J. Perinat. Med.
9 (1981) 40

Variations in some of the body fluid hormone levels currently used for endocrine monitoring during the third trimester of pregnancy

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1 Introduction

In recent years there has been an increased possibility to investigate the function of the feto-placental unit with new radioimmunological methods for several hormones, both steroids and proteins, in urine, maternal plasma and in the amniotic fluid.

In the laboratories of the Obstetric and Gynecological Clinic of the University of Genoa we have monitored third trimester of pregnancy by immunoassay of total Estriol (E_3), Estetrol (E_4), Human Placental Lactogen (HPL) and Pregnancy specific beta$_1$ glycoprotein (SP_1) in maternal plasma and of E_3 and E_4 in amniotic fluid.

We have already discussed in earlier publications why we prefer to measure plasma E_3 rather urinary, and total plasma E_3 rather than free [5, 6] and why we fell that the minimum for valid monitoring is the evaluation of HPL plus E_3 [5].

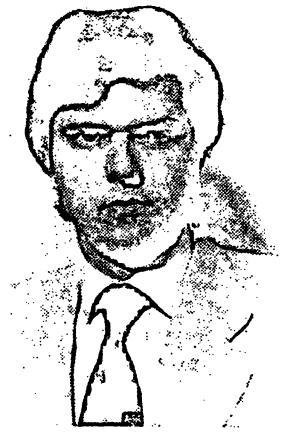
We have determinated our laboratory's mean normal values and the standard deviations of the means for E_3 and HPL and we have found that our assay procedures and values are similar to those reported in the literature for third trimester of pregnancy [5]. We have used as standard values for SP_1 those of the normal range published by TOWLER et al. [25], while we have used those of TULCHINSKY et al. [29] for our normal reference values for E_4 during pregnancy.

At the moment we are still accumulating assay values for SP_1 and E_4 for each week of pregnancy in the Ligurian women who come to our Institute. Our previous data [6] appear to have ruled out the occurrence of any circadian rhythm of E_3. Although we have only preliminary data about the concentrations of E_3 and E_4 in amniotic fluid [4], our results with SP_1 assays during normal pregnancies [7] have confirmed its usefulness as a valid test for placental function, especially in pregnancy disorders and in pregnancies with retarded intrauterine growth.

In this paper we wish to report on the circadian and day to day variations of all four hormone levels (E_3, E_4, HPL and SP_1) in plasma.

This Work was supported by research grants from the "Progetto finalizzato di Biologia della Riproduzione", Contratto del Centro Nazionale Ricerche (C.N.R.) n° 79.01144.85

Curriculum vitae



ERMANNO PEDRETTI *was born in Genoa in 1951. He studied Medicine at Genoa University, where he graduated in 1976. He specialised at the Department of Obstetrics and Gynecology (Chairmen Prof.* G. PESCETTO *and Prof.* L. DE CECCO*), University of Genoa, where he has been working since 1976. His main interests are quite different: Hormonal monitoring throughout pregnancy and contraception.*

033-5577/81/0009-0004$02.00

2 Materials and methods

Seven volunteer patients were used for the study of the circadian rhythm. Blood was taken at 8 and 12 a.m., 4, 8, 12 p.m. and again at 8 a.m. of the next day.

Eight patients took part in the study of the day to day variations. Fasting blood was taken every day for 5 days, at 8 a.m. The blood samples were immediatly centrifugated and the plasmas stored at -20 °C until analysis. All assays were performed on triplicate aliquots.

All the patients were in the third trimester of normal pregnancy between the 26th week (one case) and the 40th week after last menstruation. All the patients were clinically normal (increase in weight, blood pressure, uterine growth) had no albuminuria and normal blood glucose levels. They delivered spontaneously and without complications, all giving birth to live babies in good condition, with normal weights for their gestational ages, which had been evaluated in utero from the fetal biparietal and transthoracic diameters in bidimensional ultrasuonograms (B-scans) and were evaluated at birth as being between the 10th and the 90th percentiles [20].

