Longitudinal Assessment of Changes in Reproductive Hormones during Normal Pregnancy

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The concentrations of hormones measured in serum from maternal blood change dramatically during pregnancy. While the relative contributions of sex steroids shift from maternal ovaries and adrenals to the fetoplacental unit, other maternal tissues such as pituitary and liver respond to increasing concentrations of estrogen and secrete increasing amounts of prolactin and sex-hormone-binding globulin. To determine longitudinal changes in circulating maternal hormones, we collected blood from 60 women on three occasions during their pregnancies. We observed a 1.7-fold increase in testosterone concentration in serum; concentrations of sex-hormone-binding globulin in serum rose 5.6-fold. The major increase (6.8-fold) in estradiol in serum occurred within the first 16 weeks, followed by a further 4.8-fold increase by term. Mean concentrations of progesterone, 17-hydroxyprogesterone, and androstenedione in serum increased 11.9-, 3-, and 1.3-fold, respectively, whereas concentrations of dehydroepiandrosterone sulfate (DHEAS) fell by 50%. Mean serum prolactin concentrations increased 3.8-fold during the first trimester and by a similar amount during the final 24 weeks of pregnancy. We used these data, obtained from a cohort of women with uncomplicated pregnancies, to construct reference intervals for hormones in maternal serum.

Additional Keyphrases: sex-hormone-binding globulin  prolactin  progesterone  dehydroepiandrosterone  reference interval

During pregnancy, substantial changes occur in the circulating concentrations of binding proteins and hormones in maternal serum. In addition, changes in the secretion and metabolism of steroids by the fetoplacental unit produce a dynamic pool of hormones within maternal serum. Measurements of several maternal hormones in serum, particularly human chorionic gonadotropin (1) and, to a lesser extent, placental lactogen (2) and estriol (3) have proven useful clinically in assessing at-risk pregnancy. Because many previous reports were based on cross-sectional investigations or involved various specialized nonroutine methods, it is difficult to compare results for different hormones. The aim of this study was to measure hormones in serum in a group of women during normal pregnancy and to determine reference ranges for these analytes. The assays reported in this study were performed with commercial kits that are available in most routine laboratories.

Materials and Methods

Patients

Three blood samples were collected from 108 pregnant women during each trimester of pregnancy—at weeks 6–14, weeks 16–28, and weeks 27–39—after informed consent was obtained and the project had been approved by the Institution Ethics Committee. Gestational age was determined by ultrasound. Of the original 108 women recruited into the study, 27 withdrew voluntarily and 21 were excluded on the basis of maternal complications associated with pregnancy. Each of the 60 women who remained in the study delivered a single healthy baby with no evidence of birth defects.

Assays

Blood samples were collected into plain tubes and allowed to clot. The serum was removed within 3 h. All serum samples were stored at −20°C before assay. We used commercially available kits for sex-hormone-binding globulin (SHBG) and prolactin (by Delfia time-resolved fluoroimmunoassay; Pharmacia, Australia), dehydroepiandrosterone sulfate (DHEAS), estradiol, progesterone, 17-hydroxyprogesterone (by RIA; Diagnostic Products Corp., Los Angeles, CA), androstenedione (by RIA; Diagnostic Systems Labs., Webster, TX), and total testosterone (by RIA; Byk-Sangtec Diagnostica, Dietzenbach, F.R.G.).5 The free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG, multiplied by 100. For each analyte, we assayed the three specimens from each woman within the same batch to minimize interassay variation.

Statistical Analyses

We analyzed the data by using a simple mixed model, for which we wrote a special program to adjust for missing data and unequal measurement times. The model specifies that, for different individuals, the measurement curves over time are parallel cubic (or lower-order polynomials) and that, after allowances are made for the curve, residuals are simply random error attributable to measurement or to individual fluctuations.

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Nonstandard abbreviations: SHBG, sex-hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; and FAI, free androgen index.
This method of analysis is a special case of that proposed by Diggle (4). The reference ranges (central 95 percentile values) were estimated by taking the mean curve ± 1.96 times the estimated standard deviations (taking account of both within- and between-individual variation). For most measurements, analysis was carried out with log-transformed data, to homogenize variance. We then plotted the exponents of these data to give ranges on the original scale. We noted an apparent surplus of points outside the limits in some cases because of correlations between measurements on the same individual. Plots of the data for log(testosterone) and log(androstenedione) indicated outliers that would have undue influence on the fitting procedure. These outliers were omitted from the final statistical analysis and are therefore not identified on the plots.

