

Variability of Serum Prolactin and Progesterone Levels in Normal Women: The Relevance of Single Hormone Measurements in the Clinical Setting

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In order to delineate factors contributing to variation in hormone levels, progesterone and prolactin (PRL) levels from 28 normal women, obtained daily during one menstrual cycle and every 20 minutes during a midluteal 24-hour admission in a subgroup of five subjects, provided a data base for analysis of these variables. Pulsatile analysis of the 24-hour data was conducted using an adaptive-threshold algorithm, and normal reference ranges were generated from randomly selected daily hormone values. Our data verify that inherent variation can significantly alter single random serum levels of reproductive hormones. These variations included menstrual cycle day, circadian influence, pulsatile secretion, assay error, and biologic heterogeneity. Besides the expected day-to-day change in progesterone levels during the luteal phase, seven of ten women exhibited a significant circadian variation in progesterone; however, the time of day of the peak level was not consistent among women. Prolactin levels did not demonstrate any clinically relevant change over the menstrual cycle, but did have a consistent circadian pattern (nocturnal rise) over the 24-hour study period. Pulsatile variation occurred in both progesterone and PRL levels during the 24-hour admission. Five different reference ranges were generated from randomly selected single daily values from the 28 normal menstrual cycles. Although the mean levels calculated for each reference range were similar, the reference ranges demonstrated considerable variation due to the random sampling. In the five progesterone reference ranges, the lower limit of the range varied from 2.7–6.1 ng/mL, whereas the upper limit varied from 24.2–42.1 ng/mL. The lower and upper limits of the PRL ranges varied from 4.0–5.9 and 29.6–39.1 ng/mL, respectively. Clinicians should interpret

single hormone values in a cautious manner, tailoring their interpretation to the clinical setting and the reliability of their laboratory. (*Obstet Gynecol* 76:71, 1990)

Determination of circulating levels of reproductive hormones as an aid in the diagnostic process is a common clinical practice. The diagnoses of diseases in reproductive medicine are often supported by the determination of hormone levels in single blood samples; some examples are polycystic ovary disease (testosterone), pituitary tumors (prolactin [PRL]), luteal phase deficiency (progesterone), and primary amenorrhea (LH and FSH). When a blood sample is assayed, a specific value is reported to the clinician, usually accompanied by a reference range. An individual patient is considered to be abnormal (ie, to have a particular disease) if her serum level falls outside of the reference range. However, there are pitfalls associated with this seemingly straightforward diagnostic system that can mislead the unwary practitioner.

Difficulties can occur in both aspects of this diagnostic process; ie, in the hormone level reported and/or the reference range provided for comparison. When a serum sample is assayed for a particular hormone, the value obtained is influenced by a number of variables. These variables include menstrual cycle excursions, circadian variations, pulsatile secretion, and assay variation (besides the inherent biologic variability that exists among individuals). Each of these variables affects specific reproductive hormones to varying degrees, but they all have some effect on both the hormone value reported and the reference range.

This report addresses the ability of single serum levels of PRL and progesterone to predict clinical

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normality or abnormality and the ability of laboratories to provide accurate reference ranges for these hormones that reliably assist the diagnostic process.

Materials and Methods

Twenty-eight normal women between 22–35 years of age participated in various reproductive endocrine studies approved by the Human Subjects Committee of the University of Washington.^{1–4} All participants had a history of regular menstrual cycles, were within $\pm 10\%$ of ideal body weight (Metropolitan Life Tables, 1980), and were taking no medications. Each woman had a biphasic basal body temperature chart with a luteal phase longer than 12 days. In addition, each participant was required to have normal serum levels of testosterone (less than 40 ng/dL), PRL (less than 20 ng/mL), and progesterone (12 or more ng/mL midluteal) in a menstrual cycle preceding the study (as determined from single blood samples).

Venous blood samples were drawn between 8 and 10 AM daily throughout one menstrual cycle for measurement of progesterone, PRL, and LH. Five of the 28 women were also studied for 24 hours in the midluteal phase (cycle days 20–24, days +6 to +10 from the LH surge) during an admission to the Clinical Research Center. Each subject was kept at bed rest, with caffeine and smoking prohibited. Blood samples were drawn every 20 minutes through a heparinized indwelling intravenous line. Serum was separated, frozen at -20°C , and stored until analysis for progesterone, PRL, and LH. All samples from a given patient were analyzed in the same assay.

Serum PRL concentrations were determined in duplicate by a double-antibody radioimmunoassay (RIA)⁵ with the National Institute of Diabetes and Digestive and Kidney Diseases human PRL kit (RP-1 standard, h-PRL-3 anti-prolactin serum). The intra- and inter-assay coefficients of variation as calculated by analysis of replicate variability in the assay samples were 9.6 and 7.9%, respectively. The sensitivity of the PRL assay was 1 ng/mL at a serum volume of 200 μL .