Total E_3 was assayed with the radioimmunoassay kits obtained from the Radiochemical Center AMERSHAM (England).

A HEWLETT-PACKARD gamma-counter, model 1511, was used for counting.

The interassay variation was 12% and the intra-assay variation was less than 9%.

Unconjugated E_4 was assayed using the method of OLIVIERI and MANNA [19] using radioimmunoassay kits from BIODATA, Milan (Italy).

A BECKMAN LS 100 beta counter was used for counting.

The interassay variation was 14% and the intra-assay variation was 8.8%.

HPL was assayed by the method of GENAZZANI et al. [11] with radioimmunoassay kits obtained from C. I. S. SORIN, Saluggia, Italy.

The interassay variation was 8% and the intra-assay variation was 5%.

SP_1 was measured by BOHN's simple radial immunodiffusion method [2] using M-Partigen beta$_1$-SP_1 glycoprotein plates obtained from BEHRINGWERKE AG (Marburg, West Germany).

The diametra of precipitate rings were measured with a suitable device and concentrations read off a linear plot of diameter vs. concentration obtained with the standard.

3 Results

The concentrations of all the hormones in the plasma fell within the normal ranges.

The day to day variations for E_3 and E_4 are shown in Fig. 1 and for HPL and SP_1 in Fig. 2.

Fig. 3 and 4 show the variations within a single day of the four hormones.

4 Discussion

Statistical analysis of our data showed that none of the parameters demonstrate any circadian rhythm, which confirms previous reports about E_3 [6, 10, 16, 17], E_4 [18, 29] and HPL [24].

There were striking variations during the day in the steroids hormone levels, especially those of E_4, but much less so for the protein hormones, especially SP_1.

There were considerable differences in steroid hormone levels between different patients at the same stage of pregnancy and in the individual subjects at the same time of day over the five days, as well as a very considerable standard deviation.

These differences were at least in part due to the large interassay coefficient of variation.

The differences and the standard deviations were much less marked for the protein hormones.

The day to day differences were also more marked for the steroids (especially E_4) than for the protein hormones.

The least variable of all was SP_1.

5 Conclusions

There is no circadian rhythm of estriol, estetrol, HPL and SP_1. The within a day and day to day variations are much greater for the steroid hormones than for the protein ones. They are greater for E_4 than for E_3 and for HPL than for SP_1.

The standard deviations were great for the steroids (especially for E_4) and much less for the proteins (especially SP_1).

This implies that it is necessary to have serial assays of the steroids to have any confidence in

