Preconception stress and the secondary sex ratio in a population-based preconception cohort

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Objective: To examine the association between preconception parental stress and the secondary sex ratio, defined as the ratio of males to females at birth.
Design: A population-based preconception cohort.
Setting: Not applicable.
Patient(s): A total of 235 couples who were enrolled before conception in Michigan and Texas between 2005 and 2009 and who had a singleton birth during the follow-up period. Couples were interviewed separately at baseline to obtain information on perceived stress (Cohen's Perceived Stress Scale) and lifetime history of physician-diagnosed anxiety and/or mood disorders. Female partners were also trained to collect basal saliva samples for the measurement of salivary stress markers, alpha-amylase and cortisol.
Intervention(s): None.
Main Outcome Measure(s): Birth outcome data including infant sex were collected upon delivery. Modified Poisson regression models were used to estimate the relative risks (RRs) of a male birth for each stress marker.
Result(s): After adjusting for potential confounders, we observed a 76% increase in the risk of fathering a male infant (RR 1.76; 95% confidence interval 1.17–2.65) in men diagnosed with anxiety disorders compared with those who were not diagnosed. When lifetime history of physician-diagnosed anxiety disorders was modeled jointly for the couple, the association was slightly strengthened (RR 2.03; 95% confidence interval 1.46–2.84).
Conclusion(s): This prospective cohort study suggests that paternal lifetime history of physician-diagnosed anxiety disorders may be associated with an increase in the secondary sex ratio, resulting in an excess of male births. (Fertil Steril® 2017;107:714–22. ©2016 by American Society for Reproductive Medicine.)
Key Words: Alpha-amylases, anxiety disorders, hydrocortisone, sex ratio, stress

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Human sex ratios from birth through the lifespan have shown their modulation at the population level depending upon a variety of factors (1). The primary sex ratio (PSR), or the ratio of males to females at conception, is relatively unknown owing to the difficulty in measuring conceptions (2). Although it is conventionally recognized that the PSR is male-biased (1), a recent comprehensive study reported that the PSR is unbiased (3). On the other hand the secondary sex ratio (SSR), or the ratio of males to females at birth, is expected to range from 1.05 to 1.07 in the United States and worldwide, indicative of a slight excess of male births (4, 5). The SSR has been suggested as a possible indicator of population health and fertility despite the

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controversy over its utility, given that it is easy to measure, frequently recorded, and hardly subject to recall bias (6–9). Recent declines in the SSR observed in several developed countries have raised concerns about biological, psychological, and social factors disturbing sex selection and sex-selective survival in humans (7, 9).

Stress, which comprises multiple domains (e.g., biologic and psychological stress) that induce physiologic and behavioral responses, has been extensively evaluated in relation to human reproduction and development (10, 11). For instance, given the complex neuro-hormonal response elicited by stress, maternal stress is hypothesized to be responsible for various adverse reproductive outcomes, including preterm birth, low birth weight, and small for gestational age (12). It has also been suggested that the SSR, as a fertility endpoint, may be associated with maternal stress from various sources, such as economic contraction (13), stressful life events (14, 15), weather extremes (16), natural disasters (17–19), human-made disasters including wars (20), political upheaval (21), and terrorist attacks (22, 23), and psychiatric disorders including anxiety and depression (24, 25).

The stress response in humans is mainly regulated by the sympathetic–adrenal–medullary (SAM) system and the hypothalamic–pituitary–adrenal (HPA) axis (26). The SAM system is the primary mechanism in control of the fight-or-flight response and the secretion of catecholamines, such as norepinephrine and epinephrine, by the adrenal medulla. Meanwhile, the HPA axis is responsible for the release of glucocorticoids, such as cortisol, which is modulated by the secretion of corticotropin-releasing hormone and adrenocorticotropic hormone in hypothalamus and pituitary gland, respectively. These pathways are believed to interact with the reproductive system, wherein the hypothalamic–pituitary–gonadal axis plays an important role in the regulation of normal reproductive function (27). The physiologic role of these neuroendocrine systems in stress response is evident by several readily measurable biomarkers, such as salivary α-amylase and cortisol (28, 29), which have been investigated in the search for possible predictors of adverse reproductive endpoints in population-based studies (30, 31). To our knowledge, only one study evaluated maternal salivary stress markers, such as α-amylase and cortisol, in relation to the SSR. In a population-based preconception cohort study of 130 singleton births, the adjusted odds ratio (OR) for a male birth was decreased for women in the highest quartile of preconception salivary cortisol levels in comparison with women in the lowest quartile (adjusted OR 0.26; 95% confidence interval [CI] 0.09–0.74) (32).

