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UNCONJUGATED ESTRIOL IN MATERNAL PLASMA. RELATIONSHIP WITH EARLY FETAL DEVELOPMENT MEASURED BY ULTRASONIC MORPHOMETRY

R. DE HERTOGH and A. LUYX
Endocrinology and Nutrition Unit, University of Louvain, School of Medicine UCL 5429, 53 Avenue E. Mounier, 1200 Bruxelles, Belgium

SUMMARY

Unconjugated estriol was measured specifically by radioimmunoassay in the plasma of 95 normal pregnant women, at one to three week intervals, between the 7th to the 16th week of pregnancy. Results were correlated with the fetal size, determined by ultrasounds. Two measurements were made. The crown-rump length (CRL) up to the 11th week, and the biparietal diameter (BPD) afterwards.

It was observed that estriol levels remained below 100 pg/ml for CRL up to 30 mm. Between 30 and 42 mm, all estriol levels were above 50 pg/ml. Beyond 42 mm (CRL) or 2.1 cm (BPD) all estriol values exceeded 100 pg/ml, and increased rapidly with increasing BPD. Unconjugated "estradiol" was measured in the same plasma samples with a non-specific antibody. Relative to estradiol-17β, estrone cross-reaction was about 50% and estriol cross-reaction less than 3% with this antibody. It was observed that estriol and "estradiol" increased in a parallel way, up to a CRL value of 25 mm. The estriol to "estradiol" ratio remained constant up to that time. This ratio however increased significantly at a CRL value of 30 mm, and continued to increase thereafter.

This observation shows that additional precursors to estriol were being produced in the pregnant women as early as the 9th to 10th week of gestation. The fetal origin of these precursors is very likely since very low estriol levels were encountered in cases of molar pregnancies or of fetal death.

It is concluded that the fetal adrenals develop an increasing secretory activity when the fetus has a CRL of about 30 to 35 mm.

INTRODUCTION

Estriol is traditionally accepted as a useful parameter of the fetal well-being. Urinary analysis and more recently plasma measurements of total estriol (conjugated and unconjugated) have been used as fetal monitoring, mostly in late pregnancy. Few reports deal with unconjugated estriol measurements in early pregnancy [1–5] and this analysis has not yet been prevalent in obstetrical practice. However, unconjugated estriol increases very steeply in maternal plasma as early as the 10th–12th week of pregnancy [1] and the scatter of the levels obtained up to the sixteenth week is rather small, allowing an estimate of the gestational age with an accuracy of $\pm$ one week (S.D.) [6]. It was considered of interest to pinpoint the moment at which estriol started to increase in maternal plasma in terms of fetal development in view of the indirect evidence of the fetal origin of the estriol precursors, even in early pregnancy [1,7]. In the present work, we describe a longitudinal study conducted on 95 normal pregnant women between the seventh and the sixteenth week of gestation who were subjected to the simultaneous analysis of unconjugated estriol fluctuations in peripheral plasma, on the one hand, and of fetal development measured by ultrasonic morphometry, on the other hand.

MATERIAL AND METHODS

Ninety-five normal women, attending the outpatient clinic regularly, were selected for this study on the basis of the absence of any abnormality in the evolution of their pregnancy. Two to three blood samples were drawn at one to three week intervals, between the seventh and the sixteenth week of gestation, for estrogen measurements. Simultaneously, the fetal crown-rump length (CRL) or the biparietal diameter (BPD) were determined by ultrasounds, according to the methods of Robinson[8] and Campbell[9] respectively. The apparatus was a Diasonograf NE 4102 from Nuclear Enterprises.

Unconjugated plasma estriol was determined by radioimmunoassay, with a specific antibody to estriol-6-O-carboxymethyloxime BSA, as previously described [1,10]. The cross-reaction of estradiol or estrone with this antibody was negligible, and comparative assays made with or without a chromatographic step showed essentially the same results [11]. Hence the measurements were made on plasma extracts without chromatographic separation of the hormone.

Unconjugated estrogens were also routinely measured on crude plasma extracts with a non-specific antibody to estradiol-17β-17 hemisuccinate-BSA. Although this antibody is not suitable for direct measurements of estrogens in plasma outside pregnancy, during gestation, meaningful results can be obtained, due to the higher levels of estrogens present in the extracts [10,12]. Estrone crossreacted to about 50%, and estriol crossreacted to less than 3% [10]. Non-phenolic steroids did not cross-react to any significant extent [13]. Results obtained with this anti-

body are thus higher than those obtained for estradiol-17β, after chromatographic isolation and specific measurement [1]. However, the fluctuations of unconjugated estrogens measured by this method, paralleled and were almost quantitatively equal to the fluctuation of the sum of estrone + estradiol-17β measured separately and specifically [1, 11]. The results reported here with this method will be referred to as quoted "estradiol".