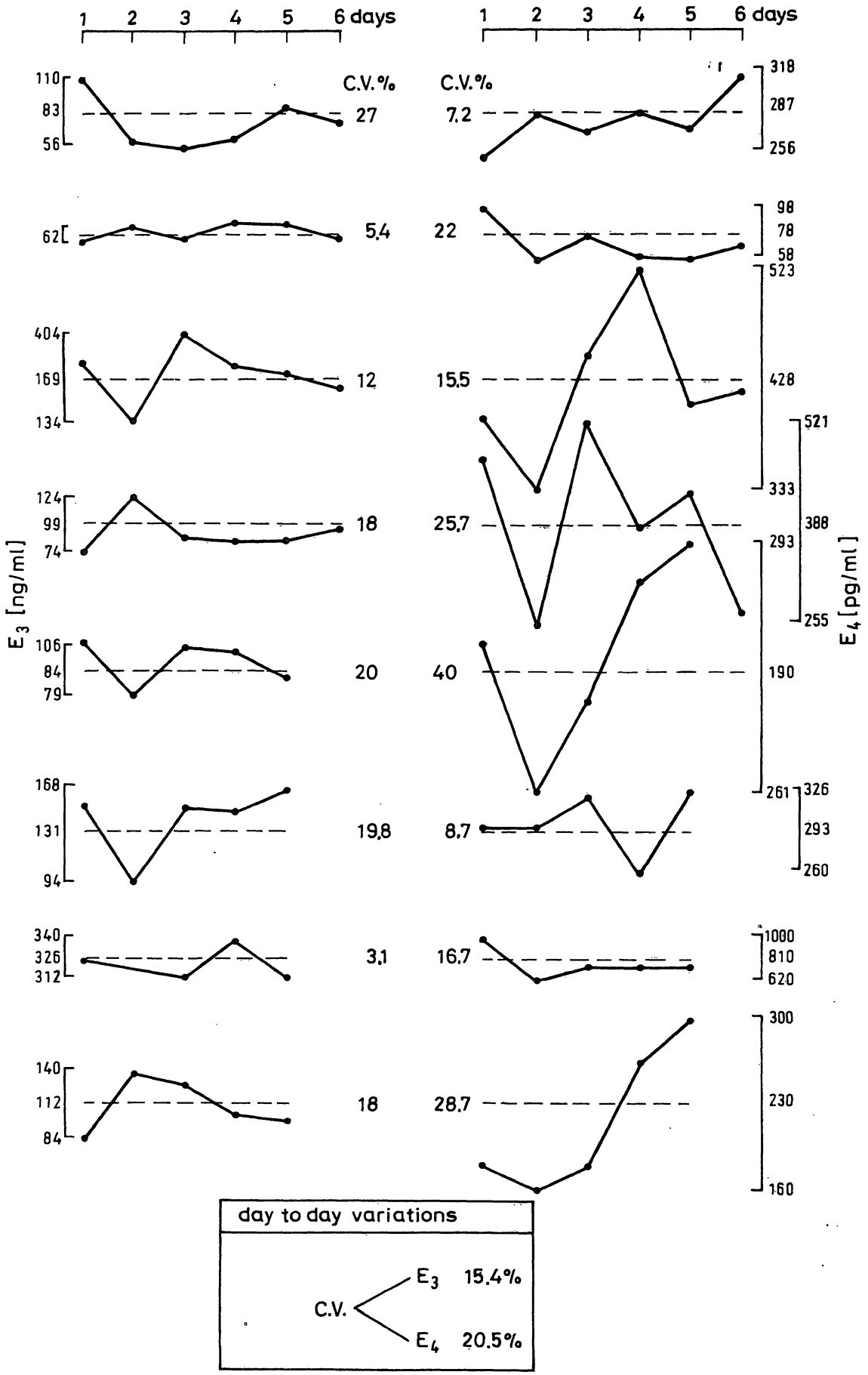


Fig. 1. Day to day variations for E_3 and E_4.