Results

Sixty infants were born to the women in this study, 27 boys and 33 girls. No clear association could be drawn between the concentrations of testosterone and SHBG in maternal serum and the sex of the baby.

Plots of the data for each analyte are shown in Figures 1–5. Included on the plots are our reference intervals for healthy nonpregnant women, obtained in each case from at least 20 women in the luteal phase of their menstrual cycles. As noted above, for many of the analytes, the variability or scatter tends to increase as the magnitude of the measured value increases; thus, we used log-transformed data for those hormones. The log-transformed data could then be reasonably fitted by sets of parallel curves for the individuals. That is, if all the points corresponding to the same individual were joined, a band of approximately parallel curves would be generated that could be fitted by low-order polynomial functions.

The mean curves assessed as appropriate for the variable (i.e., the simplest curves that adequately describe the data according to statistical testing) and the equations describing the changes in mean values are given in Figures 1–5.

Progesterone. The mean progesterone concentration in serum rose gradually from 49 (95% confidence limits, 26–91) nmol/L at week 5 to 584 (314–1087) nmol/L at 40 weeks of gestation (Figure 1a).

17-Hydroxyprogesterone. The mean concentration of 17-hydroxyprogesterone in serum at both weeks 5 and 19 was 12.2 (5.2–28.5) nmol/L. After that time, the mean concentration increased threefold to 36 (15.5–84) nmol/L at week 40 (Figure 1b).

Testosterone. The mean testosterone concentration in serum increased from 3.3 (0.9–7.4) nmol/L at week 5 to 5.7 (2.2–10.8) nmol/L at week 40 (Figure 2a).

Sex-hormone-binding globulin. The mean SHBG concentration in serum increased rapidly during the first half of gestation, from 71 (39–131) nmol/L at week 5 to 392 (214–717) nmol/L at week 25. SHBG concentrations then remained relatively constant until week 40, where the mean value was 396 (216–724) nmol/L (Figure 2b).

Free androgen index. From week 5, the mean FAI, 6.76 (2.56–17.85), fell rapidly, to plateau at week 21 at 1.22 (0.46–3.22). The FAI increased marginally toward term to a mean of 1.64 (0.62–4.32) at 40 weeks' gestation (Figure 2c).

Dehydroepiandrosterone sulfate. The mean DHEAS concentration in serum decreased from 5.8 (2.0–16.5) μmol/L at week 5 until mid-gestation, where the mean concentration was 2.7 (0.9–7.8) μmol/L. Mean DHEAS concentrations then remained relatively constant during the next 20 weeks, and were 2.3 (0.8–6.5) μmol/L at week 40 (Figure 3a).

Androstenedione. There was a gradual increase in the mean androstenedione concentration in serum, from 8.1 (3.5–18.8) nmol/L at week 5 to 10.6 (4.6–24.5) nmol/L at week 40 (Figure 3b).

Estriol. Serum estriol increased during the first trimester from a mean concentration of 1.64 (0.69–3.88)
Discussion

A high rate of attrition among participants complicates a longitudinal prospective study such as this. In

Fig. 3. Changes in maternal serum concentrations of (a) DHEAS and (b) androstenedione during pregnancy. Nonpregnant (NP) reference intervals for (a) DHEAS, 3.4–8.6 μmol/L, and (b) androstenedione, 5–10 nmol/L, are shown in the shaded areas at the left. The mean curves are described by the equations for log(DHEAS) = \exp(2.1206 - 0.0791 \times \text{week} + 0.0012 \times \text{week}^2) and for log(androstenedione) = \exp(2.0556 + 0.0076 \times \text{week})

nmol/L at week 5 to 11.13 (4.69–26.40) nmol/L at week 16. During the second and third trimesters, the mean estradiol concentration in serum rose almost fivefold, to 53.44 (22.53–127) nmol/L at week 40 (Figure 4).

