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Preconception stress and the secondary sex ratio: a prospective cohort study

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Abstract

Objective—To study the association between salivary stress biomarkers and the secondary sex ratio.

Design—Prospective, longitudinal cohort study.

Setting—Community setting in the United Kingdom.

Patient(s)—On discontinuation of contraception for purposes of becoming pregnant, 338 women aged 18–40 years with complete data (90%) were followed until pregnant or up to six menstrual cycles.

Intervention(s)—None.

Main Outcome Measure(s)—Secondary sex ratio.

Result(s)—Human chorionic gonadotropin pregnancies were detected in 207 (61%) women of whom 130 (63%) delivered singleton infants with available gender data. The adjusted odds ratio for a male birth was decreased for women in the highest quartile (AOR = 0.26; 95% confidence interval = 0.09, 0.74) of salivary cortisol relative to women in the lowest quartile during cycle 1. An inverse relation was observed between α -amylase and the 2^o sex ratio, though not statistically significant.

Conclusion(s)—Our findings are consistent with a reversal in the 2^o sex ratio with increasing preconception salivary cortisol concentrations. This relation suggests that activation of the

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hypothalamus–pituitary–adrenal axis may have implications in sex allocation and requires further study.

Keywords

Fecundity; secondary sex ratio; stress; salivary cortisol; salivary α -amylase; preconception

The secondary sex ratio is defined as the ratio of male-to-female live births and is relatively constant at the population level, ranging from 102–109 male to 100 female births (1, 2). Alterations in the 2° sex ratio may be indicative of reproductive toxicity at the time of conception, resulting in alterations in the ratio of male-to-female conceptions (1° sex ratio), or developmental toxicity during embryonic and fetal development, resulting in the selective loss of male embryos or fetuses. As such, the 2° sex ratio has been suggested to be a sentinel marker of population health or ecologic environmental patterns (3). Furthermore, the 2° sex ratio has implications for population demographics and neonatal and infant health outcomes. Adaptive and evolutionary theories argue that parental qualities and environmental resources influence offspring sex selection to ensure optimal survival and familial benefits within the confines of the organism's surrounding conditions (4).

There are few established determinants underlying the 2° sex ratio beyond parental ages and parity (5, 6). Of note is an evolving body of evidence that supports a relation between natural disasters, war, and macroeconomic disintegration and a reduction in the 2° sex ratio (7–10). Populations affected by disasters are reported to experience considerable stress (11, 12), which suggests its possible disruptive effect on sex selection. Although underlying mechanisms remain unknown, the brain may transmit perceived psychosocial stressors to various tissues by peripheral hormones released from several key pathways in the stress system, including the hypothalamic–pituitary–adrenal axis and the sympathetic–adrenal–medullary (SAM) system. These pathways further communicate via many interactions with the reproductive system, where stress signaling clearly plays a vital role in human reproduction (13–15).

A recent retrospective case–control study demonstrated a significant decline in the 2° sex ratio among African American women diagnosed with anxiety disorders in comparison with unaffected women (16). The anxiety disorders could represent a proxy for high reactivity of the stress system that may initiate a pathophysiologic cascade resulting in sex-specific pregnancy losses (16). To our knowledge, no research has assessed biomarkers of preconception stress in relation to the 2° sex ratio, despite the ability to quantify salivary cortisol and α -amylase concentrations indicative of hypothalamic–pituitary–adrenal and sympathetic–adrenal–medullary pathway activation, respectively (17–19). Therefore, we sought to evaluate the association between psychosocial stress, as measured by these salivary stress biomarkers, and the 2° sex ratio.

