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Moderate Anxiety, Whether Acute or Chronic, Is Not Associated With Ovarian Suppression in Healthy, Well-Nourished, Western Women

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ABSTRACT The relationship between psychological stress and reduced fecundity has been a matter of speculation and investigation for decades. Most previous studies have been compromised, however, by a number of problems including ambiguous direction of causation, poorly operationalized variables, and the confounding of psychological with energetic stress. We present a two-part study of the relationship between moderate anxiety, both acute and chronic, and daily measures of ovarian steroid and corticosteroid levels in saliva. Anxiety, as a particular form of psychosocial stress, was measured by the Spielberger Stait-Trait Anxiety Inventory as well as by a self-reported daily stress score. In the first part, 23 college juniors taking the Medical College Admissions Test (MCAT) were studied the month before and the month after the test, and again several months later, and compared at the same time points with 27 controls. In the second part, chronic anxiety levels were assessed in 95 women between 27 and 41 years of age and analyzed in relation to daily levels of salivary ovarian and corticosteroids over one menstrual cycle. The sample sizes are sufficient to allow for confidence in negative results. No statistically significant differences in ovarian or corticosteroid levels were observed whether between the MCAT and control subjects in part one, between the MCAT subjects before and after the MCAT test in part one, or between high and low anxiety subjects in part two. The results indicate that moderate levels of anxiety, whether acute or chronic, are not associated with suppressed ovarian function in healthy women. Am J Phys Anthropol 134:513–519, 2007.

The role of psychological stress in the natural regulation of human fertility has long been a matter of interest and conjecture (Kelly, 1942; Sandler, 1960; Eisner, 1963). Clinicians in psychology, psychiatry, and reproductive medicine often cite mood states such as anxiety as potential causes of conception difficulty (Domar et al., 2000; Smeenk et al., 2001; Boivin, 2003; Cwikel et al., 2004; Anderheim et al., 2006). Anthropologists, too, have appealed to psychological factors to explain certain features of human reproduction such as low rates of conception prior to the harvest in agricultural communities (Malina and Himes, 1977). Wasser and colleagues (Wasser and Isenberg, 1986; Wasser et al., 1993; Wasser and Place, 2001) formulated an explicit evolutionary hypothesis to explain the supposed suppressive effect of psychological stress on female fecundity by casting female psychological stress as a symptom of the lack of social support and other resources necessary for a high probability of reproductive success. Recently, Nepomnaschy et al., (2006) have documented high rates of early conception loss associated with elevated cortisol levels among Guatemalan women and included psychological stress among the possible causes of the association.

The evidence for a suppressive effect of psychological stress on female fecundity independent of other causes has, however, always been equivocal and plagued with methodological difficulties. Early clinical studies relied heavily on anecdotal data, such as reports of pregnancy following adoption (Sandler, 1965) or psychotherapy (Sharman, 1952). Later studies often confounded the directionality of causation by using infertility patients as subjects and comparing their levels of psychosocial stress with fertile controls (Harrison et al., 1981, 1986; O'Moore et al., 1983; Domar et al., 1990). A large literature has now accumulated emphasizing infertility diagnoses and treatments as causes of psychosocial stress rather than the reverse (Seibel, 1997; Schneider and Fothofer, 2005; Schmidt, 2006). Most meta-analyses conclude that clinical data are derived from designs that are inadequate to address the question of causation unambiguously (Istvan, 1986; Berg and Wilson, 1990; Wright et al., 1991; Harlow et al., 1996; Boivin, 2003).

A second problem is that psychological stress is often confounded with physiological stress, particularly with catabolic physiological states that are independently known to suppress ovarian function in women (Biller et al., 1990; Berga et al., 1997, 2000, 2003; Jasienska and Ellison, 1998; Warren et al., 1999; Meczekalski et al., 2000; Perkins et al., 2001; Ellison, 2003; Genazzani, 2005; Brundu et al., 2006; Jasienska et al., 2006). In many studies elevated cortisol levels are interpreted as a biomarker of psychological stress although they are

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more properly interpreted as markers of catabolic energy mobilization. Psychological stress is but one among a number of possible upstream causes of cortisol elevation, others of which include exercise, low blood sugar, infection, cold, hypoxia, and many other states and conditions (Larsen, 2003). Because of these multiple pathways of causation, robust inferences from cortisol levels in the upstream direction are not possible. But while the upstream associations of elevated cortisol levels are nonspecific, the downstream associations are specific and robust. Elevated cortisol levels lead to catabolic processes that raise blood glucose and inhibit fat and protein anabolism. Thus the proper interpretation of elevated cortisol is as a marker of catabolic state, not as a marker of psychological stress.