Of note, less attention has been paid to the impact of paternal stress in comparison with that of maternal stress on human reproduction and development, despite the couple-dependent nature of human conception. Although prior research on paternal stress and the SSR is lacking, several hypotheses have been proposed to explain the role of both maternal and paternal stress in offspring sex determination (8, 33–39). As theorized previously, maternal and paternal stress may affect the SSR in diverse ways, eliciting opposite effects on human sex selection. Namely, maternal stress is related to increased T secretion from the adrenal glands, whereas paternal stress is related to decreased T production by the testes (40, 41). According to one of the prevailing hypotheses on the SSR focusing on parental hormone levels around the time of conception (8, 37–39), stressed women tend to produce sons, whereas stressed men tend to produce daughters. Furthermore, maternal stress results in high circulating glucose levels, which may be related to the development of male blastocysts relative to female blastocysts, possibly owing to sex differences in the rate of glucose uptake (33, 34). However, as pregnancy continues, persistent maternal stress, especially during early pregnancy, may reduce or reverse an excess of male births, because it may be related to selective male losses relative to female losses (35, 36).

On the basis of the existing hypotheses on the SSR, the present study aimed to evaluate the impact of both maternal and paternal stress on the SSR in a population-based preconception cohort. Specifically, multiple domains of stress, which comprised both biologic (i.e., salivary stress markers) and psychological (i.e., perceived stress) stress markers, were investigated in the present study in light of possible divergent human reactivity to various stressors.

MATERIALS AND METHODS

Study Population

The Longitudinal Investigation of Fertility and the Environment (LIFE) Study is a prospective cohort study in which 501 couples discontinuing contraception and attempting pregnancy were recruited from 16 counties in Michigan and Texas between 2005 and 2009, as described previously in detail (42). Couples were followed until pregnant or up to 12 months of trying to conceive and through delivery for those becoming pregnant. The eligibility criteria for participation included the following: [1] couples in a committed relationship; [2] women aged 18–40 years and men aged $\geq$18 years; [3] female partner's self-reported menstrual cycle length of 21–42 days; [4] no use of injectable contraceptives during the past year; [5] no sterilization procedures or physician-diagnosed infertility; and [6] couples able to communicate in English or Spanish. Of the 501 couples, 237 couples (47.3%) had a live birth during the follow-up period, two of whom had twins. Our study cohort comprised 235 couples with a singleton birth.

Data Collection

Baseline and follow-up data collection. Research assistants visited the couple's home and interviewed each partner of the couple separately using standardized baseline questionnaires, allowing for ascertaining baseline characteristics of the couple, such as socio-demographic (i.e., age, sex, race/ethnicity, annual income, education level, and research site) and lifestyle factors (i.e., perceived stress) and medical (i.e., self-reported physician-diagnosed anxiety and/or mood disorders) and reproductive histories (i.e., maternal parity and number of pregnancies fathered). Upon the baseline visit, the female partner underwent a urine pregnancy test to ensure the absence of a pre-existing pregnancy. Blood was collected

and used to quantify serum cotinine (ng/mL), a metabolite of nicotine, using liquid chromatography–isotope dilution tandem mass spectrometry (43). Couples who had a live birth during the follow-up period were asked to return standardized birth announcements to ascertain information on date of birth, infant sex, birth size, and delivery mode. This study was performed in adherence with the guidelines of the Declaration of Helsinki and approved by the institutional review boards at all collaborating institutions. All study participants provided written, informed consent before any data collection.

