Method for Monitoring Plasma Progesterone Concentrations in Pregnancy

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We present a time-series model for monitoring concentrations in plasma of hormones produced in the placenta, progesterone being chosen as an example. The model, which is based on the assumption that variations in plasma progesterone concentration in pregnant subjects mainly reflect variations in the growth rate of the placenta, was applied to eight series of progesterone values measured during pregnancy in eight subjects. In the model, which was found to fit the data, it is assumed that progesterone concentration is proportional to the size of the placenta and that the growth rate of the placenta varies at random, with a mean value $\alpha$. The variation of $\alpha$ was of the same magnitude among and within the subjects. If the average of many subjects’ $\alpha$ values is used, a single subject may be used as her own reference, based on only one previous observation. When two observations are available, an individual’s own $\alpha$ value may be estimated and used for the prediction. The predictive power of the new method was found to be far superior to the conventional method in which a single sample reference material is used. Furthermore, one need not know the gestational age in order to use the method.

Additional Keyphrases: monitoring and predicting values for hormones in plasma • pregnancy, changes during • placental growth • fetal status

In evaluating the result of a laboratory test a reference interval based on data from an appropriate group of healthy subjects is often used.

For monitoring purposes (e.g.) much is gained by using the subject as his or her own reference, to eliminate inter-individual variation from the comparison. This requires the presence of one or more previous observations, obtained during a period when it was known that the subject was in a stable state, e.g., in “good health.” But it is also necessary to know how to use these observations to predict what the values for future observations should be if the former stable state still exists. Thus a study of a chronological series of biological data is necessary before we can use the subject as his (or her) own reference.

In the present investigation we used such a time-series model to analyze intra-individual changes in plasma progesterone concentration during pregnancy in a group of healthy subjects. For comparison, we computed the average time course of plasma progesterone concentrations during pregnancy, by using values for single samples obtained from healthy subjects at various stages of pregnancy.

Material and Methods

Materials

Plasma samples from each of 324 pregnant women with reliable menstrual data were used as the basis for calculating the nonparametric reference interval.

Plasma was sampled from eight pregnant women with reliable menstrual data at one- to three-week intervals during their pregnancy. A total of 72 samples were used in the consecutive series study (Figure 1).

All blood samples were drawn from the antecubital vein into heparinized glass tubes.

In all cases, pregnancy and the neonatal period were uncomplicated and birth took place in week 38 to 42. The infants’ birth weights all exceeded 2500 g.

Analytical Method

Progesterone in plasma was estimated by a competitive protein-binding method (1), which we slightly modified: after extraction with petroleum ether (Mallinckrodt, “Nanograde”) we took an aliquot (1500 μl) instead of the whole petroleum ether fraction (2000 μl) to improve the precision.

As binding protein, we used serum taken from the antecubital venous blood of postmenopausal women treated with high doses of estrogen (4 mg of estradiol, perorally, each day for eight days).

Statistical Methods

The subject as her own reference: We first examined if a simple exponential growth curve could fit the data obtained from the same subject. With this model the following assumptions are made:

(a) During later pregnancy—i.e., after the 12th week of pregnancy—the plasma progesterone value reflects the size of the placenta except for biological and analytical variation.

(b) At any given time (t) after the 12th week the growth rate of the placenta is proportional to the size it has already attained.
If the plasma progesterone value is proportional to the size of placenta, we have the following differential equation where \( X(t) \) signifies the plasma progesterone level at time \( t \): \( \frac{dX(t)}{dt} = kX(t) \). The solution of this equation is \( X(t) = Ae^{kt} \). This implies that the log of \( X(t) \) is a linear function of time. Figure 2 shows the log of the plasma progesterone as a function of time for one of the eight healthy pregnant subjects. The regression line corresponding to the data is also shown. Note that the data points are not scattered at random around the regression line and that the slope between consecutive progesterone values varies considerably. Thus the model did not fit the data. We therefore choose another model that is based on the following assumptions:

(a) The plasma progesterone concentration reflects the size of placenta.
(b) The growth rate of the placenta is proportional to the size it has already attained; however, the proportionality constant varies at random over time, a growth model first studied by Lewontin and Cohen in 1969 (2). The model implies that the slope of the log of plasma progesterone depicted as a function of time will vary with time.

