

Cortisol levels and very early pregnancy loss in humans

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Maternal stress is commonly cited as an important risk factor for spontaneous abortion. For humans, however, there is little physiological evidence linking miscarriage to stress. This lack of evidence may be attributable to a paucity of research on maternal stress during the earliest gestational stages. Most human studies have focused on "clinical" pregnancy (>6 weeks after the last menstrual period). The majority of miscarriages, however, occur earlier, within the first 3 weeks after conception (~5 weeks after the last menstrual period). Studies focused on clinical pregnancy thus miss the most critical period for pregnancy continuance. We examined the association between miscarriage and levels of maternal urinary cortisol during the first 3 weeks after conception. Pregnancies characterized by increased maternal cortisol during this period (within participant analyses) were more likely to result in spontaneous abortion ($P < 0.05$). This evidence links increased levels in this stress marker with a higher risk of early pregnancy loss in humans.

stress | miscarriage | placentation | fetomaternal conflict | evolutionary theory

Spontaneous abortions can be triggered by maternal and fetal pathologies (1–6) or immunological incompatibilities (7–11). However, pathologies and incompatibilities alone do not explain all miscarriages (12). Maternal stress is commonly cited as a potential cause for at least part of pregnancy losses that remain "unexplained" (13–21). Yet, for humans, little physiological evidence exists in support of this hypothesis (22–24).

Empirical evidence indicates that most spontaneous abortions in humans take place during the first 3 weeks after conception (or <5 weeks after the last menstrual period) (25), which, coincidentally, is the time required for the placenta's structural and functional units to develop (26). During the "placentation" period, human embryos depend heavily on their mothers for survival. For example, until the placenta is able to replace the corpus luteum as the main source of steroids, the fate of a pregnancy relies on adequate maternal production of estrogens and progestins (26–29). Thus, human embryos might be especially vulnerable to maternal challenges until the placenta matures.

Placentation is, therefore, a particularly relevant period for studying the relationship between maternal stress and pregnancy fate in humans. Previous research on the topic, however, has focused mainly on clinical pregnancy (>6 weeks after last menstrual period, equivalent to >4 weeks after conception) (23). Furthermore, except for studies on women with known fertility problems (22), past studies have rarely included physiologic measures (14–16, 30). Whereas some previous studies found stress to be associated with spontaneous abortion (15, 24, 31, 32), others did not (23, 30). Thus, whether this relationship exists in humans remains unclear.

To fill this gap, we examine the association between a physiological marker of stress (cortisol) during the first 3 weeks after conception and pregnancy fate in a nonclinical population of

nominally fertile women. Cortisol is commonly used as a stress marker because its production by the adrenal cortex tends to increase as a result of energetic, immunological, and psychological challenges (33–35). To address concerns about the use of cortisol as a stress marker (36), we account for the effects of a wide variety of confounding factors as well as for baseline differences among individuals and possible correlations within individuals.

Results

Of the 22 observed pregnancies, 9 were carried to term ("successful") and 13 were lost ("unsuccessful"). The average time from ovulation to fetal loss in unsuccessful pregnancies was 16 days (median, 14 days; range, 13–47). Mean standardized cortisol levels were higher in unsuccessful than in successful pregnancies (unsuccessful pregnancies: $\bar{x} = 0.19$; SD, 0.38; successful pregnancies: $\bar{x} = -0.20$; SD, 0.35; $F_{1,20} = 6.07$, $P = 0.02$; Fig. 1).

We calculated the comparative risk of spontaneous abortion according to cortisol exposure. Pregnancies in which the average standardized cortisol during the first 3 weeks after conception, or between ovulation and pregnancy loss (if gestation was <3 weeks), was equal to or less than the woman's overall cortisol baseline (OCB) were classified as exposed to "normal cortisol" ($n = 10$). When the 3 weeks postconception average cortisol level was above the woman's OCB ($n = 12$), the pregnancy was classified as exposed to "increased cortisol." Pregnancies exposed to increased cortisol were 2.7 times (95% confidence interval = 1.2–6.2) more likely to be unsuccessful (lost) than those exposed to normal cortisol levels (Rao–Thomas adjusted $F_{1,16} = 3.42$, $P = 0.03$). Whereas 90% of the increased cortisol pregnancies resulted in spontaneous abortions, only 33% of the normal cortisol pregnancies were lost (Table 1).

We also compared the proportion of cortisol "peaks" (values ≥ 90 th percentile of the overall distribution of standardized cortisol) between the two pregnancy outcomes. Unsuccessful pregnancies presented a larger proportion of cortisol peaks than successful ones (proportion in unsuccessful pregnancies = 0.32; SD, 0.21; proportion in successful pregnancies = 0.05; SD, 0.11; Poisson regression analysis $P = 0.01$; Fig. 2).

