The normal variabilities of the menstrual cycle

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Objective: To address conflicts in the normal variabilities of the menstrual cycle using the newest generation test methods and to establish normal ranges for use in clinical practice.
Design: Daily urine samples were collected from 167 women eager to achieve pregnancy. Samples were tested prospectively for LH and total hCG. A total of 458 nongestational and 111 gestational menstrual cycles were evaluated.
Setting: Division of Women's Health Research, University of New Mexico.
Patient(s): One hundred sixty-seven women desiring pregnancy.
Intervention(s): None.
Main Outcome Measure(s): Levels of hCG and LH.
Result(s): Menstrual cycles were 27.7 ± 2.4 days in length. The LH peak indicated the onset of the presumed ovulatory window, which occurs at 14.7 ± 2.4 days. Implantation (first day of sensitive detection of hCG) occurred in gestational menstrual cycles at 24.6 ± 3.1 days, or 4.3 ± 2.2 days before missing the expected onset of menses.
Conclusion(s): Our data confirm epidemiological studies on menstrual cycle length and variability and hormonal studies on timing of the ovulatory window and its variability. They dispute, however, the published data on the timing and variance of implantation. As shown, implantation is limited to a 10-day interval culminating in the day of the expected onset of menses. Reference range data provide guidelines for differentiating normal and problem menstrual cycles. (Fertil Steril® 2009;91:522–7. ©2009 by American Society for Reproductive Medicine.)

Key Words: hCG, LH, LH Peak, luteal phase, implantation, menstrual cycle

The range of normal variability of the menstrual cycle, length of luteal and follicular phases, and timing of ovulation and implantation are integral to elementary obstetrics and gynecology as they are taught at medical school. They apply to the normalcies of everyday medical practice. Yet relatively little research has been published in recent years on normal variability during the healthy nonconceptive menstrual cycle and during the spontaneous cycle that leads to pregnancy. Little has been published about the normal length and variability of the constituent phases of the spontaneous menstrual cycle or parameters that can be used to define normal in medical practice. We attempt here to provide these normality guidelines for practicing physicians.

The basic overview of the menstrual cycle and its timing were established between 1910 and 1960. These initial guidelines generated the concept of the 28-day menstrual cycle and repetitive constant 28-day cycle (1, 2). In the 1960s, the LH peak and its relationship to ovulation were realized (3–6). The concepts of follicular and luteal phases emerged, together with the day 14 ovulation and the concept of the 28-day menstrual cycle (3–9). From the studies of the Tremin Trust and Lenton and colleagues, menstrual cycle variability and LH peak variability became accepted (7–12), with the follicular phase of the menstrual cycle being shown to be the biggest source of menstrual cycle variability.

What remained to be further clarified was the timing of pregnancy implantation and the variability of the menstrual cycle implantation and pregnancy. These relationships were addressed by the multiple studies of Wilcox et al. (13–15). This group, however, measured timing of pregnancy and implantation using an hCG test that did not fully recognize hyperglycosylated hCG (hCG-H), a variant of hCG recently shown by multiple groups to be the principal form of hCG present in early pregnancy (16–19). This very much limited the value of these findings. Wilcox et al. also used thawed and frozen samples, a process that has been shown to diminish hCG-H concentration (20). These studies need to be repeated with fresh urine and a test that appropriately measures hCG-H (21, 22). Furthermore, all of this group's data were anchored to ovulation. Ovulation was measured using the Baird steroid metabolite urine assay (24), a method that has been described recently as the least reliable means of detecting ovulation or the presumed ovulatory window (23). Looking at the limitations of the key Wilcox data (13–15)

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with the knowledge we have today, there seemed an urgent need to reevaluate implantation criteria by prospectively measuring fresh daily urines and using optimal hCG and ovulation tests (25, 26).

In addition, we use here contemporary methods (specific immunometric LH assays) to confirm the follicular phase and luteal phase variation data of Lenton et al. (10–12) and to consider all these data with respect to keynote epidemiological studies of menstrual cycle variation as established by the Tremin Trust analyses.

While numerous statistics have been created on menstrual cycle variation (7–15), none appear to provide simple ranges (95% confidence intervals) for physicians to define normals or to clearly differentiate normal and irregular menstrual cycles. Here we describe an extensive menstrual cycle study with 184 women attempting to conceive in which we investigated 458 nonconceptive and 111 pregnancy menstrual cycles.