Assessment of stress markers. Using the baseline questionnaires, perceived stress of each partner of the couple was assessed by the four-item version of Cohen's Perceived Stress Scale (PSS-4) (44). The PSS-4 score was calculated by reversing the scores on two positive items and then summing across all four items, and ranged from 0 to 16, with higher scores indicating higher levels of perceived stress. Lifetime history of physician-diagnosed anxiety and mood disorders was determined by the answer to the following question: Have you ever been told by a doctor that you have any of the following health conditions? Physician-diagnosed anxiety disorders included agoraphobia, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder, social anxiety disorder, generalized anxiety disorder, and other anxiety disorders. Physician-diagnosed mood disorders included major depression, bipolar disorder, and other mood disorders.

In addition, the female partner was asked to provide a basal saliva sample using the Salivette collection device (Sarstedt) at two time points: [1] the morning after enrollment; and [2] the morning after the first observed menses after enrollment. The female partner was instructed to collect a basal or first-morning saliva sample immediately upon awakening before starting any daily activities, such as eating, drinking, smoking, or tooth brushing. The samples were shipped overnight to a laboratory (Salimetrics, State College, PA) and stored at −20°C until analysis. Salivary stress markers were quantified using established laboratory protocols inclusive of ongoing quality assurance and quality control procedures: salivary α-amylase (U/mL) was measured using a commercially available kinetic reaction assay (45); and salivary cortisol (μg/dL) was measured using a highly sensitive enzyme immunoassay (46).

Statistical Analysis

In the descriptive phase of analysis, various statistical methods were used to examine the completeness of data and the distributions of variables. The distributions were summarized as mean ± SD or geometric mean and accompanying 95% CI for continuous variables and frequency and percentage for categorical variables. Given the lack of statistically significant differences in salivary α-amylase and cortisol levels between the first and second samples, the average values of these salivary stress markers were used for analysis. Differences in baseline characteristics and stress markers by partner or infant sex were assessed using the nonparametric Wilcoxon test for continuous variables and Fisher's exact test for categorical variables.

In the analytic phase, modified Poisson regression models with a robust error variance (47) were used to estimate the relative risk (RR) and 95% CI of a male birth for each stress marker. Separate models were first run for each stress marker. Subsequently, select maternal and paternal stress markers (i.e., PSS-4, lifetime history of physician-diagnosed anxiety disorders, and lifetime history of physician-diagnosed mood disorders) were modeled jointly for the couple to examine each partner's stress while controlling for the other partner's stress. Maternal salivary α-amylase and cortisol were modeled both as a continuous variable (log-transformed values) and a categorical variable (tertiles). Along with the unadjusted model, we evaluated two different adjusted models while controlling for a priori confounders: [1] age (years, continuous) and serum cotinine (ng/mL, continuous); and [2] further adjusting for annual income (<$70,000/≥$70,000) and maternal parity (nulliparous/parous; for maternal stress markers only), on the basis of our review of the literature (5, 48–58). Consistent with the exploratory design of the present study, significance was set at P value <.05 without adjusting for multiple comparisons. All statistical analyses were performed by SAS version 9.3 (SAS Institute).

RESULTS

As reflected in Table 1, non-Hispanic white and college-educated couples constituted the majority of the study cohort. The mean (±SD) age of the study cohort was 29.8 ± 3.7 years for female partners and 31.5 ± 4.6 years for male partners. Approximately half of the female partners (46.8%) were nulliparous, and 42.5% of the male partners had not previously fathered a pregnancy. Among the 235 live births, the SSR was 0.97 (95% CI 0.75–1.26). A slightly higher age among men who fathered boys (mean ± SD, 32.2 ± 5.1 years) than among those who fathered girls (mean ± SD, 30.8 ± 3.9 years) was noted (P=.03). However, no significant differences were observed for the distributions of parity, race/ethnicity, annual income, education level, and research site by infant sex (Table 1).

The distributions of maternal and paternal stress markers by infant sex are presented in Table 2. The mean PSS-4 score was significantly higher among female partners (mean ± SD, 3.4 ± 2.3) than among male partners (mean ± SD, 3.0 ± 2.3) (P=.03). More female partners (7.2%) had lifetime history of physician-diagnosed mood disorders than did male partners (2.1%) (P=.01). However, none of the stress markers examined was significantly different by infant sex (Table 2).

