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Preimplantation urinary hormone profiles and the probability of conception in healthy women

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Objective: To examine hormonal predictors of conception in menstrual cycles from normal women.

Design: Longitudinal study.

Setting: Community.

Patient(s): Two hundred fifteen healthy female volunteers with no known fertility problems who were trying to conceive.

Intervention(s): Participants recorded menstrual bleeding, sexual intercourse, and collected first morning urine specimens daily from when they stopped contraception until they became pregnant or for 6 months if no clinical pregnancy was achieved. Measurements were made of urinary LH and urinary metabolites of estrogen and progesterone.

Main Outcome Measure(s): Conception was identified by a sensitive and specific immunoradiometric assay for urinary hCG.

Result(s): Statistical analyses of 189 conception and 409 nonconception cycles controlled for sexual intercourse and interdependence of cycles from the same woman. Conception was more likely in cycles with lower baseline progesterone metabolite levels, higher ovulatory LH, and higher midluteal progesterone. Midluteal estrogen also was elevated in conception cycles when examined without adjusting for other hormone levels, but this finding did not persist after multivariate adjustment.

Conclusions: Menstrual cycles in normal women vary in their hormonal quality in ways that are predictive of cycle fertility. (Fertil Steril® 1999;71:40–9. American Society for Reproductive Medicine.)

Key Words: Conception, estrogen, progesterone, LH, epidemiology

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The changes in estrogen, progesterone, and gonadotropins during the menstrual cycle have been well described (1, 2). There is substantial variability among cycles for the same woman as well as differences among women (3). It is unclear whether this variability among cycles reflects differences in cycle fertility, either differences among cycles from a given woman or differences among women. Longitudinal hormone studies usually are performed only with infertility patients. Therefore, few data are available from normal conception cycles. The available reports have small sample sizes (4–10) or examine only a few days of the cycle (11).

The North Carolina Early Pregnancy Study (NC-EP Study) was a prospective study of 221 volunteers who had no known fertility problems (12). Women enrolled when they began attempting pregnancy. They collected daily urine specimens and recorded days of sexual intercourse. Conception was identified early by a highly sensitive and specific assay for hCG in urine. Luteinizing hormone and metabolites of estrogen and progesterone were measured in daily urine specimens from each menstrual cycle of participation. With these data we describe the hormonal patterns in a large sample of natural conception cycles and compare them with nonconception cycles.

MATERIALS AND METHODS

Patients and Protocol

The NC-EP Study enrolled women with no known fertility problems who were planning a

pregnancy. Participants enrolled at or before the time they stopped using birth control to become pregnant. They collected first morning urine specimens daily until the 8th week of a clinically recognized pregnancy or for 6 months if no clinical pregnancy occurred. Women also kept a daily record of sexual intercourse (yes or no) and menstrual bleeding (number of pads and tampons) throughout participation. Menstrual cycles were identified by assigning the first bleeding day as the first day of the cycle. The field methods have been described in detail (13). The National Institutes of Health Institutional Review Board approved this study.

Assays

First morning urine specimens for each cycle were assayed for estrone 3-glucuronide (E_1G) and pregnanediol 3-glucuronide (PdG) by radioimmunoassay as described elsewhere (14, 15). For 60% of the cycles, all daily specimens were assayed to provide complete steroid profiles. Conception cycles were overrepresented deliberately in this group. Because of limited resources, assays for the remaining 40% of cycles were restricted to a midcycle window that allowed us to estimate day of ovulation but did not provide a full hormonal profile of the cycle. Initially, we assayed day 8 through the 8th day before the start of the next menses and then changed to a 17-day midcycle window, starting 21 days before the next menses (for conception cycles, days 17–3 before the first rise of hCG) (15).

Human chorionic gonadotropin was measured in urine by a sensitive and highly specific immunoradiometric assay for the intact molecule (16). The assay limit of sensitivity was 0.01 ng/mL. Conception was defined by hCG levels of >0.025 ng/mL for 3 consecutive days, levels that exceeded those found in a comparison group of women with tubal ligations (12).

