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Urinary estrogen and progesterone metabolite concentrations in menstrual cycles of fertile women with non-conception, early pregnancy loss or clinical pregnancy

Scott A.Venners[1], Xue Liu[2], Melissa J.Perry[3], Susan A.Korrick[3,4], Zhiping Li[2], Fan Yang[2], Jianhua Yang[2], Bill L.Lasley[5], Xiping Xu[1] and Xiaobin Wang[6,7]

[1]Division of Epidemiology and Biostatistics, University of Illinois at Chicago, School of Public Health, Chicago, IL, [2]Anhui Provincial Institute for Biomedicine, Anhui Medical University, Hefei, Anhui, China, [3]Department of Environmental Health, Harvard School of Public Health, [4]The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, [5]Institute for Toxicology and Environmental Health, School of Medicine, University of California, Davis and [6]Mary Ann J. Milburn Smith Child Health Research Program, Department of Pediatrics, Northwestern University Feinberg School of Medicine and Children's Memorial Hospital and Children's Memorial Research Center, Chicago, IL, USA

[7]To whom correspondence should be addressed at: Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital, 2300 Children's Plaza, Box 157, Chicago IL 60614, USA. E-mail: xbwang@childrensmemorial.org

BACKGROUND: Knowledge is limited of how estrogen and progesterone variability in fertile women are associated with achieving pregnancy. METHODS: From 1996 to 1998, we enrolled 347 textile workers without hormone treatment in Anhui, China, who provided daily urine and data upon stopping contraception for up to 1 year until clinical pregnancy. Urinary hCG was assayed to detect conception and early pregnancy losses. We compared urinary concentrations of estrone conjugates (E_1C) and pregnanediol-3-glucuronide (PdG) in 266 clinical pregnancies, 63 early pregnancy losses and 272 non-conception cycles from 347 women and also in 94 clinical pregnancy and 94 non-conception cycles from the same women. RESULTS: Using generalized estimating equations and relative to 266 clinical pregnancy cycles, log(E_1C) was lower in 272 non-conception cycles [$\beta = -0.3$ ng/mg creatinine (Cr); SE = 0.1; $P < 0.0001$]. On average, daily E_1C was 18 ng/mg Cr lower in non-conception cycles than in clinical pregnancy cycles. Relative to 94 clinical pregnancy cycles, log(E_1C) was lower in 94 non-conception cycles ($\beta = -0.4$ ng/mg Cr; SE = 0.1; $P < 0.0001$) from the same women (average difference in daily E_1C was 20 ng/mg Cr). The odds of E_1C less than the 10th percentile (<30 ng/mg Cr) were higher in early pregnancy loss cycles [odds ratio (OR) = 4.8; P = 0.0027] than in clinical pregnancy cycles in the early luteal phase. Compared with clinical pregnancy cycles, log(PdG) concentrations were lower in non-conception cycles during the follicular phase, but this analysis lacked power for multiple testing. CONCLUSIONS: Estrogen concentrations varied from cycle to cycle, and higher estrogen was associated with achieving clinical pregnancy.

Key words: estrogens/fertilization/pregnancy/progesterone/prospective study

Introduction

Our understanding of the relationships between hormone variability and fertility in female humans is still limited. Even in fertile women, there are significant differences in hormone concentrations, both between women and between different menstrual cycles from the same women. However, there have been inconsistent associations of between-women or within-woman variability in hormone concentrations with conceptions and early pregnancy losses (Baird *et al*., 1991b, 1997, 1999; Stewart *et al*., 1993; Lipson and Ellison, 1996; Li *et al*., 2001).

Differences among study findings might, in part, relate to methodological differences, including choice of hormone measures (parent compound or metabolite) and matrix for measurement (e.g. saliva, urine or serum). In addition, an important methodological issue when analysing reproductive hormone data from women is how to align menstrual cycles relative to each other to allow comparisons of hormone concentrations in different cycles. Because ovulation is hormonally regulated, predictable patterns of changes in hormone concentrations around the time of ovulation can be used to estimate the day when ovulation occurred (Baird *et al*., 1991a; Li *et al*., 2002; Chen *et al*., 2005). Previous studies used several different algorithms to estimate the day of ovulation for aligning cycles. Baird *et al*. (1991b, 1997, 1999) estimated the day of the luteal transition using the ratio of urinary estrogen

metabolites to progesterone metabolites (E/P algorithm, see *Materials and methods* for a more complete description). Stewart *et al*. (1993) aligned cycles by the peak of serum LH, whereas Li *et al*. (2001) aligned by the mid-cycle peak of urinary FSH. The accuracy of each of these methods to detect the day of ovulation was investigated by Li *et al*. (2002), who used daily ultrasound to compare the days of follicular collapse (the most precise estimate of the day of ovulation) with the days of ovulation estimated by each of the hormone algorithms used in previous studies. They reported that all of the algorithms had errors in estimating the true day of ovulation, but the distributions of errors differed for each algorithm.

