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Direct chemiluminescence immunoassay of estriol and progesterone and their ratio during pregnancy

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Abstract

Estriol (E3) and progesterone (P) concentrations in saliva were determined by direct chemiluminescence immunoassays, using solid-phase monoclonal antibodies bound to the wells of microtitre plates, and isoluminol-labelled steroid conjugates. Saliva samples were obtained from women during pregnancy, from 34 to 1 weeks before delivery. The smoothed median and mean salivary E3 : P ratios in normal pregnancy rose gradually from 0.45 at −34 weeks to 1.5 at −1 week. In twin pregnancy a similar rise was observed. However, in preterm delivery the smoothed mean ratio rose more slowly, from 0.6 at −30 weeks to 1.1 at −1 week. In pregnancy associated with intra-uterine growth retardation, the smoothed mean ratio did not reach a value of 1 towards the end of pregnancy. In all instances high and low E3 : P ratios were observed shortly before spontaneous delivery. This raises the question of whether the E3 : P ratio could be used as a predictor providing useful information in relation to the onset of labour.

Keywords: Bioluminescence; Immunoassay; Estriol; Hormones; Progesterone; Saliva

1. INTRODUCTION

Steroid hormones, particularly estrogens and progesterone, are considered important in the onset of spontaneous labour [1]. Changes in the concentrations of these hormones in the peripheral circulation, i.e., a fall of the progesterone levels or a rise of the estrogen-to-progesterone ratio in late pregnancy, have been reported [2,3]. However, in other studies no confirmation of these changes was found [4–7]. Another way to study possible changes in steroid hormone concentrations is through the analysis of saliva samples. Saliva steroid concentrations are thought to reflect the unbound (i.e., the free, biologically active) fraction of steroid hormone levels in the peripheral circulation [8,9]. In addition, saliva sampling is simple, easy and stress free, and multiple samples can be obtained. During pregnancy, good correlations between the salivary and plasma concentrations of estriol (E3) and progesterone (P) have been obtained [10–15]. The salivary P and E3 concentrations were found to reflect reliably the serum free P [16] and free, unconjugated E3, respectively [11].

Consequently, several groups have investigated the variation of salivary estriol and progesterone and the E3 : P ratio during the second half of

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pregnancy [16–20]. One group reported a slow rise in the median E3:P ratio to 1 at 3 weeks before delivery (−3 weeks) and then a rapid rise to 1.4 the day before the spontaneous onset of labour [17]. The question was raised of the role of E3 in initiating spontaneous labour in humans. In a subsequent study, the rapid rise of the E3:P ratio above 1 was found to begin at −5 weeks [16]. These results, however, could not be confirmed by several groups of investigators. Some observed a continuous rise in the E3:P ratio above 1 either during the last 9 weeks of pregnancy [18] or from 14 weeks before spontaneous delivery [19]. Others found no evidence for a rise and noticed a steady high mean E3:P ratio, well above 1, during the last 6 weeks of pregnancy [20].

These studies were mainly performed with radioimmunoassay (RIA) using extracts of saliva samples. This is a time-consuming and error-prone method. Recently, we have developed chemiluminescence immunoassays (CIA) for the determination of E3 and P in saliva [21,22]. The assays are direct (i.e., without prior extraction of the steroid), reliable and easy to perform.

In an attempt to gain more insight into this confusing matter, we set out to determine with CIA the concentrations of E3 and P in saliva during pregnancy. Because data on abnormal pregnancies are very scarce or absent, we also determined the E3:P ratio in different types of abnormal, pathological pregnancies: twin pregnancy, intra-uterine growth retardation and preterm delivery.

2. EXPERIMENTAL

2.1. Patients and sample collection

Unstimulated saliva was obtained between 08.00 and 12.00 h from pregnant women (gestational age 5–41 weeks) attending the Women's Clinic at the University Hospital, Ghent. Patients received instructions for collecting whole mixed saliva: rinse the mouth several times with tap water and start to collect saliva in a plastic vial ca. 5 min later. Samples were frozen within 10 min after collection and were stored at −20°C. Before assay, saliva was thawed and centrifuged (15 min at 3000 *g*) and the clear supernate was transferred into plastic tubes. If the supernate volume was < 0.5 ml, or it was either unclear or slightly coloured, it was discarded. Duplicate 50-μl aliquots of the clear supernatant liquid were used for direct CIA of estriol and of progesterone.