(c) The slope between consecutive progesterone values in such a semilogarithmic system reflects the growth rate over that period; i.e., we assume that the remaining biological and analytical variation is negligible in this context.

It should be noted that the model is only assumed to be valid between the 12th and 36th week of pregnancy. We may now compute an average of all slopes between consecutive progesterone values and use this average slope to predict a new progesterone value from the one previously observed. Let us focus on a smaller segment of the curve shown in Figure 2 and see how this type of prediction behaves as compared to that of the regression line. In Figure 3 we have computed the average of all the slopes of the previous curve. The prediction from one data point to the following is done as follows. Starting from one data point, one draws a line having the average slope that we just mentioned. The predicted value is found on the line. It is seen that the predicted points are much closer to the observed points than is the regression line. This is true for almost all the points shown on Figure 2. Figure 4 shows how the average slope is computed. It appears that we are only using the first and the last observation. This means that the further apart the observations are in time, the better we are able to estimate the average slope. This is an important quality of the model in that it implies that if one observation is obtained during early pregnancy one can always get the best possible estimate of the average slope. The slopes then vary at random around a

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**Fig. 1.** Consecutive plasma progesterone values in eight pregnant women.

**Fig. 2.** Plasma progesterone values of a healthy pregnant subject as a function of duration of pregnancy.
The regression line depicting the logarithm of the plasma progesterone value as a function of time is also shown. Note that the scale of the ordinate is logarithmic. The framed part of the figure is reproduced in Figure 3.

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1 We submit that our comparison between the regression model and the present model does not allow us to draw any definite conclusions, because it was only based on one example where the sample size was sufficiently large for the performance of a detailed comparison between the two models. However, our main reason for choosing the present model is that we think that the assumptions upon which this model is based are more realistic than those upon which the regression model is based.
mean value, \( \alpha \). The estimate of \( \alpha \) is \( \hat{\alpha} = (Y_n - Y_1)/t_n - t_1 \), where \( Y_n \) denotes the log of the \( n \)th or last observation of the plasma progesterone value and \( Y_1 \) the first one, \( t_n \) is the last observation time and \( t_1 \) the first observation time. The variance of the slopes (\( \sigma^2 \)) may also be estimated from the data (see the Appendix). We emphasize that although only the first and last observation are used when \( \alpha \) is estimated, all observations are used in estimating \( \sigma^2 \). The change in the log of the plasma progesterone value over the time period \( \Delta t = (t_{i+1} - t_i) \) may be predicted by the model as \( \hat{\alpha} \times \Delta t \), and the deviation from the observed change (\( Y_{i+1} - Y_i \)) computed as \( r_i = (Y_{i+1} - Y_i) - (\hat{\alpha} \times \Delta t) \). For each subject and for all pairs of consecutive values we computed \( r_i \) and divided by its variance (see Appendix) to obtain a normalized deviation from the model. To examine the validity of the model we compared the common distribution of all normalized residuals to a normal distribution with mean zero and variance one (see Figure 5).

**Single-sample analysis:** We analyzed the data from the single samples observations from the 324 pregnant women by a nonparametric method previously published by one of us (3).

### Results

The analysis of the empirical distribution of the normalized residuals showed that the model adopted for the eight subjects for whom consecutive progesterone values were available adequately described the data; that is to say, the empirical distribution based on all the 72 normalized residuals did not differ significantly from the theoretical. The two distributions are depicted in Figure 5. Evidently there is very good correspondence between the distributions. Table 1 shows the estimates of \( \alpha \) and \( \sigma^2 \) for each of the eight females. The autocorrelation coefficients in those cases where we were able to calculate them did not differ significantly from zero.2

The comparison of the variances for the eight women showed that they were significantly different (\( \chi^2_{17} = 36.5, P < 0.01 \)), mainly owing to the large variances observed in subjects 1 and 3. The variation of \( \alpha \) among the subjects was \( 9 \times 10^{-4} \), which is of the same order of magnitude as within the subjects. The common estimates of \( \sigma^2 \) and \( \alpha \) were 17.1 \( \times 10^{-4} \) and 3.46 \( \times 10^{-2} \), respectively.