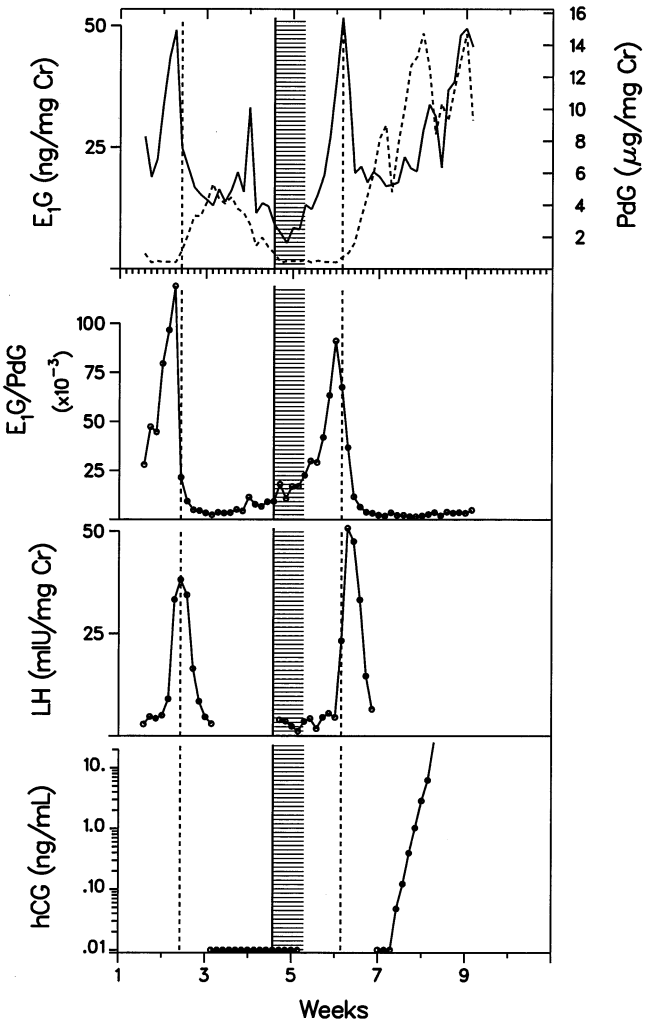
Luteinizing hormone was measured several years after the steroid assays with use of stored urine specimens that had been maintained at $-20°C$ without preservative. The LH assay is a specific and sensitive two-site, noncompetitive immunofluorometric assay (17) that measures intact LH and the LH β-subunit with 100% and 66% cross-reactivity, respectively. The LH measurements were made for a 16-day window in each cycle starting on the 10th day before estimated day of ovulation (see Data Analysis). All hormone assays of daily specimens were run in duplicate or triplicate, averaged as geometric means, and the resultant concentration was adjusted for each sample's creatinine concentration. Figure 1 shows an example of hormonal data for one woman.

Data Analysis

The ratio of urinary steroid metabolites was used to estimate the day of ovulation for each cycle (designated luteal day 0). This method takes advantage of the drop in estrogen and rise in progesterone that occurs around the time of ovulation. The estrogen-progesterone ratio drops steeply as the follicle luteinizes and remains low throughout the luteal phase of an ovulatory cycle. This method was proposed by Baker et al. (18), modified by Royston (19), and further refined and validated with urinary LH measurements by Baird et al. (20). As a marker of ovulation, it appears to have as little measurement error as plasma LH (15).

FIGURE 1

Sample data from one woman. *Top:* E_1G and PdG profiles. *Second panel*: ratio of E_1G to PdG with the designation of luteal day 0 (vertical dotted line) by the steep drop in the ratio. *Third panel*: LH. *Bottom*: hCG. The vertical shaded band shows days of menstrual bleeding.



We defined a set of summary variables a priori to describe cycle characteristics. This strategy limits the multiple comparisons problem that arises when hormonal measurements are compared on a daily basis. In this study we focus on preimplantation hormone patterns because postimplantation patterns are likely to reflect ovarian response to the conceptus. Therefore, summary variables are defined by describing ovarian hormones and LH in the follicular phase and in the

TABLE 1

Definitions of summary variables used to test for differences between hormone patterns in conception and nonconception cycles.*