MATERIALS AND METHODS

Daily urine samples were collected from 184 women ages 18–36 years eager to naturally conceive over the course of three to eight menstrual cycles. Collection ended in each case with either pregnancy or completion of the clinical trial. HCG and LH were prospectively determined in all daily urine samples. Over 2 years, from January 2005 to January 2007, LH and hCG data were accumulated on 458 nonconceptive menstrual cycles (14,197 urine hCG and LH measurements). In addition, prospective data were accumulated on 111 menstrual cycles leading to pregnancy (4075 urine hCG and LH measurements). The evaluations and analysis described here were funded by the USA hCG Reference Service at University of New Mexico and have no connection to any commercial interest.

Women were recruited to the clinical trial after completing a questionnaire. Only women with no recent history of infertility or menstrual cycle disorder were asked to volunteer. In all cases, women kept records of the date of commencement of menses. All volunteers resided in Albuquerque, New Mexico, and all parts of the study were completed according to a consenting and management protocol approved by the Human Research Review Committee of the Institutional Review Board of the University of New Mexico (protocol 04-132, reviewed May 10, 2006).

Urinary hCG was prospectively measured using the Diagnostic Products Corporation (Los Angeles) Immulite automated total hCG test. This is one of few pregnancy tests proven to detect urine hCG and one of few also shown to detect all pertinent early pregnancy isoforms—hCG, hCG-H, hCG free ß-subunit, and hCG ß-core fragment—on an equimolar basis (26). The sensitivity of this test is 1 mIU/mL; the coefficient of variation of this test is <5%. LH was measured using the Diagnostic Products Corporation Immulite automated test, which detects LH and its free ß-subunit or all pertinent forms of LH (18). The sensitivity of this test is 0.2 mIU/mL; the coefficient variation is <5%. The LH peak was defined as the day with the highest LH result.

The timing of the presumed ovulatory window was predicted biochemically from the appearance of the LH peak (23). The highest LH value in the peak period was considered as the peak. In the experience of this study, it was important to measure LH in fresh urine to obtain clear single-peak measurements. For the purposes of this study, we defined the duration of the constituent phases using self-recorded menstrual events and serially determined urinary markers. The timing from start of menses to the LH peak is considered here to be the follicular phase. The luteal phase is represented by the time interval from the presumed ovulatory window (LH peak) to the first day of onset of patient-recorded onset of next menses.

Wilcox et al. have defined the day of implantation as the day intact hCG can be first detected with a sensitive assay (13–15). In this article, we measure all variants of hCG or total hCG (hCG, free ß-subunit, ß-core fragment, nicked hCG, and hCG-H). We assume that measuring all forms of hCG is a more sensitive test for hCG production by the implanting embryo than measuring intact hCG alone. Here we define implantation as the day of detection of 1.0 mIU/mL or greater total hCG. We predicted the expected onset of menses in each pregnant individual from the mean of the length of the three preceding nongestational menstrual cycles.

Data were accumulated in a spreadsheet, and means, SDs, variances [formula: $\Sigma(x - \text{mean})^2/(n - 1)$], 95% confidence intervals, percentiles, and *t*-statistics were determined. A major objective of this article was to define parameters for normal menstrual cycles (here we define a normal menstrual cycle as one of less than 40 days in length with a clear LH peak from a patient with no evidence of anovulation or indication of polycystic ovary or other ovulatory dysfunction). As such, it was important to preemptively remove cases with clear abnormal cycles from the final data analysis. Menstrual cycles from 17 cases that failed to achieve pregnancy were excluded from the final analysis because of extraordinary hormone results and cycle timings. Six of these 17 cases were excluded because of consecutive cycles without a clear LH peak. These were considered to be cases with anovulatory menstrual cycles. Four of these 17 cases were excluded because of multiple cycles with exceptionally elevated LH concentrations (>300 mIU/mL, >95th percentile of cycles) combined with exceptionally extended menstrual cycle intervals (>40 days, >95th percentile of cycle). Menstrual cycles such as these, of >40 days, are classed as abnormal in the keynote Tremin Trust studies (7–9). The final seven of 17 cases were excluded because of a combination of intermittent anovulatory menstrual cycles with unduly high LH concentrations (>300 mIU/mL, >95th percentile of cycles). In all seven cases, the patients were later shown by gynecologists using ultrasound to have polycystic ovary syndrome or other ovarian abnormality (no further details available). No other cases were excluded for any reason.

With the exclusion of the 17 cases, the final analysis was derived from 167 cases. This provided data on 408 normal nonconceptive menstrual cycles and 111 menstrual cycles leading to pregnancy.