MATERIALS AND METHOD

Our study cohort was identified as women within the stress component of the Oxford Conception Study (20, 21) who delivered live-born singleton infants. A prospective cohort design was used to enroll 370 women from the Oxford Conception Study who were

discontinuing contraception for purposes of becoming pregnant. The study cohort was further restricted to the 338 (91%) women who collected saliva samples and completed daily diaries, of whom 207 (61%) women became pregnant and 205 (99%) had pregnancy outcome data as follows: 15 (7%) ongoing pregnancies, 54 (26%) pregnancy losses, 2 (1%) stillbirths, and 134 (65%) live births of which 131 (98%) were singletons. Pregnancy loss was defined as any loss occurring after a positive urinary hCG test irrespective of gestational age. One singleton live birth was missing gender data; thus, 130 singleton births comprised our study cohort.

Inclusion criteria for the Oxford Conception Study included planning pregnancy or trying for <3 months, aged 18–40 years, and a menstrual cycle length between 21 and 39 days. Exclusion criteria were a history of infertility, current breastfeeding, recent hormonal contraception without intervening menses, or use of injectable contraception any time in the year before enrollment. The study was approved by the appropriate ethics committee and institutional review boards, and all women gave their consent prior to enrollment.

Participants recorded lifestyle behaviors, intercourse frequency, and vaginal bleeding in daily diaries until pregnant or up to six menstrual cycles. Women also collected basal salivary samples on day 6 of each cycle using the Salivette collection device (Sarstedt AG). The specimens were stored at -20°C until shipped to an experienced salivary stress laboratory (Salimetrics, LLC) where salivary concentrations of cortisol ($\mu\text{g/dL}$) and α -amylase (U/mL) could be quantified, as previously described (21). Pregnancies were captured with home pregnancy tests performed on the days of expected menstruation and were confirmed by study personnel; thus, pregnancy was defined by a qualitative increase in urinary hCG (100% sensitivity at hCG levels $\geq 25\text{ mIU/mL}$). Women with a live birth returned pregnancy outcome cards reporting the date of delivery and sex of the infant.

Statistical analyses were performed using SAS (SAS Institute Inc.). Descriptive data were assessed using the chi-square statistic for categorical data, t -test for continuous data, and the Cochran–Armitage trend test as appropriate. Salivary stress biomarker concentrations for the entire cohort were used to establish quartiles. The association between the quartiles of salivary stress biomarkers measured during the first observed cycle and the odds ratio for a male birth was evaluated using a logistic regression model. Salivary stress biomarker measurements from the first observed cycle were chosen for analysis as our objective was to assess the impact of baseline preconception stress on the sex ratio. Adjusted odds ratios (AORs) and corresponding 95% confidence intervals (CIs) were estimated after including potential confounders, namely, maternal and partner age and gravidity. Lastly, a Monte Carlo sensitivity analysis under the null hypothesis of no association between stress and sex ratio was performed to examine the sensitivity of our model to those pregnancies with unknown gender at the time of data collection ($n = 16$). The sensitivity analysis was performed with various assumptions regarding the sex ratio for the unknown genders, including the observed ratio (0.81), population ratio (1.02), and the extremes of all males and all females.

RESULTS

Baseline characteristics and lifestyle behaviors were similar among couples irrespective of having a male versus female infant, including mothers' mean ages (30.4 ± 4.2 vs. 29.2 ± 3.5 years, $P=.08$). Most study participants were self-reported nonsmokers, with no difference in the daily number of cigarettes smoked by infant sex, although the number of smoking mothers was extremely small (1.7 ± 4.9 vs. 0.8 ± 2.9 cigarettes, respectively). In addition, no differences were observed for the distribution of male to female births by season of conception or frequency of sexual intercourse during the menstrual cycle or the fertile window (Table 1). The 2° sex ratio for the study cohort was 0.81 (58:72 males–females, CI = 0.56, 1.15) and was not statistically different from the general population.