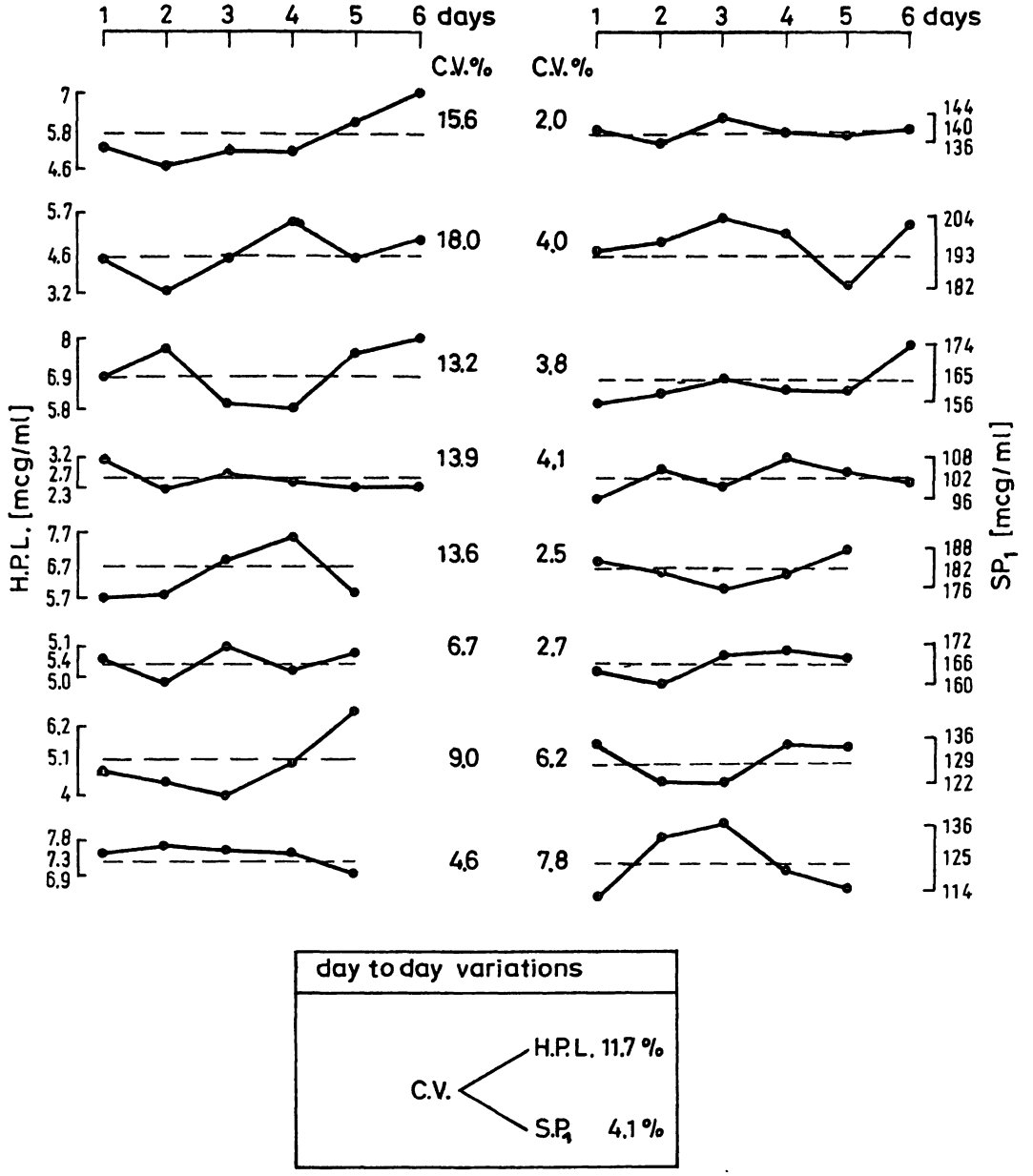


Fig. 2. Day to day variations for HPL and SP_1.

the levels while good monitoring could be guaranteed with assays for HPL and SP_1.

The evaluation of plasma concentrations of HPL and SP_1 yields similar information about placental function.

SP_1 shows less variation in the immunoassay than does HPL, but its half-life is 34 hours [3,23], against a 20 minute half-life for HPL [24]. This means that even though a single assay may give more accurate picture of placental function [26], if HPL samples taken closer together are assayed, one can have a more up-to-the-minute indication of the development of a pathological condition in the placenta.

The steroid hormones we have considered, differently from polipeptidic hormones, derive from different metabolic sources, even if both can give us useful indications about the well-being of the fetus-placental unity. In fact, we know that E_3 is sinthesized in the placenta from fetal precursors [21], while E_4 is mainly sinthesized in the fetal liver from placental precursors [13, 22, 29, 31]. Therefore, we can't overlap the theoric value of these two parameters, but we have studied the possibility of compare these two assays as clinical parameters for testing the fetus-placental unity to