Based on these two estimates, two tables were constructed. The first (Table 2) relates the minimal percentage increase in the plasma progesterone concentration to the number of weeks elapsed since the first

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2 The estimate obtained by pooling the individual \( R_i(1) \) values (for nomenclature see Appendix) is -0.27, which is significantly different from zero. By contrast the estimate obtained by pooling the \( R_i(2) \) values is 0.04, which is not significantly different from zero. Thus it appears that the differences are statistically interdependent on account of measurement error.
observation; i.e., this table is applicable if only one previous observation is available. The second table (Table 3) gives the lowest probable values of the observations (in percent of the predicted values) that is allowable at the 5% level of significance. The tolerance depends on the number of weeks elapsed since the first and the last measurements (see Appendix). This table may be used when more than one observation is available.

The single-sample plasma progesterone values are depicted in Figure 6 as a function of the duration of pregnancy together with the 0.05, 0.50, and 0.95 fractiles corresponding to the sets of observations delimited by the nonparametric method.

Figure 7 shows progesterone values observed in a patient who had an abortion during the 21st week of pregnancy. None of the values is abnormal in the sense that it is lower than the lower normal limit computed from the single sample values. However, when Table 2 is used to compute the lower 5% limit for observation number 2—i.e., when the subject is used as her own reference—it is seen that this obser-

<table>
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<th>Gestion, weeks</th>
<th>Minimum Increase, in percent of observed value</th>
<th>Lower limit, in percent of observed value</th>
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Fig. 6. The single-sample plasma progesterone values together with the 0.05, 0.50, and 0.95 fractiles corresponding to the sets of observations, with use of a nonparametric method.

<table>
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<th>1/2</th>
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<th>3</th>
<th>4</th>
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Fig. 7. Three plasma progesterone values for a patient who had an abortion during the 21st week of pregnancy. Smooth curves of the nonparametric reference interval (0.05 and 0.95 fractiles, solid lines; cf. Figure 3), and the reference interval determined by an ordinary parametric method (mean ± 2 SD) on the same set of observations (dotted lines).
ation is highly abnormal. Figure 8 illustrates that the method is also easy to use when two observations are available. The values observed in subject No. 8 are used as an example. If we want to find the value predicted for week 22 based on the two values observed during the 12th and 21st week, respectively, the latter two values are plotted on semilogarithmic paper and connected by a straight line. The predicted value is read from this line and the lower 5% value is computed by using Table 3. It is seen that the value observed during the 22nd week is well above the 5% limit.

Discussion

In the present study we have compared a method for using an individual as her own reference and compared this procedure with the use of a conventional reference interval based on single-sample values.

By comparison with earlier investigations in which comparable analytical techniques were used (competitive protein-binding, radioimmunoassay) it appears that between the 15th and 25th week of pregnancy the lower limit of the single-sample reference interval is a little lower than that found by other workers (4–6). This difference may not be attributed to our use of nonparametric statistics, because we obtained nearly the same lower limit in this period of pregnancy by using an ordinary parametric method (mean ± 2 SD; see Figure 7).

In late pregnancy (week 27–36), however, we found the lower limit to be about 30% higher by using nonparametric statistics compared to the ordinary parametric method applied to the same progesterone values.

An important aspect of the model in which a subject is used as her own reference is that the growth rate of the placenta is not assumed to be related to the size of placenta by a fixed proportionality factor. The model allows the latter factor to vary at random, which in our opinion is a more realistic concept. It should be noted, however, that the production and elimination of progesterone is assumed to be in equilibrium at time t, so that X(t) only reflects the size of placenta. While the growth rate is allowed to vary at random in the model, the production of progesterone is assumed to be related to the size of placenta by an unknown but constant proportionality factor. This concept is not realistic when the placenta is small, because the production of progesterone will probably vary from cell to cell and also within the same cell from time to time, and a considerable part of the progesterone will still come from the persisting corpus luteum. The model is not applied before the 12th week of pregnancy. Later in pregnancy, as an approximation, the assumption is a realistic one in that the number of placental cells now is so large that the law of large numbers should apply.