Discussion

Fetal loss rates reported in humans range from 31% (37) to 89% (38) of all conceptions. This high rate of miscarriages has led health scientists to describe human reproduction as "inefficient" and, therefore, an evolutionary "paradox" (12).

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Abbreviations: OCB, overall cortisol baseline; hCG, human chorionic gonadotrophin.

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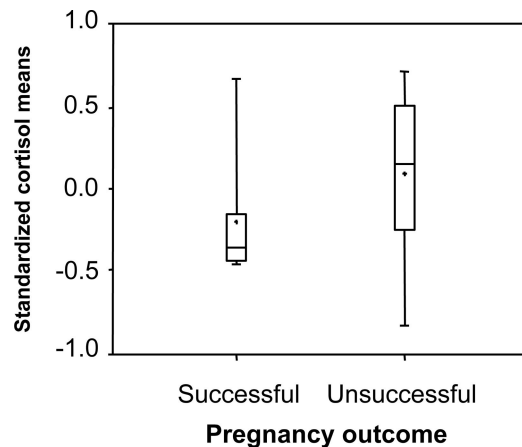


Fig. 1. Average cortisol levels and pregnancy outcome. Mean standardized cortisol was higher in unsuccessful than in successful pregnancies ($P = 0.02$) during the first 3 weeks of gestation. + indicates the mean, and the central horizontal bar indicates the median. The edges of the box indicate the 75th and 25th percentiles, respectively; error bars go to highest and lowest value that is not an outlier (<1.5 times the interquartile range above or below the edges of the box).

Evolutionary theorists, however, propose that aborting unhealthy, defective, or otherwise substandard embryos, or those gestating under “impoverished reproductive conditions,” can be reproductively advantageous (39–45). Our results indicating an association between high cortisol levels and increased risk of miscarriage should be considered within the latter context. “Impoverished reproductive conditions” refers to reductions in the quality of females’ environment and/or health status, such as droughts, infections, or social conflicts. Miscarriage under such conditions could help minimize the cost of pregnancies with diminished chances of success, preserve valuable resources to be invested in future offspring with higher fitness prospects, and free those resources to be used on a woman’s own survival and already existing offspring, which could be crucial during a crisis (43–45).

Nonetheless, current “adaptive abortion models” propose that very early abortions would be better explained by problems with the embryo’s quality, rather than environmental challenges (43, 44). They argue that to reduce the risk of terminating pregnancies that could otherwise be successful (should the reproductive context improve), gestation should be allowed to continue for as long as possible (44). The contradiction between this prediction and our results may be explained by two factors. First, these models do not consider the different costs and benefits of interrupting reproduction for species with different reproductive schedules. Whereas for seasonal breeders, interrupting a pregnancy could imply losing an

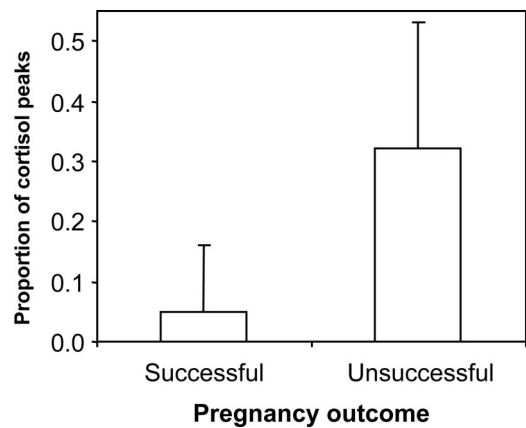


Fig. 2. Proportion of cortisol peaks (values ≥ 90 th percentile) and pregnancy outcome. Successful pregnancies had a significantly lower proportion of cortisol peaks than those that resulted in spontaneous abortions ($P = 0.01$). Bar height indicates the average proportion of cortisol peaks observed for each pregnancy outcome. Error bars are 1 SD.

entire reproductive year, for continuous breeders such as humans, the opportunity costs of very early miscarriages are comparatively lower. Second, these models are primarily based on maternal cost–benefit analyses and ignore the potential constraints that fetal development imposes on maternal reproductive strategies. The threshold of conditions under which it would be beneficial for a fetus to be born should be lower than that for the mother. Each genetically unique fetus has but one opportunity to be born. Thus, it is not surprising that within hours of fertilization conceptuses begin secreting a battery of metabolites that reduce the risk of miscarriage (46). The physiologic maturation of the fetus, however, is necessarily gradual; therefore, fetuses should be most vulnerable to stress-triggered abortive mechanisms during the first weeks of gestation.