An additional problem that often plagues the study of psychological stress and its effects on human fertility regulation is the poor operationalization of the concept of psychological stress itself. Differences that are attributed to the effects of "stress" can variously refer to differences in a "stressor," i.e., the kind and degree of stress applied; differences in "stress response," i.e., the physiological consequences of the stressor; or differences in "perceived stress," i.e., the reported experience of the subject. Yet many studies fail to adequately distinguish among these different aspects of the concept of stress. For example, studies of the effects of stressful life events are essentially studies of different stressors. Studies that show different responses to a common stressor, like public speaking, depending on sex or menstrual status are essentially studies of differences in stress response. Studies based on reported stress levels are essentially studies of differences in perceived stress that might be modified by personality, coping style, or personal history.

Psychological stress is also an overly general and heterogeneous category, potentially including emotions and states as disparate as anxiety, depression, pain, shock, confusion, and remorse, among others. At the very least it is incumbent on researchers to be as specific as possible about the aspect of stress they are studying (stressor, stress response, perceived stress), the nature and specificity of the operational variables they are using to represent and measure psychological stress, and to be as specific as possible about the variety of stress (anxiety, depression, etc.) that they are focusing on.

Studies of the relationship of psychological stress to female fecundity often suffer from one or more of the difficulties listed above. As a result, despite a history of interest and speculation, there is little hard evidence of an effect of psychological stress *alone*, independent of the confounded effects of catabolic state or other known suppressors of ovarian function, on female reproductive physiology. This might be because previous research designs have been inadequate, or it might be because psychological stress by itself does not affect female fecundity. We have attempted to address this problem by designing a study of the effect of moderate chronic and acute anxiety, a specific psychological stressor, on ovarian steroid levels among Boston women. We have limited our study to anxiety because it is one of the aspects of psychological stress most consistently proposed as having a negative effect on female fecundity in both clinical and anthropological literature. We take advantage of the Spielberger State-Trait Anxiety Inventory (STAI, Spielberger, 1983, 1989) to operationalize perceived anxiety since it is well validated and widely used. We use salivary steroid levels to index ovarian function since they have been demonstrated to be very sensitive to other regulators of female fecundity, such as metabolic stress (Ellison, 2003), and because ovarian steroids have been shown to correlate with fecundity within and between women (Lipson and Ellison, 1996; Venners et al., 2006). Our subjects are blind to their own steroid levels, reducing the possibility of reverse causation, i.e., low fecundity causing anxiety rather than the reverse. We incorporate multiple levels of control into our design, i.e., between subjects and controls and between and within subjects over time. And we use sample sizes sufficient to render negative results meaningful. We thus hope to avoid many of the problems we outline above while addressing the question, "Are moderate levels of anxiety, such as women might be expected to experience with some regularity, either chronic or acute, associated with suppressed levels of ovarian steroids independently of the confounding effects of catabolic state?"

The study consists of two parts. The first part examines the relationship of moderate acute anxiety to ovarian steroid levels using the Medical College Admissions Test (MCAT) as an acute situational stressor. The second part examines the relationship of chronic stress to ovarian steroid levels by comparing women with different trait anxiety scores on the STAI.

MATERIALS AND METHODS

Subjects

For Part I, focusing on acute anxiety, 23 subjects were recruited from among Harvard College juniors planning to take the Medical College Admissions Test (MCAT). In addition, 27 controls not planning to take the MCAT were recruited from the same undergraduate class. All participants reported regular menstrual cycles at the time of recruitment, were not using oral contraception, were of normal weight-for-height, and were not engaged in regular physical exercise. In addition to saliva samples and information on anxiety described below, subjects and controls provided information on age and menarcheal age. Height and weight were measured at the beginning of two sample collection periods, one in the spring before and after the MCAT exam, the other the following fall.

In Part II, focusing on chronic anxiety, 95 subjects were recruited from the Cambridge/Boston area. All subjects were between the ages of 21 and 40, reported regular menstrual cycles, were not using oral contraception, and had not been pregnant or lactating within 6 months at the time of recruitment. In addition to saliva samples and information on anxiety described below, subjects reported on their daily hours of aerobic exercise during the period of sample collection. Height and weight were measured at the beginning and end of the sample collection period.