Nevertheless, we want to emphasize that in all probability the model only represents a very crude approximation to the biological mechanisms regulating plasma progesterone. Thus for a given population of cells the total production of progesterone may vary over time. Also it should be remembered that progesterone is stored in the fatty tissue and the transport of progesterone between water and fat may be slow and subject to the influence of many varying factors. It is also assumed in the model that the analytical error is negligible, although there are some indications that this assumption may be incorrect.3 However, our purpose was not to study the biological mechanisms regulating the progesterone concentration, but rather to devise a method that, although based on crude assumptions, is of value for predictive purposes.

The application of the model implies that the gestational age is between 12 and 36 weeks, but no further assumptions are made. Thus it is not necessary to know the exact gestational age to apply the model. Therefore, in cases where the gestational age is uncertain the model might be used to predict the time of birth, in that the model should not apply just before birth.

We found a statistically significant variation among the intra-individual variances, but this does not preclude that an estimate of the common variance may be used. This approach is probably better.

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3 The assumption that the differences between consecutive observations are independent was not valid, because the measurement error was not negligible. In the present study we ignored this violation. Alternative approaches would be to include this dependency in the model or to decrease the measurement error. The former approach is very complicated even if measurements are obtained at equidistant times, and almost untractable if they are not. Thus, to impose this restriction severely limits the practical applicability of the model. Therefore we would recommend that either the measurement error be decreased or that one ignore the dependency as we did. But some clinical experience with the model is needed before it is possible to decide this issue.
than to estimate the variance separately for each subject, because such an estimate usually tends to be very poor, owing to the small number of samples. Furthermore, with this method it is necessary to have at least two observations before a prediction can be made.

However, we want to emphasize that our estimate of the common variance, which is based only on data from eight subjects, may be unreliable. Therefore Tables 2 and 3, which we used for the predictions, should only be taken as an illustrative example.

The method presented here is useful in practice, and in our opinion it is based on assumptions that are more realistic than those of the regression analysis.

We emphasize that the present work is a methodological study. Human placental lactogen and estriol (or its derivatives) are probably more valuable quantities in the clinical context than is progesterone (7). Because the production of estriol involves both the fetus and the placenta, the present model may not apply. However, placental lactogen is produced solely in the placenta and may therefore follow the same model as progesterone. We now are exploring this possibility.

References


Appendix

Stephen L. Lauritzen

Stochastic Model

As the stochastic model we choose an approximation to a model for cell growth in a random environment, suitable when the total number of cells is large. The model was first studied by Lewontin and Cohen (A1). See also Capocelli and Ricciardi (A2), Keiding and Nielsen (A3) and Keiding (A4).

We assume that the concentration $X(t)$ of plasma progesterone at time $t$ is a stochastic process satisfying the differential equation

$$\frac{dX(t)}{dt} = a(t)X(t),$$

where $a(t)$ is "white noise," i.e., all $a(t)$'s are independent and identically normally distributed. Let $Y(t) = \log X(t)$. It follows that $Y(t)$ is a Wiener process with linear drift; in other words, the $Y(t)$'s are normally distributed with mean and variance proportional to $t$ and with increments over disjoint time-intervals independent.

Statistical Model

For patient $i$, let $t_{i1}, \ldots, t_{in_i}$ denote the points in time at which measurements were made, and let $Y(t_{i1}), \ldots, Y(t_{in_i})$ denote these measurements. Let, for $i = 1, \ldots, 8$, $j = 1, \ldots, n_i - 1$, $\Delta_{ij} = Y(t_{ij+1}) - Y(t_{ij})$ and $d_{ij} = t_{ij+1} - t_{ij}$.