Table 3 presents the RRs of a male birth by maternal and paternal stress markers when modeled separately. Neither paternal PSS-4 nor maternal PSS-4 was significantly associated with the SSR. No significant associations were observed for maternal salivary α-amylase and cortisol, when analyzed continuously and categorically. However, after adjustment for age, serum cotinine, and annual income, we observed a 76% increase in the risk of fathering a male infant (RR 1.76; 95% CI 1.17–2.65) in men diagnosed with anxiety disorders (2 with panic disorder; 2 with generalized anxiety disorder; 1 with agoraphobia; and 3 with other anxiety disorders)

TABLE 1

Baseline characteristics of the study participants by infant sex, 2005–2009.

Characteristic	Male (n = 116)	Female (n = 119)
Maternal		
Age (y), mean ± SD	30.0 ± 4.0	29.5 ± 3.4
Serum cotinine (ng/mL), GM (95% CI)	0.03 (0.02–0.04)	0.04 (0.02–0.08)
Parity		
Nulliparous	58 (50.4)	51 (43.2)
Parous	57 (49.6)	67 (56.8)
Annual income ($)		
<70,000	27 (23.9)	32 (27.6)
≥70,000	86 (76.1)	84 (72.4)
Education		
≤High school graduate/GED	5 (4.4)	4 (3.4)
Some college/technical school	13 (11.4)	14 (11.8)
College graduate or higher	96 (84.2)	101 (84.9)
Race/ethnicity		
Non-Hispanic white	92 (80.7)	102 (85.7)
Non-Hispanic black	2 (1.8)	1 (0.8)
Hispanic	13 (11.4)	7 (5.9)
Other	7 (6.1)	9 (7.6)
Paternal		
Age (y), mean ± SD	32.2 ± 5.1[a]	30.8 ± 3.9[a]
Serum cotinine (ng/mL), GM (95% CI)	0.08 (0.04–0.15)	0.12 (0.06–0.23)
No. of pregnancies fathered		
0	46 (43.0)	48 (42.1)
≥1	61 (57.0)	66 (57.9)
Annual income ($)		
<70,000	25 (21.9)	36 (30.5)
≥70,000	89 (78.1)	82 (69.5)
Education		
≤High school graduate/GED	3 (2.6)	4 (3.4)
Some college/technical school	34 (29.6)	23 (19.5)
College graduate or higher	78 (67.8)	91 (77.1)
Race/ethnicity		
Non-Hispanic white	91 (79.1)	105 (88.2)
Non-Hispanic black	3 (2.6)	2 (1.7)
Hispanic	13 (11.3)	8 (6.7)
Other	8 (7.0)	4 (3.4)
Couple characteristic		
Research site		
Michigan	21 (18.1)	26 (21.9)
Texas	95 (81.9)	93 (78.2)

Note: Values are number (percentage) unless otherwise noted. GED = general equivalency diploma; GM = geometric mean; SD = standard deviation.
[a] $P<.05$.

Bae. Stress and the secondary sex ratio. Fertil Steril 2016.

compared with those who were not diagnosed (Table 3). When lifetime history of physician-diagnosed anxiety disorders was modeled jointly for the couple, the association was slightly strengthened (RR 2.03; 95% CI 1.46–2.84) (Table 4).

DISCUSSION

In our analysis of multiple domains of preconception stress markers and the SSR in a population-based prospective cohort, we identified evidence suggesting that paternal lifetime history of physician-diagnosed anxiety disorders may influence sex allocation in offspring, resulting in an excess of male births. However, the significant association between paternal lifetime history of physician-diagnosed anxiety disorders and the SSR was observed depending upon model specification or statistical methods used, possibly reflecting an uncertain association between this stress marker and the SSR. Given that the observed significant association was sensitive to the adjustment for annual income, we examined the effects of other sociodemographic factors that may be related to this important covariate, such as health insurance and employment status. We found that health insurance (for female partners, $P<.0001$; for male partners, $P=.03$) and employment status (for female partners, $P=.0009$; for male partners, $P=.03$) were significantly associated with annual income. We also undertook sensitivity analyses in which we further adjusted for these variables in the multivariate-adjusted model. We observed that results on stress markers including paternal lifetime history of physician-diagnosed anxiety disorders and the SSR were similar (data not shown), although we cannot rule out the possibility of residual confounding. Besides, our findings on paternal lifetime history of physician-diagnosed anxiety disorders conflict with the influential hormonal hypothesis proposed by James (8, 37–39), which theorizes that decreased T production by the testes caused by paternal stress around the time of

TABLE 2

Distributions of maternal and paternal stress markers by infant sex, 2005–2009.