Four groups of patients were considered, as follows:

(1) Normal, uncomplicated pregnancy. This group included 102 patients from whom 212 usable saliva samples were obtained. Pregnancy terminated after 38–41 weeks of gestation. Labour was spontaneous (54 patients, 122 saliva samples) or was induced with prostaglandins (48 patients, 90 saliva samples). In all instances a healthy baby was born, with a birthweight above the 10th centile.

(2) Twin pregnancy, including 7 patients from whom 44 saliva samples were analyzed. Pregnancy terminated after 36–39 weeks with spontaneous labour (4 patients, 17 samples), induction of labour with prostaglandins (1 patient, 3 samples) or with Caesarean section (2 patients, 24 samples).

(3) Preterm delivery including 15 patients and 53 samples. Pregnancy terminated after 34–37 weeks with spontaneous labour (13 patients, 46 samples) or induction of labour with prostaglandins (2 patients, 7 samples).

(4) Intra-uterine growth retardation (IUGR). This group consisted of 14 patients who delivered 28 usable saliva samples. Pregnancy terminated after 36–41 weeks with spontaneous labour (7 patients, 15 samples) or was induced in 7 others (13 samples). The birthweight of the newborns was below the 10th centile.

2.2. Chemicals and reagents

Chemicals and different reagents, including the chemiluminescent conjugates estriol-6-carboxymethyloxime aminopentylethylisoluminol (E3-6-APEI) and progesterone-11α-hemisuccinate aminobutylethylisoluminol (P-11-ABEI), have been described previously [21,22].

2.3. Chemiluminescence immunoassay (CIA)

The CIAs for E3 and P have been described in detail previously [21,22]. Briefly, they were performed in microtitre plates. These were coated with second rabbit antimouse IgG antibody, to which the primary monoclonal antisteroid antibody was subsequently bound. To the wells were added 50 μl of standard or assay buffer, 50 μl of the saliva sample to be analysed or of male saliva for the standards [21,22] and 100 μl of the chemiluminescent conjugate in assay buffer. The plates were incubated for 10 min (E3) or 3.5 h (P) at room temperature on a horizontal shaker. Incubation was stopped by emptying and washing the wells. The chemiluminescence of the antibody-bound E3-6-APEI or P-11-ABEI was measured in a Lumac Biocounter M 2000 or M 2010 [23].

2.4. Calculation of results

Non-specific blank values were subtracted from the Biocounter readings and B/B_0 vs. log (dose of standards) was plotted as a polynomial using the RIA-CALC program from Pharmacia–LKB. Saliva steroid concentrations were expressed as nmol l^{-1}. A Statview program from Macintosh was used to calculate and draw a best-fit second-order polynomial regression line through the points in the graphs of E3:P ratio vs. weeks before delivery. As a result, two types of best-fit curves were calculated: a smoothed median through the median E3:P ratios and a smoothed mean through all E3:P values. In normal pregnancy the number of E3:P ratios was sufficient to calculate a smoothed median. In pathological pregnancy a smoothed mean curve was calculated. For comparative purposes a smoothed mean was also calculated for normal pregnancy.

3. RESULTS AND DISCUSSION

3.1. Reliability of the assays

In the present series of immunoassays, the within- and between-assay precision, calculated as the relative standard deviation (R.S.D.), was essentially as observed previously [21,22]. For E3, the within- and between-assay R.S.D.s were 5–11% and 6–14%, respectively. For P the within- and between-assay R.S.D.s were 3–10%. The recovery of added steroids ranged between 91 and 105%. These CIAs were sufficiently sensitive to need only 50 μl of saliva sample for the direct determination of E3 and P. They use microtitre plates as a solid phase for binding antibodies and for easy separation of antibody-bound and free fractions. This resulted in simple and rapid assays that were easy to perform. This was very convenient for the analysis of a large number of saliva samples.