RESULTS

Four Fifty Eight Nonconceptive Menstrual Cycles in 167 Women: 12,687 Urine hCG and LH Measurements (Table 1)

The mean menstrual cycle (±SD), that is, day of commencement until day of recommencement of bleeding, was 27.7 ± 2.4 days. As a guideline of normality, the 95% confidence interval of the normal distribution of menstrual cycle length was 23–32 days. We investigated individual variance in length of menstrual cycles in cases with five or more consecutive menstrual cycles. Variance was 2.7 ± 1.6 days. As a guideline for normality, the 95% confidence interval of the normal distribution of individual variances in menstrual cycle length was 0.8–5 days. The between-individual variance in these same cases was 4.7 days. A representative example of a case with eight menstrual cycles is shown in Figure 1.

The timing of the presumed ovulatory window was predicted from the appearance of the LH peak (days since start of previous menstrual bleeding). The mean LH peak occurred at 14.7 ± 2.4 days. As a guideline of normality, the 95% confidence interval of the normal distribution of LH peak timing was 10–20 days. The individual variance in the timing of the LH peak was 3.9 ± 3.7 days; the 95% confidence interval of the normal distribution of individual variations was 1–13 days. The between-individual variance in these same cases was 6.6 days. A representative example of a case with eight menstrual cycles is shown in Figure 1.

The mean luteal phase was calculated as 13.2 ± 2.0 days. The 95% confidence interval of the normal distribution was 9–17 days. The individual variance in the timing of the luteal phase was 2.6 ± 3.1 days; the 95% confidence interval of the normal distribution of individual variation was 0.3–9 days. The between-individual variance in these same cases was 4.0 days.

One Hundred Eleven Menstrual Cycles Leading to Pregnancy: 4075 Urine hCG and LH Measurements (Table 2)

Of 111 pregnancies, 70 were term pregnancies, 13 ended in spontaneous abortions, and 28 were early pregnancy losses. The LH peak or presumed ovulatory window was extremely similar in nonconceptive menstrual cycles and cycles leading to gestation, 14.7 ± 2.4 days and 15.2 ± 2.4 days, respectively. The mean timing of implantation was 24.6 ± 3.1 days, and the 95% confidence interval of the normal distribution of 20–30 days. The between-individual variance in the timing of implantation was 10.1 days. It was calculated that implantation occurred at 4.3 ± 2.4 days before the expected onset date of menses, with a range of 10 days before the expected onset

TABLE 1

Experience with 458 healthy menstrual cycles from 167 women (12,687 urine hCG and LH measurements).

	Timing of menstrual cycle, days	Within-individual variance in timing of menstrual cycle, days	Timing of ovulatory window (length of follicular phase), days	Within-individual variance in timing of ovulatory window, days	Length of luteal phase, days	Within-individual variance in length of luteal phase, days
Mean ± SD	27.7 ± 2.4	2.8 ± 1.6[a]	14.7 ± 2.4	3.9 ± 3.7[b]	13.2 ± 2.0	2.6 ± 3.1[c]
Range	20–34	0.8–6.7	9–20	0.5–18	9–20	0.2–15
95% limits	23–32	0.8–6.2	10–20	1–13	9–17	0.3–9

Note: The timing of the presumed ovulatory window is reported as the day of the LH peak value. The number of days to LH peak is assumed to be the length of the follicular cycle. The luteal phase is calculated as the length of the menstrual cycle minus the day to the LH peak value. The timing of menstrual cycle is the time of the reported start of menses compared with the start of previous menses. Within-individual variance is the mean statistical variance among 31 individuals with five monitored sequential menstrual cycles. Between-individual variance is the total variance within this group of 31 individuals. All means are ±SD. The 95% limits are the 95% confidence interval of a normal distribution (2.5th percentile to 97.5th percentile).

[a] The between-individual variance for timing of the menstrual cycle is 5.2 days.

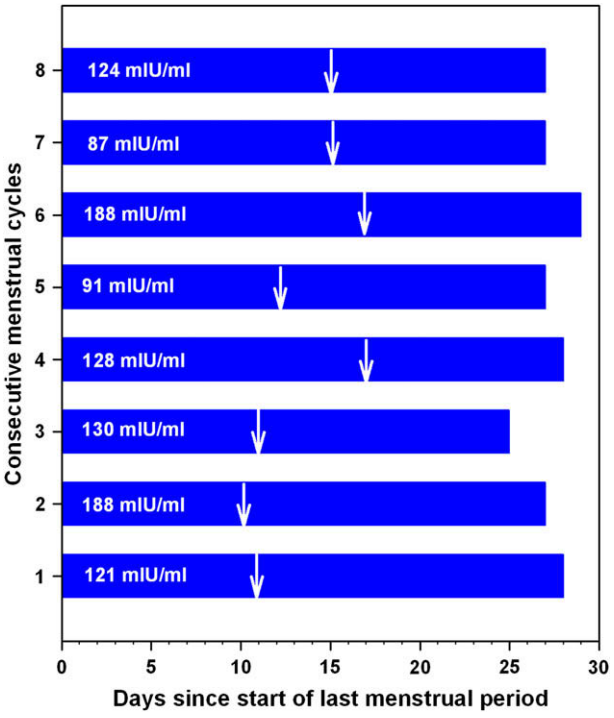
[b] The between-individual variance for timing of the presumed ovulatory window (follicular phase) is 6.6 days.

