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Original Research Article

Stress and Female Reproductive Function: A Study of Daily Variations in Cortisol, Gonadotrophins, and Gonadal Steroids in a Rural Mayan Population

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ABSTRACT We report here on a longitudinal study of stress and women's reproduction in a small Kaqchikel Mayan community in rural Guatemala. Current understanding of the effects of stress on the reproductive axis in women is mostly derived from clinical studies of individual stressors. Little is known, however, about the cumulative effects of "real life" stress. Cortisol increases in response to a broad variety of individual stressors (Tilbrook et al., 2002). In this article, we evaluate the association between daily fluctuations in women's urinary cortisol and reproductive hormones: estrone conjugates (E_1C), pregnandiol glucuronide (PdG), luteinizing hormone (LH), and follicle stimulating hormone (FSH). To assess the association between daily changes in cortisol levels and changes in the profiles of the reproductive hormones, we used a random coefficients model based on polynomial regression. The sample includes 92 menstrual cycles provided by 24 participants over a year-long prospective study. Increases in urinary cortisol levels were associated with significant increases in gonadotrophin and progestin levels during the follicular phase. Also, in a time window between days 4 and 10 after ovulation, increased cortisol levels were associated with significantly lower progestin levels. These results are significant because untimely increases in gonadotrophins and low midluteal progesterone levels have previously been reported to impinge on the ovulatory and luteinization processes and to reduce the chances of successful implantation (Ferin, 1999; Baird et al., 1999). Future research should consider the possibility that stress may affect fecundability and implantation without necessarily causing amenorrhoea or oligomenorrhoea. Am. J. Hum. Biol. 16:523–532, 2004.

Female reproductive function occurs in a graded continuum from fully "competent" menstrual cycles (eumenorrhoea) to the complete absence of cyclic ovarian activity (amenorrhoea) (Prior, 1985; Ellison, 1990). Energetic, health, or psychological stressors may affect the reproductive axis, causing it to depart from eumenorrhoea and move towards amenorrhoea (Ellison and Lager, 1986; Keay, 1998; Ferrin, 1999). The adaptive value of reproductive suppressive mechanisms is clear: in unfavorable circumstances, avoiding reproduction allows females to focus scarce resources on survival, improvement in overall condition, and investment in existing offspring. Animal studies have confirmed the existence of reproductive suppression mechanisms and provided experimental evidence on the hormonal pathways through which several isolated, intense stressors impact reproductive function (Sapolsky, 2000; Tilbrook et al., 2000). The mechanisms through which stress suppresses reproduction in humans, however, are still poorly understood (Tilbrook et al., 2002).

Every day, women face multiple stress challenges of various types, intensities, and durations that can act synergistically. This makes "real life" stress (i.e., the combination of stressors faced by individuals in the course

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of their daily lives) extremely difficult to replicate in clinical or laboratory settings. Thus, most research has been narrowly focused on population subgroups, whose individuals are known to share specific stressors, such as athletes (e.g., Bonen, 1994) or women within a given profession (e.g., Schenker et al., 1997). These studies are valuable as they build upon and help validate the knowledge gained from animal studies for the human case, but they do not evaluate the effects that real life stress has on reproduction at a population level.

Based on data collected in a population of Kaqchikel Mayan women in Guatemala, we evaluate here the association between women's real life stress and reproductive function. We use urinary cortisol as a proxy for stress, and changes in the profiles of gonadotrophins (LH and FSH) and gonadal steroids (E_1C, and PdG) to assess reproductive function. Small traditional communities are a good alternative to urban settings for population-based longitudinal research, as they permit the study of real life stress without having to rely on particular subgroups or resort to experimental manipulations. In addition, researchers can achieve a more intimate and thorough knowledge of their participants (Flinn and England, 2003). The Kaqchikel Maya in our study population are socially, economically, and ethnically more homogeneous than people living in industrial societies, which reduces the number of confounding variables to be controlled (Pike, 2001). Furthermore, the lifestyles of women living in small, traditional populations are arguably more similar to those of ancestral populations (Jasienska, 2001) in which the human reproductive axis evolved some of its current characteristics.

