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Amniotic Fluid 17-Hydroxyprogesterone in Early Pregnancy

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The results of measurement of 17-hydroxyprogesterone (17-OH-P) in 125 samples of amniotic fluid (AF) from early amniocenteses are presented. The fetuses from all pregnancies studied were unaffected by congenital adrenal hyperphasia caused by 21-hydroxylase deficiency. The AF 17-OH-P level increases slightly but significantly between the 11th and 15th week of gestation, with a maximum in the 14th week. There is no difference between the values measured in male and female fetuses. The AF 17-OH-P levels from the early gestation were compared with those from the 16th–22nd week of pregnancy (published previously). The overall differences of AF 17-OH-P concentrations when considered in all gestational age groups in the whole period 12–22 weeks were statistically insignificant. Thus, the biochemical prenatal diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency and control of its early fetal treatment could be carried out starting from the end of the first trimester in the same way as at the later period of gestation.

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INTRODUCTION

17α-Hydroxyprogesterone (17-OH-P) is the main biochemical diagnostic indicator of steroid 21-hydroxylase deficiency, which is the most frequent cause of congenital adrenal hyperplasia (CAH). Determination of 17-OH-P in amniotic fluid (AF) is widely used for prenatal diagnosis of CAH [1–8] as originally suggested by Frasier *et al.* [9]. Further study showed a relatively wide variation in the levels of 17-OH-P in AF with normal fetuses unaffected by CAH and the importance of carrying out as far as possible a great number of measurements for the estimation of the range of normal values [5, 6, 10].

It was further shown that between the 15th and 24th week of pregnancy the levels of 17-OH-P in AF are not dependent on the gestational age or the sex of the fetus [1–3, 5].

In recent years attempts have been made to shift the prenatal diagnosis to the early stage of pregnancy, i.e. before the 15th week [11]. Raux-Demay *et al.* [12] published the results of the assay of 17-OH-P in AF from the 10th–11th week of pregnancy in comparison with the results from the 16th–18th week. The relevant data on the period between the 11th and 16th week have so far appeared in the literature only in isolated cases or in a preliminary form [13].

The following study reports levels of 17α-OH-P in AF from 125 pregnancies with fetuses unaffected by CAH from the 11th–15th week of gravidity and their dependence on the gestational age and the sex of the fetuses.

MATERIALS AND METHODS

AF was obtained by transabdominal amniocentesis from 125 pregnant women with a genetic risk in the fetus, of which, however, none had signs of CAH caused by an enzyme defect in the family and who had given birth to children without symptoms of CAH. The AF was taken during the 11th–15th week of pregnancy beginning from the date of the last menstruation. The AF was centrifuged and the supernatant kept at −20°C until the analysis was carried out.

The determination of 17-OH-P was carried out by radioimmunoassay with the use of a highly specific antiserum prepared in our laboratory [14].

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Table 1. Normal concentrations (nmol/l) of 17α-OH-P in AF during early pregnancy (11–15 weeks), the influence of fetal sex

Fetal sex	*n*	Mean	SD	Median	95% Confidence limits	Range
M	58	8.54	2.95	7.9	5.6–13.2	3.9–15.6
F	56	8.69	2.22	8.4	5.0–12.0	4.1–13.9
M + F	114	8.61	2.41	8.4	5.0–12.2	3 9–15.6

M, male; F, female.

Antiserum

As an antigen, the conjugate 17-OH-P-3-CMO-BSA was used. Rabbits were immunized with a primary dose of 0.5 mg antigen and with booster doses of 0.1 mg, which were given weekly for 3 months. Antiserum was collected 10 days after the last boost. The cross-reactions of 17-hydroxypregnenolone was 2.0%, of progesterone 0.3% and of pregnenolone 0.5%; cross-reactions of the other structurally related steroids to 17-OH-P did not exceed 0.1% [14].

Radioligand

[1,2,6,7-^{3}H]17α-OH-P, sp. act. 2.52 TBq/mmol (Radiochemical Centre, Amersham) was purified by thin layer chromatography before use.