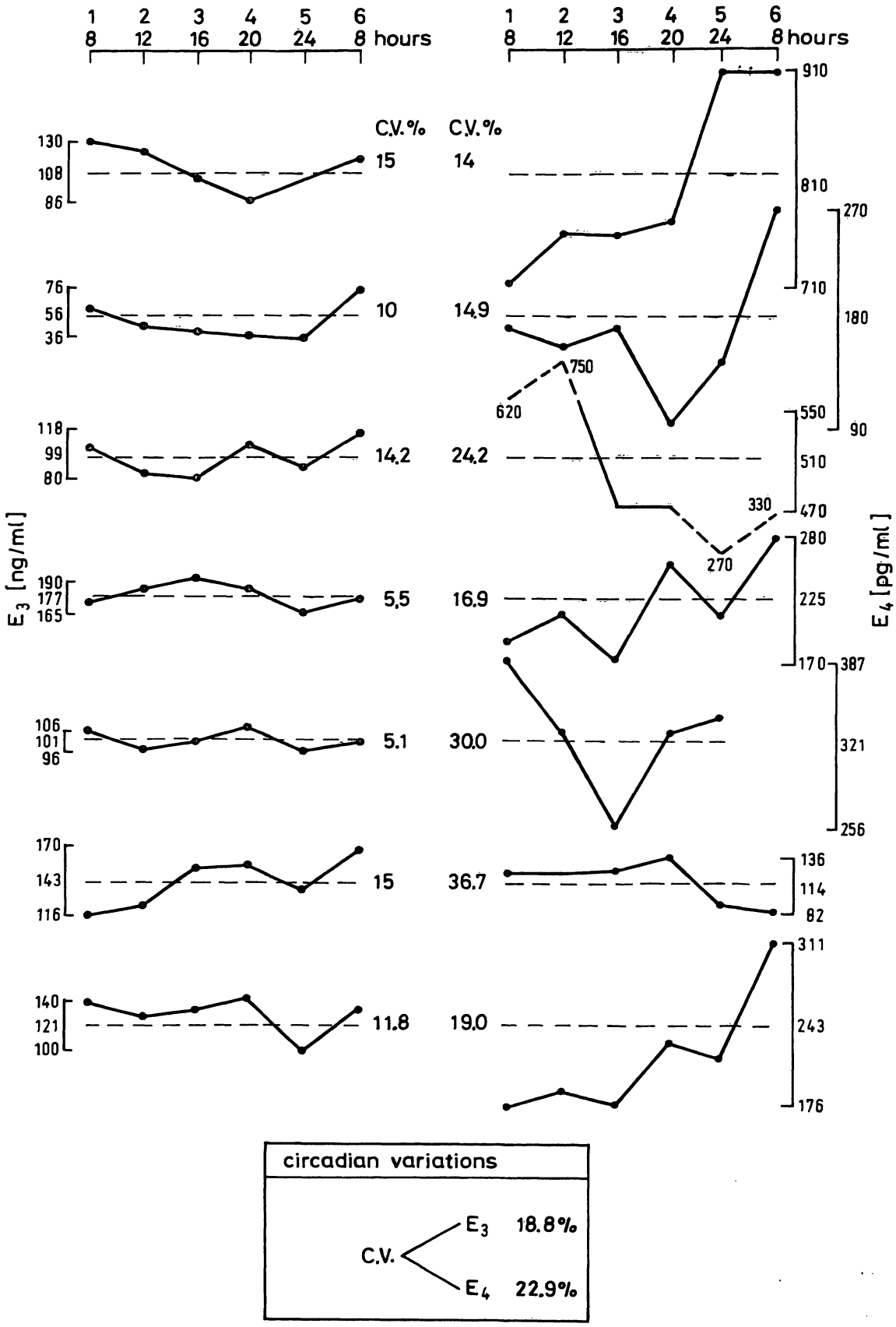


Fig. 3. Circadian variations of E_3 and E_4.