For this article we define stress as a disruption of homeostasis (Rivier and Rivest, 1991). The secretion of glucocorticoids, including cortisol, increases in response to a variety of energetic, immunological, and psychological challenges to homeostasis and has, therefore, been widely used as a stress marker (e.g., Kanaley and Hartman, 2002; Padgett and Glaser, 2003; Altemus, 2001). Earlier concerns about the use of cortisol as a stress marker were primarily related to lack of control over changes in this metabolite unrelated to stress, baseline cortisol differences between individuals, and the lack of independence between multiple observations collected from the same individual (Pollard, 1995). Here we address these concerns by controlling for the effect of a wide variety of confounding factors and by using statistical methods that account for differences between individuals as well as for the possible correlations among samples provided by the same woman (see Subjects and Methods).

SUBJECTS AND METHODS

Study population

Fieldwork was conducted between November 2000 and November 2001 in a rural Kaqchikel Mayan community composed of 1,159 inhabitants in the southwestern highlands of Guatemala. In this village, 99% of the individuals are Kaqchikel Mayan. The local economy is primarily based on small-scale familial agriculture. The diet of the population consists mainly of corn, beans, and local fruits, complemented by freshwater fish and crabs, the occasional consumption of chicken, and, rarely, beef. The social structure is strongly based on family ties and migration is rare. Thus, women's social networks are mostly composed of family members. Women's primary activities consist of raising children, helping during the harvest, weaving fabric, and performing household chores. Potential everyday stressors that could trigger increases in cortisol levels include demanding physical activities, low energy intake, exposure to infectious diseases, and psychosocial stressors such as quarrels with family members and neighbors, worries about economic problems, and the personal health of close relatives.

Sample selection criteria and sample characteristics

All women in the study population who met the following sample selection criteria were invited to participate: Married with co-resident husband, parity ≥ 1, not using any form of contraception, not pregnant, and last birth >6 months prior to the onset of the study. During the first half of the study recruitment was restricted to women age 18–32 years, but later the upper age limit was expanded to 40 years to increase the sample size. Throughout the year, 61 women (about three-quarters of those eligible) volunteered to participate. Twenty-four of the 61 participants cycled at least once during the study and experienced a total of

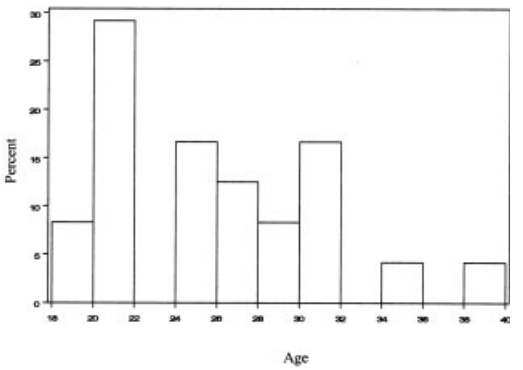

Fig. 1. Age distribution of cycling women.

TABLE 1. Assay quality control data

Metabolite	Intra CV	Inter CV
Creatinine range = 0.05–1.4 mg/mL.	5.4%	9.8%
E_1C range = 5.10–408.0 ng/mL	3.8%	6.5%
PdG range = 0.005–25.5 μg/mL	3.6%	11.6%
FSH range = 0.3–144.0 mIU/mL	2.3%	5.8%
LH range = 0.1–53.1 mIU/mL	3.5%	5.4%
Cortisol range = 0.2–75 μg/dl	2.0%	6.5%
hCG range = 0.003–1.0 ng/mL	3.5%	5.8%

92 menstrual cycles (mean = 4.0, SD = 3.17, median = 2, mode = 1). The ages of these 24 women ranged from 18–39 years, but the age distribution was heavily weighted toward the mid-20s (mean = 25.4 years, SD = 5.3 years, median = 25 years) (Fig. 1). This research was approved by the Institutional Review Board of the University of Michigan.