Summary variable	Definition
Follicular phase	
Length	Number of days from first day of menses up to, but not including, estimated day of ovulation.
Long	Follicular phase length was dichotomized with the top 10% designated long (>23 days) and coded "1" versus the remaining 90% that was coded "0."
Estrone 3-glucuronide (E_1G)	
Midfollicular E_1G†	Geometric mean of E_1G for the interval starting on day 5 of the cycle and ending 3 days before luteal day 0 (the estimated day of ovulation).
Peak E_1G	Geometric mean of E_1G for 3 days centered on the day of highest midcycle E_1G (the day of highest E_1G in a 16-day window ending on luteal day 3).
Rate of E_1G rise	Slope of 3 days of E_1G ending on the day before the highest midcycle level.
Early luteal E_1G	Geometric mean of E_1G for luteal days 1–4.
Midluteal E_1G	Geometric mean of E_1G for luteal days 5 and 6.
Pregnanediol 3-glucuronide (PdG)	
Baseline PdG†	Geometric mean of PdG for the interval starting on day 5 of the cycle and ending 3 days before luteal day 0.
Rate of PdG rise	Slope of the PdG for luteal days 1–5.
Midluteal PdG	Geometric mean of PdG for luteal days 5 and 6.
Low midluteal PdG	Midluteal PdG was dichotomized with <2 μg/mg of Cr designated as low and coded "1," versus higher midluteal PdG levels that were coded "0."
Ratio (E_1G-PdG)	
Rate of ratio descent	The 3-day slope of the ratio values around luteal day 0. For each cycle, slopes for luteal days $-1,0,1$ and $0,1,2$ relative to luteal day 0 were calculated. The more negative (steeper) of the two was chosen to reflect the rapidity of the midcycle transition from the primarily estrogen-producing follicle to the primarily progesterone-producing corpus luteum.
Midluteal ratio	Ratio of midluteal E_1G (as defined above) and midluteal PdG (as defined above).
LH	
Baseline LH	Geometric mean of LH for days -4 to -8 relative to estimated luteal day 0.
Peak LH	Geometric mean of LH for 3 days centered at the day of the highest midcycle LH (the highest in a 16-day window ending on luteal day 5).
Preovulatory LH	Geometric mean of LH for 3 days ending on luteal day 0.

* We required complete data for the specified intervals with the following exceptions: midfollicular E_1G, early luteal E_1G, and rate of PdG rise required at least 3 days to be nonmissing; baseline PdG and LH required at least 2 days to be nonmissing.
† Midfollicular E_1G and baseline PdG were defined for periods of variable length depending on follicular phase length. Other variables were defined for a fixed number of days.

luteal phase up to time of detectable hCG, specifically through luteal day 6. Two percent of the conception cycles had the first sustained elevation of hCG ($\geq$0.025 ng/mL) on luteal day 6, 4% on luteal day 7, and the remaining 94% on luteal day 8 or after; most occurred on luteal day 9 or 10. The summary hormone variables are calculated as mean concentrations or rates of change for specified intervals in the cycle. Definitions are shown in Table 1.

Of 724 menstrual cycles in the NC-EP Study, we were able to estimate a day of ovulation for 696 cycles (96%). Of these, we excluded 98 from the present analyses. Fifty-four were excluded because they did not have sexual intercourse in the observed fertile window, a 6-day window ending with luteal day 0 (21). Other cycles were excluded because of missing data: 10 were missing critical intercourse data, 26 were missing hCG data needed to ascertain occult pregnancy, and 8 were missing all hormone variables.

The remaining 598 cycles from 215 of the 221 total women were the basis for this analysis. They included 189 of the 199 conceptions in the study. Of the 598 cycles, 138 were missing at least one of the defined hormone variables so that sample sizes were smaller than 598 cycles for analysis of specific hormonal variables. Multivariate modeling requires nonmissing data for all variables examined simultaneously; 460 cycles had complete data for our final multivariate model. Most of the cycles excluded from the multivariate model were those in which only a midcycle window was assayed so that hormone data were missing at the beginning and end of the cycle.

Two factors complicate the analysis of conception and nonconception cycles from longitudinal studies. First, conception depends highly on the pattern of intercourse around the time of ovulation. Therefore, controlling for intercourse pattern is required. Second, cycles from the same woman are not independent, and this dependence must be taken into account to estimate valid confidence intervals. Appropriate

statistical methods have been developed recently with use of an approach designed to address both these problems (22). This approach yields adjusted fecundability ratios that provide a measure of each hormone variable's impact on the probability of conception. For example, a fecundability ratio of 1.2 for a given hormone variable indicates that the probability of conception increases 20% for each unit increase in the hormone variable.

The statistical approach is labor- and computer-intensive. Therefore, we did a preliminary analysis using an intercourse-adjusted time-to-pregnancy model. This model provides partial adjustment for sexual intercourse and the dependence among cycles from the same woman. The results from the time-to-pregnancy model were then checked with the approach allowing for further adjustment. Descriptions of the time-to-pregnancy model, as well as the generalized estimating equation (GEE) approach that allows for further adjustment, are given in the Appendix.

RESULTS

The 215 participants who contributed hormonal data for this analysis were healthy volunteers with no known fertility problems. Basic demographic and reproductive characteristics of these participants are shown in Table 2. Nearly all were white, most had some college education, and most were between the ages of 25 and 35 years. Although participants in the study were not representative of the general population, their reproductive characteristics appear typical in terms of fecundability and recognized spontaneous abortion rates (12).