RESULTS

1. *Plasma levels of unconjugated estriol*

Figure 1 shows the individual values of unconjugated estriol measured in the 95 subjects, during the 7th to the 16th week of pregnancy. The gestational age on the abscissa has been corrected on the basis of the fetal morphometry (CRL or BPD) shown as additional scales.

By the seventh week (CRL 10 mm), estriol levels (32 ± 18 pg/ml (S.D.)) already exceeded by a factor of at least 2–4, the values usually obtained outside pregnancy. In the latter case indeed the levels rarely exceeded 15 pg/ml [11, 14] and were very often close to zero (mean level: 6 pg/ml) [11]. Up to a CRL value of 30 mm, estriol levels remained below 100 pg/ml. For CRL values between 30 and 42 mm, estriol levels were all above 50 pg/ml. Beyond a CRL of 42 mm

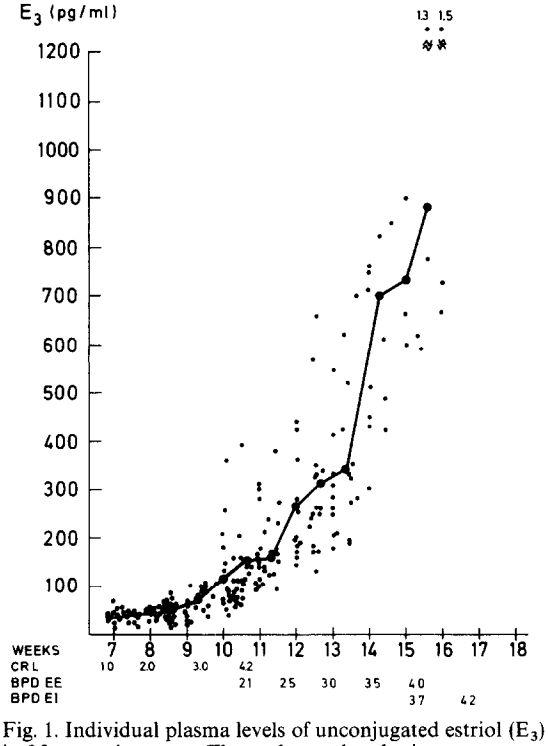

Fig. 1. Individual plasma levels of unconjugated estriol (E_3) in 95 normal women. The scale on the abscissa represents the gestational age in weeks elapsed from the first day of the last menstrual period, corrected for fetal size, measured by ultrasounds. CRL = crown-rump length (cm); BPD = biparietal diameter (cm); EE = external–external; EI = external–internal.

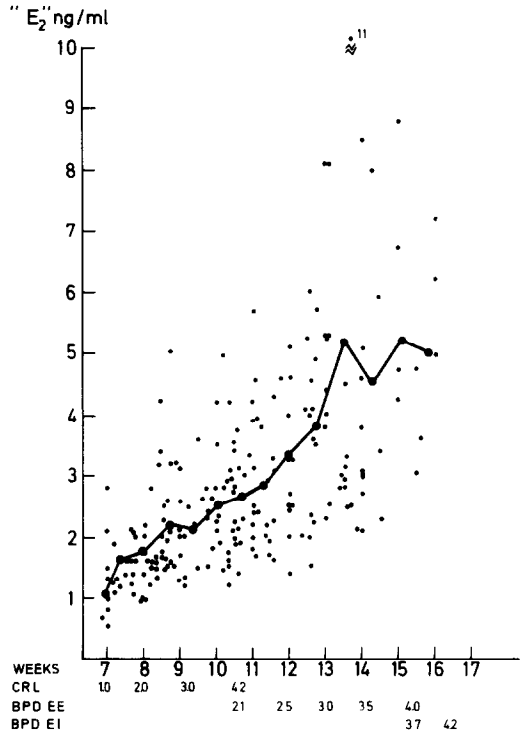

Fig. 2. Individual plasma levels of unconjugated "estradiol" ("E_2") (see Methods). Legends as in Fig. 1.