Data and specimens collection

Six trained Kaqchikel Mayan female assistants were in charge of the collection of personal data and urine specimens. Every other day, for a total of three times each week, participants collected their first morning urine specimens in a clean, dry, nonreactive plastic container. The assistants gathered the samples on the same morning that the participants produced them. Samples were kept on ice until the assistant returned to the laboratory (≈2 hours from the urinary void). Two-ml aliquots from the original specimens were stored frozen at –10°C in the field. They were shipped on dry ice to the laboratory at the University of Michigan, where they were stored at –80°C until analysis. Participants were followed from the time of their inclusion in the study until 6–8 weeks after a clinical pregnancy was detected in the field through commercial pregnancy tests (e.p.t® or Clear Blue Easy®).

Hormonal assays

The concentrations of urinary free cortisol, FSH, LH, E_1C, PdG, and human chorionic gonadotrophin (hCG) were determined using immunoassays developed in our laboratory for use on the Bayer Automated Chemilum-inescence System (ACS-180) immunoassay analyzer. Creatinine was assayed using a spectrophotometric assay. All samples from a single participant were run on the same assay and in duplicate. Outliers were identified and the samples rerun. Intra- and interassay coefficients of variation were within acceptable ranges (Table 1).

Data analysis

Characterization of hormonal profiles. The concentration of each hormone was divided by the concentration of creatinine in the same sample and results were expressed as the concentration of analyte per mg of creatinine. Hormone levels were plotted longitudinally by date and participant and visually inspected. Menstrual cycles were considered to begin on the first day of vaginal bleeding and end the day before the next bleeding. If the report of vaginal bleeding was unreliable, the last day of the cycle was the day the PdG level fell to 40% of its luteal peak and remained low for ≥2 days. Cycles presenting a 3-fold rise in PdG levels above baseline were considered ovulatory (Kassam et al., 1996). The time of ovulation was inferred using an algorithm based on the urinary ratio of E1C/PdG (modified from Baird et al., 1991) and verified using the presence of LH and FSH surges. Menstrual cycles were aligned to the estimated day of ovulation which was designated "day 0." Follicular days were given negative numbers and luteal days positive numbers.

Confounding factors. Cortisol secretion can be affected by circadian rhythms, physical

activity, food consumption, smoking, caffeine, alcohol, and steroid medications (Pruessner et al., 1997; Weitzman et al., 1971; Meulenberg and Hofman, 1990; Flinn and England, 1995; Bonen, 1976). None of the participants smoked or consumed alcohol. To reduce the influence of the rest of the confounding variables mentioned above, participants in our study were requested to collect their urine samples as soon as they woke up each morning and before they consumed food or performed any major physical activity. Occasionally, women forgot to collect their first morning urine before they began their daily chores or consumed one of the substances mentioned above within 12 hours of producing their sample. In such cases the specimen was discarded (20.7% of 1,645 samples).

Cortisol and gonadal steroid levels begin to increase shortly after conception (McLean and Smith, 1999), so to avoid bias, conceptive cycles (n = 18) were truncated at luteal day +4 for analysis. Women were considered to be pregnant when urinary hCG >0.025 ng/ml for at least 3 days (Wilcox et al., 1999). Nulliparous women have been reported to present higher cortisol levels than parous women (Vleugels et al., 1986). This confound was avoided in our study by the exclusion of nulliparous women.

Cortisol levels might also be affected by age (Kudielka et al., 2004), but in our sample age did not affect cortisol (mixed model ANOVA, $P > 0.05$). Age is also known to affect reproductive function (Lipson and Ellison, 1992; Harlow and Ephross, 1995). We therefore evaluated the effect of age and the interaction between age and cortisol by including them as variables in all regression analyses. In these analyses, however, age did not have a significant relationship to the urinary levels of reproductive hormones ($P > 0.05$), with follicular PdG as the only exception (Table 2). Nor did age affect the relationship between cortisol and reproductive hormones ($P > 0.05$). It is likely that the lack of a significant effect of age on cortisol and reproductive function was due to the youthfulness of our sample (Fig. 1).