We investigated whether within-woman and between-women variability in urinary estrogen and progesterone metabolite concentrations were associated with non-conception, early pregnancy loss and clinical pregnancy (lasting at least 6 weeks from the beginning of the last menstrual period) in a large and uniquely homogenous Chinese sample. We measured urinary metabolites of estrogen and progesterone and used two hormone algorithms to estimate the day of ovulation: (i) the E/P algorithm previously used by Baird *et al*. (1991b, 1997, 1999) and (ii) an algorithm previously used by Chen *et al*. (2005) that estimates the day when urinary progesterone metabolites begin to rise rapidly in the early luteal phase [pregnanediol-3-glucuronide (PdG)-rise algorithm, see *Materials and methods* for a more complete description]. To ensure that our results were robust when we used different ovulation algorithms, we did analyses using both the E/P and the PdG-rise algorithms for estimating ovulation and compared our results after aligning cycles by the day of ovulation estimated by each.

Materials and methods

Study population and procedures

Our analysis used daily, first morning urine samples collected as part of a prospective study of environmental influences on reproductive health in young, newly married Chinese women who were trying to conceive (Wang *et al*., 2003). Briefly, we recruited women from 1996 to 1998 who were employed in a large textile mill in Anhui, China. All women were newly married, aged 20–34 years, nulliparous and had obtained state permission to have a child. We excluded women if they smoked (<1%), were already pregnant before enrolment, had previously attempted to become pregnant without success for 1 year or more or would not be available for the 1-year course of follow-up. Beginning from the date of stopping use of contraceptive methods and continuing for up to 12 months or until pregnancy was clinically confirmed, whichever came first, each woman kept a daily diary to record sexual intercourse and other relevant factors and collected a first morning void urine specimen for hormone assay. We assayed each urine sample for estrone conjugates (E_1C) and PdG, the major urinary metabolites of estrogen and progesterone, and hCG, a sensitive and specific marker of conception (see *Laboratory assays of urinary PdG, E_1C and hCG*). Our protocols were approved by the Institutional Review Boards of Children's Memorial Hospital (Chicago, IL, USA) and the Institute of Biomedicine, Anhui Medical University (Hefei, China).

As we reported previously in detail (Wang *et al*., 2003), 387 women provided adequate daily urine and diaries for 1484 menstrual cycles, 804 of which we selected for urinary hormone analysis. When selecting cycles, we preferentially chose cycles with clinical pregnancy or early pregnancy loss and the cycles immediately preceding them regardless of outcome. We also included a sample of non-conceptive cycles with the fewest missing urine samples. We used two methods to determine the day of ovulation during the menstrual cycle. We first used the 'E/P algorithm', which was identical to that used previously by Baird *et al*. (1991b, 1997, 1999). This algorithm scanned for 5-day sequences in which the ratio value for the first day was the highest of the five, and the ratio values for each of the last 2 days were 40% or less of the first-day value. The second day in this sequence was designated as the day of ovulation for that cycle. We also used the 'PdG-rise algorithm', which was identical to that used in a previous report (Chen *et al*., 2005). This algorithm used a two-piece regression model for daily PdG levels and applied a 'best fit' (maximum R^2) criterion to identify the turning point when PdG started to rise and assigned this as the day of ovulation. The E/P algorithm sometimes identified multiple 5-day blocks. When this occurred, we used the estimated day of ovulation that was closest to that identified by the PdG-rise algorithm. Of 804 cycles, we excluded 16 cycles because they were determined not to have had ovulation. Also, we excluded 12 because no ovulation day was identified by the PdG-rise algorithm, 20 because no ovulation day was identified by the E/P algorithm and 26 because neither algorithm identified an ovulation day. Additionally, to select cycles with more robust estimates of the day of ovulation, we excluded 57 cycles in which the estimates by the two algorithms differed by >3 days, leaving 673 cycles from 371 women.

To focus our analysis on fertile women, we excluded 19 women with 35 cycles who never conceived during the period of prospective observation. We also wanted to exclude cycles in which the reason for non-conception might have been lack of sexual intercourse because these cycles might have been hormonally adequate and thus obscured hormonal patterns related to non-conception. We used daily diary data to investigate timing of sexual intercourse in non-conception cycles and excluded cycles in which no sexual intercourse was reported during the 6-day window starting 5 days before ovulation and ending on the day of ovulation. Wilcox *et al*. (1995) reported that among healthy women who were planning to become pregnant, they could observe conceptions that clearly resulted from intercourse on each of the days of this 6-day window. Because the day of ovulation estimated by the two algorithms sometimes differed, we only included those cycles in which intercourse was reported at least once during the 6-day window for both the algorithms. With this criterion, we excluded 37 non-conception cycles, which resulted in five women being completely excluded from the analysis. Our report includes 601 cycles with ovulation from 347 healthy women who were planning to become pregnant and conceived at least once during prospective observation.

Laboratory assays of urinary PdG, E_1C and hCG

We previously published our laboratory methods for PdG, E_1C and hCG (Wang *et al*., 2003; Chen *et al*., 2005). Briefly, we measured urinary concentrations of PdG and E_1C using enzyme-based immunoassays (Munro *et al*., 1991). We analysed urinary hCG concentrations by the immunoradiometric assay (IRMA) (O'Connor *et al*., 1988) and measured urine creatinine (Cr) levels according to the method of Jaffe (Husdan and Rapoport, 1968). We normalized all PdG, E_1C and hCG values by Cr values to adjust for urine concentration. We assayed all urine specimens in duplicate during a single-run and repeated assays if there were discrepancies of more than 3-fold between duplicates. We used the geometric mean of the duplicates from each sample for analyses.