Serum progesterone was measured in duplicate with reagents supplied by Diagnostic Products Corporation (Los Angeles, CA). Cross-reactivity data supplied by the producer specified less than 2.4% cross-reaction with all steroids tested, including 0.3% with 17 alpha-hydroxyprogesterone and less than 0.01% with testosterone, estradiol, pregnenolone, and cortisol. The intra- and inter-assay coefficients of variation were 5.7 and 8.5%, respectively. Sensitivity of the assay was less than 0.5 ng/mL at a serum volume of 100 μL . Confirmation of the above variabilities and the detection of no significant bias (greater than 10%) were

established using the World Health Organization program, an external quality control involving monthly measurements of unknown samples.

An adaptive-threshold method was used to determine the occurrence and amplitude of hormone pulses.⁶ A pulse was defined as an increase from local minimum to local maximum that was greater than a threshold value. The correct threshold was determined in an iterative manner. Initially, the threshold was set at 2.5 times the SD of the sample replicates, and the number of pulses in the data set was determined. Based on the estimated number of pulses, the threshold was readjusted according to the following formula:

$$T = S \cdot (5.518 + F \cdot [-0.3519 + F \cdot (0.01339 - 0.0002478 \cdot F)]),$$

in which T = threshold; S = the SD of the replicates; and $F = 100 \cdot (\text{number of pulses detected last time}) / (\text{number of samples in the data set})$. The analysis was then repeated with the new threshold. If the number of pulses detected was different from the number found on the previous pass, a new threshold was calculated according to the above formula and the procedure was repeated. This iterative procedure was continued until the number of pulses detected stabilized. The formula for threshold was based empirically on computer simulations.

Daily serum PRL and progesterone data were arranged relative to the day of the LH surge (day 0) for all subjects. Normal reference ranges were generated from the daily hormone values of all 28 women in our study population in the following manner: A sample was randomly selected from each individual (across the entire menstrual cycle for PRL and between days -4 and -11 from the next menstrual period for progesterone), the log of each value was determined, the mean and 2 SD were calculated, and thereby (after anti-log transformation) a reference range was established. Five separate normal ranges were derived in this fashion for both progesterone and PRL.

An hourly mean hormone concentration was calculated for each of the five women who underwent multiple sampling during admission. A 24-hour cosine regression analysis was performed on these hourly means to detect a possible circadian rhythm.⁷

Results

Each subject demonstrated considerable day-to-day variation in serum levels of progesterone and PRL (Figure 1). Progesterone did not follow a smooth, parabolic curve over the luteal phase when a single individual underwent consecutive daily samples. (The smooth curve we are accustomed to seeing is a compi-

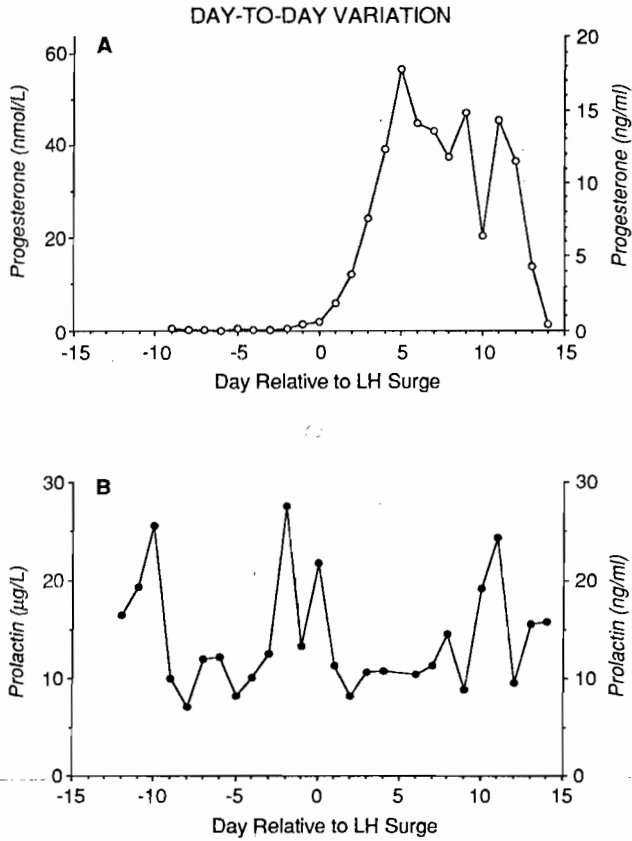


Figure 1. Daily (8–10 AM) serum hormone concentrations in two separate normal women. A = progesterone; B = PRL.

lation of daily mean levels.) Quite large variations occurred in samples collected on consecutive days. Prolactin exhibited large, apparently random variations when sampled daily. To understand what causes these variations, we will describe the inherent sources of variability.

Levels of some reproductive hormones vary dramatically across the menstrual cycle. Figure 2A shows mean progesterone levels as determined from daily blood samples collected across the menstrual cycle in 28 normal women. Progesterone remained quiescent throughout the follicular phase, rose rapidly to relatively high levels in the midluteal phase, and fell back to low levels during the late luteal phase. Figure 2B illustrates the daily mean PRL levels over the menstrual cycle in 28 normal women. Prolactin did not show any clinically relevant changes over the menstrual cycle (however, there was a significant but subtle mid-cycle peak when mean daily levels were determined in a group of normal women).