If urinary cortisol excretion varied with the phase of the menstrual cycle, then such a pattern could generate a spurious association between cortisol and the reproductive hormones. Previous studies (Kanaley et al., 1992; Stewart et al., 1993; Kirschbaum et al., 1999) report no variation in cortisol levels between the follicular and the luteal phase. We found no significant differences in daily mean urinary cortisol levels within menstrual cycles with luteal phases of standard length (<15 days). However, cycles with prolonged luteal phases (n = 9) had significantly higher cortisol levels after luteal day 14 (mixed model ANOVA, adjusted $P = 0.03$). We therefore restricted our evaluation of the relationship between cortisol and the reproductive hormones to days –15 to +15 of the menstrual cycle.

Cortisol standardization. To make meaningful comparisons regarding changes in cortisol levels with respect to each woman's baseline and overall variability, cortisol was standardized using:

$$\text{Std cort}_{ij} = \frac{(\text{obs}_{ij} - \text{geomean}_i)}{\text{SD}_i}$$

Where obs_{ij} is the value of cortisol/creatinine for participant "i" on day "j," geomean (i) is the geometric mean of cortisol/creatinine for participant "i" while cycling, and SD_i is the standard deviation of cortisol for individual "i."

Statistical analyses

Data from each hormone were analyzed using the natural logarithm transformation to achieve a more normal distribution. We used mixed model analyses in Proc Mixed, SAS release 8.2 (Cary, NC) in all statistical analyses to take into account both fixed (e.g., day of the menstrual cycle) and random effects (individual participant). By considering individual participants as a random effect, the model controls for the possible correlations between samples collected from the same woman and captures the effects of unmeasured variables that might explain some of the differences between women that are not related to variations in their cortisol levels. Throughout the analyses, $\alpha = 0.05$ was used as the threshold for statistical significance.

Association between cortisol and the reproductive hormones. Associations between the reproductive hormones, time, and cortisol were examined using a random coefficients regression model (RCRM) (Brown and Prescott, 1999). The RCRM calculates a polynomial regression for the level of each

metabolite (the dependent variable) as predicted by time (day of the menstrual cycle) and each woman's cortisol levels and an overall polynomial for all the women. The degrees of freedom in the model are calculated based on the number of individuals included in the analyses adjusted to account for daily values missing from the record of each individual. We checked the adequacy of the model using residual diagnostic plots, influence statistics, and plots of predicted versus observed values. The RCRM method is more accurate for establishing whether the independent variable (cortisol) has an effect over time on the dependent variable (the reproductive hormone) than statistical methods that use the area under the curve (AUC). Whereas AUC models can mask changes that are opposite over time in the relationship between metabolites (as they cancel each other out), the RCRM captures daily variations in the relationship between metabolites, preventing this problem.

To evaluate the results obtained from the RCRM, we graphed the profiles of each metabolite as predicted by time and cortisol. To do so, we replaced cortisol in the polynomial equation with three values: the geometric mean of cortisol and ±2 SD. We present the graphs of the most interesting associations. These graphs do not represent the profile of any given participant, but are instead a model of the overall daily association between the variables of interest.

RESULTS

Urinary cortisol and the gonadotrophins throughout the follicular phase

LH and FSH. Both LH and FSH were positively associated with standardized urinary cortisol levels. The regression equation for follicular LH is: LH = 0.7006 + 0.2338 Std cort ($P < 0.0001$ for both coefficients; SD = 0.09 and 0.04, respectively; df = 22; Std cort = standardized cortisol). The regression equation for follicular FSH is: FSH = 2.376 + 0.3242 Std cort ($P < 0.0001$ for both coefficients; SD = 0.12 and 0.05, respectively; df = 22). Neither of these gonadotrophins (LH and FSH) exhibited a significant association with time, age, or the interaction between age and cortisol during the follicular phase. Time and age were therefore not included as predictors in the final versions of these two models.

TABLE 2. Regression model for the relationship between PdG, time, and cortisol in the follicular phase

Effect	Coefficient (β)	Std. Error	DF	*P*-values
Intercept	0.7569	0.1133	22	<.0001
Time	0.03084	0.01473	22	0.0481
$Time^2$	0.005461	0.00226	22	0.0244
$Time^3$	0.000251	0.0001	22	0.0203
Std cort	0.02786	0.007516	22	0.0012
Age	−0.01199	0.004238	21	0.01

Regression equation: Follicular PdG = $\beta_0 + \beta_1$Time + $\beta_2 Time^2 + \beta_2 Time^2 + \beta_3$ Cortisol + β_4 Age.