3.2. E3:P ratio

In each of the four groups of patients no significant differences were observed in the saliva E3:P ratios from −34 weeks to −1 week, obtained from patients with spontaneous onset of labour on the one hand and from treated patients (prostaglandins for induction of labour or Caesarean section) on the other. All E3:P ratios in a group could therefore be considered as one population.

The E3:P ratio of the saliva samples and a smoothed median and mean through these ratios are presented in Figs. 1 and 2. In Fig. 1A median values of the E3:P ratio in normal, uncomplicated pregnancy (212 samples from 102 patients) are shown. In Fig. 1B the smoothed mean of all E3:P ratios is presented. In both instances the median and mean E3:P ratios rose gradually from 0.45 at −34 weeks to 1.5 at −1 week, reaching a value of 1 at −12 weeks (median) and at −16 weeks (mean). Evidently an abrupt rise in this ratio near delivery was absent. Lewis et al. [20] reported only on the last 6 weeks of pregnancy. The E3:P ratio in their study fluctuated between 2.8 and 1.4 during this period, but showed no rise. Perry et al. [19] observed a continuous rise of the E3:P ratio from −14 weeks until birth and Zubke et al. [18] found an increase in the ratio above 1 approximately 10 weeks before delivery. These observations failed to confirm the abrupt rise above 1 of the E3:P ratio 3–5 weeks before delivery observed by McGar-

rigle and co-workers [16,17]. A comparable controversy is encountered in earlier studies on E3 and P concentrations in the peripheral circulation. A decrease in blood progesterone concentrations and a rise in the estrogen : P ratio in late pregnancy were reported [2,3], but this could not be confirmed [4–7].

Methodological differences may be responsible for the observed discrepancies which up to now have remained unexplained. It has been accepted that the biological activity of the steroid hormones present in the peripheral circulation is not reflected by the total steroid concentration but rather by the unbound (i.e., free) fraction [24], and that steroid hormone concentrations in saliva represent a true estimate of this free fraction in the circulation [8,9]. If an increase in the concentration of E3 (and not of progesterone) were to play a role in initiating spontaneous labour in humans, as was recently suggested, this should be reflected by the biologically active fraction and thus by an increase in the salivary E3 : P ratio [17]. Such a rise was indeed observed in this study and also by others [16–19]. In one study a high E3 : P ratio was seen but a rise was not reported [20].

In Fig. 2A the E3 : P ratios in twin pregnancy are presented (44 saliva samples from 7 patients). The smoothed mean of E3 : P is almost identical with that observed in normal pregnancy (Fig. 1B). In contrast, the slope of the graph representing the smoothed mean E3 : P ratio observed in preterm delivery (53 saliva samples from 15 patients) is shallow (Fig. 2B). The graph reaches