Statistical analysis

Conception status in each ovulating cycle

The use of sensitive and specific urinary hCG assays makes it possible to detect conception close to the time of implantation (Wilcox *et al*., 1985). We previously reported our application of a Bayesian model to determine conception status in this population based on daily urinary hCG values in 1561 menstrual cycles from 518 women (Wang *et al*., 2003). In the present analysis, we used the results regarding conception status from our prior analysis. Briefly, using Markov Chain Monte Carlo methods, we calculated a probability of conception for each observed cycle. Our previous analysis showed that this model was highly sensitive and specific. Among cycles without clinical pregnancy, 97% had a conception probability of exactly either 0 or 1. Two percent had a probability $P \geq 0.9$ but less than 1. We defined conception as probability $P \geq 0.9$.

Menstrual cycle outcomes

The definitions of menstrual cycle outcomes were (i) non-conception—determined using urinary hCG assays (see *Laboratory assays of urinary PdG, E_1C and hCG*), (ii) early pregnancy loss—pregnancy loss (detected by urinary hCG assay) occurring <6 weeks (42 days) after the onset of the last menstrual period and (iii) clinical pregnancy—pregnancy continuing without loss to at least 6 weeks (42 days) after the onset of the last menstrual period. A total of 32 of 266 clinical pregnancy cycles ended in spontaneous abortion defined as a pregnancy loss occurring 6 weeks or more but no later than 20 weeks after the onset of the last menstrual period. We initially modelled spontaneous abortions as a separate cycle outcome using the models described below. Because the differences in concentrations of E_1C and PdG in clinical pregnancy cycles with or without spontaneous abortion were not statistically significant, we combined all clinical pregnancies regardless of pregnancy losses occurring after the sixth week.

Modelling menstrual cycle outcomes and urinary hormone metabolite concentrations

All analyses were done twice, once using the ovulation days determined by the E/P algorithm and once using those determined by the PdG-rise algorithm. We aligned the cycles according to the days of ovulation from each algorithm and focused on a 15-day window starting from 9 days before ovulation to 5 days after ovulation. Although we show mean E_1C and PdG concentrations in Figures 1 and 2 for days 6–9 after ovulation, we did not include these days in our models because of concerns about potential implantation-related changes in hormone concentrations. Because the distribution of urinary PdG and E_1C concentrations were skewed with an upper tail, we transformed them to the log scale for analysis.

We used all observed menstrual cycles to investigate the overall association of urinary hormone metabolite concentrations and cycle outcomes (combining both between-women and within-woman variability in one analysis). Using continuing pregnancy cycles as the reference, we modelled mean log(E_1C) and log(PdG) concentrations by cycle outcomes using linear regression. All of our models included a class variable for cycle day relative to ovulation to adjust for normal changes throughout the cycle. We used generalized estimating equations with the SAS procedure, GENMOD, assuming an exchangeable correlation structure to adjust for intrawoman correlation in multiple urine samples and cycles. If a day had missing hormone data, we excluded it from analysis. We had hormone data for 10 432 days (82%) for log(E_1C) and 10 567 days (83%) for log(PdG). We reported the results of our models for the entire cycle and also stratified by cycle phases defined by days relative to ovulation as follows: mid-follicular (days −9 to −6), late-follicular (days −5 to −2), periovulation (day −1 to 1) and early luteal (days 2 to 5). For significance testing at $\alpha = 0.05$, we applied a conservative Bonferroni correction for 10 tests (four menstrual cycle phases plus all combined for two ovulation algorithms) and considered *P*-values of 0.005 in individual models to give us an overall *P*-value of 0.05 for all analyses combined. We adjusted all of our models for covariates (based on report at entry into the study) that we thought could potentially confound our associations including age (linear and squared terms), BMI (linear and squared terms), passive smoke exposure from husband (binary), rotating work shifts (binary), perceived life stress (binary, ≤low or ≥moderate) occupational noise exposure (binary, ≤low or ≥moderate) and dust exposure (binary, ≤low or ≥moderate).

To specifically focus on the associations between cycle outcomes and differences in urinary hormone metabolite concentrations in different cycles from the same women (within-woman variability), we reanalysed our data using 94 women who could provide both clinical pregnancy and non-conception cycles. We also conducted an analysis using 21 women who could provide both clinical pregnancy and early

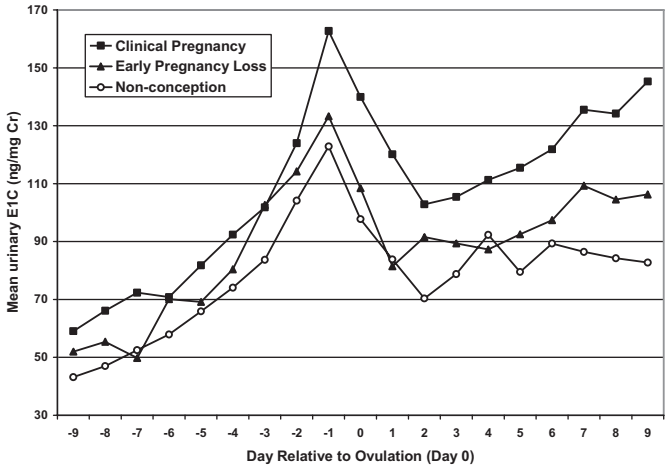

Figure 1. Daily mean urinary estrone conjugates (E_1C) concentrations by cycle outcome for 601 cycles of 347 fertile women who were attempting to become pregnant without hormone treatment.

