically between dexamethasone treated (partial- and full-term)

Fig. 7 Diagram depicting Prader stages of affected female infants in monitored, dexamethasone prenatally treated pregnancies, in relation to gestational age when dexamethasone was started. Affected untreated sibs are shown attached by a dotted line.

pregnancies from untreated. As previously reported [23], prenatally treated (partial- and full-term) newborns do not differ significantly in birth weight from untreated newborns. Mean birth weight for dexamethasone prenatally treated fetuses was 3.39 kg, while for untreated it was 3.46 kg ($P = 0.17$; Table 1). The birth weight of 59 affected infants treated with dexamethasone is significantly lower than affected infants not prenatally treated with dexamethasone ($P = 0.02$). Despite statistical significance, it is unlikely that there is any clinical significance between the birth weights of the treated (mean wt. 3.28 kg) and untreated affected (mean wt. 3.60 kg) groups. Infants not affected with CAH whether prenatally treated with dexamethasone (mean wt. 3.42 kg) or not (mean wt. 3.44 kg), had similar birth weights ($P = 0.74$). The birth length and head circumference (data not shown) were normal in offspring of dexamethasone-treated pregnancies compared to those not treated, which is consistent with other studies where patients and physicians adhered to the recommended therapeutic protocol [22–24,27,28]. A large quantitative follow-up study is currently in progress regarding cognition, gender, temperament, and

Table 1 Birth weights (kg) in dexamethasone treated vs. untreated fetuses at risk for classic CAH. Excludes premature infants, twins with suspected feto-fetal transfusion.

	Dexamethasone	No dexamethasone	T-test
Affected	3.28 ($n = 59$)	3.60 ($n = 20$)	$P = 0.02$
Not Affected	3.42 ($n = 171$)	3.44 ($n = 143$)	$P = 0.74$

handedness (an indicator of prenatal androgen effect) in children and adults who were prenatally treated with dexamethasone.

The authors did not find significant differences in side effects between the mothers who were treated with dexamethasone and the mothers who were not treated, except in weight gain, edema, and striae. By report, mothers who were not treated with dexamethasone gained an average of 29.7 lbs., while treated mothers gained an average of 36.8 lbs., which was statistically significant ($P < 0.005$). There was a statistically significant difference found for the presence of striae ($P = 0.01$) and edema ($P = 0.02$). There was not a statistically significant difference found for hypertension ($P = 0.5$), or gestational diabetes ($P = 0.34$) in the treated or untreated pregnancy groups. All mothers who took prenatal dexamethasone (partial- and full-term) treatment stated they would take dexamethasone again in the event of a future pregnancy.

DISCUSSION

Controversy of prenatal treatment

One report in 1997 questioned the safety of long-term prenatal glucocorticoid treatment of fetuses potentially affected with congenital adrenal hyperplasia [31]. The authors claimed that prenatal treatment with dexamethasone contributes to low birth weight, fetal demise, serious maternal complications, and cognitive and developmental deficiencies. However, the cited references are predominately based on animal studies, in which excess glucocorticoid dosages are used. We find these claims to be unfounded based on our experience with the largest number of treated human pregnancies in the world, in addition to the results of other large studies [27,32,33].

The risk-to-benefit ratio in view of no enduring side effects in mother or child favors prenatal treatment. Additionally, males and unaffected females treated with short-term dexamethasone show no side affects [28]. Treatment of affected females alleviates potential sex mis-assignment, repeated genital surgeries that cannot easily recreate natural genital structures, and psychological effects. We agree that long-term studies are needed to conclusively determine outcome of treatment, which are currently in progress.

Based on the experience at New York Presbyterian Hospital–Weill Cornell Medical College, proper prenatal diagnosis and treatment of 21-OHD is safe and is effective in significantly reducing or eliminating virilization in the affected female. Of the monogenic disorders, steroid 21-OHD is one of the few in which prenatal treatment is effective and influences postnatal life.

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