Stress marker	Male (n = 116)	Female (n = 119)
Maternal stress marker		
PSS-4, mean ± SD	3.4 ± 2.3	3.4 ± 2.4
Salivary α-amylase (U/mL), GM (95% CI)	15.1 (12.3–18.6)	14.8 (12.3–17.8)
Salivary cortisol (μg/dL), GM (95% CI)	0.40 (0.36–0.45)	0.36 (0.33–0.40)
Lifetime history of physician-diagnosed anxiety disorders		
No	111 (95.7)	107 (89.9)
Yes	5 (4.3)	12 (10.1)
Lifetime history of physician-diagnosed mood disorders		
No	107 (92.2)	111 (93.3)
Yes	9 (7.8)	8 (6.7)
Paternal stress marker		
PSS-4, mean ± SD	2.9 ± 2.4	3.0 ± 2.3
Lifetime history of physician-diagnosed anxiety disorders		
No	111 (95.7)	116 (97.5)
Yes	5 (4.3)	3 (2.5)
Lifetime history of physician-diagnosed mood disorders		
No	114 (98.3)	115 (97.5)
Yes	2 (1.7)	3 (2.5)

Note: Values are number (percentage) unless otherwise noted. None of the stress markers differed significantly by infant sex (all $P>.05$). Physician-diagnosed anxiety disorders included agoraphobia, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder, social anxiety disorder, generalized anxiety disorder, and other anxiety disorders. Physician-diagnosed mood disorders included major depression, bipolar disorder, and other mood disorders.

Bae. Stress and the secondary sex ratio. Fertil Steril 2016.

conception is associated with an excess of female births. In light of the lack of prior research findings, the association between paternal lifetime history of physician-diagnosed anxiety disorders and the SSR observed in the present study needs to be corroborated through further investigation. Of note, although not significant, paternal lifetime history of

TABLE 3

Stress markers and the RRs of a male birth by partner, 2005–2009 (n = 235).

Stress marker	Unadjusted	Adjusted[a]	Adjusted[b]
Maternal stress marker			
PSS-4	1.00 (0.95–1.06)	1.01 (0.95–1.06)	1.00 (0.95–1.06)
Salivary α-amylase (U/mL)			
Log-transformed	1.01 (0.88–1.17)	1.01 (0.87–1.16)	1.00 (0.86–1.17)
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	1.06 (0.74–1.52)	1.06 (0.74–1.51)	1.07 (0.74–1.55)
3rd tertile	1.13 (0.80–1.59)	1.11 (0.79–1.57)	1.10 (0.77–1.58)
Salivary cortisol (μg/dL)			
Log-transformed	1.09 (0.98–1.21)	1.08 (0.97–1.21)	1.08 (0.97–1.21)
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	1.22 (0.86–1.74)	1.21 (0.85–1.72)	1.19 (0.83–1.71)
3rd tertile	1.24 (0.87–1.77)	1.22 (0.86–1.75)	1.22 (0.85–1.75)
Lifetime history of physician-diagnosed anxiety disorders			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	0.58 (0.27–1.22)	0.65 (0.31–1.38)	0.67 (0.32–1.39)
Lifetime history of physician-diagnosed mood disorders			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	1.08 (0.68–1.72)	1.11 (0.69–1.77)	1.04 (0.62–1.75)
Paternal stress marker			
PSS-4	1.00 (0.94–1.05)	1.00 (0.94–1.06)	0.98 (0.92–1.05)
Lifetime history of physician-diagnosed anxiety disorders			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	1.28 (0.74–2.22)	1.32 (0.84–2.07)	1.76 (1.17–2.65)[c]
Lifetime history of physician-diagnosed mood disorders			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	0.80 (0.27–2.37)	0.72 (0.27–1.94)	0.52 (0.10–2.86)

Note: Values are RR (95% CI). Modified Poisson regression models were used to estimate the RRs of a male birth (47). All point and interval estimates were rounded to two decimal places. Physician-diagnosed anxiety disorders included agoraphobia, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder, social anxiety disorder, generalized anxiety disorder, and other anxiety disorders. Physician-diagnosed mood disorders included major depression, bipolar disorder, and other mood disorders.