Prolactin. Prolactin results are expressed as milli-international units per liter, with use of standards calibrated against the WHO 1st International Reference Preparation (75/504). The mean prolactin concentration in serum rose gradually during the first trimester, from 294 (101–857) milli-int. units/L at week 5 to 1106 (379–3227) milli-int. units/L at week 16. There was a rapid increase in the serum prolactin concentrations during weeks 16–35, with a mean prolactin concentration of 4092 (1402–11944) milli-int. units/L. This was followed by another small rise to 4293 (1471–12531) milli-int. units/L at week 40 (Figure 5).
addition to the large numbers of voluntary withdrawals, we were conservative in selecting subjects for inclusion so as to maximize the probability that this reference population would be representative of women completing uncomplicated pregnancies and delivering single healthy babies.

During the first nine weeks of pregnancy, the corpus luteum and, to a lesser extent, the maternal ovary and adrenal cortex contribute to circulating concentrations of maternal steroids (5, 6). After that time, the placenta becomes the predominant source of maternal steroids (7). Increased production of estradiol, in particular, affects hepatic synthesis of binding proteins such as thyroxin-binding globulin and SHBG as well as pituitary gland secretion of prolactin (8–10). We undertook this longitudinal study of the latter 35 weeks of gestation to define appropriate hormone ranges for several analytes known to alter markedly during pregnancy.

**Progesterone and 17-Hydroxyprogesterone**

Whereas estrogen production during pregnancy derives from precursors formed within the fetal and maternal compartments, placental progesterone production is derived mainly from the maternal pool of circulating cholesterol (11, 12). The progesterone values in serum obtained in this study follow a pattern similar to those previously reported (13–15) but we found considerably higher values, especially toward term, than those reported by Dorr et al. (15). Whether this reflects a difference in methods is not clear. Progesterone is considered vital for maintaining mammalian pregnancy, but its precise role remains obscure (16).

17-Hydroxyprogesterone is generally accepted to indicate ovarian steroidogenesis, particularly during the first trimester of pregnancy (6, 7). However, during the later stages of gestation, fetal and maternal adrenal glands as well as the placenta contribute to the maternal concentration of circulating 17-hydroxyprogesterone (17). Our longitudinal study confirms that the increase in 17-hydroxyprogesterone concentrations in maternal serum occurs predominantly during the second half of pregnancy, as was previously reported in cross-sectional (5) and longitudinal studies (15).

**SHBG and Testosterone**

The concentration of SHBG in serum reportedly increases by about sixfold throughout pregnancy, as a result of high concentrations of circulating estrogen, which stimulate synthesis of the binding protein within the maternal liver (18, 19). However, our longitudinal data demonstrate that the major increase in SHBG concentration occurs during the first half of pregnancy, whereas the estradiol concentration increases somewhat later, during weeks 20–35. Therefore, hepatic synthesis of SHBG must be sensitive to relatively small changes in circulating estrogens. During weeks 5–40 of pregnancy, testosterone concentrations in serum increase by ~29%. Some direct hormone assays, involving coated-tube techniques, may underestimate the testosterone concentration, particularly in the presence of high concentrations of SHBG (20). However, the testosterone radioimmunoassay used in our longitudinal study did not appear to be significantly affected by the high concentrations of SHBG seen in pregnancy sera (20). In further studies we have demonstrated that SHBG concentrations between 20 and 200 nmol/L do not affect the recovery of added testosterone (21). However, at higher SHBG concentrations, such as those in late pregnancy, testosterone recovery is diminished to 80–85%.

Therefore, our results for testosterone concentrations in maternal serum may underestimate the true circulating concentrations. In absolute terms, our results are consistent with those found in sera from the third trimester of pregnancy by previous investigators (22,
Testosterone values that remain within the range found in nonpregnant women have been associated with spontaneous abortions. Previous reports have suggested that the abnormal conceptus does not produce sufficient estrogen to stimulate SHBG production and increase testosterone concentrations (18, 19).

Ordinarily, 98% of the total circulating testosterone is bound to serum binding proteins (SHBG and albumin), whereas 2% is free (18, 24). It is generally believed that SHBG-bound testosterone is not biologically active. The relative bioavailability of free and albumin-bound testosterone is debated (25, 26), whereas the non-SHBG-bound testosterone is considered to be the biologically active moiety (27). The FAI is commonly used as an index of biologically active testosterone in men and nonpregnant women (28, 29). Assuming that the affinity of SHBG for testosterone does not change during pregnancy (we are not aware of any such evidence), the FAI would reasonably reflect the non-SHBG-bound fraction of total testosterone.