During the study, 162 of the 215 women conceived; 189 pregnancies were detected; 47 of these were early losses (lost within 6 weeks of last menstrual period), and the remaining 142 survived to be clinically recognized. Of the clinically recognized pregnancies, 15 were lost before 28 weeks of gestation: 13 spontaneous abortions, one ectopic pregnancy, and one molar pregnancy. Twenty-six women conceived one (n = 25) or two (n = 1) early losses before becoming clinically pregnant in the study. Fifty-three women contributed only nonconception cycles to the study either because they dropped out of the study (n = 19) or because they did not become pregnant during the 6-month participation period (n = 34).

Urinary estrogen, progesterone, and LH profiles for conception and nonconception cycles are shown in Figure 2. The means (±SD) for variables used to describe the hormonal patterns of each cycle are shown in Table 3. The graphs and means are not adjusted for pattern of intercourse or the dependence among menstrual cycles from the same woman.

When each cycle variable was considered by itself in time-to-pregnancy analyses, those most strongly related to conception ($P<.20$) were long follicular phase length (those in the top 10% of our sample, i.e., >23 days), midfollicular E_1G, rate of E_1G rise, early luteal E_1G, midluteal E_1G, baseline PdG, low midluteal PdG (<2 μg/mg of Cr), ratio descent, midluteal ratio, baseline LH, peak LH, and preovulatory LH (Table 4).

TABLE 2

Demographic and reproductive characteristics of 215 women from the North Carolina early pregnancy study.

Variable	No. of women	(%)
Age (y)		
20–24	22	(10)
25–29	104	(48)
30–34	70	(33)
35–39	16	(7)
40–42	3	(1)
Race		
White	206	(96)
Other	9	(4)
Education		
High school	17	(8)
Some college	44	(20)
College graduate	84	(39)
Postgraduate	70	(33)
Smoking		
Never	149	(69)
Past	53	(25)
Current	13	(6)
Prior pregnancies		
None	74	(35)
1	77	(36)
≥2	63	(29)
Prior spontaneous abortions (among gravid)		
None	108	(77)
1	29	(21)
≥2	3	(2)

Multivariate, time-to-pregnancy modeling to assess the independent effects of a given menstrual cycle variable while controlling for other menstrual cycle variables revealed four variables associated with the probability of conception. Cycles with a long follicular phase, higher baseline PdG, lower preovulatory LH, or low midluteal PdG were less fertile. The importance of these four variables was confirmed with the GEE approach (Table 5).

The association between long follicular phase and conception was of borderline statistical significance ($P<.10$), but the other three variables were all strongly associated with conception ($P<.02$ for each). Cycles with a midluteal PdG of <2 μg/mg of Cr had only about one-half the likelihood of conception as those with higher midluteal PdG. A 1 μg/mg of Cr higher baseline PdG was associated with approximately a 40% reduction in the probability of conception. A unit increase in the preovulatory LH variable was associated with a 30% increased likelihood of conception.

TABLE 3

Mean values of the cycle characteristics for nonconception and conception cycles.

Cycle characteristic	Nonconception		Conception	
	No. of cycles	Mean no. of cycles (± SE)	No. of cycles	Mean no. of cycles (± SE)
Follicular phase				
Length	407	17.2 (±0.3)	189	17.3 (±0.4)
Long follicular phase* (>23 days)	409	0.10 —	189	0.07 —
Estrone-3-glucuronide				
Midfollicular	352	27.5 (±1.1)	179	24.1 (±1.0)
Peak	382	61.5 (±1.8)	178	63.1 (±2.5)
Late follicular rise	376	9.5 (±0.5)	177	10.3 (±0.8)
Early luteal	371	37.4 (±1.2)	175	38.3 (±1.6)
Midluteal	364	37.0 (±1.3)	173	39.6 (±1.8)
Pregnanediol-3-glucuronide				
Baseline	379	0.61 (±0.03)	184	0.53 (±0.03)
Early luteal rise	392	0.81 (±0.04)	186	0.80 (±0.05)
Midluteal	365	4.7 (±0.2)	174	4.4 (±0.3)
Low midluteal* (<2 μg/mg of Cr)	365	0.19 —	174	0.12 —
Ratio (E_1G-PdG)				
Ratio descent	399	−59.1 (±2.1)	182	−69.0 (±5.1)
Midluteal ratio	368	11.3 (±0.6)	175	12.3 (±1.0)
LH				
Baseline	381	5.5 (±0.3)	174	6.2 (±0.4)
Peak	365	27.3 (±0.9)	166	28.9 (±1.3)
Preovulatory	368	13.4 (±0.7)	163	15.2 (±0.9)

* A dichotomized variable coded "1" for presence and "0" for absence of defined characteristic. Therefore, the mean is the percentage of the cycles in which the defined characteristic is present.