Urinary cortisol and the gonadal steroids throughout the follicular phase

E_1C and PdG. After controlling for the effects of time and participant, there was no significant association between cortisol or age and E_1C during the follicular phase ($P > 0.05$). However, age and cortisol had a significant association with PdG levels during this phase (Table 2). Increased age predicted lower PdG levels and elevated cortisol was associated with higher PdG levels throughout the follicular phase. The interactions between time and cortisol and age and cortisol did not have a significant effect on PdG levels. In sum, increased cortisol levels predicted higher PdG levels, but this association was not affected by time or age within the follicular phase.

Urinary cortisol and the gonadotrophins throughout the luteal phase

FSH. There was a significant positive association between cortisol and FSH in the luteal phase after controlling for the polynomial effect of time (Table 3). Age, and the interactions between age and time, and age and cortisol were not significant for FSH ($P > 0.05$).

LH. The association between cortisol and LH varied with time during the luteal phase, as seen in the significant interactions

TABLE 3. Regression model for the relationship between FSH, time, and cortisol in the luteal phase

Effect	Coefficient (β)	Std. Error	DF	*P*-values
Intercept	3.1276	0.1157	23	<.0001
Time	−0.3666	0.0330	22	<.0001
$Time^2$	0.02041	0.0025	18	<.0001
Std cort	0.1958	0.0493	18	0.0009

Regression equation: Luteal FSH = $\beta_0 + \beta_1$Time + $\beta_2 Time^2 + \beta_3$ Cortisol.

TABLE 4. Regression model for the relationship between LH, time, and cortisol in the luteal phase

Effect	Coefficient (β)	Std. Error	DF	*P*-values
Intercept	1.5641	0.1075	23	<.0001
Time	−0.3884	0.0597	22	<.0001
$Time^2$	0.0458	0.0107	17	0.0005
$Time^3$	0.0016	0.0005	17	0.005
Std cort	0.6623	0.0994	18	<.0001
Time*Std cort	−0.2653	0.0654	17	0.0008
$Time^2$*Std cort	0.0348	0.0119	17	0.009
$Time^3$*Std cort	−0.0013	0.0006	17	0.0452

Regression equation: Luteal LH = $\beta_0 + \beta_1$*Time + β_2*$Time^2$ + β_3*$Time^3$ + β_4*Cortisol + β_5*Time*Cortisol + β_6*$Time^2$*Cortisol + β_7*$Time^3$*Cortisol.

TABLE 5. Regression model for the relationship between E_1C, time, and cortisol in the luteal phase

Effect	Coefficient (β)	Std. Error	DF	*P*-values
Intercept	4.613	0.07958	23	<.0001
Time	−0.2446	0.03526	22	<.0001
$Time^2$	0.03973	0.006414	17	<.0001
$Time^3$	−0.00196	0.000318	16	<.0001
Std cort	0.1362	0.05249	18	0.0183
Time*Std cort	−0.04433	0.01863	17	0.0293
$Time^2$*Std cort	0.003243	0.001443	16	0.0391

Regression equation: Luteal E_1C = $\beta_0 + \beta_1$*Time + β_2*$Time^2$ + β_3*$Time^3$ + β_4*Cortisol + β_5*Time*Cortisol + β_6*$Time^2$*Cortisol.

between time and cortisol levels in the regression model (Table 4). The strongest association between cortisol and LH was around the time of ovulation (day "0") and towards the end of the luteal phase (>day +10). The association appeared to be weakest in the middle of the luteal phase (days +4 to day +8), where LH levels predicted by the different levels of cortisol were more similar to each other than to those at the beginning and end of the luteal phase (Fig. 2). After controlling for the effect of time and cortisol, age was not a significant predictor of changes in LH levels.