[a] Adjusted for age (continuous) and serum cotinine (continuous).

[b] Adjusted for age (continuous), serum cotinine (continuous), annual income (<$70,000/≥$70,000), and maternal parity (nulliparous/parous; for maternal stress markers only).

[c] $P<.05$.

Bae. Stress and the secondary sex ratio. Fertil Steril 2016.

TABLE 4

Couples' stress markers and the RRs of a male birth, 2005–2009 (n = 235).

Stress marker	Unadjusted	Adjusted[a]	Adjusted[b]
PSS-4 (maternal)	1.00 (0.95–1.06)	1.00 (0.94–1.06)	0.97 (0.91–1.03)
PSS-4 (paternal)	1.00 (0.94–1.05)	1.00 (0.95–1.06)	0.99 (0.93–1.05)
Lifetime history of physician-diagnosed anxiety disorders (maternal)			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	0.57 (0.27–1.22)	0.65 (0.30–1.43)	0.65 (0.30–1.39)
Lifetime history of physician-diagnosed anxiety disorders (paternal)			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	1.31 (0.78–2.19)	1.35 (0.87–2.10)	2.03 (1.46–2.84)[c]
Lifetime history of physician-diagnosed mood disorders (maternal)			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	1.10 (0.68–1.76)	1.17 (0.69–1.98)	1.04 (0.55–1.96)
Lifetime history of physician-diagnosed mood disorders (paternal)			
No	1.00 (referent)	1.00 (referent)	1.00 (referent)
Yes	0.78 (0.26–2.31)	0.67 (0.23–1.91)	0.51 (0.08–3.06)

Note: Values are RR (95% CI). Modified Poisson regression models were used to estimate the RRs of a male birth (47). All point and interval estimates were rounded to two decimal places. In combination with other covariates, both maternal and paternal stress markers were included simultaneously in the model. Physician-diagnosed anxiety disorders included agoraphobia, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder, social anxiety disorder, generalized anxiety disorder, and other anxiety disorders. Physician-diagnosed mood disorders included major depression, bipolar disorder, and other mood disorders.

[a] Adjusted for both partners' age (continuous) and serum cotinine (continuous).

[b] Adjusted for both partners' age (continuous), serum cotinine (continuous), and annual income (<$70,000/≥$70,000), and maternal parity (nulliparous/parous).

[c] $P<.05$.

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physician-diagnosed mood disorders was found to be associated with an excess of female births (adjusted RR 0.52; 95% CI 0.10–2.86; Table 3). Differences in the steroid metabolome have been reported between men with anxiety disorders and men with mood disorders, providing a possible explanation for the varying effects of these psychiatric disorders on the SSR (59).

In contrast to a previous population-based prospective cohort study assessing maternal preconception salivary α-amylase and cortisol in relation to the SSR (32), the present study did not show any significant associations between maternal preconception salivary stress markers and the SSR. Although not significant, we found that the adjusted RR of a male birth was increased for women in the third tertile of preconception salivary cortisol levels in comparison with women in the first tertile (adjusted RR 1.22; 95% CI 0.85–1.75; Table 3). This finding conflicts with an inverse association between maternal preconception salivary cortisol levels and the odds of a male birth (4th vs. 1st quartile: adjusted OR 0.26; 95% CI 0.09–0.74) observed in a cohort of 130 singleton births from the Oxford Conception Study (OCS) (32). According to the hormonal hypothesis, an increased T secretion from the adrenal glands caused by maternal stress around the time of conception is associated with an excess of male births (8, 37–39). Furthermore, it has been proposed that high circulating glucose levels caused by maternal stress favor the development of male blastocysts rather than female blastocysts, owing to sex differences in the rate of glucose uptake (33, 34). However, persistent maternal stress during early pregnancy, which is recognized as a predictor for spontaneous abortion that disproportionately affects male conceptuses, may compensate for the male-biased PSR, or even result in an excess of female births (35, 36). Given that salivary cortisol is a marker for chronic stress, which represents HPA axis activity, rather than a marker for acute stress (26), the inverse association between maternal preconception salivary cortisol levels and the odds of a male birth observed in the OCS cohort may not be considered unexpected. It is worth noting that the median salivary cortisol concentration (μg/dL) for the OCS cohort was 0.41 (interquartile range, 0.29–0.51), which was similar to that for the present study cohort (0.39; interquartile range, 0.28–0.51). However, the basal saliva samples of the two cohorts were collected at different time points (i.e., day 6 of the first observed cycle for the OCS cohort; the first day after enrollment and day 1 of the first observed cycle for the present cohort). Given the equivocal findings regarding longitudinal variations in basal cortisol secretion throughout the menstrual cycle (60–62), it is challenging to know how best to model stress in the absence of validation studies. We averaged the salivary stress markers in light of few differences between the first and second measurements. Future research may identify other options for considering multiple measurements. Also of note, maternal preconception salivary cortisol levels were not found to be significantly correlated with maternal preconception salivary α-amylase levels or other stress markers obtained from the baseline questionnaires among female partners (correlation coefficient range, −0.04 to 0.10), reflecting the importance of assessing markers for multiple domains of stress (e.g., biological and psychological stress markers) and multiple pathways of stress response (e.g., the SAM system and the HPA axis).