DISCUSSION

In this large data set of menstrual cycles from women with no known fertility problems, hormonal patterns were related to the probability of conception. Low midluteal progesterone, lower preovulatory LH, and higher baseline progesterone were associated with failure to conceive in a given cycle. In addition, the probability of conception appeared to be reduced in cycles with long follicular phases, but our analysis included only 45 such cycles, and the result was of borderline statistical significance.

Although this is the largest set of natural cycles to be intensively studied, there are limitations. Participants are self-selected volunteers, not a random sample of women. Most were white, well-educated, nonsmokers, although their reproductive characteristics appear representative (12). Second, because our multivariate models required complete data for the hormonal variables involved, nearly one-fourth of our cycles were excluded from the final statistical analyses. This may have affected our results for midluteal estrogen.

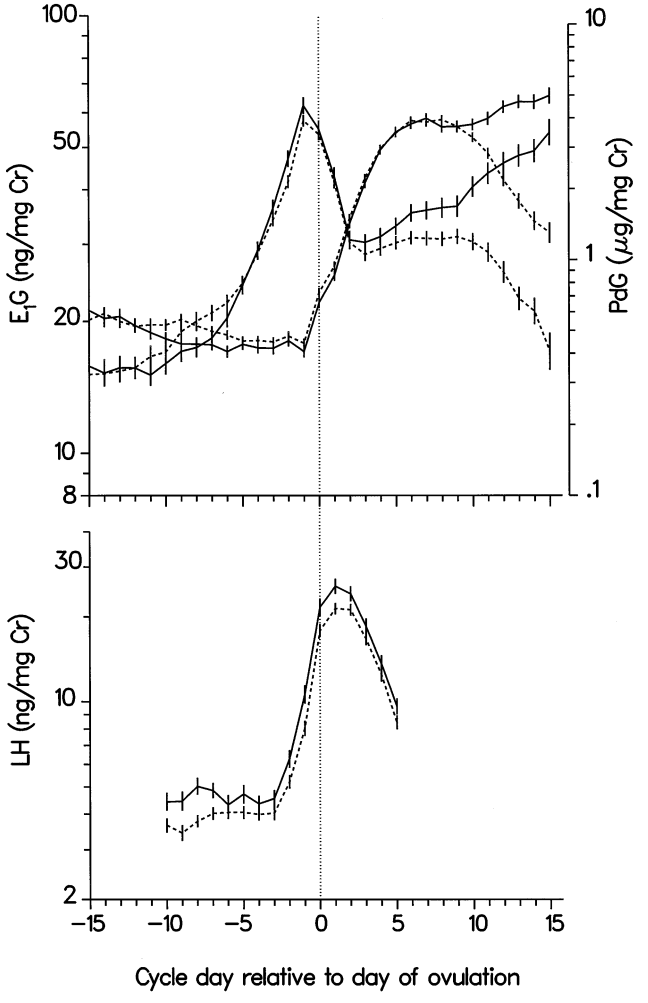
Both Figure 2 and the single-factor analysis (Table 4) suggest that higher midluteal estrogen is associated with conception. Yet, this variable was not as important in the restricted data set of 460 cycles, and midluteal estrogen was not significantly associated with conception in the final model (Table 5). Three prior studies (8–10) have noted higher midluteal estrogen in conception cycles, but none included multivariate analyses.

Approximately one-fourth of the pregnancies in our analysis were lost early, before clinical recognition of the pregnancy. Without the sensitive test for hCG, these cycles would have been considered nonconception cycles. When these conception cycles resulting in early loss are grouped with the nonconception cycles, as they would be in most previous studies, then higher baseline progesterone and low midluteal progesterone remain strongly associated with a decreased probability of conception in the given cycle. The association between preovulatory LH and conception becomes weaker (fecundability ratio [FR] = 1.22; confidence interval [CI] = 0.98,1.52), and the association with long follicular phase length becomes much weaker (FR = 0.88; CI = 0.49,1.59).

Few hormonal data have been published on conception cycles from samples of couples with no known fertility problems. Most studies that include such women reported only a few cycles (4–7) or had hormone data for only a few days of the cycle (11). Three studies are the exception in that they reported at least 10 conception cycles in normal women.

FIGURE 2

Daily geometric means of urinary hormone data for conception cycles (solid lines) and nonconception cycles (dotted lines) shown on a log scale. The standard error for each day is shown by vertical lines. *Top*: E_1G and PdG. *Bottom*: LH.



Stewart et al. (8) described serum estrogen, progesterone, and LH profiles for 14 conception cycles and 13 nonconception cycles with data starting a few days before ovulation. They found higher midluteal progesterone and estrogen in conception cycles. Peak LH was also higher in conception cycles but not significantly so. Lipson and Ellison (9) reported salivary estrogen and progesterone levels for 17 conception cycles versus 81 nonconception cycles. They found no differences in progesterone between conception and nonconception cycles, but conception cycles had higher salivary estrogen throughout, especially during the midfollicular days.

