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DIAGNOSTIC RELEVANCE OF OESTROGEN ESTIMATIONS IN HUMAN PREGNANCY

R. E. OAKEY
Division of Steroid Endocrinology, Department of Chemical Pathology, School of Medicine, University of Leeds, Leeds, England

SUMMARY

The application of measurements of urinary oestrogen excretion and plasma oestrogen concentration for the prediction of fetal death have been compared. It is concluded from a review of published results, that estimation of plasma oestrogens cannot yet be recommended as a sole alternative to measurement of urinary oestrogen excretion for this purpose. The simplicity, robustness and precision of urinary techniques makes them particularly attractive. Evidence for an increased incidence of abnormal development of infants delivered as a consequence of subnormal oestrogen excretion has not yet been provided.

Introduction

Oestrogen estimations are widely used in the management of those pregnancies where there is a greater risk than usual of perinatal death. The relative insensitivity of early methods [1, 2] necessitated analysis of urine rather than of blood. Interpretation was based upon the quantity of oestrogen excreted in unit time (usually 24 h) which is analogous to a production rate. Radioimmunoassay has the sensitivity for analysis on small volumes of blood and provides the result as a concentration. This is not necessarily related to production. Replacement of analysis of urine by analysis of blood therefore requires careful consideration.

The principle concern of the obstetrician is to avoid perinatal death in an individual patient. His dilemma can be represented by two questions: (a) Will this fetus die if the pregnancy is managed conservatively? (b) Should one intervene to avoid perinatal death? Much of the literature on the application of oestrogen estimations is of little relevance to these questions, since it concentrates on differences between populations with various complications of pregnancy and a normal population.

In this review, the value of estimations of oestrogens in urine and plasma will be related to the prediction of imminent intrauterine or perinatal death in pregnancies of more than 30 weeks' duration. In these circumstances, there is opportunity for intervention to obtain a surviving infant. A proportion of perinatal deaths will not be avoidable by use of biochemical monitoring, for example, deaths associated with infection, lethal abnormalities, rhesus disease and sudden haemorrhage.

There is no accepted role for oestrogens in late pregnancy. Subnormal oestrogen production is not a cause of intra-uterine death since thriving babies can be delivered after oestrogen excretion only 2% of normal, as in placental sulphatase deficiency. The success of oestrogen estimations probably depends on the sensitivity of the intricate biosynthetic pathways to those factors which predispose to, or precede, fetal death.

Urinary oestrogen estimations for the prediction of impending fetal death

Oestrogen excretion rises in normal pregnancy to 140 μmol/24 h or more near term, but is about 3 μmol/24 h after fetal death. It was reasoned that it might be possible on this basis to identify those living fetuses which were likely to die if left *in utero*. Greene and Touchstone[1] were among the first to test this hypothesis. In 14 pregnancies, 12 surviving infants were delivered by caesarean section undertaken following subnormal oestriol excretion. Similarly, Wray and Russell[3] described 11 pregnancies which ended in stillbirth where subnormal oestriol excretion was recorded in association with live fetuses. The need for rapid analysis and reporting has been emphasised [4, 5]. Heys *et al.*[6] reported a retrospective study of 403 pregnancies. Of the 16 stillbirths, 12 were preceded by subnormal oestrogen excretion. Beischer *et al*[7] found the incidence of stillbirth in patients with subnormal oestrogen excretion was 4.4% but only 0.9% when excretion was normal. Bjoro[8] found 12 perinatal deaths among 203 patients with normal oestrogen excretion, but 35 among 97 patients in whom oestrogen excretion was subnormal. Subnormal oestrogen excretion preceded all 5 stillbirths reported by Hull *et al.*[9] and 21 of 23 perinatal deaths described by MacLeod *et al.*[10]. More recently, Rao[11] reported that 34 stillbirths (in which the fetus was not abnormal) among 5429 deliveries (after 32 weeks) were preceded by oestrogen excretion below the 10th centile. Most of the 11 stillbirths following normal oestrogen excretion were associated with haemorrhage or asphyxia.

There is therefore abundant evidence that perinatal deaths may be predicted by regular and frequent estimation of urinary oestrogens. Modern techniques (see

below) enable daily analysis of high precision to be carried out on all patients considered to be at risk.

Subnormal values are important in predicting imminent fetal death. The converse also applies in that fetal death occurs only infrequently when maternal oestrogen excretion is normal. Only 3 intrauterine deaths following normal oestrogen excretion were found among 403 patients but assays were relatively infrequent [6]. In a more recent survey of 101 perinatal deaths at Leeds Maternity Hospital between 1968 and 1976 none of the 31 deaths preceded by normal oestrogen excretion was associated with chronic placental inadequacy [12]. Congenital malformation, rhesus disease or abprubtio placenta were the common causes of death in these patients. Similarly, fetal death occurred in only 0.4% patients with normal oestrogen excretion compared with 43% of those with subnormal oestrogen excretion [11]. Therefore, provided oestrogen excretion remains within the normal range avoidable fetal death is most unlikely, even in the face of disturbing clinical signs. Intervention can be left until conditions warrant it.