Besides the familiar menstrual cycle changes that occur in hormone secretion, most hormones also display 24-hour rhythms (circadian variation). When blood samples were obtained every 20 minutes over a 24-hour period during the luteal phase, seven of ten normal women experienced a significant 24-hour progesterone rhythm.² Figure 3 represents an example of this rhythm. The unusual aspect regarding the circa-

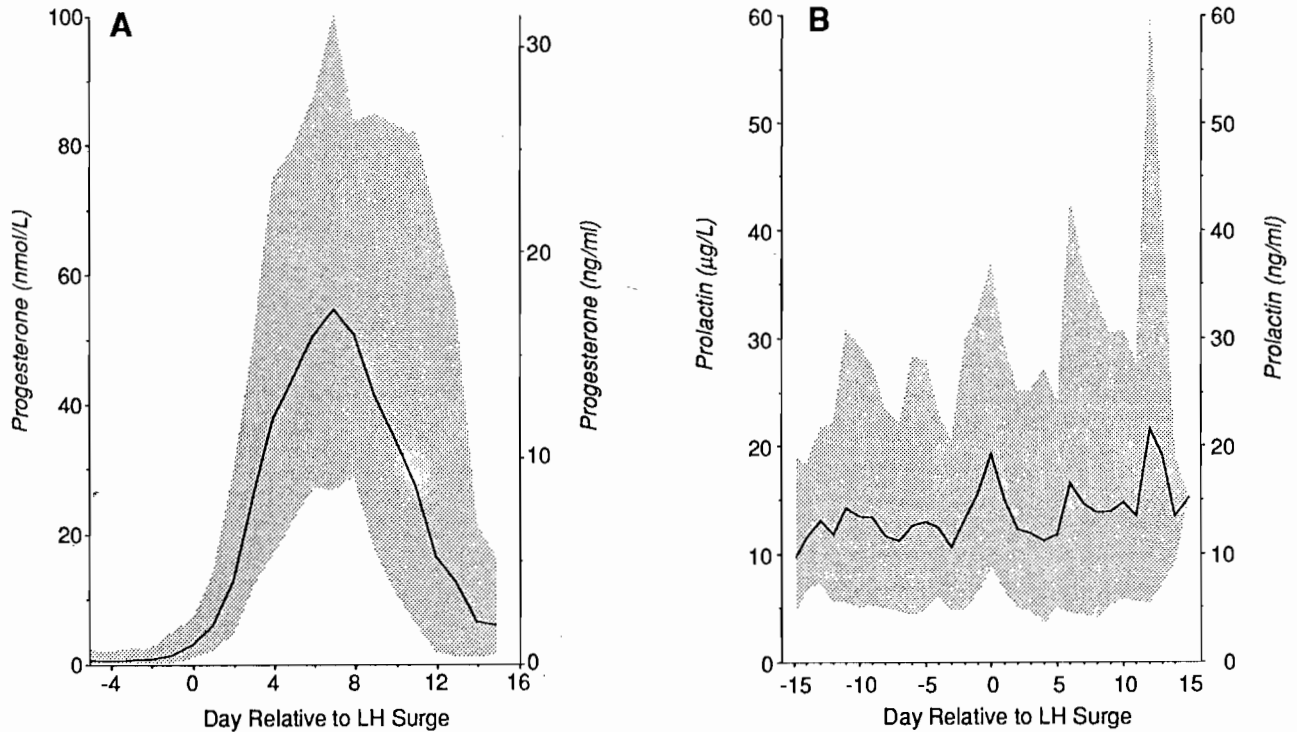


Figure 2. Menstrual cycle variation. Mean \pm 2 SD of daily hormone concentrations over the luteal phase (A; progesterone) and the menstrual cycle (B; PRL) in 28 normal women.