[c] The between-individual variance for length of luteal phase is 4.0 days.

Cole. Normal variabilities of the menstrual cycle. Fertil Steril 2009.

FIGURE 1

Bar graph showing representative example of case and eight consecutive normal menstrual cycles. Bar length is menstrual cycle length in days (start of first bleeding to start of first bleeding). The mean menstrual cycle length in this case was 27.3 days, and the within-individual variance was 1.4 days. Arrows show the day of LH peak for each cycle (highest LH concentration, value is concentration) or presumed ovulatory window. The mean day of LH peak was 13.5 days, and the within-individual variance was 8 days.



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of menses and a 95% confidence interval of 9–0 days before the day of the expected onset of menses.

DISCUSSION

Using fresh samples and optimal assays, we determined the variability of the menstrual cycle. We excluded any cases with apparent infertility issues and tested total hCG and LH in 18,272 urine samples to define normal menstrual cycle parameters for clinical practice. We used LH peak determination to divide the major phases of the menstrual cycle. This we consider the timing of the presumed ovulatory window. However, the chronological relationship between the LH peak and ovulation timing as addressed by ultrasound and other methods was not specifically addressed.

Variance of Menstrual Cycle

The Tremin Trust epidemiology studies report a mean menstrual cycle length of 28.6 ± 5.0 days (7–9), and the Lenton

TABLE 2

Experience with 111 menstrual cycles leading to pregnancy (4,075 urine hCG and LH measurements).

	Timing of ovulatory window, days	Timing of implantation, days	Implantation relative to timing of ovulatory window, days	Day of missing expected onset of menses, days	Implantation relative to day of missing start of expected onset of menses, days
Mean ± SD	15.2 ± 2.4	24.6 ± 3.1[a]	9.4 ± 2.0	28.7 ± 2.5	−4.3 ± 2.2
Range	10–20	16–30	4–14	24–34	−10 to 0
95% limits	11–20	20–30	5–14	24–34	−9 to 0

Note: The timing of the presumed ovulatory window is reported as the day of the LH peak value. The timing of implantation is calculated as the day of the first total hCG detection (>1 mIU/mL), according to Wilcox et al. (13–15). The day of the missing expected onset of menses is calculated from the average length of the menstrual cycle in the previous three menstrual cycles. The minus sign means before the expected onset of menses, and the plus sign means after missed menses. The timing of the presumed ovulatory window is reported as the day of the LH peak value. Values are means ± SD. The 95% limits are the 95% confidence interval of a normal distribution (2.5th percentile to 97.5th percentile).

[a] The between-individual variance in the timing of implantation was 10.1 days.

Cole. Normal variabilities of the menstrual cycle. Fertil Steril 2009.

studies indicate a mean of 27.1 ± 2.7 days (10–12); the data presented here are in the middle of these ranges, or 27.7 ± 2.4 days. All of these values have a component of subjective variance because they are based on volunteer reporting of the day of commencement of menstrual bleeding. The between-individual variances reported here and in the Tremin Trust and Lenton studies are similar, 5.2, 5.8, and 4.7 days, respectively. Only this study and the Tremin Trust studies investigate within-individual variances, which were 2.9 and 5.4 days, respectively. The tighter between-individual variances and within-individual variances recorded in this study compared with other studies may be due to the systematic exclusion of cases with infertility issues and menstrual cycle disorders. If we include the 17 cases with menstrual cycle disorders in our analyses, it increases the within-individual variance threefold to 8.3 days. We infer that a variance of 2.9 days is more representative of healthy menstrual cycles and that it was important to exclude abnormal cycles.