Modern methods of urinary oestrogen estimation

Early U.K. investigators used methods [2] based on those developed for measuring oestriol excreted by non-pregnant or ovariectomized women [13]. Gradually, these techniques were superseded by methods which measured conjugated oestrogens without separation [14–16]. These methods were valuable for the prediction of intrauterine fetal death (see above). Besides permitting a rapid analysis, their simplicity encouraged smaller laboratories to offer the test. As demand increased, continuous flow systems, largely based on Technicon ® modules, were introduced [17–20]. In these, the Kober reaction [21] is performed directly on diluted urine. The product is extracted from the urine with organic solvent and estimated by fluorimetry. Twenty to 40 samples can be assayed each hour but even more rapid analysis (~60 samples/h) is possible if the final two-phase purification is avoided [22].

Radioimmunoassay techniques have also been applied. Estimation of oestriol liberated by acid hydrolysis from the urinary conjugates was described by Anderson and Goebelsmann[23]. Davis and Loriaux[24] incorporated antisera which recognized oestriol-16-glucosiduronate into polyacrylamide to measure the most abundant conjugate. Use of antisera with substantial recognition of oestriol-16-glucosiduronate has also been reported [25–28].

Day-to-day variation in urinary oestrogen excretion

Interpretation of oestrogen excretion is often stated to be complicated by the high variability in daily output. Variations may arise from imprecise analysis and from pathological or non-pathological changes in oestrogen production and excretion. Frandsen[29] reported daily variation rarely exceeded 40% whilst others [30] reported a coefficient of variation of 15.8%. Neither group defined their analytical precision. We noted (unpublished results) that the mean (±S.D.) difference in oestrogen content of 305 consecutive pairs of urine specimens (containing 50–140 μmol/24 h) collected from 51 women (in hospital or attending outpatient clinics during March–May 1978) was 14 ± 12 μmol. Analytical precision (C.V.) was 4%. The shorter the collection period, the greater the variation [30]. Measurements made using only short collections cannot be extrapolated to the standard 24-h period [31–33].

Oestrogen–creatinine ratios

To overcome the supposedly large variation in oestrogen output efforts have been directed to detection of and correction for incomplete collections by estimation of creatinine although its excretion is not invariable within or between individuals [34]. The coefficient of variation in daily total oestrogen excretion was reduced when expressed as an oestrogen–creatinine ratio measured on a 24-h collection [35–36]. Others [37, 38] found significant discrepancies between daily excretion and oestrogen–creatinine ratios measured on 24-h or early morning samples. Creatinine measurement will serve to detect grossly incomplete samples [39] which can be discarded. It seems doubtful whether measurement of oestrogen–creatinine ratios on small urine voidings collected at different times of day will improve the value of the test. Our own protocol is to collect 24-h specimens, measure the vol. (by wt.) and assess the results in the light of any marked alterations in urine vol.

Materials which vitiate urinary oestrogen estimations

(a) *By chemical interference.* The most common material is glucose. Its effect can be eliminated by incubating the urine (5 ml) with $NaBH_4$ (0.5 ml, 50% w/v in 0.1 M NaOH) for 1 h at 37°C [40]. Mandelamine® or Hiprex® used for treatment of urinary tract infection interferes seriously [41, 42] due to release of formaldehyde. There appears to be no way of overcoming this. Hydrochlorothiazide interferes in techniques using hot acid and can be avoided by enzyme hydrolysis [43]. Aspirin has been alleged to interfere [44] but this is probably due to inefficient enzyme hydrolysis [45].

(b) *By interference with oestrogen production or excretion.* Corticosteroids, given for treatment of coexisting disease [46, 47] or to induce maturation of the fetal lung [48] suppress oestrogen production in a dose-dependent manner [47]. Ampicillin, phenoxypenicillin and neomycin reduce the quantity of oestrogen excreted in the urine [49–51]—but not in all patients [52, 53]. The effect is mediated by alteration of the bacterial flora of the gut with a consequent alteration of hepatic–intestinal metabolism of oestrogens [54, 55].

Estimation of oestrogens in peripheral plasma

Radioimmunoassay permits measurement of

several forms of oestrogen in small samples of the peripheral circulation. Although "any competent laboratory can now provide measurements on blood", results from the U.K. Quality Control Survey illustrate the difficulties of reaching and maintaining adequate standards of accuracy and precision.

Before the introduction, for purposes of management, of estimations of oestrogens in blood, consideration must be given to which oestrogen should be measured and whether there are circadian or day-to-day variations in oestrogen concentrations which complicate interpretation.