Some previous studies have reported that psychiatric disorders such as anxiety and depression are associated with the SSR (24, 25). In a time-series analysis of Swedish data for the 276 months beginning January 1974, dispensing of anxiolytics and antidepressants, as a marker for population stress, was observed to be associated with the SSR (24). Specifically,

an increase of one defined daily dose of anxiolytics or antidepressants per 1,000 Swedish women predicted [1] an increase of approximately seven male conceptions 1 month before gestation; and [2] a loss of approximately five male embryos in the first month of gestation at the time of the increase. Although not statistically significant, an increase of one defined daily dose of anxiolytics or antidepressants per 1,000 Swedish men predicted a decrease of approximately four male conceptions 1 month before gestation (24). These findings based on monthly data seem to be congruent with several existing hypotheses on the effects of parental stress on the SSR, as described above (8, 33–39). Because we have data on couples' prescription medication use, including psychotropic drugs, we undertook sensitivity analyses to evaluate any potential impact of prescription medication use on the SSR. However, no significant association between prescription medication use, whether it is any prescription drug use or psychotropic drug use, and the SSR was noted, possibly owing to a relatively small sample size. In addition, our findings on stress markers and the SSR did not change significantly after further adjustment for prescription medication use in the multivariate-adjusted model (data not shown). As such, we cannot rule out potential confounding by indication, nor can we assume that couples complied with prescription medications. A retrospective case–control study conducted in California found that infants born to African American women diagnosed with anxiety disorders exhibited a significantly lower SSR compared with those born to African American women with other psychiatric disorders (odds for a male birth 0.89; 95% CI 0.79–0.99; $P=.04$) or to African American women without mental health diagnoses (odds for a male birth 0.88; 95% CI 0.78–1.00; $P=.04$) (25). However, no significant associations between anxiety disorders and a decreased SSR were observed for non-Hispanic white, Hispanic, and Asian women (25). Likewise, the present study did not show any significant associations between maternal lifetime history of physician-diagnosed anxiety disorders and the SSR. The literature argues that high reactivity to stress manifests clinically as anxiety-related symptoms (63). Individuals with anxiety disorders may exert greater autonomic and neuroendocrine responses to stressors in comparison with unaffected counterparts (63, 64). As yet, little is known about the biologic mechanisms that underlie the effects of anxiety disorders on the SSR, but these mechanisms deserve more research attention.