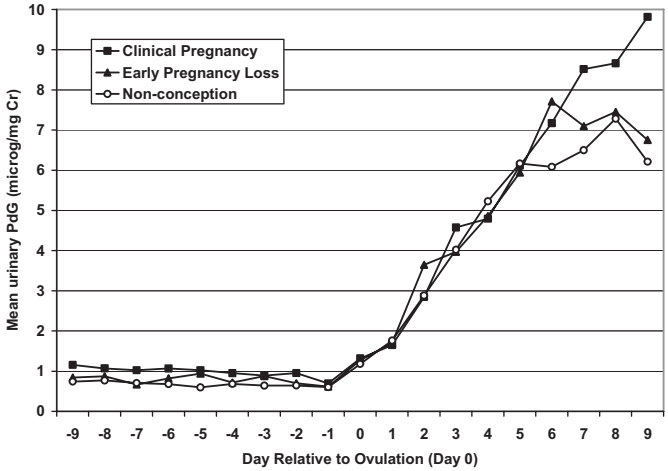

Figure 2. Daily mean urinary pregnanediol-3-glucuronide (PdG) concentrations by cycle outcome for 601 cycles of 347 fertile women who were attempting to become pregnant without hormone treatment.

pregnancy loss cycles, but this analysis lacked power and is not reported here. If a woman had more than one non-conception or early pregnancy loss cycle, we chose the first one that had been observed. Because comparison groups were balanced with regard to important covariates, we only estimated models without adjustment for covariates using generalized estimating equations to accommodate intrawoman hormone correlations in multiple urine samples and cycles.

Baird *et al*. (1999) reported that low luteal PdG (<2 μg/mg Cr on days 5 and 6) was associated with failure to conceive, even though there was no association with the entire PdG distribution. To test whether high or low hormone concentrations were associated with cycle outcomes, we first calculated the average concentration of each hormone in each cycle phase for each observed cycle. If hormone data were missing, then we excluded that day from the average. We next created binary variables for high E_1C or PdG in each cycle phase that were coded 1 if the average concentration was higher than the 90th percentile in that phase and coded 0 otherwise. We also created binary variables for low E_1C or PdG in each cycle phase that were coded 1 if the average concentration was less than the 10th percentile in that phase and coded 0 otherwise. To accommodate non-independence of observations from the same woman, we used generalized estimating equations assuming an exchangeable correlation structure for each cycle phase to model the odds of high (or low) hormone concentrations in early pregnancy loss or non-conception cycles relative to clinical pregnancy cycles.

Results

As summarized in Table I, this population was young (mean age 24 years, range 21–34 years). Most participants had husbands who smoked (63%), and almost all had rotating work shifts (96%). Most participants reported moderate or heavy occupational exposure to dust (68%) and/or noise (74%). None of the participants smoked, and all conceived at least once during prospective observation. None of the participants had used hormone contraceptives or an intrauterine device before prospective follow-up. Figures 1 and 2 show the mean concentrations of urinary E_1C (using E/P ovulation day algorithm) and PdG (using PdG-rise ovulation day algorithm) for each day of the cycle relative to ovulation by cycle outcomes: non-conception, early pregnancy loss or clinical pregnancy.

Table I. Characteristics of 347 non-smoking women who were trying to become pregnant without exogenous hormone treatment and who conceived at least once

	Mean ± SD
Age (years)	24.8 ± 1.5
Height (m)	1.58 ± 0.05
Weight (kg)	49.3 ± 5.9
BMI (kg/m^2)	19.8 ± 2.1
	n (%)
Husband currently smokes	217 (63)
Rotating work shifts	333 (96)
Occupational dust exposure	
Low	112 (32)
Medium	127 (37)
High	108 (31)
Occupational noise exposure	
Low	91 (26)
Medium	124 (36)
High	132 (38)
Self-reported life stress	
Low	222 (64)
Moderate	107 (31)
High	18 (5)

Urinary E_1C using all observed cycles

Analyses that used all observed cycles investigated the overall association of urinary hormone metabolite concentrations (combining both between-women and within-woman variability in one analysis) and cycle outcomes. As summarized in Table II, using the E/P algorithm to estimate the day of ovulation, mean daily log(E_1C) was lower in 272 non-conception cycles relative to 266 clinical pregnancy cycles in every cycle phase after Bonferroni correction for 10 tests (days −9 to 5, $\beta = -0.29$ ng/mg Cr; SE = 0.06; $P < 0.0001$). On average, daily E_1C was 18 ng/mg Cr lower in non-conception cycles than in clinical pregnancy cycles using the E/P algorithm. The results when using the PdG-rise algorithm to determine the day of ovulation were similar in all cycle phases to those when using the E/P algorithm except in the late-follicular phase (days −5 to −2), which was of smaller absolute magnitude and not significant after Bonferroni correction for 10 tests ($\beta = -0.15$ ng/mg Cr; SE = 0.07; $P = 0.0292$). Mean daily log(E_1C) tended to be lower in 63 early pregnancy loss cycles relative to 266 clinical pregnancy cycles in all cycle phases by both ovulation day algorithms (average difference in daily E_1C across all phases using the E/P algorithm was 13 ng/mg Cr), but these differences were not significant after Bonferroni correction for 10 tests. We did not show crude parameter estimates in Table II, which were similar to those that we showed for the adjusted models.