The third report is from our own NC-EP Study. We previously (10) compared urinary estrogen and progesterone in paired conception and nonconception cycles from each of 32 study participants (those 64 cycles are a subset of the 598 cycles described in this study). All 32 conceptions were pregnancies that continued to term live birth. In this previous within-woman comparison, conception cycles had higher midluteal urinary estrogen and progesterone metabolites. Conception cycles also had a steeper rise in early luteal progesterone and tended to show a steeper ratio descent compared with nonconception cycles from the same woman.

The present analyses differ from these previous studies. First, we include conceptions that were lost early, not just clinically recognized conceptions. Second, our large sample size allowed us to conduct multivariate analyses to evaluate the relative influence of several hormonal characteristics. Finally, our current findings assess differences in fertility from woman to woman, as well as differences from cycle to cycle for the same woman. This was also the case for Stewart et al. (8) and Lipson et al. (9), but not for our previous analysis of 32 conceptions from the NC-EP Study (10) in which we examined only within-woman differences.

Our previous analysis and this current one used measure-

TABLE 4

Association between cycle characteristics and conception expressed as fecundability ratios with 95% confidence intervals.

Cycle characteristic	Fecundability ratio (95% confidence interval)	*P* value
Follicular phase		
Length	1.00 (0.98,1.02)	>.20
Long follicular phase (>23 days)	0.70 (0.43,1.14)	<.20
Estrone-3-glucuronide		
Midfollicular	0.99 (0.98,1.00)	<.20
Peak*	1.16 (0.92,1.46)	>.20
Rate of rise	1.01 (1.00,1.02)	<.20
Early luteal*	1.15 (0.92,1.42)	>.20
Midluteal*	1.26 (1.01,1.56)	<.05
Pregnanediol-3-glucuronide		
Baseline	0.81 (0.61,1.08)	<.20
Rate of rise	0.99 (0.83,1.17)	>.20
Midluteal	0.99 (0.95,1.03)	>.20
Low midluteal (<2 μg/mg of Cr)	0.68 (0.46,1.01)	<.05
Ratio (E_1G-PdG)		
Ratio descent*	1.12 (0.97,1.29)	<.20
Midluteal ratio*	1.14 (0.98,1.33)	<.10
LH		
Baseline*	1.16 (1.01,1.34)	<.05
Peak*	1.20 (0.99,1.45)	<.10
Preovulatory*	1.21 (1.05,1.40)	<.01

Note: The fecundability ratio is the estimated change in the probability of conception associated with a one-unit increase in the cycle characteristic, partially adjusted for pattern of intercourse and dependence among cycles from the same woman using a time-to-pregnancy model (see Appendix). The *P* values shown are those for the difference in deviance between a model with and without the cycle characteristic.

* The log transformed variable was used. For ratio descent all initial values were negative, so the log transform of the absolute value was used.

TABLE 5

Adjusted fecundability ratios and 95% confidence intervals for the cycle characteristics important in the final adjusted model.

Cycle characteristic	Adjusted fecundability ratio (95% confidence interval)
Baseline PdG (μg/mg of Cr)	0.57 (0.36,0.89)
Preovulatory LH* (mIU/mg of Cr)	1.31 (1.13,1.52)
Low midluteal PdG ($\leq$2 μg/mg of Cr)	0.52 (0.35,0.79)
Long follicular phase (>23 days)	0.63 (0.37,1.08)

Note: This analysis included 460 cycles, 150 of which were conception cycles. Each fecundability ratio is adjusted for pattern of sexual intercourse and the dependence among cycles from the same woman, as well as for the effects of the other cycle characteristics shown.

* The log transformed variable was used.

ments of E_1G and PdG, urinary metabolites of E_2 and progesterone to describe hormonal patterns. Because total plasma E_2 and progesterone are excreted through several metabolic pathways, not just as E_1G and PdG, respectively, our hormonal measures can vary with changes in metabolism as well as with true hormonal differences. However, unless hormone metabolism is related systematically to the probability of conception, the urinary hormone differences due to metabolism will add noise to the data, making it more difficult to identify hormonal predictors of conception. Lipson and Ellison (9) measured salivary E_2 and progesterone, which are the free hormones. Variation in free hormone arises from changes in plasma-binding proteins as well as changes in total plasma concentration. Only Stewart et al. (8) measured plasma concentrations of E_2 and progesterone directly.