Urinary cortisol and the gonadal steroids throughout the luteal phase

E_1C. The relationship between E_1C and cortisol appeared to be greatest at the beginning and end of the luteal phase (days < +3 and days > +10, respectively) where higher levels of cortisol predicted higher levels of E_1C. Even when

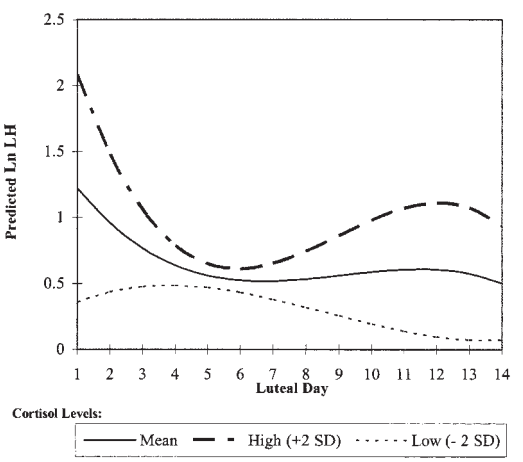

Fig. 2. LH predicted by time and cortisol throughout the luteal phase. The three LH profiles were obtained by replacing cortisol in the RCRM polynomial equation with three values: Mean (the geometric mean of cortisol), High (2 standard deviations above the mean), and Low (2 standard deviations below the mean). Although the intensity of the relationship varied with time, cortisol and LH levels remained positively associated throughout the luteal phase.

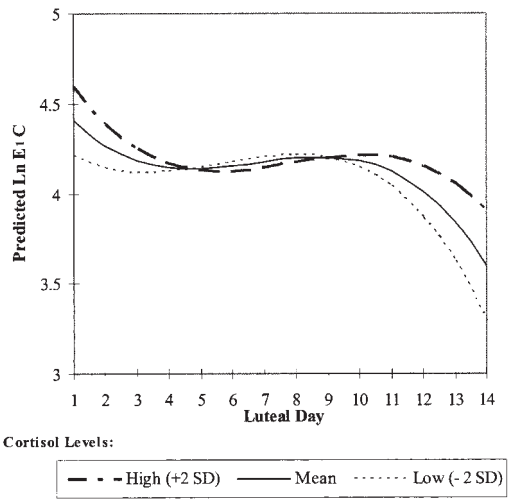

Fig. 3. E_1C predicted by time and cortisol throughout the luteal phase. The three E_1C profiles were obtained by replacing cortisol in the RCRM polynomial equation with three values: Mean (the geometric mean of cortisol), High (2 standard deviations above the mean), and Low (2 standard deviations below the mean). High cortisol levels predicted higher E_1C levels for a few days after ovulation (day 0) and in the last days of the menstrual cycle. High levels of cortisol were associated with lower levels of PdG between days 4 and 10 after ovulation (day 0).

TABLE 6. Regression model for the relationship between PdG, time, and cortisol in the luteal phase

Effect	Coefficient (β)	Std. Error	DF	*P*-values
Intercept	0.5297	0.05258	23	<.0001
Time	0.2104	0.03834	21	<.0001
$Time^2$	0.000606	0.006533	16	0.9273
$Time^3$	−0.00111	0.00035	15	0.0062
Std cort	0.1442	0.05491	17	0.0177
Time*Std cort	−0.06009	0.01942	16	0.007
$Time^2$*Std cort	0.004422	0.001546	15	0.0119

Regression equation: Luteal PdG = $\beta_0 + \beta_1$*Time + β_2*$Time^2$ + β_3*$Time^3$ + β_4*Cortisol + β_5*Time*Cortisol + β_6*$Time^2$*Cortisol.

it was significant, the relationship between E_1C and cortisol was not very pronounced in the midluteal phase (Table 5, Fig. 3). Age was not significantly associated with E_1C, nor did it have a significant effect on the relationship between this metabolite and cortisol.