A two- to threefold increase in SHBG-binding capacity during pregnancy, mainly in the first trimester, has been reported previously (9). In our longitudinal study, we observed a 5.5-fold increase in the mean values for immunoreactive SHBG concentrations during pregnancy. An assay artifact is unlikely, because this increase is reproducible in several assay systems (18, 22, 30). Although clinical manifestations of androgen excess are uncommon in pregnant women, any change should be of concern. Testosterone does not usually cross the placental membranes because of the high capacity of the aromatase enzyme system to convert androgens to estrogens (31).

DHEAS and Androstenedione

Fetal androgens as well as androgens derived from maternal adrenal tissue can undergo aromatization to form estrogens (31). The major substrate is DHEAS; once the sulfate moiety is removed, dehydroepiandrosterone is then converted via androstenedione and testosterone to produce the estrogens (23). We observed a 50% reduction in maternal DHEAS concentrations during the first half of pregnancy, similar to that observed previ- ously (32); this reflects, in part, a six- to 10-fold increase in the clearance rate of DHEAS (33). We observed only a slight increase in androstenedione concentrations in our longitudinal study of normal pregnancy, consistent with previous reports (34, 35).

Estradiol

After the first five to six weeks of pregnancy, the major source of estradiol is the placenta, where enzymes convert maternal and fetal DHEAS to estradiol, estrone, and estriol (5). Near term, 50% of the estradiol synthesized in the placenta is derived from precursors in the fetal circulation; the remainder comes from precursors in the maternal circulation (36). The estradiol concentrations we report are similar to those reported by others (34, 37, 38). A direct assay of estradiol may be affected by high SHBG concentrations during the latter half of pregnancy in a manner similar to that described previously for testosterone (20) and estradiol (39). However, our data are consistent with those of Sparre et al. (38), who used an ether extraction procedure before radioimmunoassay and observed a fivefold increase in maternal estradiol concentrations during weeks 6–16 of pregnancy. Buster et al. (23) also observed a similar trend, i.e., a twofold increase in maternal serum estradiol concentrations during weeks 26–40 of pregnancy.

We observed a rapid increase in estradiol concentrations, particularly between weeks 20 and 35, which is most likely due to the enhanced aromatization of circulating DHEAS by the increasing mass of placental tissue (23, 31, 37). Low concentrations of estradiol measured in maternal serum during the first trimester are suggested to be an indicator of poor prognosis in cases of threatened abortion (1). However, because of the large overlap between low and normal values during the third trimester, estradiol measurements do not appear to offer useful information to predict clinical outcome (40–42).

Prolactin

Our longitudinal data for prolactin in maternal serum are consistent with earlier studies that showed that prolactin concentrations increase significantly during pregnancy (10, 43) because of estrogen-induced hyperplasia and hypertrophy of pituitary lactotrophs (44). We observed an increase in the mean prolactin concentrations in maternal serum that strongly correlates with the mean circulating estradiol concentrations (r = 0.9997, P < 0.05; Spearman rank correlation coefficient).

Despite the many technical advances in measuring hormone concentrations in serum, there is no clear consensus as to why production of these hormones is increased during pregnancy or what their function is. Why does the system require seemingly excessive amounts of circulating steroids and protein hormones in the mother? If, as it would appear reasonable to assume, gestation represents a coordinated process, there must be an integrated control of information across the placenta from fetus to mother. Further analysis of hormone fluxes may lead to a greater understanding of the hormonal control of fetal and placental growth.

Many previous studies have reported changes in hormone concentrations in serum during pregnancy, but few have attempted to define "normal" limits based on prospective studies. There is clearly a need to examine those changes that occur during normal pregnancy so that unusual or unexpected trends can be identified. Furthermore, because hormone measurements in serum are most likely to be performed in laboratories that use commercially available reagents, at least for the first measurement, we used such methods to obtain the data presented here to detail hormone changes in serum during normal pregnancy.

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References