The strong association in our data between low midluteal progesterone and failure to conceive is consistent with previous data from Stewart et al. (8) and Baird et al. (10). Low luteal progesterone has been reported to be associated with failure to conceive in stimulated cycles as well. Luteal progesterone is necessary for maturation of the endometrium of the estrogen-primed uterus (23). A low luteal progesterone may also reflect other problems with the cycle, such as an unruptured follicle (24). Stewart et al. (8) suggest that a higher midluteal progesterone in conception cycles may reflect an early preimplantation response of the corpus luteum to preimplantation signals from a developing conceptus, but analyses by Baird et al. (10) support the alternative hypothesis that higher progesterone reflects a higher quality cycle more able to support implantation.

We hypothesized that high LH would be associated with reduced likelihood of conception, because high baseline LH has been associated with reduced conception in many studies of patients undergoing ovulation induction for treatment of infertility (reviewed in Chappel and Howles 25 and Shoham et al. 26), as well as in a large sample of unstimulated cycles (27) contributed by a sample of women, most of whom had a history of miscarriage. However, we found the opposite in our data. High LH was associated with increased probability of conception.

The fact that our analyses focused on preovulatory LH rather than baseline cannot explain the difference because higher baseline LH in our data was also associated with increased fertility, although not as strongly as preovulatory LH. Our LH findings are consistent with data presented by Stewart et al. (8) showing higher peak LH in conception cycles compared with nonconception cycles. However, in their small sample, the association was not statistically significant.

We measured urinary LH, so we are not able to make direct comparisons with the serum levels previously reported in the literature. However, it may be that the high LH levels associated with failure to conceive in infertility patients and women with a history of pregnancy problems are considerably higher than those commonly found in a nonclinical population.

An association between high preovulatory progesterone and low probability of conception, as we see in our sample of women, also has been reported in IVF patients (28–30), but these studies measured progesterone late in the follicular phase at time of hCG administration. Baseline progesterone was not higher in salivary measures reported by Lipson and Ellison (9) nor in Baird et al. (10); Stewart et al. (8) did not measure progesterone much before ovulation. Baird et al. (10) report only on cycle-to-cycle differences within women, so if the association between high baseline PdG and reduced likelihood of conception is real, it may reflect hormonal differences among women that are associated with their fertility.

High baseline progesterone has some biological plausibility as a marker of fertility problems. Baseline progesterone levels usually reflect adrenal production rather than ovarian production (31), but regardless of origin, higher levels of baseline progesterone might have direct antifertility effects. Progesterone can reduce levels of estrogen and progesterone receptors in endometrial tissue (32), and poor endometrial maturation could result, despite adequate luteal progesterone levels. Alternatively, high baseline progesterone may simply be a marker for general hyperstimulation of the adrenals, with concomitant reduction in fertility.

Obesity can be associated with abnormal adrenal and hypothalamic-pituitary function (33), but when we controlled for body mass (kg/m^2), the effect of baseline progesterone was still present. Smoking has been associated with increased baseline progesterone concentrations (34), but smoking does not account for our findings. Only 13 women in our sample smoked, and when these were dropped, the association between high baseline progesterone and failure to conceive remained.

In summary, this study describes the largest sample to date of natural conception cycles for women with no known

fertility problems. It is the first to examine hormonal associations with fertility that adjust for patterns of intercourse and correlations among hormonal variables. In addition to the multivariate analyses, we present single-factor analyses (Table 4) so that future studies of conception cycles can examine these same variables to test the generalizability of our findings. Our findings indicate that menstrual cycles from women with no known fertility problems vary hormonally in ways that are predictive of cycle fertility.

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APPENDIX

We applied the statistical model for fertility first proposed by Schwartz et al. (1) and further developed by others (2, 3). This model was used recently to estimate the probability of conception associated with intercourse on particular days (4). Mathematically, the model specifies that the probability of conception can be written as follows for a particular ovulatory menstrual cycle:

$$\Pr[\text{conception} \mid \text{intercourse pattern}] = A\{1 - \prod_{k} (1-p_k)^{X_{ijk}}\},$$

where A corresponds to the probability that cycle j for couple

i is "viable," the X_{ijk} are 0/1 indicator variables denoting whether there was not or was intercourse on day k, where the indexing of days is relative to ovulation, and the p_k are probabilities of fertilization associated with day k.

The model can only be fitted to ovulatory cycles, because the indexing of days with intercourse depends on the identifiability of a day of ovulation. This model embodies the fact that timing of intercourse relative to ovulation is important for conception but recognizes that good timing is not all that is required. The notion of cycle viability aggregates the female factors that must be favorable if pregnancy is even to be possible in a given cycle: a viable egg must be released, must be picked up and properly transported by the oviduct, the uterus must have been prepared adequately in the right hormonal milieu, the woman's immune system must function properly, and so on. A cycle in which all these factors act together in such a way that conception could potentially occur is said to be "viable." (Cycle viability, as embodied in the A parameter, also implicitly includes the probability that a conceptus would implant and survive long enough to be detected.)