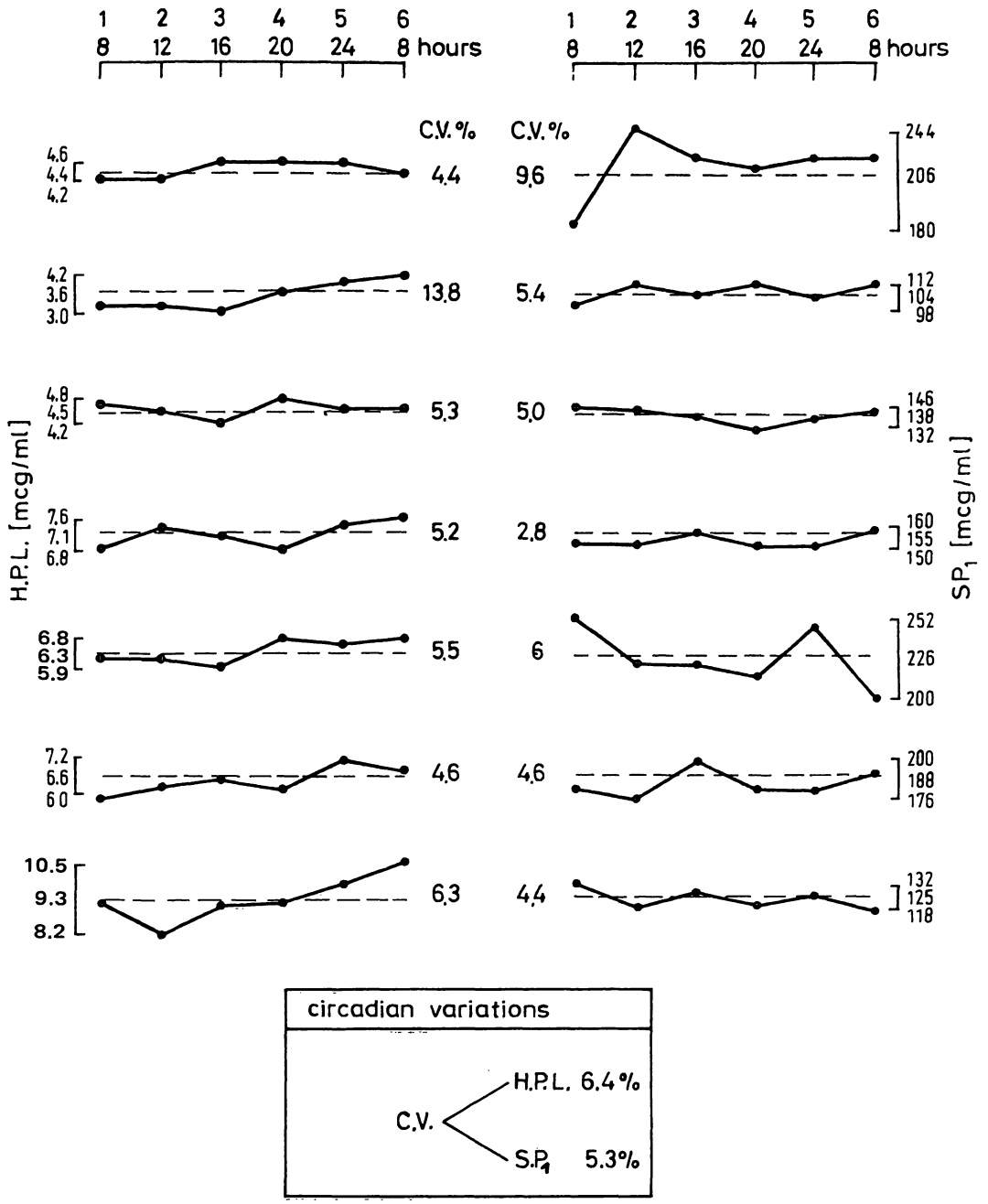


Fig. 4. Circadian variations of HPL and SP_1.

have better informations about the well-being of the fetus [1, 18, 29]. Moreover, in contrast with the first announcements about E_4 as the most specific parameter for monitoring some pathological pregnancies (i.e. isoimmunization Rh, anencephaly, preeclampsia) [12, 14, 22, 27, 28], most of the authors now suggest that the E_4 assay can be useful only in few cases (i.e. gestosis) [15, 29]. At last, various authors have very different opinions about the serial assay of E_4 after intravenous injection of DHAS [1, 8, 30].