Anxiety measures

The STAI was administered to all participants in both parts of the study. As noted earlier, the STAI is a widely used and fully validated instrument for the assessment of acute and chronic anxiety. The instrument consists of two panels of questions, one focused on a subject's perception of her current psychological state, the other on the subject's perception of her customary state of mind. Answers to the former set of questions provide an index

TABLE 1. Mean (SE) values for age, height, and weight variables characterizing the subjects in the study of acute anxiety

Group	Age (yrs)	Menarcheal age (yrs)	Height (cm)	Beginning weight spring (kg)	Change in weight spring (kg)	Beginning weight fall (kg)
MCAT	20.0 (0.1)	12.5 (0.5)	164 (2)	57.0 (2.0)	0.7 (0.5)	57.0 (2.0)
Controls	20.0 (0.2)	12.4 (0.2)	163 (1)	60.0 (2.0)	0.2 (0.3)	60.0 (2)
P values	0.81	0.83	0.83	0.31	0.34	0.36

of "state" or acute anxiety; answers to the latter set of questions provide an index of "trait" or chronic anxiety. The STAI was administered twice to participants in Part I, once the day before the MCAT exam, and once at the beginning of the sample collection period the following fall. The STAI was administered once to participants in Part II, at the beginning of the sample collection period.

In addition to the STAI, all study participants were asked to score their perceived stress level on a 5-point scale (1, much less than usual; 2, somewhat less than usual; 3, usual; 4, somewhat more than usual; 5, much more than usual) on every day of saliva sample collection at the time of sample collection. While the STAI asks questions about stress on an absolute scale, the daily stress score asks about stress relative to each subject's individual average level.

Salivary steroid measures

Saliva samples were collected each morning soon after waking and stored using previously validated procedures (Lipson and Ellison, 1989). Participants in Part I collected samples daily for a two-month period centered on the day of the spring administration of the MCAT exam, and again for one full menstrual cycle, beginning and ending on first day of menstrual bleeding, the following fall. The inclusion of the fall sampling period allowed us to consider whether there were any seasonal effects that both the MCAT and Control groups might be subject to in the spring. It also allowed us to compare the MCAT group in the month immediately after the MCAT test with a month much more distantly removed to determine whether effects of the MCAT test might continue in the immediate post-test month and only attenuate with more time. Participants in Part II collected saliva samples for one full menstrual cycle. All participants recorded the time and day of sample collection on the sample tube.

Saliva samples were assayed for estradiol, progesterone, and cortisol using previously described methods (Ellison, 1988; Lipson and Ellison, 1996). Inter-assay variability was 15% for estradiol, 9% for progesterone, and 8% for cortisol. Intra-assay variability was 7% for estradiol, 10% for progesterone, and 7% for cortisol. Ovarian steroids were assayed from all daily samples. Values were aligned on the day of the midcycle estradiol drop as described elsewhere (Lipson and Ellison, 1996). From these daily values the following indices were calculated: mid-luteal progesterone, being the average of values from days −5 to −9 relative to menses; mid-follicular estradiol, being the average of values from days −6 to −10 relative to the day of the midcycle estradiol drop; and late follicular estradiol, being the average of the values from days −1 to −5 relative to the day of the midcycle estradiol drop. Cortisol was assayed in one-third of the daily samples, the sample from every third day being assayed unless the sample that day was collected after 9 a.m., in which case the next daily sample was used. All subjects provided the same number of cortisol samples for analysis. Cortisol values from these samples were averaged over relevant time periods for analysis.

Statistics

The design of Part I allows for comparisons between groups (MCAT vs. control) at three points in time (spring before test, spring after test, and fall) as well as comparisons within subjects between these three time points. Comparisons are made using one-tailed, paired and unpaired *t*-tests with the Bonferroni correction for multiple contrasts applied where warranted. One-tailed tests are used since the direction of group differences is posited *a priori*. Significance is recognized at an alpha level of 0.05. The sample size provides for detection of moderate effect sizes (Cohen's $d = 0.70$) with a power coefficient (beta) of 0.80 and smaller effect sizes ($d = 0.50$) with a power coefficient of 0.50. Pearson product-moment correlation is used to assess the relationship of cortisol levels to anxiety measures and to ovarian steroid levels, with significance recognized at an alpha level of 0.05. The sample size provides for the detection of moderate correlations ($r = 0.30$) with a power coefficient of 0.80, and smaller correlations ($r = 0.20$) with a power coefficient of 0.50.

In Part II subjects were assigned to one of four groups on the basis of their trait anxiety scores on the STAI. Group A was composed of subjects with scores lower than the reported normal range (<26, $N = 14$). Group B was composed of subjects with scores within the reported normal range (26–35.9, $N = 41$). Groups C and D were composed of subjects with scores above the reported normal range (>36), subdivided into two equal groups. Group C had scores from 36 to 45.9 ($N = 20$), and Group D had scores greater than 46 ($N = 20$). Comparison between groups is made using one-way ANOVA with significance recognized at an alpha level of 0.05. The sample size provides for the detection of moderate effect sizes ($f = 0.45$) with a power coefficient >0.95, and for smaller effect sizes ($f = 0.23$) with a power coefficient of 0.50.