PdG. Higher levels of cortisol were associated with increased levels of PdG at the beginning and end of the luteal phase (days 0 to +3 and days +10 to 14, respectively). However, in the middle of the luteal phase (~days +4 to +10), higher levels of cortisol were associated with lower levels of PdG (Table 6, Fig. 4). This graph also suggests that at increased cortisol levels there might be a small delay in the timing of the luteal peak of PdG. Age was not significantly associated with PdG, nor did it have a significant effect on the relationship between this metabolite and cortisol.

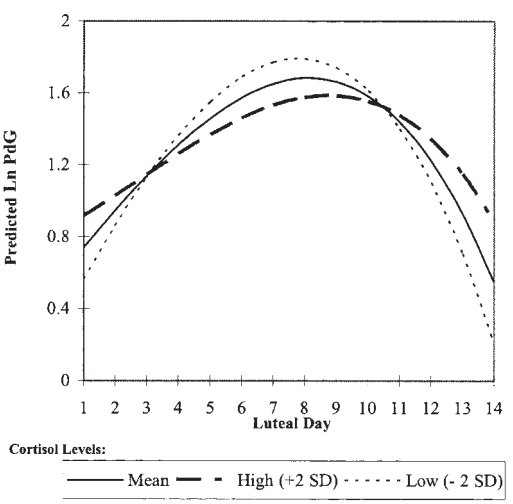

Fig. 4. PdG predicted by time and cortisol throughout the luteal phase. The three PdG profiles were obtained by replacing cortisol in the RCM polynomial equation with three values: Mean (the geometric mean of cortisol), High (2 standard deviations above the mean), and Low (2 standard deviations below the mean). High levels of cortisol were associated with lower levels of PdG between days 4 and 10 after ovulation (day 0).

DISCUSSION

It has long been demonstrated that exposure to severe stress challenges can affect female reproduction (Judd, 1992; Ferin, 1999). Experimental and clinical studies show, for example, that intense physical, psychological, and immune challenges can activate the hypothalamic–pituitary–adrenal axis (HPA), hampering reproduction both in humans and nonhuman mammals (Tilbrook, 2002; Arena et al., 1995; Laatikainen, 1991; Wasser et al., 1993). Nonetheless, very little data have been produced that validate the notion that stress can affect the functioning of women's reproductive axis in real life. Here we provide evidence that increases in urinary cortisol, as measured during the regular daily life of Mayan women, are associated with changes in the menstrual patterns of their reproductive hormones. Our results, however, do not fully conform to the physiological pathways through which intense stressors are commonly proposed to impinge on the reproductive axis.

Traditionally, stress is believed to affect reproductive function through a reduction in gonadotrophins, which subsequently leads to a reduction in gonadal steroids. This pathway involves the stress activation of the HPA axis triggering the hypothalamic release of corticotrophin-releasing hormone (CRH) and arginine-vasopressin. The rise in these neurohormones leads to an increase in pituitary ACTH and a consequent surge in adrenal cortisol. The increase in CRH negatively affects hypothalamic GnRH pulsatility and the cortisol surge causes a reduction of sensitivity to GnRH at the pituitary, leading to a reduction in the release of gonadotrophins (Rivier and Vale, 1990; Kalra, 1990; Feng et al., 1991; Chorusos et al., 1998). Ultimately, the reduction in gonadotrophin levels alters maturation of the follicle delaying or preventing ovulation, dampening luteal function, and therefore affecting the production of gonadal steroids and reducing the chances of a successful implantation and pregnancy (Ferin, 1999).

Alternatively, recent studies present evidence suggesting that, although always accompanied by a raise in glucocorticoids, at different intensities stress might affect reproductive function through different pathways. A study by Xiao et al. (2000), for example, showed that while intense levels of inflammatory stress can inhibit the secretion of LH in female monkeys, under estrogenic conditions milder inflammatory stress levels can actually promote LH secretion. They also reported that the adrenal stimulation caused by low stress levels was accompanied by an increase in the secretion of adrenal progesterone during the follicular phase (Xiao et al., 2000). Puder et al. (2000) demonstrated the existence of a similar mechanism in humans by comparing the effects of low versus high levels of inflammatory stress on LH levels in postmenopausal women after estrogen replacement. As in nonhuman primates, low levels of inflammatory stress led to a significant increase in cortisol, progesterone, and LH. While at high concentrations progesterone is known to inhibit the secretion of LH, the mild rise in progesterone, of apparent adrenal origin, synergizes with circulating estrogen enhancing LH secretion (Ferin, 1999). These hormonal changes are similar to the ones we observed. In our sample, daily increases in urinary cortisol levels predicted higher levels of gonadotrophins throughout the menstrual cycle and increased progestin levels during the follicular phase. These findings are significant because untimely increases in LH can affect follicular development, ovulation, luteinization, fertilization, and even early pregnancy (Stanger and Yovich, 1985; Watson et al., 1993; Paulson et al., 1992).