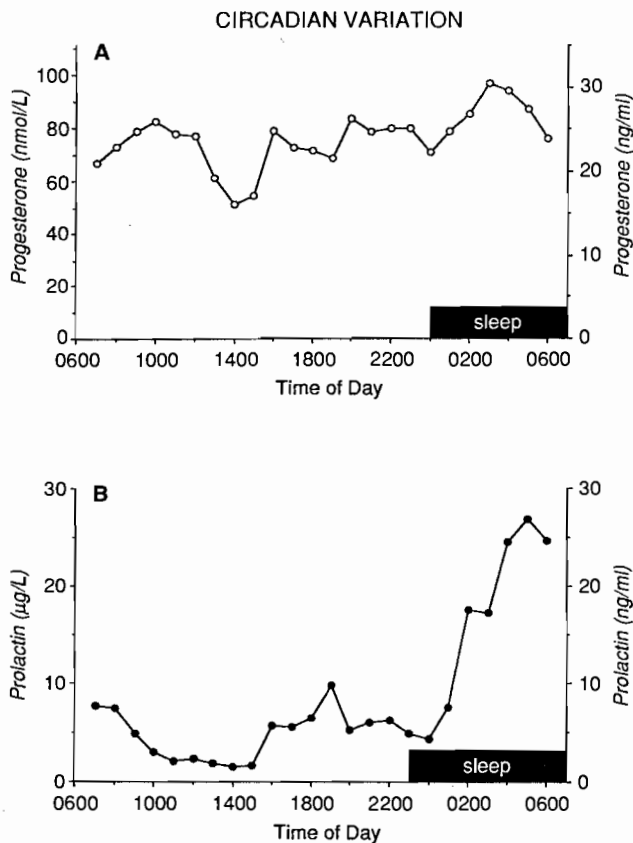


Figure 3. The mean serum hormone concentrations were calculated for each hour over a 24-hour sampling period (blood samples were obtained every 20 minutes) in two different normal women. For progesterone (A), the hourly levels vary in a subtle circadian pattern that corresponds to a sine-cosine curve. For PRL (B), there is a dramatic increase at night associated with the onset of sleep.

dian rhythm for progesterone was the lack of consistent timing among women. The peak progesterone level (acrophase) occurred at different times in different women (eg, at 2 AM in one subject and at 1 PM in another). Using the same frequent sampling protocol, we observed a consistent 24-hour PRL rhythm—low during the day and higher during the night.

Pulsatile variation can be clearly seen in the data we obtained from blood samples collected from normal women at 20-minute intervals over 24 hours. Regardless of sampling time, a single sample drawn from a patient during the 24-hour period shown in Figure 4A could have yielded a progesterone value anywhere from 4.8 to 15.3 ng/mL. Similarly, a single daytime PRL measurement from the patient shown in Figure 4B could have indicated a level anywhere from 3.5 to 25.7 ng/mL. Figure 5 illustrates the range in progesterone and PRL levels that occurred over a 24-hour period in each of five normal women.

One other relatively small, but real, source of vari-

ability in single-sample hormone measurement is assay error. No method of hormone measurement achieves absolute accuracy. Table 1 shows the results of measuring the same sample two or three times in the same assay and in ten different assays. For example, concerning the performance of the progesterone RIA in Table 1, assay 10 shows that the progesterone values reported by the assay for the same sample measured three times ranged from 3.12–3.99 ng/mL, roughly a 25% difference between the highest and lowest reported values for the sample. The mean value reported for this sample by each progesterone assay also showed considerable variation, reflecting both within- and between-assay variations. The lowest mean progesterone value was reported by assay 7 (2.72 ng/mL) and the highest by assay 4 (3.54 ng/mL). Similar variations occurred in the PRL assay (Table 1).

To make a highly accurate assessment of hormone levels in an individual, it is necessary to average a large number of samples collected over a period of time. We achieved this for progesterone and PRL in five women

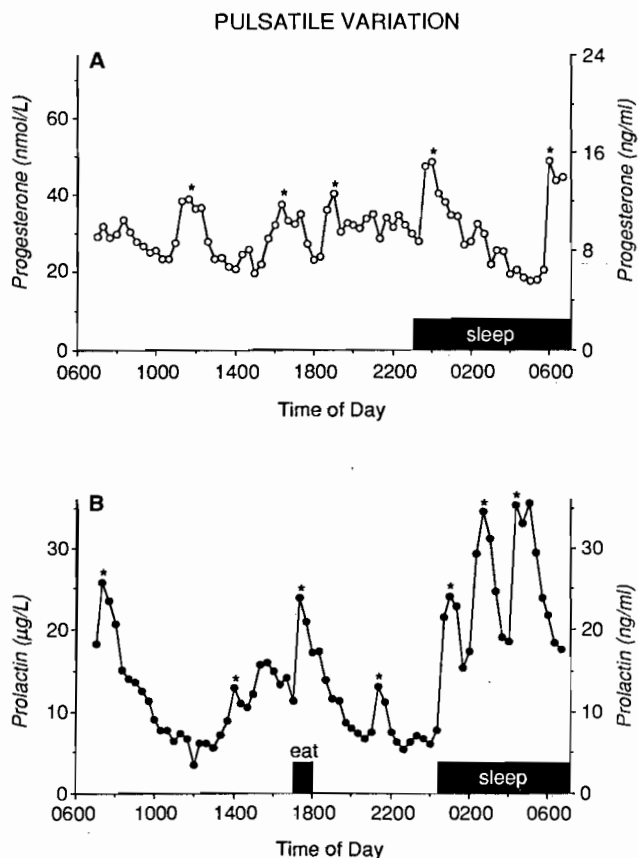


Figure 4. The secretion patterns of progesterone (A) and PRL (B) in the midluteal phase as determined by frequent blood sampling (every 20 minutes) over 24 hours in two different normal women. Each secretory episode (pulse) is indicated by an asterisk.