Urinary E_1C using non-conception and clinical pregnancy cycles from the same women

This analysis focused on the association between cycle outcomes and differences in urinary E_1C concentrations in different cycles from the same woman (within-woman variability). There were 94 women who had one each of non-conception and clinical pregnancy cycles (Table III). Using the E/P algorithm to estimate the day of ovulation and relative to clinical pregnancy cycles, mean daily log(E_1C) was lower in non-conception cycles in every phase of the menstrual cycle after Bonferroni correction for 10 tests except the late follicular (days −5 to −2), which was almost significant ($\beta = -0.26$ ng/mg Cr; SE = 0.09; $P = 0.0059$). On average, daily E_1C was 20 ng/mg Cr lower in non-conception cycles than in clinical pregnancy cycles using the E/P algorithm.

Low (< 10th percentile) or high (>90th percentile) urinary E_1C using all observed cycles

Using the E/P algorithm, the 10th and 90th percentiles for average E_1C by phase were mid-follicular (17 and 113 ng/mg Cr), late-follicular (30 and 177 ng/mg Cr), periovulation (39 and 212 ng/mg Cr) and early luteal phase (30 and 181 ng/mg Cr), respectively. The 10th and 90th percentiles were similar using the PdG-rise algorithm. Using the E/P algorithm and relative to clinical pregnancy cycles, the odds of having low E_1C in non-conception cycles were significantly higher after

Table II. Relative adjusted[a] mean log(urinary E_1C) by menstrual cycle outcomes for 601 cycles of 347 fertile women who were attempting to become pregnant without hormone treatment

	Number of cycles	Algorithm for day of ovulation Log(E_1C) (ng/mg creatinine)					
		E/P algorithm			PdG-rise algorithm		
		β	SE	*P*	β	SE	*P*
Mid-follicular phase (days −9 to −6)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	−0.15	0.10	0.1183	−0.13	0.08	0.0969
Non-conception	272	−0.30	0.07	<0.0001	−0.31	0.06	<0.0001
Late-follicular phase (days −5 to −2)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	−0.07	0.10	0.4734	−0.06	0.12	0.6125
Non-conception	272	−0.21	0.06	0.0012	−0.15	0.07	0.0292
Periovulation phase (days −1 to 1)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	−0.20	0.08	0.0126	−0.21	0.08	0.0135
Non-conception	272	−0.37	0.05	<0.0001	−0.36	0.06	<0.0001
Early luteal phase (days 2 to 5)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	−0.17	0.10	0.0744	−0.15	0.09	0.0846
Non-conception	272	−0.34	0.06	<0.0001	−0.35	0.06	<0.0001
All phases (days −9 to 5)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	−0.15	0.09	0.1186	−0.13	0.09	0.1667
Non-conception	272	−0.29	0.06	<0.0001	−0.27	0.06	<0.0001

E_1C, estrone conjugates; PdG, pregnanediol-3-glucuronide.
[a]All models adjusted for day of cycle relative to ovulation and for non-independence of observations from multiple days and menstrual cycles from the same woman. Other covariates in models included age, age^2, BMI, BMI^2, husband's smoking, rotating work shifts, occupational dust and noise exposure and self-reported stress. The estimated parameters and *P*-values for hormone concentrations by cycle outcomes were very similar in models that did not include other covariates (not shown).

Table III. Mean log(urinary E_1C) in 94 non-conception cycles with sexual intercourse during the fertile period of the menstrual cycle relative to 94 clinical pregnancy cycles from the same women

	Number of cycles	Algorithm for day of ovulation Log(E_1C) (ng/mg creatinine)					
		E/P algorithm			PdG-rise algorithm		
		β	SE	*P*	β	SE	*P*
Mid-follicular phase (days −9 to −6)							
Clinical pregnancy	94	Ref			Ref		
No conception	94	−0.44	0.10	<0.0001	−0.43	0.09	<0.0001
Late-follicular phase (days −5 to −2)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.26	0.09	0.0059	−0.20	0.10	0.0384
Periovulation phase (days −1 to 1)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.42	0.08	<0.0001	−0.44	0.08	<0.0001
Early luteal phase (days 2 to 5)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.42	0.08	<0.0001	−0.40	0.08	<0.0001
All phases (days −9 to 5)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.38	0.07	<0.0001	−0.36	0.08	<0.0001

E_1C, estrone conjugates.
All models adjusted for day of cycle relative to ovulation and for non-independence of observations from multiple days and menstrual cycles from the same woman. Other covariates were not included in models because non-conception and clinical pregnancy cycles were from the same women.