We were unable to make direct comparisons with the Wilcox and Lenton studies data regarding the mean timing of the presumed ovulatory window (±SD) (10–15) and the between-individual and within-individual variances owing to the absence of published data. However, we could compare our data on 95% confidence intervals with those reported by the Lenton group (10–12). The Lenton publications indicated an interval in the follicular phase of 8.2–21 days and in the luteal phase of 9–20 days. Here we report 10–20 days and 9–17 days, respectively. We again attribute the tighter limits indicated here to the exclusion of disordered cases. We confirm that the greater between-individual variance in the menstrual cycle occurs within the follicular phase (10–12).

Variance of Implantation in Menstrual Cycles Leading to Pregnancy

Wilcox and colleagues (14), using their methods, determined that the mean date of implantation occurred at 9.2 days after their estimate of ovulation (no standard deviation), with a range 6–18 days after ovulation. Here we report a similar detection interval for implantation, 9.4 ± 2.0 days after the presumed ovulatory window, with a range of detection of implantation occurring 2 days earlier and ending 4 days earlier, 4–14 days after the presumed ovulatory window. It is noteworthy that using a different hCG test that equally detects hCG and hCG-H, we detect the first production of hCG or implantation earlier than the Wilcox group (4 days compared with 6 days), even though our test is notably less sensitive (1 mIU/mL compared with 0.13 mIU/mL). As published, hCG-H is the principal or only hCG form produced at the time of implantation (18, 19, 27). The use by Wilcox and colleagues of an hCG test that poorly detected hCG-H may have methodologically limited the earliest detection of hCG.

As published by Wilcox and colleagues (13, 14), implantation can occur as late as 11 days after a women has her expected onset of menses (range, 7 days before expected onset of menses up to 11 days after expected menses), with a reported 10% of women implanting later than the time of the expected onset of menses. Given what is known about the duration of corpus luteum activity in the LH-only dependent nonconceptive menstrual cycle, it is difficult to conceive how LH production by the pituitary and the resulting P production by the corpus luteum can maintain the menstrual decidua beyond the expected onset of menses. The data presented here conflict with the data of Wilcox and colleagues (13, 14), who observed implantation much earlier, at 10 days before the date of expected menses. We again believe that appropriate detection of hCG-H explains the difference in our results and the earlier and more realistic detection of implantation. We infer that Wilcox et al.'s conclusion that implantation occurs as much as 11 days after the expected onset of menses is invalid. We also infer that their claims of a limitation in pregnancy testing close to the timing of missing the onset of menses are invalid (13, 14).

In support of these inferences, we note that the findings of Wilcox and colleagues are inconsistent. If one assumes the ovulatory window occurs around, on average, day 14 and that the menstrual cycle is on average 28 days long, taking into account their findings of implantation at 6–18 days of ovulation, then one might anticipate implantation occurring at or around days 20–32 after the last menstrual period, or 8 days before 4 days after missing the expected onset of menses. This is not what is reported (7 days before 11 days after missing the expected onset of menses). If one does the same with our data (implantation 4–14 days after the presumed ovulatory window), then one might anticipate implantation occurring at or around 18–28 days after last menstrual period or 10–0 days before the day of missing the expected onset of menses. This is exactly what is observed.

Overall, we observe here the presumed ovulatory window occurring at 14.7 days (6.6 days variance) and implantation of pregnancy occurring at 24.6 days (10.1 days variance). Considering the wide variability of the presumed ovulatory window and of timing for fertilization and early embryo development, implantation could occur anywhere from day 16 to day 30 of a menstrual cycle.

Defining Normals in Clinical Practice

This research with noncontracepted menstrual cycles that did and did not result in pregnancy provides prospective data from which 95% confidence intervals can be constructed for the follicular phase, luteal phase, the integrated menstrual cycle, and the interval for the time after the presumed ovulatory window in which implantation can be first detected. Care was taken to exclude cases with fertility problems, ovarian cysts, or anovulatory cycles, avoiding abnormalities.

We propose the use of the following 95% confidence intervals for the contributing phases of a normal menstrual cycle as defined in this study (the 95% of cases with values closest to the mean) as means to define the normal reproductive physiology and readily identify potential problem cases

that warrant further investigation. According to this study, a normal menstrual period occurs every 23–32 days (95% confidence interval). The follicular phase concludes with the presumed ovulatory window within 10–20 days after the start of the last menstrual period (95% confidence interval). The luteal phase lasts 9–17 days after the presumed ovulatory window (95% confidence interval).

On the basis of the broad range of total hCG tests, implantation seems to occur 20–30 days after the start of the last menstrual period (95% confidence interval) or 5–14 days after the presumed ovulatory window (95% confidence interval) or from 9 days before the day of missing the next menses (95% confidence interval).

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