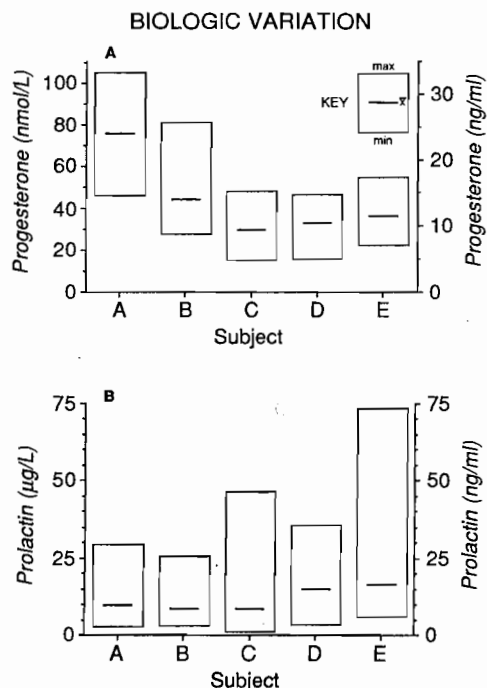


Figure 5. To illustrate biologic variation among normal individuals, data from five normal women in the midluteal cycle phase are presented. Each bar graph contains the mean level over a 24-hour sampling period as well as the minimum and maximum value (range) attained by that individual during that time interval. A = progesterone; B = PRL.

by collecting samples every 20 minutes over a 24-hour period. The persistent variation in mean levels among normal women for both hormones in Figure 5 reflects inherent biologic variation among individuals.

Table 1. Assay Variation

Assay	Progesterone					Prolactin			
	Sample*			Mean	CV	Sample*		Mean	CV
	A1	A2	A3			A1	A2		
1	3.06	2.98	3.08	3.04	0.017	5.9	6.4	6.2	0.057
2	2.99	2.84		2.92	0.036	5.5	7.9	6.7	0.253
3	3.16	3.08	2.93	3.06	0.038	6.6	6.1	6.4	0.056
4	3.15	3.54	3.92	3.54	0.109	6.1	6.2	6.2	0.011
5	2.90	2.80	3.13	2.94	0.057	6.2	5.7	6.0	0.059
6	2.93	3.01	3.01	2.98	0.015	5.2	6.2	5.7	0.124
7	2.72	2.88	2.55	2.72	0.061	6.1	7.0	6.6	0.097
8	3.50	3.11	3.07	3.23	0.074	5.5	6.1	5.8	0.073
9	3.28	3.41	3.43	3.37	0.024	4.8	5.7	5.3	0.121
10	3.12	3.99	3.27	3.46	0.134	5.0	5.8	5.4	0.105
Mean intra-assay CV					5.7%	9.6%			
Mean ± SD				3.13 ± 0.26 ng/mL		6.03 ± 0.48 ng/mL			
Inter-assay CV†				8.5%		7.9%			
Overall assay variation‡					10.2%	12.4%			

CV = coefficient of variation.

* A1, A2, and A3 represent three aliquots of the quality control pool run in each assay.

† SD divided by mean of assay means.

‡ $\sqrt{[(\text{intra-assay CV})^2 + (\text{inter-assay CV})^2]}$.

Different reference ranges were generated from single daily samples randomly selected from the same population of normal women in a single menstrual cycle. Random sampling variation is illustrated in Figure 6 (menstrual cycle, circadian variation, and assay variation were controlled). The means (geometric) and reference ranges were determined by appropriate statistical methods. As expected, the mean levels were quite similar for each hormone, yet the reference ranges demonstrated considerable variation due to random sampling.

We conducted a survey among laboratories in three pertinent areas: 1) assay methodology, 2) assay variability, and 3) source of reference range (internally generated or externally applied). Table 2 is a survey of these differences as self-reported by different clinical laboratories. For PRL, there was a similar reference range among all the laboratories surveyed. The reference ranges reported for progesterone were quite wide, with the upper limit exceeding 30 ng/mL in four of the five examples. Furthermore, most of the laboratories did not establish their own reference ranges, but obtained them from the manufacturer of the RIA kits.

Discussion

The diagnostic process in reproductive endocrinology and infertility, as in other phases of medicine, is based on a compilation of information obtained from history, physical examination, and laboratory measurements. The latter is an important, but sometimes overempha-

REFERENCE RANGES

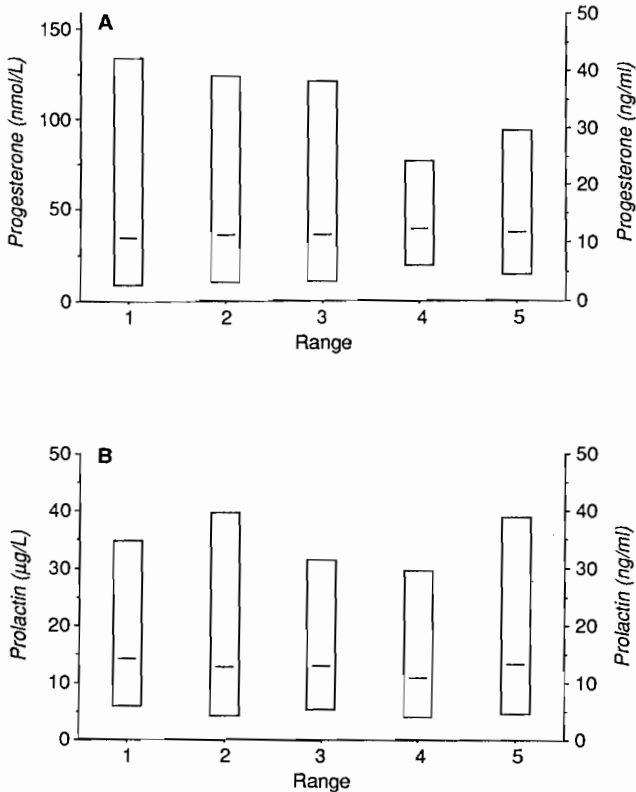


Figure 6. Five separate reference ranges, generated by randomly selecting a daily hormone level from each woman on five occasions, were calculated from 28 women. For progesterone (A), only cycle days -4 to -11 from the next menstrual period were used; all cycle days were used for PRL (B).