Bonferroni correction in all phases: mid-follicular [odds ratio (OR) = 7.6; $P < 0.0001$], late-follicular (OR = 3.8; $P < 0.0001$), periovulation (OR = 3.4; $P < 0.0001$) and early luteal (OR = 6.9; $P < 0.0001$) phases. Using the E/P algorithm and relative to clinical pregnancy cycles, the odds of having high E_1C in non-conception cycles were significantly lower after Bonferroni correction in the periovulatory phase (OR = 0.3; $P < 0.0003$). The odds of having low E_1C were also significantly higher in

early pregnancy loss cycles (OR = 4.8; $P = 0.0027$) in the early luteal phase. The results for low and high E_1C were similar using the PdG-rise algorithm.

Urinary PdG using all observed cycles

As summarized in Table IV, mean daily log(PdG) tended to be lower in 272 non-conception cycles relative to 266 clinical pregnancy cycles in the mid- and late-follicular phases using either ovulation day algorithm, but these differences were not statistically significant after Bonferroni correction for 10 tests. [On average, daily PdG was 262 μg/mg Cr lower in non-conception cycles than in clinical pregnancy cycles across the mid- and late-follicular phases (days –9 to –2) using the E/P algorithm.] Using the PdG-rise algorithm to estimate the day of ovulation and relative to clinical pregnancy cycles, mean daily log(PdG) tended to be higher in the early luteal phase for both early pregnancy loss and non-conception cycles, but these differences were not significant after Bonferroni correction for 10 tests and were not observed when the E/P algorithm was used to identify the ovulation day. We did not show crude parameter estimates in Table IV, which were similar to those that we showed for the adjusted models.

Urinary PdG using non-conception and clinical pregnancy cycles from the same women

When we focused only on urinary PdG variability in different cycles from the same woman (Table V), the magnitudes of the estimated associations in the mid-follicular and early luteal phases were similar to those when we analysed all observed cycles. But the results were not significant after Bonferroni correction for 10 tests. The magnitudes of the estimated associations in all other phases of the menstrual cycle were attenuated relative to the effects estimated using all cycles, and none was statistically significant.

Low (<10th percentile) or high (>90th percentile) urinary PdG using all observed cycles

Using the PdG-rise algorithm, the 10th and 90th percentiles for average PdG by phase were mid-follicular (0.2 and 2.0 μg/mg Cr), late-follicular (0.2 and 1.8 μg/mg Cr), periovulation (2.5 and 2.7 μg/mg Cr) and early luteal (1.2 and 9.5 μg/mg Cr) phase, respectively. The 10th and 90th percentiles were similar using the E/P algorithm. Using the PdG-rise algorithm and relative to clinical pregnancy cycles, there were statistically significant differences after Bonferroni correction in the odds of low PdG (OR = 2.5; $P = 0.0044$) and high PdG (OR = 0.4; $P < 0.0005$) in non-conception cycles in the late-follicular phase. Using the E/P algorithm and relative to clinical pregnancy cycles, there were statistically significant differences after Bonferroni correction in the odds of high PdG (OR = 0.4; $P = 0.0019$) in non-conception cycles in the late-follicular phase and low PdG (OR = 2.4; $P = 0.0049$) in the mid-follicular phase.

Discussion

The participants in our sample had many characteristics that made them ideal for investigating whether variability in concentrations of urinary metabolites of estrogen and progesterone

Table IV. Relative adjusted[a] mean log(urinary PdG) by menstrual cycle outcomes for 601 cycles of 347 fertile women who were attempting to become pregnant without hormone treatment

	Number of cycles	Algorithm for day of ovulation Log(PdG) (ng/mg creatinine)					
		E/P algorithm			PdG-rise algorithm		
		β	SE	*P*	β	SE	*P*
Mid-follicular phase (days –9 to –6)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	–0.06	0.12	0.6114	0.01	0.12	0.9092
Non-conception	272	–0.26	0.10	0.0089	–0.24	0.10	0.0114
Late-follicular phase (days –5 to –2)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	0.14	0.12	0.2281	0.09	0.13	0.4701
Non-conception	272	–0.20	0.12	0.0830	–0.24	0.12	0.0363
Periovulation phase (days –1 to 1)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	0.14	0.13	0.2909	0.23	0.13	0.0686
Non-conception	272	0.07	0.10	0.5290	0.13	0.10	0. 1920
Early luteal phase (days 2 to 5)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	0.15	0.14	0.2699	0.29	0.14	0.0383
Non-conception	272	0.13	0.09	0.1631	0.25	0.09	0.0062
All phases (days –9 to 5)							
Clinical pregnancy	266	Ref			Ref		
Early pregnancy loss	63	0.11	0.13	0.3743	0.18	0.13	0.1606
Non-conception	272	–0.01	0.10	0.8899	0.04	0.10	0.7063

PdG, pregnanediol-3-glucuronide.

[a]All models adjusted for day of cycle relative to ovulation and for non-independence of observations from multiple days and menstrual cycles from the same woman. Other covariates in models included age, age^2, BMI, BMI^2, husband's smoking, rotating work shifts, occupational dust and noise exposure, and self-reported stress. The estimated parameters and *P*-values for hormone concentrations by cycle outcomes were very similar in models that did not include other covariates (not shown).