Cortisol production rises in response to energetic, immunological, and psychosocial challenges (47, 48). Thus, cortisol increases could serve as a physiological cue to women’s bodies that conditions for reproduction are deteriorating. Beyond its utility as a stress marker, cortisol may also be directly or indirectly involved in some of the proximate mechanisms mediating the putative association between maternal stress and pregnancy loss. For example, cortisol may affect the production of luteal progesterone. Low progesterone levels can affect uterine maturation and pregnancy maintenance (12). Consistent with this “mediation” hypothesis, we have previously reported a negative association between cortisol and progesterone around the time of implantation (49). The discovery of corticotrophin-releasing factor receptors on the

Table 1. Cortisol exposure and pregnancy outcome

| Cortisol exposure | Pregnancy outcome | | Total |
|---|--------------------------|----------------------------|-------|
| | Successful, <i>n</i> (%) | Unsuccessful, <i>n</i> (%) | |
| Increased cortisol: average > baseline | 1 (10) | 9 (90) | 10 |
| Normal cortisol: average \leq baseline | 8 (66.67) | 4 (33.33) | 12 |
| Total | 9 | 13 | 22 |

Relative risk of miscarriage given increased cortisol exposure was 2.7 (95% confidence interval = 1.2–6.2; Rao–Thomas adjusted $F_{1,16} = 3.42$, $P = 0.03$). Average is the mean of all cortisol values obtained for a given pregnancy from the estimated time of ovulation until the pregnancy was lost or 3 weeks after ovulation, whichever came first. Pregnancies exposed to increased cortisol were 2.7 times more likely to result in miscarriage (unsuccessful) than those exposed to normal cortisol (Rao–Thomas $F_{1,16} = 3.4$, $P = 0.03$).

ovary (50) is also consistent with the possible existence of a down-regulatory effect of stress on steroidogenesis exerted at the gonadal level (51). Additionally, immune challenges in mice appear to promote a shift in the Th1/Th2 cytokine ratio, which has been associated with low progesterone levels and early spontaneous abortion (24, 52). Another possible mechanism is suggested by cell-culture studies in rabbits showing that glucocorticoids can cause degeneration and premature aging of the trophoblast (53, 54).

Alternative explanations for our results should also be considered. First, an impending early loss could hypothetically lead to an increase in cortisol levels. Any cortisol increases during the first 3 weeks after conception would have to be maternal because embryos cannot produce glucocorticoids during that period (55). Although no conditions have been reported to date in which an impending loss could trigger an increase in maternal cortisol during the first 3 weeks after conception, the potential existence of such a condition cannot be discounted. Second, human chorionic gonadotrophin (hCG) can occasionally increase in the absence of pregnancy (56), which, if combined with a simultaneous raise in cortisol, could lead to spurious results. *In vitro* studies, for instance, have shown that exogenous hCG can stimulate cortisol production in adrenal tissue from individuals with Cushing's syndrome (57, 58). In healthy women of reproductive age, however, hCG stimulation tests do not lead to increases in adrenal cortisol (59). Furthermore, both sustained high hCG levels in nonpregnant women and Cushing's syndrome are rare conditions. Monthly medical examinations did not detect symptoms of either condition in any of our participants. Thus, although we cannot completely rule out alternative explanations, maternal stress appears to be, at this time, the most parsimonious interpretation for the observed relationship between increased cortisol and spontaneous abortion.

Our finding of an association between increased maternal cortisol and higher risk of miscarriage within the first 3 weeks of conception, together with the failure of previous research to find such an association later during gestation (23), suggests that pregnancy may be particularly sensitive to maternal stress during the placentation period. Future longitudinal studies with larger samples will be necessary to both replicate our results and further test this hypothesis by comparing cortisol levels and risk of miscarriage across the entire duration of gestation. Further research will also be necessary to explore the physiological pathways that might mediate the observed association.

Methods

Study Population. Data were collected over 12 months in a rural Kaqchikel Mayan community in the southwestern highlands of Guatemala. According to a census conducted in collaboration with the State's Health Ministry, 1,159 inhabitants lived in this village in the year 2000. Women who met all five of the following criteria were invited to participate: (i) not pregnant at the onset of the study, (ii) cohabitating with husband, (iii) parity ≥ 1 , (iv) not using any form of contraception, and (v) last birth > 6 months before the onset of the study (49).

Sixty-one women ($\approx 75\%$ of those eligible in the entire population) volunteered to participate. All of the participants were breastfeeding previously born children throughout the duration of this study. Of the 61 women, 24 cycled and the rest experienced lactational amenorrhoea throughout their participation. Over the course of the year, 16 of the 24 eumenorrhoeic (cycling) women conceived 22 pregnancies. Ten of the women conceived once, and six women lost their first pregnancy but then conceived again. Participants were medically examined once a month by a local medical doctor or professional nurse to determine their health status.