Conjugated forms of oestrogens predominate in blood; those of oestriol are more abundant than those of oestrone. Oestradiol conjugates are present in the lowest concentration [56, 57]. The 3-sulphate-16-glucuronoside and the 3-sulphate appear to be the most abundant forms of oestriol [58]. The predominance of sulphates in the blood and of glucuronosides in the urine (see above) reflects the faster clearance of the glucuronosides [59–61]. The mean concentrations of unconjugated oestrogens appears to decrease in the order oestradiol > oestriol > oestrone [62–64], although more unconjugated oestrone than oestriol has been recorded [65]. There are large differences in concentrations between individual subjects.

Early assay techniques based on gas–liquid chromatography or the Kober reaction have been superseded and will not be considered further. Binding assays have been developed for unconjugated oestradiol using uterus cytosol [66] or antisera [67]. Unconjugated oestriol can be measured by competitive protein binding after purification by column chromatography [68] or by solvent partition [69]. To avoid chromatography, others have used antisera with properties selective for oestriol [70–72]. Assay of conjugated oestriol in plasma can be carried out by competitive protein binding [73] or by radioimmunoassay [74–76] on the free oestriol obtained after acid hydrolysis of conjugates. In search of even simpler techniques Standefer[77] and Kerr *et al.*[78] used antisera capable of recognizing oestriol and its conjugates to analyse diluted sera without extraction.

Diurnal variations in plasma oestrogen concentrations

Large sporadic variations in oestrogen concentration might vitiate the use of the assay for obstetric purposes. Regular variations would require standardization of the time of sampling.

(a) *Variation in unconjugated oestradiol.* Large variations in the concentrations of unconjugated oestradiol were reported [79], for example, from 94 nmol/l. at 8.00 to 34 nmol/l. at 22.00 or from 18 nmol/l. at 8.00 to 42 nmol/l. at 22.00. Similar large variations were also recorded by others [67, 80]. Mean concentrations were highest near midday and lowest near midnight [79–81].

(b) *Variations in unconjugated oestriol.* Eight patients showed diurnal variations of 20–40% [68]. Mean concentrations tended to be highest at midnight. Goebel and Kuss[82] found the lowest mean concentration in another 8 patients at 8.00 h. Variation with individuals (30–70%) were higher.

(c) *Variation in conjugated oestriol.* The concentration of total (conjugated plus free) oestriol varied by up to 130% within 22 individuals [83, 84] without a consistent diurnal pattern. In another study using the same method diurnal variation was less than 25% [85]. In 22 patients changes of up to 150% were found, but these were smaller than those for unconjugated oestradiol in the same patients [80]. Highest mean values were found at noon.

For all 3 oestrogen fractions there are large variations during the day within individuals. The magnitude of these changes must be considered when applied to patient management. Unconjugated oestriol appears to show the least variation.

Day-to-day variations in oestrogen concentrations

Day by day assessment of trends in oestrogen concentrations may be important in practice so that daily variations merit consideration. The individual maximum variation (as C.V.) for unconjugated oestradiol was 14% [86], for total oestriol was 25% [87] or 22% [88] and for unconjugated polar oestrogens was 46% [89]. Thus, although unconjugated oestriol shows the lowest variation within the day unconjugated oestradiol is least variable from day-to-day. These conclusions are not easily reconcilable.

Materials which lower plasma oestrogen concentrations

Ampicillin was reported to lower the concentration of conjugated oestrogens in plasma [50]. This does not occur in all patients or affect all oestrogen fractions [52, 53, 90]. Large doses of natural or synthetic corticosteroids decrease the concentration of unconjugated oestrone, oestradiol and oestriol in maternal plasma [48, 91].

Plasma oestrogen estimations for the prediction of fetal death

Relatively few studies have been concerned with this aspect, although low concentrations after fetal death are often recorded. Table 1 lists reported perinatal deaths (excluding rhesus disease and congenital malformation) in relation to the last estimation before fetal death or delivery. The information shown suggests low oestrogen concentrations precede many fetal

Table 1. Perinatal deaths after 30 weeks of gestation: prediction by measurement of the concentration of oestrogens in maternal plama

Oestrogen measured	P/N deaths	Subnormal Oe	Normal Oe	Ref.
Conj. E_3	12	9	3	[92]
Conj. E_2	11	7	4	[92]
Conj. E_3	4	3	1	[93]
Conj. Oe	4	3	1	[94]
Unconj. E_3	12	10	2	[95]
Unconj. E_3	7	4	3	[96]

deaths, but provides little assistance in deciding which oestrogen should be measured. For example, unconjugated oestriol appears to predict 10 out of 12 or 4 out of 7 deaths, depending on the centre. On the other hand, in studies of the same patients conjugated oestriol had a better predictive value than conjugated oestradiol.