If we assume that the measurement error is negligible as compared to the random variations of the concentrations themselves, then $\Delta_{ij}$, $i = 1, \ldots, 8$, $j = 1, \ldots, n_i - 1$ are independent normally distributed with mean $E[\Delta_{ij}] = a_i d_{ij}$ and variance $V[\Delta_{ij}] = \sigma^2 a_i d_{ij}$, where $\sigma^2 > 0$ and $-\infty < a_i < \infty$ are unknown.

Analysis of the Model

By standard linear methods (A5) we get the estimates

$$\hat{\sigma}^2_i = \frac{\sum_{j=1}^{n_i-1} \Delta_{ij} - Y(t_{in_i}) - Y(t_{i1})}{n_i - 2}$$

and

$$\hat{\sigma}^2_i = \frac{1}{n_i - 2} \sum_{j=1}^{n_i-1} \Delta_{ij}^2 - \frac{\left(\sum_{j=1}^{n_i-1} \Delta_{ij}\right)^2}{\sum_{j=1}^{n_i-1} d_{ij}}$$

the $\alpha_i$'s, and $\hat{\sigma}^2_i$ being statistically independent, $\hat{\sigma}_i$ normally distributed with mean $\alpha_i$ and variance $\hat{\sigma}^2_i/(t_{in_i} - t_{i1})$, and $\hat{\sigma}^2_i$ following and $\chi^2/f$ distribution with $f = n_i - 2$ degrees of freedom.

Under the hypothesis that $\hat{\sigma}^2_i = \hat{\sigma}^2_2 = \ldots = \hat{\sigma}^2_8 = \hat{\sigma}^2$ (which is tested by Bartlett’s test), the common estimate of $\sigma^2$ is

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{8} (n_i - 2)\hat{\sigma}^2_i}{\sum_{i=1}^{8} n_i - 16} \quad (1)$$

Under the assumption of identical variances and of

$$\alpha_1 = \alpha_2 = \ldots = \alpha_8 = \alpha \quad (2)$$

the common estimate of $\alpha$ is

$$\hat{\alpha} = \frac{\sum_{i=1}^{8} \hat{\alpha}_i (t_{in_i} - t_{i1})}{\sum_{i=1}^{8} n_i - 1} \quad \frac{\sum_{i=1}^{8} \sum_{j=1}^{n_i-1} \Delta_{ij}}{\sum_{i=1}^{8} (t_{in_i} - t_{i1}) \sum_{j=1}^{n_i-1} d_{ij}}$$

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The hypothesis (2) is tested by an $F$-test comparing the estimate (1) of $\sigma^2$ with

$$\hat{\sigma}^2 = \frac{1}{8} \sum_{i=1}^{8} (\hat{\alpha}_i - \bar{\alpha})^2 (t_{i2} - t_{i1})$$

Under (2), $\sigma^2$ is estimated by

$$\hat{\sigma}^2 = \frac{1}{8} \sum_{i=1}^{8} n_i - 16 \hat{\sigma}^2 + 7 \hat{\sigma}^2$$

and analogous here to

$$R'(k) = \frac{1}{8} \sum_{i=1}^{8} (n_i - k - 1)R_i(k)$$

is approximately normal, with mean zero and variance $1/\sum_{i=1}^{8} n_i - 8(k + 1)$. An alternative to the $\Delta_{ij}$'s being independent could be caused by a nonnegligible measurement error; i.e., suppose that we instead of $Y(t)$ observe $Y_{*}(t) = Y(t) + \epsilon(t)$, where the $\epsilon$'s are independent of the $Y$'s, the $\epsilon(t)$'s being i.i.d. v. following a normal distribution with mean 0 and variance $\tau^2$. In that case we should have

$$R_i(1) \approx \frac{\tau^2}{\alpha^2 + \tau^2} = \frac{E(\Delta_{ij} - \alpha u_{ij})^2(\Delta_{ij} + \alpha u_{ij})}{\sqrt{E(\Delta_{ij} - \alpha u_{ij})^2 E(\Delta_{ij} + \alpha u_{ij})}}$$

and analogous here to

$$R_i(k) \approx 0 \text{ for } k \geq 2.$$