RESULTS

Part I: Acute anxiety

Subject characteristics. Table 1 presents means and standard errors for selected characteristics of subjects and controls in Part I. The two groups show no significant differences in age, menarcheal age, height, weight in the fall, weight in the spring, or change in weight between fall and spring. There is no significant difference between the mean trait anxiety score for the two groups for either the spring or fall administration of the STAI.

The MCAT as a stressor. Stress and anxiety scores are compared before and after the MCAT to determine whether the test was indeed perceived as a stress and

TABLE 2. State anxiety and trait anxiety score means (SE) for MCAT and Control subjects in the spring at the time of the MCAT exam, and in the following fall

Group	State anxiety; spring	Score; fall	Trait anxiety; spring	Score; fall
MCAT	49.3 (2.4)[a,b]	34.6 (1.7)	38.0 (2.0)	34.7 (1.6)
Controls	38.3 (2.2)	40.6 (2.6)	38.9 (1.8)	37.8 (1.6)

[a] $P = 0.002$, MCAT state anxiety spring vs. control state anxiety spring.
[b] $P < 0.0001$, MCAT state anxiety spring vs. MCAT state anxiety fall. All other relevant comparisons are statistically non-significant.

TABLE 3. Mean (SE) daily stress scores before and after the MCAT exam in the spring and again the following fall for MCAT and control subjects

Group	Before	After	Fall
MCAT	3.7 (0.2)[a,b]	2.9 (0.1)	2.8 (0.1)
Control	2.9 (0.1)	3.0 (0.1)	2.7 (0.1)

[a] $P < 0.0001$, MCAT before vs. MCAT after.
[b] $P < 0.0001$, MCAT before vs. control before.

associated with increased levels of anxiety. Table 2 presents means and standard errors for state and trait anxiety scores for the MCAT and control groups in the spring and fall; Table 3 presents means and standard errors for average daily stress scores for the month before the MCAT, the month after the MCAT, and the fall. State anxiety scores are significantly higher in the MCAT group than the control group in the spring at the time of the MCAT ($P = 0.02$), but not in the fall. Within subjects, the MCAT group shows a significant drop in state anxiety between the spring and the fall ($P < 0.0001$). The control group shows no significant change in state anxiety scores over the same period. The average daily perceived stress score is significantly higher among the MCAT takers than among the controls during the month prior to the MCAT ($P < 0.001$) but not during the month following the test or the following fall. Within subjects, there is a significant drop in average daily perceived stress score among the MCAT group from the month preceding the MCAT to the month after ($P < 0.0001$), but not for the control group.

There are no significant differences between the MCAT and control groups in average cortisol levels at any of the three periods, the month before the MCAT, the month after the MCAT, or the following fall. Nor are there significant correlations between average cortisol and either state anxiety scores or daily stress scores, within or between individuals, at any time period.

Effects on ovarian steroids. Table 4 summarizes average ovarian steroid levels for the MCAT and control groups at all three periods. There are no significant differences in the ovarian steroid levels between the two groups at any time period. Nor are there any significant changes in ovarian steroid levels within subjects between time periods.

There are no significant correlations between cortisol and ovarian steroid levels at any time period.

Part II: Chronic anxiety

Table 5 summarizes the mean values for selected subject characteristics by group. There are no significant differences between groups for age, height, weight, change in weight, cycle length, or reported exercise. Subjects do differ significantly ($P < 0.0001$) in state anxiety scores, but not in daily stress scores. These results are consistent, given that the daily stress score is standardized on what each subject perceives as her "usual" level of stress, whereas the STAI purports to measure non-standardized levels of anxiety. Subjects who are chronically anxious should experience an elevated level of absolute, non-standardized anxiety as "usual."

TABLE 4. Mean (SE) values for steroid indices for MCAT and control groups in the month before and the month after the MCAT exam in the spring, and the following fall

	Spring		
Group	Before MCAT	After MCAT	Fall
Mid-follicular estradiol (pmol/L)			
MCAT	23 (1)	25 (3)	24 (2)
Controls	25 (2)	23 (1)	25 (1)
Late follicular estradiol (pmol/L)			
MCAT	31 (3)	32 (3)	32 (3)
Controls	31 (2)	32 (3)	31 (2)
Mid-luteal progesterone (pmol/L)			
MCAT	235 (25)	244 (27)	279 (34)
Controls	258 (25)	257 (37)	227 (17)
Cortisol (nmol/L)			
MCAT	10.7 (1.7)	8.5 (0.8)	9.6 (0.8)
Controls	9.1 (1.1)	9.0 (1.2)	9.5 (0.8)

None of the relevant pair-wise differences are statistically significant.

There are no significant differences between the groups in any of the steroid indices (Table 6).