The inverse association we found between cortisol and progestin levels in the midluteal phase is also interesting. Previously, it had been suggested that the stress-led inhibition of progesterone during the luteal phase was triggered by a reduction in the levels of follicular gonadotrophins (Williams et al., 2001). Our results, however, show that while at the midluteal phase elevated cortisol levels predicted low progestin levels, during the follicular phase higher cortisol levels were associated with higher, not lower, gonadotrophin levels. Likewise, working on a nonhuman primate model, Xiao et al. (2002) found that a combination of psychogenic and health stressors caused luteal deficiencies without the previous inhibition of follicular gonadotrophin secretion. This new evidence combined with the finding of CRF (corticotrophin releasing factor) receptors on the ovary (Ghizzoni et al., 1997) supports the possible existence of pathways involving a downregulatory effect of stress on steroidogenesis exerted directly at the level of the ovaries (Chorusos et al., 1998; Tilbrook et al., 2002).

Energetic and psychosocial stress challenges have been previously reported to cause poor luteal progesterone levels in human and nonhuman primates (e.g., Ellison and Lager, 1986; Loucks et al., 1989; Xiao et al., 2002). This type of luteal deficiency tends to be intermittent and mostly asymptomatic. Thus, insufficient progesterone levels often pass unnoticed to the women experiencing them and, consequently, are not as commonly diagnosed as more obvious types of reproductive dysfunction (De Souza et al., 2003). However, luteal deficiencies may be one of the most common forms of reproductive suppression (Soules, 1989) and are perhaps as deleterious as the more obvious oligomenorrhea or amenorrhoea (De Souza et al., 1998).

The inverse association we observed between cortisol and progesterone levels in the midluteal phase suggests a possible mechanism through which stress may adversely affect the implantation process. Low progesterone levels may result in a degenerative endometrium (Soules, 1989) and are known to impinge on implantation (Soules, 1989; Baird et al., 1997, 1999). Significantly, we were able to pinpoint the negative association between cortisol and progesterone to a narrow window between luteal days +4 to +10. This is important because successful implantations take place approximately between days +8 and +10 and high midluteal progesterone levels are critical for the success of this process (Wilcox et al., 1999; Baird et al., 1999).

Implantation is a logical time for mechanisms of reproductive suppression to spring into action. Three weeks after conception the embryo is capable of synthesizing all the steroid hormones needed for pregnancy (Johnson and Everitt, 2000). Consequently, as gestation advances its maternal disruption by hormonal mechanisms becomes more difficult. In addition, after implantation the maternal investment and risks associated with pregnancy increase dramatically. Thus, implantation is a critical point in terms of the mother's physiological ability

to interrupt gestation, minimizing maternal risks and the wasteful allocation of energetic resources.

In summary, after controlling for the effect of a variety of confounding factors as well as differences in HPA functioning within and between individuals, we found a time-varying association between fluctuations in daily urinary cortisol levels and the profiles of reproductive hormones (FSH, LH, E_1C, and PdG) during the menstrual cycle. Changes in gonadotrophin and gonadal steroid levels similar to the ones we found have been reported to impinge on follicular maturation, ovulation, and implantation (Puder et al., 2000; Soules, 1989; Baird et al., 1999). Thus, our results raise the possibility that real life stress, as assessed by urinary cortisol, may adversely impact women's reproductive function. Further research is needed to demonstrate a link between specific stressors and the activation of the HPA axis in our study population.

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