sized, facet of the diagnostic process. In reproductive medicine, the laboratory measurement of serum hormone concentrations is common practice. Considering cost and patient convenience, these hormone measure-

ments are usually performed on single blood samples. However, a number of variables remain uncontrolled when random single samples are used. This study explored the relative effects of these inherent variables on the results obtained. Table 3 summarizes the relative contributions of these variables for PRL and progesterone measurements.

Inherent variability makes the determination of any single hormone concentration only a rough approximation of the secretory status of that hormone. When measuring a hormone that changes across the menstrual cycle, the timing of the sample is important. The sample must be taken within the same time frame (eg, midluteal) as are the reference range values against which it is to be compared. For progesterone, it is common practice to obtain a blood sample between days -11 and -4 from the estimated next menstrual period. For PRL, samples can be obtained without reference to menstrual cycle phase.

Concerning circadian rhythms, our progesterone data did not demonstrate a consistent rhythm in terms of a peak or nadir at a particular time of day. Other investigators have reported a more consistent circadian rhythm for progesterone.⁸ Based on our data, the lack of reproducible circadian patterns for progesterone precludes the necessity to control for the time of day in obtaining relevant samples. For PRL, which showed a marked and consistent circadian rhythm, it is important for the clinician to take the rhythm into account when obtaining diagnostic blood samples. It is also important to verify the time of day the laboratory collects its blood samples to determine its normal ranges. The common practice is to measure PRL in blood samples obtained between 8 and 10 AM.

For some hormones, the 24-hour rhythm occurs in the absence of any external stimulus, but for others it is

Table 2. Survey of Reference Ranges Reported by Clinical Laboratories

Laboratory	PRL normal range (ng/mL)	Progesterone normal range (luteal phase) (ng/mL)	Assay technique	Normal range source
National commercial lab	0-20	3-36	PRL—kit* Prog—kit	Use kit ranges
Large regional commercial lab	3.8-23.2	3-25	PRL—use their own kit Prog—developed their own assay, not a kit	PRL—N = 39 screened normals Prog—N = 10 screened normals
Large private hospital lab	2-26	3-36	PRL—kit Prog—kit	PRL—combination of kit and normals Prog—kit range only
Small private hospital lab	0-18.5	2.5-28.2	PRL—kit Prog—sent out	PRL—combination of kit and normals
University medical center lab	0-20	0-35	PRL—kit Prog—kit	PRL—N = 48 screened normals Prog—N = 12 screened normals

PRL = prolactin; prog = progesterone.

* A "kit" is a commercially supplied set of reagents for a radioimmunoassay.

Table 3. Summary of Variables That Affect Prolactin and Progesterone

	Prolactin	Progesterone
Menstrual cycle	+	++++
Circadian rhythm	+++	+
Environmental factors (eg, food, stress, sleep)	+++	---
Pulsatile release	++++	++++
Assay	+	+
Biologic variability	+++	+++

synchronized to an external cue such as light. These rhythms adjust slowly when the timing of the cue is changed. Such is the case with PRL, which has been shown to increase during sleep.⁹ If sleep is withheld, PRL does not rise; when sleep occurs during the day instead of at night, PRL is high during the day and low at night.¹⁰ Therefore, clinicians must be aware of their patient's schedules and sleep patterns (eg, working a night shift).

Environmental factors can influence the secretion of some hormones. Prolactin is much more susceptible than progesterone in this regard. Factors that affect PRL include eating, drinking, smoking, and many different types of stress (eg, exercise).⁹

Most serum hormone levels fluctuate from minute to minute because of pulsatile secretion.^{1,2,6} This is true for gonadal as well as pituitary hormones. Large swings in serum PRL and progesterone levels associated with these pulses can cause substantial variation in the apparent concentration of hormone present when a single sample is taken. Pulsatile hormone secretion is the chief source of the acute variation seen in daily levels of progesterone and PRL. Depending on when the sample is taken in relation to a pulse, the value could range widely. For example, in Figure 1A, samples taken on days 4, 9, and 11 of the luteal phase were probably obtained near a peak of a progesterone pulse and are thus high values, whereas the sample taken on day 10 was probably close to a nadir. Likewise, in Figure 1B, the higher values of PRL probably reflect sampling at the peak of a PRL pulse.