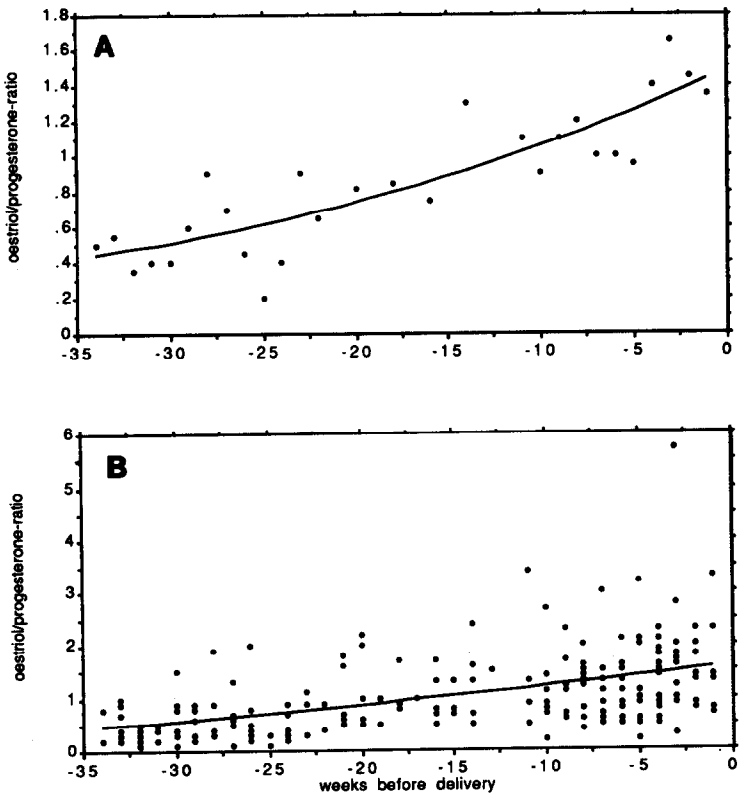

Fig. 1. Smoothed (A) median and (B) mean estriol-to-progesterone ratio in normal pregnancy.

only a value of 1.1 at −1 week. In pregnancy associated with IUGR the smoothed mean graph of the E3 : P ratios does not reach a value of 1.

It is remarkable that in the groups of pathological pregnancy, e.g., the group preterm delivery and the group IUGR, spontaneous onset of labour was noticed in patients whose saliva E3 : P ratios varied from 0.1 to 1.9 1–2 weeks before delivery. If indeed E3 is assumed to play a role in initiating spontaneous labour, the very low ratios (below 1.0) shortly before delivery cannot be explained. It is therefore our opinion that, although the salivary E3 : P ratio may in most instances provide interesting information concerning foetal well be-