(or a BPD of 2.1 cm) all estriol levels exceeded 100 pg/ml and the sharp rise of estriol continued. The first significant increase of estriol beyond the seventh week level was observed at a CRL value of 21 to 25 mm ($P < 0.02$) (Fig. 3).

For a CRL value of 26–30 mm, a significant increase was again observed, above the preceding level ($P < 0.001$).

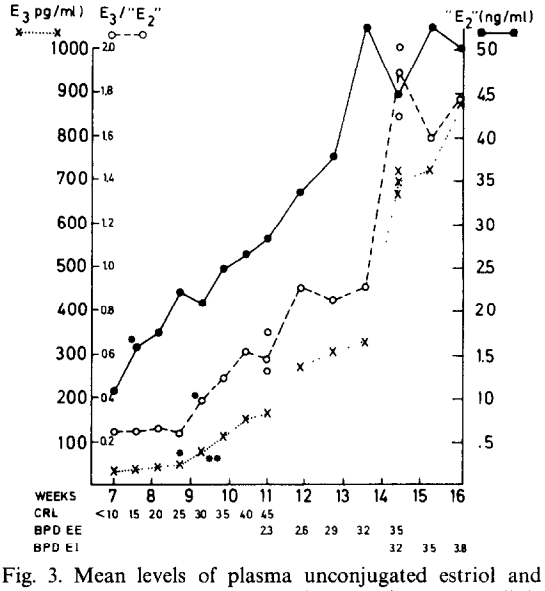

Fig. 3. Mean levels of plasma unconjugated estriol and "estradiol" and mean of the individual estriol to "estradiol" ratios. Legends as in Fig. 1. *: first significant increase beyond the seventh week level. **: significant increase beyond the preceding level.

Table 1. Plasma unconjugated estriol and "estradiol" in cases of fetal death and molar pregnancies

Subjects	Gestational age (Weeks)	"Estradiol" ng/ml	Estriol ng/ml	Estriol / "Estradiol"
Fe...(fetal death)	27	9.7 (6–30)*	0.047 (3–8)	0.0048
Cr...(fetal death)	38	12.4 (11–50)	<0.1 (6–30)	<0.0080
An ..(fetal death)	16	2.4 (2.3–12)	0.020 (0.5–2.0)	0.0083
Pe...(mole)	20	6.4 (4–18)	0.018 (1.5–4.0)	0.0028
Be...(mole)	20	0.6 (4–18)	0.012 (1.5–4.0)	0.020
J....(mole)	16	3.5 (2.3–12)	0.035 (0.5–2.0)	0.010

* Normal values under brackets.

2. *Plasma levels of unconjugated "estradiol"*

Figure 2 shows the individual values for unconjugated "estradiol" from the 7th to the 16th week of pregnancy. The gestational age is indicated as for Fig. 1. The mean increase (Fig. 3) and the scatter of the individual results are comparable to previous measurements made with the same technique in 500 other pregnancy plasma samples [12]. As already mentioned under "Material and Methods", these results are higher than those obtained for specific estradiol-17β, although they parallel the increase of the latter during this period of pregnancy [1].

3. *Comparison between the fluctuations of unconjugated estriol and "estradiol" levels*

Figure 3 shows the fluctuation of the mean estriol to "estradiol" ratio, calculated from the individual measurements. The ratio remained stable up to a CRL value of 21–25 mm. A significant rise ($P < 0.005$) of the ratio occurred at a CRL value of 26–30 mm. This rise of the estriol to "estradiol" ratio continued thereafter.

4. *Unconjugated estriol and "estradiol" in a few cases of fetal death or molar pregnancies*

Table 1 shows the levels of unconjugated estrogens in three cases of molar pregnancies and three cases of fetal death. In the latter three cases, the fetuses died for reasons unrelated to placental insufficiency; HCS (human chorionic somatomammotropin) levels were still normal when plasma estrogens were measured. Plasma "estradiol" levels were normal (low normal range) in the three cases of fetal death and in two of the molar pregnancies. Plasma estriol levels were below 50 pg/ml in five out of six cases. In patient Cr..., the level was below 100 pg, although the exact figure could not be determined due to the excessive dilution of the plasma sample. Hence, in five out of these six subjects, the estriol levels were below those encountered when the CRL of the fetus exceeds 30 mm.