Along with anxiety disorders, maternal stress from various sources has been reported to be associated with the SSR. In contrast to our findings on perceived stress as measured by PSS-4, previous studies have reported that stress life events (14) and psychological distress as measured by the General Health Questionnaire (15) were significantly associated with a female-biased SSR. In addition, various population stressors, such as economic contraction (13), natural disasters (17–19), and human-made disasters (20–23), have been reported to be associated with a decrease in the SSR. With the accumulated evidence on maternal stress and the reversal of the SSR, some of these studies have examined whether the decline in the odds of a male birth resulted from an excess of male fetal loss or reduced male conceptions, suggestive of the fetal death sex ratio as a sentinel indicator of population stress reactivity (22, 23). Furthermore, subsequent research has examined whether stressful times make male fetuses less fit or whether male fetuses need greater fitness to avoid spontaneous abortion during stressful times, supporting that the latter mechanism is responsible for reducing the SSR (36). Still, controversy remains over underlying biological mechanisms that link stressors to spontaneous abortion. For instance, preconception salivary cortisol levels were not found to be associated with an increased risk of hCG-confirmed pregnancy loss in the OCS cohort, despite the significant association between this salivary stress marker and the SSR observed in this cohort (32). This finding indicates that the decline in the SSR may have resulted from alterations in the PSR or sex-selective losses of preimplantation embryos rather than postimplantation losses (32). Other potential mechanisms by which preconception stress alters the SSR or the PSR include reduced coitus (8, 24), sperm abnormalities (17, 65), and perturbations in the female reproductive tract (66). Of note, when we evaluated the correlation between stress markers and frequency of sexual intercourse, paternal lifetime history of physician-diagnosed mood disorders (correlation coefficient −0.13; $P=.04$), but not anxiety disorders, was significantly correlated with frequency of sexual intercourse.

The present study is strengthened by its population-based prospective cohort design, both partners' preconception measurements of multiple domains of stress, and the use of a couple-based analytic approach for the assessment of a couple-dependent outcome. However, our study is limited by its relatively small sample size for the detection of variability in the SSR, which should be taken into account when interpreting our results. For instance, we observed a relatively low SSR of 0.97 in comparison with the SSR observed in the US general population, approximately ranging from 1.05 to 1.07 (5). This may be due to our small sample size, resulting in the estimate of the SSR with a wide 95% CI (0.75–1.26). In light of our sampling of couples planning pregnancies, our findings may not be generalizable to the general population or among couples with unplanned pregnancy. The external validity of our study may be compromised, if couples planning pregnancies have unique mood or stress profiles relative to unplanners. Still, the majority of births in the United States are reported planned (67). Some unique but unmeasured characteristics of our study cohort may be an explanation for a slightly higher age among men who fathered boys than among those who fathered girls noted in our study, which is in contrast to existing literature (51, 54). Additionally, selection bias is a consideration, if couples with higher or lower stress levels disproportionately participated in the study. Compared with the US general population, our study participants may be less likely to be stressed, coupled with the lower prevalence of psychiatric disorders such as anxiety and mood disorders (68). Given that we performed multiple statistical tests to assess the effects of multiple stress markers on the SSR, chance may be an explanation for our results. However, when we adjusted for multiple comparisons in our analysis, we found that our findings on

paternal history of physician-diagnosed anxiety disorders remained significant ($P<.05/8$ [the number of stress markers examined]). Because of our inability to measure the PSR for all conceptions and the fetal death sex ratio, our findings only speak to the SSR. In addition, the lack of data on hormone levels of each partner of the couple prevents us from proving underlying mechanisms with regard to hormone reactivity to stressors. Because of fiscal and logistical concerns, we were unable to obtain repeated saliva samples across the full cycles of pregnancy attempt, preventing us from addressing possible changes in salivary stress markers over time in our analysis. The incompleteness of self-reported measures is a consideration, if couples did not feel comfortable disclosing their medical history. Given the uncertainty as to factors affecting the SSR, we cannot eliminate residual confounding or model misspecification in the interpretation of our results.

In summary, this population-based prospective cohort study suggests that paternal lifetime history of physician-diagnosed anxiety disorders may be associated with an increase in the SSR, resulting in an excess of male births. However, given the relatively small sample size, our findings require cautious interpretation and await future corroboration. Furthermore, the pathways through which paternal stress influences the SSR remain to be established. Previous research on paternal stress suggests that fathers may transmit neurobiologic, metabolic, and behavioral phenotypes induced by stress to their offspring through inherited epigenetic variation (69). Meanwhile, the varying effects of maternal stress on the SSR underscore the need for a more comprehensive study that includes multiple stress markers across different reproductive stages, such as preconception and early pregnancy, and assesses changes in stress levels across the spectrum of gestation (34). These research efforts would provide a more complete investigation regarding the impact of parental stress on sex selection and sex-selective survival in humans.

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