Assay

To 200 μl of AF supernatant the same volume of redistilled H_2O was added and extraction with 4 ml of dichlormethane (Merck) was carried out; the analyzed aliquot corresponded to 50 μl of AF. As a medium sodium phosphate 0.05 mol/l buffer, pH 7.4, containing 0.9% NaCl, 0.1% NaN_3 (Merck) and 0.1% gelatine was used. To the residues of AF extracts aliquots of 0.1 ml buffer, 0.1 ml antiserum (diluted 1:30,000) and 0.1 ml radioligand (about 28,000 dpm) were added and incubated for about 20 h at 4°C. The separation of free from bound fraction was performed using 0.5 ml suspension of charcoal (Norit A, Serva).

Specifically bound radioactivity was measured and results were calculated from the calibration curve.

Statistics

For the statistical interpretation of the influence of fetal sex on the level of 17-OH-P in AF the Student's *t*-test was used.

The influence of gestational age upon the AF 17-OH-P concentration was evaluated by non-parametric analysis with the Kruskal–Wallis H-test (age groups: 12–22; 12–15; and 16–22 weeks) and the differences between any two of the gestational age groups (weeks) were evaluated by the Wilcoxon U-test. The measured 17-OH-P concentrations in AF resolved in time were fitted by linear regression analysis and the results were tested.

RESULTS

Table 1 shows the levels of 17-OH-P in AF from the 11th–15th weeks of pregnancy in connection with the fetal sex. In this period of gestation the sex of the fetus has no influence on the concentration of 17-OH-P in AF. The difference between the values measured in male and female fetuses was not statistically significant.

Table 2 shows the variation of 17-OH-P concentrations in AF with gestational age during early pregnancy. The mean level of 17-OH-P in AF increases slightly between the 11th and 15th week with a maximum in the 14th week; this increase is statistically significant.

The regression analysis shows that the increase between the 11th and 14th week is highly statistically significant ($n = 67$, regression coef. = 0.4266, $P \leqslant 0.01$).

In Fig. 1 the values of AF 17-OH-P from the 11th–15th week of gestation (Table 2) are compared with the results from midpregnancy. By statistical evaluation of differences in gestational age groups we found that in the whole period 12–22 weeks differences in AF 17-OH-P concentrations were insignificant. Similar results were obtained by linear regression analysis (12–22 weeks: $n = 336$, regression

Table 2. Normal concentrations (nmol/l) of 17α-OH-P in AF during early gestation (11–15 weeks)

Week of gestation	*n*	Mean	SD	Median	95% Confidence limits	Range	Statistics 12[a]	13[a]	14[a]	15[a]
11	1	6.1	—	—	—	—				
12	8	6.9	1.8	5.9	5.7–8.4	4.8–10.2	—	0	+	+
13	31	7.5	2.4	7.1	4.4–10.4	4.2–15.6	0	—	+ +	+ +
14	27	9.6	2.7	9.7	6.0–14.2	3.9–16.1	+	+ +	—	0
15	58	8.9	2.1	8.7	5.6–12.0	4.1–13.9	+	+ +	0	—
11–15	125	8.5	2.3	8.2	5.0–12.2	3.9–16.1				

Statistics: [a]weeks; $^+P < 0.05$; $^{++}P < 0.01$, 0, not significant.