DISCUSSION

The design of the present study allows for a sensitive exploration of the relationship between moderate anxiety and ovarian steroid production with minimal potential for a number of possible confounding effects. Most importantly, there is little chance of impaired ovarian function being itself a source of anxiety among the study subjects. All subjects reported regular menstrual cycles and no history of gynecological pathology or infertility. At the time of the study they were blind to their own ovarian steroid levels. The comparisons made between groups in both parts of the study were also free of confounded differences in age, weight, weight change, or exercise levels. Hence the potential for psychological stress and metabolic stress to be confounded was minimized.

Psychological anxiety was identified as the independent variable in both parts of the study. In Part I the MCAT was used as a situational stressor. Both the STAI and subjects' daily stress scores confirm that MCAT subjects perceived themselves as more anxious and more stressed during the month leading up to the MCAT than the control group, and also than they themselves were in the month after the MCAT and the following fall. The acute nature of this difference in anxiety levels is under-

TABLE 5. Mean (SE) values for age, height, weight, change in weight between beginning and end of cycle, cycle length, and exercise variables characterizing the subjects in the study of chronic anxiety

Group	Trait anxiety score range	Age (yrs)	Height (cm)	Weight (kg)	Change in weight (kg)	Cycle length (days)	Hours of exercise per week
A	20–25.9	32 (1)	161 (1)	57 (1)	0.0 (0.2)	29.5 (1.6)	3.6 (0.8)
B	26–35.9	29 (1)	164 (1)	61 (1)	0.0 (0.2)	29.4 (0.5)	2.8 (0.4)
C	36–45.9	29 (1)	164 (1)	61 (1)	0.1 (0.2)	29.9 (0.9)	2.5 (0.4)
D	46–74	29 (1)	163 (1)	59 (2)	−0.1 (0.3)	29.2 (1.0)	2.3 (0.8)
P values		0.27	0.54	0.35	0.95	0.97	0.55

TABLE 6. Means (SE) of hormonal indices for subjects in the chronic anxiety study group by level of chronic anxiety score on the STAI

Group	Mid-follicular estradiol (pmol/L)	Late-follicular estradiol (pmol/L)	Mid-luteal progesterone (pmol/L)	Cortisol (nmol/L)
A	25 (2)	35 (2)	265 (26)	7.8 (0.8)
B	26 (1)	35 (2)	244 (17)	7.5 (0.5)
C	28 (2)	35 (2)	278 (26)	8.6 (0.7)
D	26 (2)	37 (3)	320 (27)	8.0 (0.8)
P values	0.82	0.94	0.10	0.67

No two-way comparisons were statistically significant.

scored by the lack of any difference in trait (chronic) anxiety levels between the MCAT subjects and the controls either in the spring or in the fall.

In Part II subjects were assigned to groups on the basis of their trait anxiety scores. The groups differed significantly in their state anxiety scores, as expected. Subjects who are chronically anxious should be more acutely anxious than less chronically anxious subjects at any given point in time. The chronic nature of this anxiety difference between groups was underscored, however, by the absence of any group differences in daily stress scores. Although chronically anxious subjects should report greater "absolute" levels of anxiety than less chronically anxious subjects on any given day, they should also perceive this higher level of absolute anxiety as "usual" for themselves.

The absence of group differences in cortisol levels indicates that the differences in anxiety levels that were established by the study design were not reflected in differences in HPA axis activity. This is consistent with the interpretation of elevated cortisol levels as a marker of catabolic mobilization of energy reserves. Such a mobilization is usually a transient feature of acute situational stress. It is possible, even likely, that the cortisol levels of the MCAT subjects were elevated on the morning of the test itself. But there is no evidence that the more moderate anxiety experienced for the preceding month was associated with catabolic activation of this kind. Nor is there any evidence that the differences in chronic anxiety isolated in Part II are associated with differences in average cortisol levels. We cannot say whether transitory elevations of cortisol may have occurred in our subjects, though it would be very unusual if they did not. Our study, however, was designed to detect sustained cortisol elevation corresponding either to sustained periods of situational stress (Part I) or to chronic stress (Part II). Sustained cortisol elevation has been demonstrated to be effective in disrupting pituitary gonadotropin and ovarian steroid profiles in experimental studies with sheep, whereas episodic cortisol does not produce a definitive effect (Breen et al., 2005). Ovarian responses can only be properly assessed over entire ovarian cycles, due to the changing pattern of ovarian steroid production within cycles. Our design for Part I included assessment of ovarian function both in the month immediately following the MCAT exam and again 6 months later. At least one study of ovarian responses to weight loss indicated that effects of acute stress in 1 month could carry over into the succeeding month (Lager and Ellison, 1990). Repeating our assessment of ovarian function 6 months after the MCAT test allowed us to control for this possibility.