The model further supposes that batches of sperm introduced on various days of the cycle compete in a statistically independent way in trying to fertilize the ovum. Thus, the p_k parameters have a simple interpretation: p_k is the probability that with intercourse only on day k, conception would occur, provided that the cycle is viable. In summary, the above model incorporates two factors: one that depends on the timing of intercourse and corresponds to the event of fertilization and one that depends jointly on all factors other than timing that may influence the likelihood of conception.

Royston (2) and Weinberg et al. (3) extended the above model to allow for the possibility that factors such as age may have an effect on cycle viability. Clearly, hormones also could potentially affect the probability that a given cycle is viable, so that instead of being a single number, A would be a function of the hormonal patterns for the cycle. Suppose $H_1, H_2, \ldots H_n$ are hormonal variables measured for a particular ovulatory menstrual cycle. We model the potential effect of these variables on cycle viability by extending the above formulation as follows:

$$\Pr[\text{conception} \mid \text{intercourse pattern and } H_1, H_2, \ldots H_n]$$
$$= A(H_1,H_2,\ldots H_n)\{1-\prod_k (1-p_k)^{X_{ijk}}\}$$
$$= \exp(\mu + \beta_1 H_1 + \beta_2 H_2 + \ldots + \beta_n H_n)$$
$$\{1 - \prod_k (1-p_k)^{X_{ijk}}\}.$$

The dependence of A on the hormonal variables is thus multiplicative (similar to what is done in the usual proportional hazards model). This results in a simple interpretation for the β coefficients: exponentiating the estimated β_p yields the estimated ratio of the conception probabilities (and the ratio of cycle viabilities) for two cycles that only differ by one unit in the corresponding variable, H_p. Thus, $\exp(\beta_p)$ can be thought of as a fecundability ratio with adjustment for intercourse. When β_p is positive, the fecundability ratio is greater than 1.0, suggesting that cycles with higher levels of H_p have a higher likelihood of viability, hence higher fecundability.

Fitting this model in a statistically rigorous way is computationally intensive (5), so a simplification becomes useful. Taking the logarithm in the above model, we see that the log of the conception probability is a linear function of the hormonal variables, plus the log of the factor involving the intercourse data, which is constrained to have coefficient 1. If we knew the p_k parameters, we could easily fit such a model using standard software with a fixed "offset" defined by

$$\text{Off} = \log\{1 - \prod_k (1-p_k)^{X_{ijk}}\}.$$

This can be done using standard statistical packages such as GLIM (6) or SAS, using the new GENMOD procedure.

To do our "crude" exploratory modeling, we assumed the estimated p_k parameters taken from the Early Pregnancy Study (4) were fixed and known, and we fitted the log-linear model just described, using the above offset. To adjust crudely for heterogeneity among couples (which induces statistical dependency in the outcomes), we incorporated an additional categorical variable for cycle number. This allows the baseline cycle viability (the "constant" intercept) to decline with cycle number, reflecting the fact that couples with a relatively low proportion of viable cycles will tend to remain at risk longer, because of self-selection due to their failure to conceive. Preliminary analyses were conducted with this simplified model, referred to in the text as the "time-to-pregnancy" model because of its similarity to a model previously used to estimate relative fecundability from time-to-pregnancy data where detailed data on intercourse were not available (7).

We first identified a subgroup of cycle characteristics (including hormonal measures) that seemed to influence cycle viability ($P<.20$) in exploratory modeling. This subset of characteristics then was tested to find those that remained important in multivariate models that included other cycle characteristics. To do this, we started with the variables with the strongest relationship to conception and added others one at a time, in a step-up process, retaining additional variables that contributed to predicting conception. We continued this process until no other significant variables remained ($P<.05$). The cycle characteristics not included in the initial subgroup were then given another opportunity to be included in the model, by adding them one

at a time and retaining them if they significantly improved the fit to the conception data.

The variables identified in this way then were considered in the more statistically rigorous models that allowed for the uncertainty in the p_k and also allowed for statistical dependency among cycles within couples (due to heterogeneity across couples in cycle viability) by use of the generalized estimating equation approach (5). In this way, our overall strategy allowed the exploratory data analysis to proceed efficiently while ensuring that the more valid model was ultimately fit to the data. In fact, empirically, the differences between the "crude" results and the statistically more correct results were negligible.

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