Table V. Mean log(urinary PdG) in 94 non-conception cycles with sexual intercourse during the fertile period of the menstrual cycle relative to 94 clinical pregnancy cycles from the same women

		Algorithm for day of ovulation Log(PdG) (ng/mg creatinine)					
		E/P algorithm			PdG-rise algorithm		
	Number of cycles	β	SE	*P*	β	SE	*P*
Mid-follicular phase (days −9 to −6)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.26	0.14	0.0681	−0.25	0.13	0.0512
Late-follicular phase (days −5 to −2)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.13	0.16	0.4212	−0.17	0.16	0.2826
Periovulation phase (days −1 to 1)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	0.14	0.15	0.3492	0.25	0.15	0.1052
Early luteal phase (days 2 to 5)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	0.20	0.14	0.1412	0.32	0.14	0.0214
All phases (days −9 to 5)							
Clinical pregnancy	94	Ref			Ref		
Non-conception	94	−0.01	0.13	0.9365	0.05	0.13	0.7150

PdG, pregnanediol-3-glucuronide.
All models adjusted for day of cycle relative to ovulation and for non-independence of observations from multiple days and menstrual cycles from the same woman. Other covariates were not included in models because non-conception and clinical pregnancy cycles were from the same women.

was associated with non-conception, early pregnancy loss and clinical pregnancy. In particular, our cohort was both large and uniquely homogenous with regard to important characteristics that could have affected hormone cyclicity and confounded the associations that we investigated. They were young, healthy, nulliparous women who were recently married and trying to conceive by natural methods and who were enrolled in the study at the time that they stopped using contraceptive methods. None had a history of infertility or were receiving exogenous hormone therapies nor had any used hormone contraceptives or intrauterine devices. None of the women smoked or drank coffee or alcohol. Women provided daily histories of sexual intercourse that allowed us to exclude menstrual cycles in which the reason for non-conception might have been lack of sexual intercourse during the fertile window around the day of ovulation. Daily urine samples allowed us to measure concentrations of E_1C and PdG throughout menstrual cycles as well as hCG to accurately measure conception and early pregnancy losses.

When we modelled all observed cycles together, we combined variability in hormone concentrations between different women with that from different cycles from the same women. Our results showed that compared with cycles that resulted in clinical pregnancy, cycles in which there was no conception had lower estrogen in each phase of the menstrual cycle (Table II). These results were consistent regardless of the hormone algorithm that we used to estimate the day of ovulation and align the cycles and were significant even after Bonferroni correction for 10 tests. When we limited our analyses to model only variability between different cycles from the same women, the magnitudes of the estimated associations became stronger (Table III). Our results showed that in naturally occurring menstrual cycles in these healthy, fertile women, estrogen concentrations varied from cycle to cycle, and higher estrogen was associated with achieving clinical pregnancy.

Our results for E_1C were very similar to those of Lipson and Elison (1996), who found that salivary estradiol concentrations were significantly lower in 81 non-conception cycles compared with 17 conception cycles during the follicular (days −10 to −1 relative to ovulation) and luteal (days 0 to 5) phases. When they limited their analysis to different cycles from the same women, their results were strengthened for both the follicular and luteal phases. Stewart *et al*. (1993) found higher daily average concentrations of serum estradiol in 14 conception compared with 13 non-conception cycles beginning on day 2 after the LH peak, which were statistically significant (without correction for multiple testing) beginning on day 6 to the end of the cycle. Baird *et al*. (1997) found higher average oestrone-3-glucuronide (E_1G) concentrations in 32 conception cycles on days 5 and 6 after ovulation compared with 32 non-conception cycles from the same women. In another study, they found a higher probability of conception associated with higher mean E_1G concentrations on days 5 and 6 in 189 conception cycles and 409 non-conception cycles from 215 women (Baird *et al*., 1999). However, when they modelled this simultaneously with other hormones that were statistically significant in the original model, E_1G was no longer associated with the probability of conception. Li *et al*. (2001) reported results for daily urinary E_1C in matched conception and non-conception cycles from 14 women from day −6 to 6 and daily serum estradiol for 6 women from day −1 to 6. They found that serum estradiol concentration was statistically significantly higher in 6 conception cycles on the day of the urinary FSH peak compared with 6 non-conception cycles from the same women. There were no statistically significant differences in urinary E_1C concentrations between 14 conception and 14 non-conception cycles.

We speculated that higher urinary E_1C concentrations in cycles that lead to clinical pregnancy might indicate a hormonal effect

on libido. To test this hypothesis, we analysed 523 cycles for which daily information regarding sexual intercourse was complete for the fertile period—cycle days −5 to 0 relative to ovulation. The total number of days with intercourse in a single cycle ranged from 1 to 6. Using generalized estimating equations assuming an exchangeable correlation structure, there were no statistically significant differences in mean concentrations of log(E_1C), E_1C, log(PdG) or PdG in cycles with different total number of days with intercourse, suggesting that the associations we observed were not due to hormonal effects on libido. An alternative explanation is that higher E_1C concentrations indicated the presence of higher quality dominant follicles in clinical pregnancy cycles. We found that low (<10th percentile) or high (>90th percentile) E_1C in the mid-follicular phase was strongly and significantly associated (respectively) with low or high E_1C in each of the late-follicular, periovulatory and early luteal phases (data not shown). These results show that cycles with high or low E_1C 5–9 days before ovulation continue to have high or low E_1C throughout the cycle, a finding that is consistent with the hypothesis regarding dominant follicle quality. We found that relative to clinical pregnancy cycles, the odds of an average E_1C concentration below 17 ng/mg Cr (10th percentile) in the mid-follicular phase was much higher (OR = 7.6; $P < 0.001$) in non-conception cycles. Our selection of the 10th percentile concentration as the threshold for this analysis was not based on any physiological considerations. An appropriate clinical threshold would need to be determined empirically. But our results demonstrate the potential for a clinically relevant marker of dominant follicle quality in the mid-follicular phase that could be utilized to predict the potential for achieving clinical pregnancy in a specific cycle.