DISCUSSION

The fetal adrenals are able to realize the synthesis of steroids *in vitro*, as early as the tenth week of pregnancy [15–18]. By this time, all the necessary enzymes are present except for a relative lack of 5-ene-3β-hydroxysteroid dehydrogenase [17], necessary for the "de novo" synthesis of cortisol. Johannisson[19] has given cytological evidences of a secretory potentiality of the fetal adrenals during the first trimester of pregnancy.

Siiteri and MacDonald[20] have shown that the production of estriol in pregnant women was realized by a biosynthetic route independent of estradiol, and that this route was largely of fetal origin, already by the end of the first trimester of pregnancy.

This route is now well known and involves the secretion of dehydroepiandrosterone sulphate by the fetal adrenals, the 16-α hydroxylation of this precursor within the fetal liver [21] and the aromatization of the latter product into estriol at the placental level. A maternal contribution to the production of estriol by the estradiol independent route was described by Siiteri and MacDonald[20], as well as by Barlow *et al.* [22], very early in pregnancy.

With the present data, we can confirm the minor contribution of the estradiol dependent route to the production of plasma unconjugated estriol. Indeed, in cases of fetal death or of molar pregnancies, despite the presence of large amounts of estradiol and estrone ("estradiol" in Table 1), the estriol levels were very low, and the estriol to "estradiol" ratios were much lower than those encountered even in early pregnancy. Hence, the estriol levels encountered as early as the seventh week, significantly higher than those obtained outside pregnancy, may represent a maternal contribution by a route independent of estradiol. The latter might involve, as suggested by Barlow, the hydroxylation of maternal dehydroepiandrosterone in the maternal liver, and the aromatization into estriol by the placenta.

In a previous longitudinal study [1], we described the steep increase of plasma unconjugated estriol by the 10th to 12th week of pregnancy, in a group of 22 normal women, followed throughout pregnancy. The steep increase of the estriol to estradiol-17β ratio and the fall of the HCS to estriol ratio after the twelfth week lead us to conclude that the fetal adrenals were increasing their secretory activity by that time. This estriol increase was not observed in one case of molar pregnancy [7]. In the present work, we correlated the appearance of increasing amounts of estriol in maternal plasma with the size of the fetus

measured by ultrasonic morphometry. Although the latter measurement involves some technical error [23], it is more precise than the gestational age calculated from the first day of the last menstrual period. With this technique, it appeared that a significant increase of unconjugated estriol together with a significant increase of the estriol to "estradiol" ratio occurred as early as the 9th to 10th week of pregnancy (CRL of 30 mm). The increase continued thereafter, confirming the preceding findings [1]. The increase in the estriol to "estradiol" ratio implies that a new source of estriol precursors develop an increasing activity by that time. The fetal origin of this source is very likely, in view of the low estriol levels encountered in a few cases of molar pregnancies and of fetal death (Table 1). Although these cases may not be representative of their respective pathology, they show that even late in pregnancy, a functioning placenta, lacking precursors from fetal origin, may not be able to maintain plasma levels of estriol exceeding those encountered before the tenth week of pregnancy.

In vivo [24] and *in vitro* [25,26] experiments have directly demonstrated the existence of a stimulatory activity of ACTH on the fetal adrenals, early in pregnancy. On the other hand, administration of corticosteroids to the pregnant woman produces a considerable drop in the urinary estrogens [24,27] confirming previous evidences for an ACTH mediated regulatory mechanism of fetal adrenal function [28]. Evidences have been provided for the presence of ACTH in fetal pituitary as soon as the tenth week of gestation [29,30]. Recently, Isherwood and Oakey [18] showed that ACTH could stimulate *in vitro* an 18 week fetal adrenal to incorporate acetate into steroids, but not a 10 week gland. Other peptides, like growth hormone or human chorionic somatomammotropin were also able to exert a stimulatory activity [18]. Growth hormone has been the subject of controversy concerning its trophic activity on the fetal adrenals [28]. A short report by Chochinov *et al.*[31] describes a steep increase of the growth hormone levels in the amniotic fluid by the eleventh week of pregnancy. Other less defined pituitary peptides [32], as well as human chorionic gonadotrophin [17,28] have been, or still are candidates as fetal adrenal corticotrophins.

The present data, strongly suggesting an increasing secretory activity of the fetal adrenals as early as the tenth week of pregnancy when the mean crown-rump length of the fetus is of the order of 30–35 mm, emphasize the importance of this early period of gestation in the setting up of the adrenal function.

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