Urinary Specimens, Hormonal Assays, and Hormonal Profiles. First-morning urine specimens were collected every other day, for a total of three times each week. Collection, preservation, transportation, and assay protocols have been published previously (49, 60). To control for urinary dilution, the concentrations of free cortisol, estrone conjugates, pregnandiol glucuronide, luteinizing hormone, follicle-stimulating hormone, and hCG were divided by the concentration of creatinine in the same sample (38). Timing of ovulation was inferred by using the urinary ratio of estrone conjugates to pregnandiol glucuronide as well as luteinizing hormone and follicle-stimulating hormone surges (49, 61). Chemical pregnancy was inferred when urinary hCG was > 0.025 ng/ml for at least 3 days (i.e., in two consecutive samples) (62). Pregnancy loss was inferred when pregnandiol glucuronide or hCG values declined to follicular levels, whichever was first, or by personal reports after the collection of samples had ceased (approximately the 8th week after the last menstrual period). If a participant who became pregnant during the study experienced a spontaneous abortion after having left the study (i.e., > 8 weeks after last menstrual period), she was invited to resume participation.

Confounding Factors and Cortisol Standardization. Cortisol secretion can be affected by circadian rhythms, physical activity, food consumption, smoking, caffeine, alcohol, and steroid medications (63–67). None of the participants smoked or consumed alcohol. To reduce the influence of the other confounding variables, participants were requested to collect their urine samples as soon as they woke up each morning, before they consumed food or performed any major physical activity. Sometimes women forgot to collect their first-morning urine before they began with their daily chores or consumed one of the substances mentioned above (besides tobacco or alcohol) before producing their sample. In such cases, the specimen was discarded (20.7% of 1,645 samples).

Nulliparous women tend to present higher cortisol levels than parous women (68); our protocol, however, included only parous women. Cortisol levels may be affected by age (69), but in our sample, age was not associated with cortisol (mixed-model ANOVA, $P > 0.05$). The lack of an effect of age on cortisol may be due to the youthfulness of our sample. The 16 women who conceived during the study ranged from 18 to 34 years, but the age distribution was heavily weighted toward the early 20s ($\bar{x} = 23.5$ years; median, 21 years; SD, 4.5 years). Later stages of pregnancy are known to be accompanied by mild hypercortisolemia (70). We controlled for pregnancy stage by focusing on the first 3 weeks after conception.

Cortisol production varies both between and within individuals. Thus, to make meaningful comparisons, we standardized the concentrations of this metabolite with respect to each woman's cortisol baseline using the following equation:

$$\text{standardized cortisol}_{ij} = [(\text{observation}_{ij} - \text{OCB}_i) / \text{SD}_i],$$

where observation_{ij} is the value of cortisol/creatinine for participant i on day j , OCB_i is the OCB for participant i , which is calculated as the mean of cortisol/creatinine for all of the cortisol values available for that woman, and SD_i is the SD of cortisol for individual i .

Cortisol does not vary significantly across the menstrual cycle (49, 71) except, perhaps, after luteal day 14 in unusually long luteal phases (37). To prevent cortisol from prolonged luteal phases from introducing a bias in our calculation of individual OCBs, we restricted our calculations to days -15 to $+15$ of the menstrual cycle.

It is possible that some of the cycles with prolonged luteal phases (9 of 92 cycles) could mask "hidden" pregnancies (i.e., conceptive cycles that did not fulfill all of the requirements to be

classified as a pregnancy). Considering those “cycles” as pregnancies would have provided potentially spurious support for the proposed hypothesis because cycles with prolonged luteal phases presented higher-than-average cortisol levels. To avoid this bias, we excluded the above-mentioned data not only from our calculations of OCBs but also from our analyses involving cortisol and miscarriage.

Statistical Analyses. Some participants contributed more than one pregnancy, so we controlled for individual effects in all statistical analyses. All analyses were based on standardized cortisol. We compared cortisol means between pregnancy outcomes using a linear mixed model (Proc Mixed in SAS 8.2; SAS Institute, Cary, NC) with a random effect term for “woman” to account for possible correlations among observations within women. Comparisons of cortisol levels between successful and unsuccessful pregnancies included all cortisol values obtained within the first 3 weeks of gestation (counting from the day of ovulation onward) or until the pregnancy was lost, whichever came first.

We estimated the relative risk of pregnancy loss in relation to cortisol exposure using the Rao–Thomas modified *F* test (Svytab in Stata 7.0; Stata Corporation, College Station, TX). Each woman was treated as a “cluster” to adjust for individual

effects. We compared the proportion of cortisol peaks experienced by women during successful and unsuccessful pregnancies using a Poisson regression analysis. In this case, individual effects were taken into account with Generalized Estimating Equations (Proc Genmod in SAS). We defined “cortisol peaks” as all values in the 90th percentile of the overall distribution of standardized cortisol (across women). In all cases, we used $\alpha = 0.05$ as the threshold for statistical significance. This research was approved by the Institutional Review Board of the University of Michigan.

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