We had far fewer early pregnancy loss cycles than non-conception cycles, so had limited power in our models. There were trends toward lower urinary E_1C concentrations in all phases of 63 early pregnancy loss cycles compared with 266 clinical pregnancy cycles, especially around the day of ovulation (Table II). These results were consistent regardless of the hormone algorithm that we used but were not statistically significant after Bonferroni correction for 10 tests. We found that the odds of E_1C less than the 10th percentile were significantly higher in early pregnancy loss cycles relative to clinical pregnancy cycles in the early luteal phase. In contrast to our results, Baird *et al*. (1991b) compared 20 cycles that ended in early pregnancy loss to 20 cycles from the same women that ended in a live, term birth. They reported that urinary E_1G and PdG concentrations in cycles with early pregnancy losses were very similar to those in successful conception cycles, but it is likely that this study had insufficient statistical power. Our results suggest that future studies with more early pregnancy loss cycles are warranted to confirm or refute the trends seen in our study.

The most robust of our PdG results appeared to be those for the mid-follicular phase in which we found lower urinary PdG concentrations in non-conception cycles relative to clinical pregnancy cycles. The magnitudes of the estimated associations did not change when we used different algorithms to determine the day of ovulation, all cycles observed from all women or pairs of non-conception and conception cycles from the same women. However, none of these results were statistically significant after Bonferroni correction for 10 tests, and they were opposite to those from a previous large study. Baird *et al*. (1999) compared 189 conception and 409 non-conception cycles from 215 women and found higher probabilities of conception in cycles that had lower baseline PdG [geometric mean of PdG for the interval starting on day 5 (from menses) of the cycle and ending 3 days before the day of ovulation]. In contrast, relative to clinical pregnancy cycles, we found significantly lower odds of PdG above the 90th percentile and significantly higher odds of PdG below the 10th percentile in non-conception cycles in the late-follicular phase.

Our other results for PdG in linear models were less robust than those in the mid-follicular phase because in addition to not being statistically significant after Bonferroni correction for 10 tests, the magnitudes of estimated associations were attenuated when we used pairs of non-conception and conception cycles from the same women rather than all cycles observed from all women. There were no consistent patterns of association of PdG with cycle outcomes in the periovulation or early luteal phases (Tables IV and V; Figure 2). Our most statistically significant result was higher PdG concentrations in non-conception cycles relative to clinical pregnancy cycles in the early luteal phase, but this was dependent on the algorithm that we used to determine the day of ovulation. Furthermore, our findings were opposite to those of two previous studies. Stewart *et al*. (1993) found higher daily average concentrations of serum progesterone in 14 conception cycles compared with 13 non-conception cycles (from 18 women) beginning on day 2 after the LH peak. They were statistically significant (without correction for multiple testing) beginning on day 7 to the end of the cycle. Baird *et al*. (1997) found that 32 conception cycles had a steeper early luteal rise in PdG and higher PdG concentrations on days 5 and 6 after ovulation than did 32 non-conception cycles from the same women. In our results, apparent differences in PdG concentrations in the later luteal phase (days 6–9, Figure 2) in conceptive, early pregnancy loss and non-conception cycles likely reflect an implantation effect rather than an effect of PdG on conception. Baird *et al*. (1999) reported that low mid-luteal PdG (<2 μg/mg Cr on days 5 and 6) was associated with non-conception. Two other previous studies did not find any differences in concentrations of salivary progesterone (Lipson and Ellison, 1996) or urinary PdG (Li *et al*., 2001) in non-conception relative to conception cycles.

A limitation of our study was that we relied on daily self-reports of sexual intercourse to exclude non-conception cycles in which no sexual intercourse occurred during the fertile period of days −5 to 0 relative to the estimated day of ovulation. Self-reports of sexual intercourse might have been incomplete; so we possibly excluded some non-conception cycles in which sexual intercourse had occurred during the fertile period. However, because urinary E_1C and PdG concentrations should not have systematically differed between non-conception cycles with accurately or inaccurately reported sexual intercourse during the fertile period, the hormone concentrations in non-conception cycles that we included should have been representative of this sort of cycle.

In conclusion, our study investigated the hormonal characteristics associated with achieving clinical pregnancy among a large and uniquely homogenous group of fertile young women who were attempting to become pregnant through natural methods without exogenous hormone treatment. We confirmed the results of previous studies which showed that estrogen concentrations vary from cycle to cycle in healthy, fertile women and that higher estrogen concentrations are associated with achieving clinical pregnancy. These results further our understanding of the natural history of hormonal patterns associated with fertility in the general population.

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