The design of the study can thus be judged to have successfully isolated acute and chronic anxiety differences between subjects who are otherwise comparable and blind to their own ovarian steroid levels and without confounding effects of differences in catabolic state. This allows for a clear test of the hypothesized association between anxiety and ovarian steroid production with sufficient statistical power to render negative findings persuasive. No evidence was found for an effect of either acute or chronic anxiety on ovarian steroid levels. Given the power of the study design, the negative findings provide a basis for rejecting the notion that moderate anxiety by itself, disassociated from confounded effects of metabolic stress, causes significant suppression of ovarian steroid production.

The implications of these results must be limited, however, to the focused domain that was the target of the research: a causal pathway leading from moderate anxiety to suppressed ovarian steroid production. A number of important possibilities beyond this narrow domain remain unchallenged. Among them are the following.

Moderate anxiety may be associated with suppressed ovarian steroid production when causation is in the opposite direction, from ovarian function status to anxiety level. A number of studies document elevated anxiety among women with diagnoses of infertility or who are seeking infertility treatment (Mazure and Greenfield, 1989; Laffont and Edelmann, 1994; Hsu and Kuo, 2002), observations that are consistent with this direction of causation. Similarly, cortisol responses to experimental situations involving psychosocial stress vary between phases of the menstrual cycle and between sexes (Kirschbaum et al., 1999; Kajantie and Phillips, 2006). But in these cases, as well, there is evidence that stress

responses are affected by ovarian steroids, not the other way around (Kirschbaum et al., 1995, 1996).

Moderate anxiety may be associated with potential causes of reduced fertility other than suppressed ovarian steroid production. The effects of anxiety on male fecundity are not assessed in this study. Nor are the effects of moderate anxiety on other aspects of female reproductive function or behavior. Studies that report lower success rates of IVF associated with high anxiety levels in women prior to the procedure are consistent with mechanisms other than differences in ovarian steroid production. For example, differences in tubal transport or trophoblast attachment may be associated with anxiety levels, perhaps mediated by catecholamine levels or other proximal causes. Nepomnaschy et al. (2006) have recently reported that early pregnancy loss in Guatemalan women is associated with elevated salivary cortisol levels, suggesting the possibility of post-implantation effects of HPA axis activity. Differences in anxiety levels may also be associated with behavioral differences in the frequency of intercourse that can contribute to fertility differentials in couples not undergoing ART intervention.

The conclusions of the present study apply only to conditions of moderate anxiety. More extreme anxiety levels, such as occur during lifethreatening or other extreme situations, may trigger significant HPA axis activity and simultaneously inhibit ovarian function. The potential impact of higher levels of psychological stress must be separately assessed. It is also possible that the subjects in our study, well-nourished residents of a major US city, may not represent the full range of potential human responses to moderate anxiety. Subjects who are under chronic energetic stress, for example, or who have had different developmental histories, might have different set-points for HPA axis activation and/or ovarian response (Jasienska and Ellison, 2006).

Most importantly, the conclusions of the present study must be limited to situations in which moderate anxiety is not confounded by evidence of metabolic stress. In many situations, moderate anxiety may lead to metabolic stress, either by influencing patterns of sleep, eating, or exercise, or by directly activating endogenous systems of catabolic activation such as the HPA axis. A number of studies have found associations between functional hypothalamic amenorrhea and elevated levels of psychological stress (Berga et al. 1997, 2000; Brundu et al., 2006), but in these instances there is also evidence of elevated catabolic state, usually in the form of elevated cortisol levels. The association can sometimes be subtle, requiring direct evidence of individual metabolic state. For example, restrained eating, or consciously eating below appetite, has been associated with suppressed ovarian steroid levels even when total caloric intake is the same as in controls (Warren et al., 1999; Berga et al., 2003). However, cortisol levels are also elevated in these subjects, indicating that the subjects displaying restrained eating are also experiencing a catabolic state. In the study by Nepomnaschy et al. (2006) referred to above, the authors note that many factors, including both psychological stress and energetic stress, may have contributed to the observed elevations in cortisol that are associated with early pregnancy loss.

The conclusion of this study can be stated, then, as follows: moderate anxiety in well-nourished, Western women, whether acute or chronic, that is not associated with signs of a catabolic state shows no evidence of causing suppressed levels of ovarian steroids. Within this domain the conclusion is relatively free of many of the confounders that have plagued much prior research.

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LITERATURE CITED

Anderheim L, Holter H, Berg C, Moller A. 2006. Does psychological stress affect the outcome of in vitro fertilization? Hum Reprod 20:2969–2975.

Berg RG, Wilson JF. 1990. Psychiatric morbidity in the infertile population: a reconceptualization. Fertil Steril 53:654–661.