Moreover, the only study we know about the RIA of conjugated E_4 does not seem to demonstrate any advantages of this assay in comparison with the RIA of unconjugated E_4 [9].

On the ground of our studies and our clinical experiences then, for want of a conclusive test, we think that it's better to assay total E_3 instead of unconjugated E_4 for its less biochemical variability and its easier and faster method of assaying.

Summary

We have investigated the variability of four hormonal parameters used for the endocrine monitoring of the third trimester of pregnancy (E_3, E_4, HPL, SP_1).
We have observed no circadian rhythm of Estriol, Estetrol, HPL and SP_1. The within-a-day and the day-to-day variations are much greater for E_4 than for E_3 and for HPL than for SP_1.
The standard deviations were great for the steroids, especially for E_4 and much less for the protein hormones, especially SP_1.
This implies that it is necessary to have serial assays of the steroids to have any confidence in the levels, while good monitoring could be guaranteed with a smaller number of assays for HPL and SP_1.

Keywords: Estetrol, estriol, human placental lactogen, pregnancy-specific-beta$_1$-glycoprotein, pregnancy, third trimester.

Zusammenfassung

Schwankungsbreiten bei Hormonbestimmungen während der endokrinen Überwachung im letzten Schwangerschaftstrimester
Uns interessierten die Schwankungsbreiten bei 4 Hormonbestimmungen, die als Parameter für die endokrine Überwachung während des letzten Schwangerschaftstrimesters gebräuchlich sind (E4, E3, HPL, SP1).
Bei keinem der 4 Hormone ließ sich ein zirkadianer Rhythmus feststellen. Die Schwankungsbreite innerhalb von 24 Stunden wie auch Variationen von Tag zu Tag sind jedoch bei E_4 größer als bei E_3 sowie bei HPL größer als bei SP_1.
Hohe Standardabweichungen fanden wir bei den Steroidhormonen, speziell bei E_4, während Proteohormonbestimmungen, speziell bei E_4, während Proteobestimmungen, und hier besonders SP_1-Messungen, geringere Standardabweichungen aufwiesen. Das bedeutet, daß für Steroidhormonbestimmungen Serienmessungen unbedingt notwendig sind, während man bei HPL- und SP_1-Bestimmungen auch mit weniger Proben eine ausreichende Genauigkeit erhält.

Schlüsselwörter: HPL, letztes Schwangerschaftstrimester, Östetrol (E_4), Östriol (E_3), SP_1.

Résumé

Variations de quelques taux hormonaux dans les liquides du corps usuellement utilisés pour la surveillance endocrinienne pendant le troisième trimestre de grossesse
Nous avons exploré la variabilité des quatre paramètres hormonaux utilisés dans la surveillance endocrine du troisième trimestre de grossesse (E_3, E_4, HPL, SP_1).
Nous avons observé l'absence de rythme circadien pour l'oestradiol. l'oestétrol, l'HPL et la SP_1. Les variations au cours d'une journée et d'une journée à l'autre sont plus importantes pour l'E_4 que pour l'E_3 et pour la HPL que pour la SP_1.
L'ecart-type était important pour les stéroides, specialement pour la E_4, alors qu'il était faible pour les hormones protéiques, notamment la SP_1.
Ceci signifie qu'il est nécéssaire de pratiquer des dosages en série des stéroides afin d'obtenir des valeurs fiables alors qU'une surveillance de qualité peut être assurée au moyen d'un petit nombre de dosages d'HPL et de SP_1.

Mots-clés: p_1-glycoprotéine spécifique de grossesse, grossesse, hormone placentaire lactogène humaine, oestetrol, oestriol, troisième trimestre.

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Received March 23, 1980. Revised June 26, 1980. Accepted September 1, 1980.

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