Another source of variability in a single-sample hormone measurement is assay error. For the RIAs we used to measure progesterone and PRL, the intra-assay coefficient of variation was usually around 10%. This means that for a sample that really contains 10 ng/mL of hormone, the assay will, 95% of the time, report values that range anywhere from 9–11 ng/mL. The relative importance of the assay-related variation as shown in Table 1 depends on the accuracy of the assay and the intrinsic variability of the hormone. For

example, for PRL, minute-to-minute and 24-hour variability is approximately 55%, compared with an assay coefficient of variation of around 10%. The total variability is calculated by taking the square root of the sum of the squares of the separate variabilities. For this example, total variability is $(55^2 + 10^2)^{1/2} = 55.9\%$. The total variability would be 55% if the assay were perfect. From this example, it is clear that assay error makes only a minor contribution to the overall variability seen in the results of a single clinical sample.

What the clinician usually wants to know when a sample is taken and sent to the laboratory for a hormone determination is, "How does this woman's hormone level(s) compare to those of normal women?" Unfortunately, a single sample provides only a rough approximation of a patient's overall hormonal status because of the variability introduced into the samples by the above factors. Figure 5 illustrates the diversity (biologic variation) that still exists in overall hormone values among women even after circadian and pulsatile variations have been removed by averaging a large number of samples, assay variation has been minimized by measuring all samples from the same patient in one assay, and the menstrual-cycle variation has been minimized by sampling during the same cycle phase.

Although this study did not specifically address the ability of single random levels of progesterone and PRL to predict pathologic states, we can make some general comments. Major abnormalities such as anovulation (eg, progesterone less than 3 ng/mL) and a lactotrophic pituitary adenoma (eg, PRL more than 100 ng/mL) would generally not be misdiagnosed by single serum hormone levels. However, more subtle abnormalities, such as luteal phase deficiency (low postovulatory serum progesterone levels) and infertility states thought to be associated with minor elevations of PRL, would be very difficult to diagnose accurately using single random hormone levels.

When reference ranges are defined by clinical laboratories, a number of considerations need to be taken into account. First, the subjects must be certified as normal by use of objective criteria. Next, blood samples must be obtained from these normal individuals in a manner similar to the sampling protocol that will be used for unknown subjects. The time of day (eg, 8–10 AM) is the only variable (circadian) that is commonly controlled when single samples are obtained in the clinical setting. Therefore, single samples should be obtained from a reasonably large number of normal subjects in that time interval. However, these samples from normal women remain subject to all of the other variables that are known to affect the patient samples. In Figure 6A, the progesterone reference ranges are so

wide that clinical usefulness would be precluded. The fallacy of attempts to diagnose luteal phase deficiency by single progesterone values becomes apparent here. The PRL reference ranges were all similar (Figure 6B); however, the upper limits of these reference ranges would call into question whether a random PRL level between 20–40 ng/mL is abnormal.

The use of single samples is so widespread that this sampling frequency appears to be ingrained in medical practice. Based on the wide variations in hormone levels observed in our data, we can make several recommendations. First, physicians should interpret results in a cautious and knowledgeable manner; it is important to realize the approximate nature of any result. Whereas the reported value may be a very specific number (eg, 13.73 ng/mL), it should be applied only in a more general sense (ie, the value is low or medium or high).

Second, clinical laboratories that assay samples and thereby determine hormone concentrations must be cognizant of the clinical situation in which these results are applied. Laboratories should use the best assay materials and procedures available. We recommend that they report their results without using a decimal point, because such seemingly high degrees of accuracy contradict the approximate nature of the result. Concerning reference ranges, clinical laboratories should each establish their own normal ranges. Techniques and results may vary from laboratory to laboratory even when the same reagents are used. The normal subjects should be screened by history (and physical examination when necessary) to clarify their normal status objectively. The common practice of sampling any subject in a certain age group who volunteers (without further clarification of his or her relevant health status) is counterproductive. Use of a relatively large number of normal subjects to establish reference ranges would help decrease variability and result in narrower and more valid ranges. In the calculation of reference ranges, valid statistical techniques should be applied to appropriately transformed data. Application of all of these recommendations would result in more useful reference ranges. Finally, we recommend that laboratories include a cautionary statement warning against overinterpretation when they report results.

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clarify that we have observed only one case of a 45,X abortus with a high CA 125 level.

We have subsequently found other cases that have also had chromosomal abnormalities, including another trisomy 21 and a trisomy 10. We are presently engaged in a prospective study aimed at determining whether elevated CA 125 levels in the early first trimester indicate an increased risk of fetal chromosomal abnormalities or whether the association is merely coincidental.

We apologize for any confusion with the reported case and want to reiterate that we described only one case of elevated CA 125 and subsequent abortion in a fetus of karyotype 45,X.