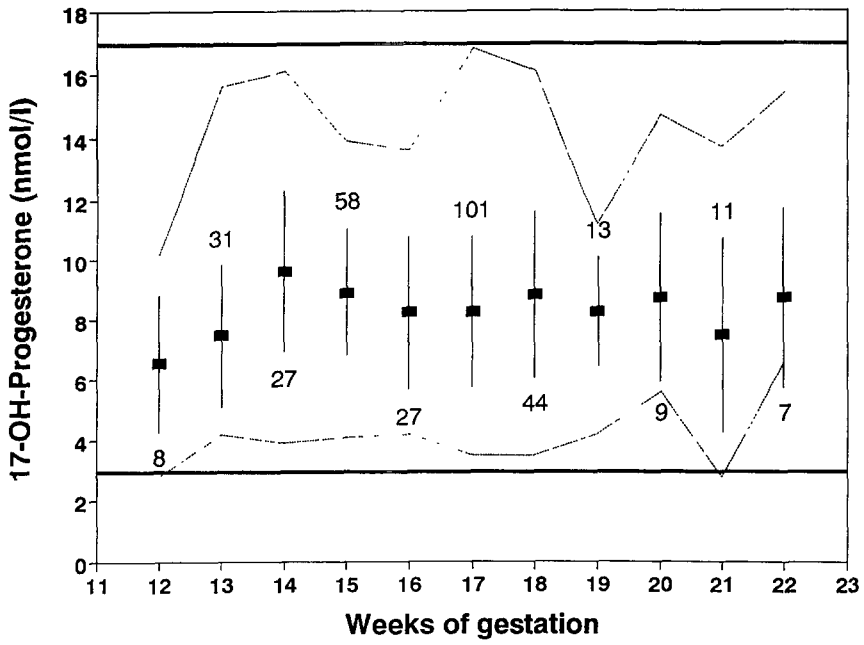

Fig. 1. 17-OH-P levels in normal AF during pregnancy. Square, mean; vertical line, ±SD; dotted line, communication of minimal or maximal values measured (range); number near the vertical line, number of examinations; horizontal bars, normal range for prenatal diagnosis.

coef. = 0.0281; 16–22 weeks: $n = 212$, regression coef. = 0.0054).

Furthermore, from Fig. 1 it is clear that there are wide and variable ranges of 17-OH-P levels in the individual weeks of pregnancy.

DISCUSSION

The levels of 17-OH-P in the AF from early pregnancy as well as in the later period are unaffected by fetal sex [1–3, 5].

The 17-OH-P levels in AF from the 11th–15th week of pregnancy show a slight but significant increase, with a peak in the 14th week.

For the 10th–11th week period of gestation Raux-Demay *et al.* [12] reported lower AF 17-OH-P concentrations than for weeks 16–18, however, the values for the 11th–16th week period were not given. According to Sippel *et al.* [15] on the basis of a lower number of observations, AF 17-OH-P levels increased after the 14th week until the 18th week. Our present results demonstrate that there is no significant change in AF 17-OH-P after the increase in the 11th–14th week up to the 22nd week.

The physiological cause of the peak in the 14th week of pregnancy is unclear. It may be speculated that the content of 17-OH-P in AF increases disproportionally to the increasing volume of AF up to the 14th week or that it serves as a precursor for metabolic processes in fetal membranes during midpregnancy.

The relation between concentration and gestational age is significant in early gestation (11–15 weeks) but this significance was not found when the whole period 12–22 weeks was evaluated. This contradiction can be explained by a large number of observations, a wide range of individual values and by differences in the frequency distributions of round values in each of the age groups (weeks). These facts also confirm the necessity for a sufficient number of controls for reliable estimation of the normal range of 17-OH-P levels in AF.

The independence of the concentration of 17-OH-P in the AF, regardless of gestational age and sex of the fetus, is very advantageous for diagnostics. For this purpose it is necessary, to take into consideration the relatively high differences in values found in the individual specimen samples of normal AF (see Fig. 1).

In evaluating the results of the 17-OH-P determinations for the prenatal diagnosis of steroid 21-deficiency we used the normal range of 3–17 nmol/l, for the 13th–22nd week period of pregnancy. The AF 17-OH-P levels measured in our laboratory in 8 CAH affected fetuses from the 16th–17th week resulted in 19.1–74 nmol/l; from a pregnancy which had the lowest level of 17-OH-P (19.1 nmol/l), a baby boy was born with a severe salt-losing form of CAH. There is, therefore, no direct connection between the degree of enzyme disorder in the fetus and the concentration of 17-OH-P in the AF.

As shown here the normal levels of 17-OH-P in the AF from early pregnancy (12th–15th week) are comparable with the levels from the later period. A block of 21-hydroxylase in the fetus caused elevation of the AF 17-OH-P level as early as 10–11 weeks of gestation [12]. An early prenatal diagnosis of the classical form CAH with 21-hydroxylase deficiency can,

therefore, be carried out with the help of the simple determination of 17-OH-P in the AF. It should not be forgotten, however, that the molecular genetic method will provide more precise and complete information for prenatal diagnosis of CAH and that HLA typing also has its place here [16].

Apart from prenatal diagnosis, the determination of 17-OH-P in the AF can also be used to monitor prenatal treatment of CAH [16–21]. The possibility of shifting this determination from the second trimester to the end of the first trimester is especially important here, since treatment must be started as soon as possible during pregnancy.

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