Berga SL, Daniels TL, Giles DE. 1997. Women with functional hypothalamic amenorrhea but not other forms of anovulation display amplified cortisol concentrations. Fertil Steril 67: 1024–1030.

Berga SL, Loucks-Daniels TL, Adler LJ, Chrousos GP, Cameron JL, Matthews KA, Marcus MD. 2000. Cerebrospinal fluid levels of corticotropin-releasing hormone in women with functional hypothalamic amenorrhea. Am J Obstet Gynecol 182:776–781.

Berga SL, Marcus MD, Loucks TL, Hlastala S, Ringham R, Krohn MA. 2003. Recovery of ovarian activity in women with functional hypothalamic amenorrhea who were treated with cognitive behavior therapy. Fertil Steril 80:976–978.

Biller BM, Federoff HJ, Koenig JI, Klibanski A. 1990. Abnormal cortisol secretion and responses to corticotropin-releasing hormone in women with hypothalamic amenorrhea. J Clin Endocrinol Metab 70:311–317.

Boivin J. 2003. A review of psychosocial interventions in infertility. Soc Sci Med 57:2325–2341.

Breen KM, Billings HJ, Wagenmaker ER, Wessinger EW, Karsch FJ. 2005. Endocrine basis for disruptive effects of cortisol on preovulatory events. Endocrinology 146:2107–2115.

Brundu B, Loucks TL, Adler LJ, Cameron JL, Berga SL. 2006. Increased cortisol in the cerebrospinal fluid of women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 91:1561–1565.

Cwikel J, Gidron Y, Sheiner E. 2004. Psychological interactions with infertility among women. Eur J Obstet Gynecol 117:126–131.

Domar AD, Clapp D, Slawsby EA, Dusek J, Kessel B, Freizinger M. 2000. Impact of group psychological interventions on pregnancy rates in infertile women. Fertil Steril 73:805–811.

Domar AD, Seibel MM, Benson H. 1990. The mind/body program for infertility: a new behavioral treatment approach for women with infertility. Fertil Steril 35:489–499.

Eisner BG. 1963. Some psychological differences between fertile and infertile women. J Clin Psychiatry 19:391–395.

Ellison PT. 1988. Human salivary steroids: methodological considerations and applications in physical anthropology. Yearb Phys Anthropol 31:115–142.

Ellison PT. 2003. Energetics and reproductive effort. Am J Hum Biol 15:342–351.

Genazzani AD. 2005. Neuroendocrine aspects of amenorrhea related to stress. Pediatr Endocrinol Rev 2:661–668.

Harlow CR, Fahy UM, Talbot WM, Wardle PG, Hull MG. 1996. Stress and stress-related hormones during in-vitro fertilization treatment. Hum Reprod 11:274–279.

Harrison RF, O'Moore AM, O'Moore RR, McSwweney JR. 1981. Stress profiles in normal infertile couples: pharmacological and psychological approaches to therapy. In: Insler V, Bettendorf G, editors. Advances in diagnosis and treatment of infertility. New York: Elsevier/North Holland. p 143–157.

Harrison RF, O'Moore RR, O'Moore AM. 1986. Stress and fertility: some modalities of investigation and treatment in couples with unexplained infertility in Dublin. Int J Fertil 31:153–159.

Hsu YL, Kuo BJ. 2002. Evaluations of emotional reactions and coping behaviors as well as correlated factors for infertile couples receiving assisted reproductive technologies. J Nurs Res 10:291–302.