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VARIABILITY OF SERUM PROLACTIN AND PROGESTERONE LEVELS IN NORMAL WOMEN: THE RELEVANCE OF SINGLE HORMONE MEASUREMENTS IN THE CLINICAL SETTING

To the Editor:

Having had the opportunity to formally critique the presentation by Fujimoto at the 38th Annual Clinical Meeting of The American College of Obstetricians and Gynecologists on May 9, 1990, I feel that some comments are necessitated by the appearance of the manuscript in the July 1990 issue of *Obstetrics and Gynecology*.¹

For years clinicians have relied on laboratory values to enhance their clinical impressions (diagnoses) and subsequent treatment of their patients. Unfortunately, many physicians have given more importance to the laboratory value itself rather than the history (which

usually reveals the diagnosis in 90% of the cases) and the physical examination (which usually reveals the diagnosis in 8% of the cases). Laboratory studies ordered are adjuncts to the history and physical examination.

One must always be cautious about a single laboratory value, but even more so when discussing hormone levels. Physicians have always wanted "exact" results to enhance or document their diagnostic impressions, but unfortunately, as this manuscript so aptly demonstrates in the examples cited (progesterone and prolactin), such exactness is not possible.

Errors inherent in laboratory values include differences in clinical settings, the reliability of the laboratory, the range of "normal" values, use of "approximations," assay errors, and assay variations.

When assaying for certain hormones, one must also take into account the menstrual cycle day, circadian influences, biologic heterogeneity, pulsatile secretion of the specific hormone, and environmental factors.

In 1976, Goldzieher et al² reported that when one determines plasma hormone levels that undergo rapid and large oscillations, such as testosterone, FSH, and LH, sampling should be multiple. Computer analysis demonstrated that three equally spaced samples taken at 6-18-minute intervals and pooled before the radioimmunoassay provided the optimum schedule to increase the reliability of the test result.

One cannot disagree with the conclusions of Fujimoto et al, ie, that clinicians should interpret single hormone values in a cautious manner, thereby tailoring their interpretation to the clinical setting and the reliability of their laboratory. However, I would add that the suggestion made by Goldzieher et al² to pool three plasma samples and then perform the hormonal assay would certainly help to normalize the result obtained.

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Ann Arbor, MI 48109-0718

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In reply:

We appreciate Dr. Ansbacher's comments, particularly his support of our thesis regarding the inherent difficulties in interpreting single hormone values. In his letter, he suggests an alternative of pooling three plasma samples to more closely approximate the "true" value. We purposely chose not to focus on this approach because of the predominance of single hormone sampling in the clinical setting.

Goldzieher et al¹ concluded that three-sample pooling increases the probability of estimating the true mean plasma hormone level at a standardized time of day. That study assessed LH, FSH, and testosterone profiles in four men drawn every several minutes over 8 hours, with the best estimates of gonadotropin values occurring for sampling intervals up to 20 minutes. For clinical purposes, they proposed pooling three plasma samples and then performing the assay, even though this technique was not used in their study per se. However, they reported only a modest increase in accuracy with pooling (eg, for testosterone the three-sample technique yielded a result within $\pm 50\%$ of the true mean 85% of the time, as opposed to 68% for the single random sample). We encourage the performance of a study that would assess the actual effectiveness of pooling in the clinical setting.

Pooling separate specimens should decrease the variables inherent in single serum hormone analysis. However, to do so in the clinical setting would appear to be tedious. One must pose the question, "Is the improvement in efficiency enough to warrant three serum specimens from the patient over a period of time, which would be costly in technician time and patient inconvenience and discomfort?" Furthermore, despite such efforts, the final value obtained is still only an approximation of the true hormone value and must be integrated with caution.

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levels by optimized multiple-sampling techniques. *J Clin Endocrinol Metab* 1976;43:824-30.

AMOXICILLIN THERAPY FOR *CHLAMYDIA TRACHOMATIS* IN PREGNANCY

To the Editor:

Although the results of the study, "Amoxicillin therapy for *Chlamydia trachomatis* in pregnancy"¹ are impressive, they should be qualified because of the nonrandomized method of selection. Women with positive cultures for chlamydia were asked to enter an amoxicillin study. If they agreed, they were entered into the amoxicillin arm of the study. Otherwise they were assigned to the second arm and received erythromycin, the standard therapy for *C trachomatis*. The authors state that "Although less desirable methodologically, we chose an open study because we estimated that it would have taken far longer to accumulate the same numbers of patients in a randomized trial."

A randomized study may take longer, but the importance of randomization in a clinical trial should not be underestimated, for the following reasons²: 1) It prevents accidental bias. Randomization will on average balance not only known demographic and prognostic variables, but also those variables that are not measured or known. 2) It prevents selection bias, including bias of either the investigators or the patient. Volunteer bias is well documented; volunteers differ in health status, socioeconomic status, and compliance with drug and therapy regimens. 3) It helps provide a foundation for statistical testing, in that it guarantees that certain assumptions are valid.³

It would appear that the two study groups were not handled in an identical manner. The amoxicillin group was asked to keep medication diaries and to record side effects, but no mention of a diary is made with respect to the other group.

While one may argue as to whether the lack of randomization affected the outcome of this particular study, one cannot ignore the consequences of a poorly designed study: 1) Patients may be placed at risk or inconvenience for little or no benefit; 2) other researchers may be encouraged to adopt similar inappropriate methods; and/or 3) the potential to perform future studies may be compromised by poorly supported conclusions.⁴ Proper randomization in a clinical trial may require some extra work on the part of the