Comparative studies of urine and plasma

The prediction rates from assay of conjugated oestriol in urine or plasma were virtually identical [92]. Total oestriol in serum was compared with urinary total oestrogens in 31 complicated pregnancies [97]. Serum conjugated oestriol was not recommended as a replacement urinary excretion measurements, because in pregnancies with diminished renal clearance *and* diminished oestrogen production, plasma concentrations remain high though urinary excretion is low. In the same patients serum unconjugated oestradiol provided a poorer assessment than did urinary conjugated oestriol [98]. After 454 parallel estimations of total oestrogen in urine and plasma from 166 high-risk patients, Aickin *et al.*[94] concluded that both approaches were of equal value in predicting fetal risk. The similarity of trends of serum unconjugated oestriol and urine conjugated oestriol was emphasised by Miller *et al.*[99].

After a study of unconjugated oestriol, unconjugated oestriol, total oestriol in plasma and urinary total oestrogens in 58 patients, Allen and Lachelin[81] conclude that plasma total oestriol and urinary total oestrogen provided comparable information.

It is concluded from this review that the place of plasma measurements in the prediction of fetal death has not yet been established to a degree that justifies their use, at present, without concomitant urinary oestrogen measurements.

Alternative methods

(a) *Assay of oestetrol.* Since oestetrol biosynthesis occurs mainly in the fetus, it was considered that its measurement might provide a useful indicator of the condition of the fetus. Methods for oestetrol estimation in urine [100] and in blood [101–103] were devised. Measurement of urinary conjugated oestetrol or oestriol provided equivalent information in 89 pregnancies [104]. Although subnormal concentrations of unconjugated oestetrol in plasma preceded all 7 cases of fetal death [105], estimation of serum unconjugated oestetrol in 41 pregnancies was found by others [106] not to provide any extra information from that obtained by assay of serum oestriol.

There does not yet appear any real value in the more difficult assay of oestetrol.

(b) *Precursor challenge tests.* Lauritzen[107] investigated changes in urinary oestrogen excretion following infusions of dehydroepiandrosterone sulphate and related the fetal condition to the quantity of oestrogen produced. More recently, changes in plasma oestrogen concentrations were measured. Initial reports were encouraging (*e.g.* [108, 109]). After more detailed investigation it was concluded that the variations of response found in normal pregnancy were too great to offer a discriminating test of fetal condition [110–112]. Normal responses were found after fetal death. The response of 100 women in this test has recently been reported [113]. The test is of real value in antepartum detection of placental sulphatase deficiency (reviewed in [114]). Injection of dehydroepiandrosterone sulphate in these cases is not followed by increases in oestrone concentration as it is in normal pregnancy.

Tulchinsky *et al.*[115] found that measurement of plasma oestetrol after DHAS injection enabled confirmation of fetal risk. Others noted that the rate of increase of plasma oestrogen concentration after dehydroepiandrosterone infusion appeared to identify pregnancies with increased fetal risk in labour [116]. Further developments are awaited with interest.

Infant development after subnormal oestrogen excretion

It would be a poor reward for obstetrician and biochemist if implementation of the test reviewed led to a higher incidence than necessary of babies who were more severely handicapped due to intervention. Several reports examine infant development after subnormal oestrogen excretion in pregnancy.

Wallace and Michie[117] reported on 14 such children. Although almost all were of adequate height and weight in the 2nd year, there was a high incidence of neonatal morbidity and of later motor and psychological/neurological abnormalities. A study of 5000 children showed that the incidence of severe handicap was 27% following low oestrogen excretion and only 2% following normal oestrogen excretion (Jergensen, quoted in [118]). Thirty-four children ($1\frac{1}{2}$–8 years) were investigated by Greene *et al.*[119] who concluded that the frequency of defects after subnormal oestrogen excretion was insufficient to warrant undue pessimism. In another study of 16 hypertensive women, Yogman *et al.*[120] noted that development defects were found most frequently in children born after chronic low oestrogen excretion. Children delivered after precipitous falls in oestrogen excretion did well.

Premature delivery after subnormal oestrogen excretion does not, in itself, necessarily involve subsequent handicap. Chronic subnormal oestrogen excretion appears to reflect an underlying intra-uterine disadvantage, the effects of which persist after birth. Efforts should be made to rectify this, perhaps along the lines suggested in France [121].

Practicability

The practicalities of urine and blood assays need consideration. Performance of either test must interlock with the particular local form of obstetric practice. Out-patients and in-patients will require testing, the latter possibly on a daily basis. Blood samples

need the presence of the patient and skilled assistance, whereas urine can be collected without help and can be delivered by others to the laboratory. For two practical reasons–(i) robustness and precision of the method and (ii) simplicity of collection of multiple specimens—urine assays must be considered superior.

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