Istvan J. 1986. Stress, anxiety, and birth outcomes: a critical review of the evidence. Psychol Bull 100:331–348.
Jasienska G, Ellison PT. 1998. Physical work causes suppression of ovarian function in women Proc R Soc Lond B Biol Sci 265:1847–1851.
Jasienska G, Thune I, Ellison P. 2006. Fatness at birth predicts adult susceptibility to ovarian suppression: an empirical test of the "Predictive Adaptive Response" hypothesis. PNAS 103: 12759–12762.
Jasienska G, Ziomkiewicz A, Thune I, Lipson SF, Ellison PT. 2006. Habitual physical activity and estradiol levels in women of reproductive age. Eur J Cancer Prev 15:439–445.
Kajantie E, Phillips DL. 2006. The effects of sex and hormonal status on the physiological response to acute psychological stress. Psychoneuroendocrinology 31:151–178.
Kelly K. 1942. Sterility in the female with special reference to psychic factors: a review of the literature. Psychosom Med 4:211–228.
Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary, adrenal axis. Psychosom Med 61:154–162.
Kirschbaum C, Pirke KM, Hellhammer DH. 1995. Preliminary evidence for reduced cortisol responsivity to psychological stress in women using oral contraceptive medication. Psychoneuroendocrinology 20:509–514.
Kirschbaum C, Schommer N, Federenko I, Gaab J, Neumann O, Oellers M, Rohleder N, Untiedt A, Hanker J, Pirke KM, Hellhammer DH. 1996. Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to psychosocial stress in healthy young men. J Clin Endocrinol Metab 81:3639–3643.
Laffont I, Edelmann RJ. 1994. Psychological aspects of in vitro fertilization: a gender comparison. J Psychosom Obstet Gynaecol 15:85–92.
Lager C, Ellison PT. 1990. Effect of moderate weight loss on ovarian function assessed by salivary progesterone measurements. Am J Hum Biol 2:303–312.
Larsen PR, editor. 2003. Williams textbook of endocrinology, 10th ed. Philadelphia: W.B. Saunders.
Lipson SF, Ellison PT. 1989. Development of protocols for the application of salivary steroid analyses to field conditions. Am J Hum Biol 1:249–255.
Lipson SF, Ellison PT. 1996. Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. Hum Reprod 11:2090–2096.
Malina RM, Himes JH. 1977. Seasonality of births in a rural Zapotec Municipio, 1945–1970. Hum Biol 49:125–133.
Mazure CM, Greenfeld DA. 1989. Psychological studies of in vitro fertilization/embryo transfer participants. J In Vitro Fert Embryo Transf 6:242–256.
Meczekalski B, Tonetti A, Monteleone P, Bernardi F, Luisi S, Stomati M, Luisi M, Petraglia F, Genazzani AR. 2000. Hypothalamic amenorrhea with normal body weight: ACTH, allopregnanolone and cortisol responses to corticotropin-releasing hormone test. Eur J Endocrinol 142:280–285.
Nepomnaschy PA, Welch KB, McConnell DS, Low BS, Strassmann BI, England BG. 2006. Cortisol levels and very early pregnancy loss in humans. Proc Nat Acad Sci USA 103:3938–3942.
O'Moore AM, O'Moore RR, Harrison RF, Murphy G, Carruthers ME. 1983. Psychosomatic aspects in idiopathic infertility: effects of treatment with autogenic training. J Psychosom Res 27:145–151.
Perkins RB, Hall JE, Martin KA. 2001. Aetiology, previous menstrual function and patterns of neuro-endocrine disturbance as prognostic indicators in hypothalamic amenorrhoea. Hum Reprod 16:2198–2205.
Sandler B. 1960. Emotional stress and infertility. Practitioner 184:355–361.
Sandler B. 1965. Conception after adoption. Practitioner 194: 505–510.
Schmidt L. 2006. Psychosocial burden of infertility and assisted reproduction. Lancet 4:379–380.
Schneider MG, Forthofer MS. 2005. Associations of psychosocial factors with the stress of infertility treatment. Health Soc Work 30:183–191.
Seibel MM. 1997. Infertility: the impact of stress, the benefit of counseling. J Assist Reprod Genet 14:181–183.
Sharman A. 1952. Therapeutic experiments in female infertility. JAMA 148:603–605.
Smeenk JM, Verhaak CM, Eugster A, van Minnen A, Zielhuis GA, Braat DD. 2001. The effect of anxiety and depression on the outcome of in-vitro fertilization. Hum Reprod 16:1420–1423.
Spielberger CD. 1983. Manual for the state-trait anxiety inventory. Palo Alto, CA: Consulting Psychologists Press.
Spielberger CD. 1989. State-trait anxiety inventory: a comprehensive bibliography. Palo Alto, CA: Consulting Psychologists Press.
Venners SA, Liu X, Perry MJ, Korrick SA, Li Z, Yang F, Yang, J, Lasley BL, Xu X, Wang X. 2006. Urinary estrogen and progesterone metabolite concentrations in menstrual cycles of fertile women with non-conception, early pregnancy loss, or clinical pregnancy. Hum Reprod 21:2272–2280.
Warren MP, Voussoughian F, Geer EB, Hyle EP, Adberg CL, Ramos RH. 1999. Functional hypothalamic amenorrhea: hypoleptinemia and disordered eating. J Clin Endocrinol Metab 84:873–877.
Wasser SK, Isenberg DY. 1986. Reproductive suppression: pathology or adaptation. J Psychosom Obstet Gynecol 5:153–175.
Wasser SK, Place NJ. 2001. Reproductive filtering and the social environment. In: Ellison PT, editor. Reproductive ecology and human evolution. New York: Aldine de Gruyter. p 137–158.
Wasser SK, Sewall G, Soules MR. 1993. Psychosocial stress as a cause of infertility. Fertil Steril 59:685–689.
Wright J, Allard M, Lecours A, Sabourin S. 1991. Psychosocial distress and infertility: a review of controlled research. Int J Fertil 34:126–142.