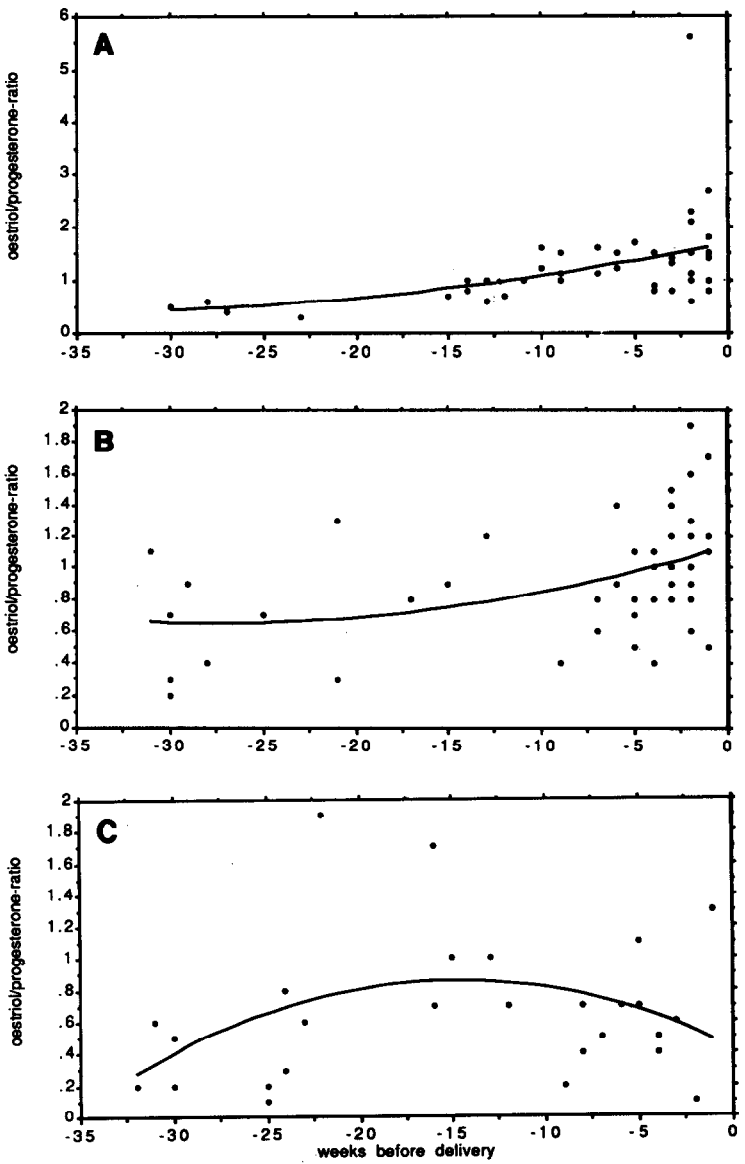

Fig. 2. Smoothed mean estriol-to-progesterone ratio in (A) twin pregnancy, (B) preterm delivery and (C) intra-uterine growth retardation.

ing, it cannot be used as a predictor providing useful new information in relation to the onset of labour.

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REFERENCES

[1] G.C. Liggins, R.G. Fairclough, S.A. Grieves, J.Z. Kendall and B. Knox, Recent Prog. Horm. Res., 29 (1973) 111.
[2] A.I. Csapo, E. Knobil, H.J. van der Molen and W.G. Wiest, Am. J. Obstet. Gynecol., 110 (1971) 630.
[3] A.C. Turnbull, P.T. Patten, A.P. Flint, M.J. Keirse, J.Y. Jeremy and A.B. Anderson, Lancet, i (1974) 101.
[4] M.M. Shaaban and A. Klopper, J. Obstet. Gynaecol. Br. Commonw., 80 (1973) 210.
[5] R.S. Boroditsky, F.I. Reyes, J.S. Winter and C. Faiman, Obstet. Gynecol., 51 (1978) 686.
[6] R.S. Mathur, S. Landgrebe and H.O. Williamson, Am. J. Obstet. Gynecol., 136 (1980) 25.
[7] A.L. Hartikainen-Sorri, A. Kauppila, R. Tuimala, L. Vinikka and O. Ylikorkla, Acta Obstet. Gynaecol. Scand., 60 (1981) 497.
[8] F.H. Katz and I.L. Shannon, Acta Endocrinol., 46 (1964) 393.
[9] R.F. Vining, R.A. McGinley and R.G. Symons, Clin. Chem., 29 (1983) 1752.
[10] P.L. Truran, G.F. Read and S. Walker, Clin. Chem., 28 (1982) 2393.
[11] R.F. Vining, R. McGinley and B.V. Rice, J. Clin. Endocrinol. Metab., 56 (1983) 454.
[12] N. Kundu, N. Novak and L.P. Petersen, Steroids, 41 (1983) 145.
[13] I.J. Evans, A.R. Wilkinson and D.R. Aickin, Clin. Chem., 30 (1984) 120.
[14] L.A. Perry, N. Wathen and T. Chard, Horm. Metab. Res., 19 (1987) 444.
[15] P.M.M. Meulenberg and J.A. Hofman, Clin. Chem., 35 (1988) 168.
[16] J. Darne, H.H.G. McGarrigle and G.L. Lachelin, Br. J. Obstet. Gynaecol., 94 (1987) 227.
[17] H.H.G. McGarrigle and G.C.L. Lachelin, Br. Med. J., 289 (1984) 457.
[18] W. Zubke, D. Colic, H. Franz and R. Haeckel, J. Clin. Chem. Clin. Biochem., 27 (1989) 237.
[19] L. Perry, N. Wathen and T. Chard, Br. Med. J., 289 (1984) 1004.
[20] P.R. Lewis, P.M. Galvin and R.V. Short, J. Endocrinol., 115 (1987) 177.
[21] J. De Boever, F. Kohen, W. Ulrix and D. Vandekerckhove, Anal. Chim. Acta, 205 (1988) 215.
[22] J. De Boever, F. Kohen and D. Vandekerckhove, Anal. Chim. Acta, 227 (1989) 119.
[23] J. De Boever, F. Kohen, C. Usanachitt, D. Vandekerckhove, D. Leyseele and L. Vandewalle, Clin. Chem., 32 (1986) 1895.
[24] R.L. Rosenfield, J. Clin. Endocrinol., 32 (1971) 717.