The median (IQR) salivary cortisol concentration for the entire cohort was 0.41 (0.29–0.55) $\mu\text{g/dL}$ and that of α -amylase was 5.30 (2.9–10.3) U/mL. Of note was the absence of significant changes in concentrations of salivary stress biomarker concentrations across time (up to six observed cycles) among women who did not conceive during the first cycle. When comparing women who delivered a male versus a female infant, the median cortisol concentration was higher and the median α -amylase concentration was lower in women who deliver a female infant (Table 2). The Cochran–Armitage trend test demonstrated a significant decreasing trend ($P=.022$) in sex ratio by cortisol quartile, and a significant increasing trend ($P=.026$) in sex ratio by α -amylase quartile (Table 2). Further evaluation of the distribution using a chi-square test of homogeneity confirmed the significant difference seen with α -amylase ($P=.029$) but not with cortisol concentration ($P=.069$).

The association between preconception basal salivary stress biomarkers, as measured during the first observed cycle, and the 2° sex ratio was evaluated using a logistic regression model. Odds of a male birth were decreased for women in the third (AOR = 0.32; CI = 0.11, 0.95) and highest (AOR = 0.26; CI = 0.09, 0.79) quartiles of salivary cortisol relative to the lowest quartile (Table 3). Conversely, odds of a male birth were increased for women in the third (AOR = 2.98; CI = 1.10, 8.09) quartile of salivary α -amylase relative to the lowest quartile. To further test the robustness of our findings, we performed a binomial regression, which also demonstrated that the third (adjusted RR = 0.61; CI = 0.38, 0.96) and highest (adjusted RR = 0.56; CI = 0.35, 0.91) quartiles of salivary cortisol concentration were significantly associated with a decreased risk for a male birth.

The AOR for the highest quartile of cortisol remained significant in our sensitivity analyses (AOR = 0.30; CI = 0.11, 0.82) across all varied sex ratio assumptions (data shown for assumption of our observed sex ratio). Thus, the sensitivity analysis affirmed the statistically significant association between only the highest quartile of cortisol and the odds of a male infant.

DISCUSSION

In this population-based prospective cohort, preconception salivary cortisol was associated with decreased odds of a male birth. Our findings are novel in that they reflect the first attempt to assess individual stress biomarkers in relation to the 2° sex ratio. Furthermore, we

were able to quantify stress biomarkers prior to conception, thereby, establishing a more defined temporal order with the secondary sex ratio. As we are unaware of previous studies evaluating this relation, we are unable to interpret our findings in the context of similar past research. However, our finding is congruent with the existing body of literature, which reports a reversal in the 2° sex ratio with perceived psychosocial stress as measured by exposure to population-level stressors (11, 12).

Several hypotheses have been suggested for alterations in the 2° sex ratio indicative of its likely heterogeneous etiology. Byrne and Warburton first suggested that altered maternal hormone milieu secondary to stress may result in pregnancy losses that disproportionately affect male conceptuses, resulting in a female excess of live births (22). Most recently, an increase in the male fetal death rate was reported for subpopulations affected by the September 11, 2001, terrorist attacks on the World Trade Towers (9). Given that we identified pregnancies by the earliest increases in urinary hCG while following women in the cohort, we were able to assess the association between salivary stress biomarkers and pregnancy loss, defined as any loss after having had a positive urinary hCG. Neither salivary cortisol (adjusted HR = 0.56, CI = 0.09, 3.35) nor α -amylase (adjusted HR = 0.99, CI = 0.95, 1.04) concentrations were associated with an increased risk of hCG-confirmed pregnancy loss. Therefore, the association between stress, as measured by salivary cortisol, and a decreased odds of a male birth cannot be explained by postimplantation losses in this cohort, and instead may result from either alterations in the 1° sex ratio or sex-specific losses of preimplantation embryos. Stress may alter the 1° sex ratio, which denotes the ratio of male-to-female conceptions, possibly through sperm abnormalities or perturbations in the female reproductive tract. A reduction in sperm motility shortly after the Kobe earthquake was followed by a decline in the 2° sex ratio 280 days later, suggesting a role of altered sperm function (23, 24). On the contrary, couples with male factor infertility demonstrated no difference in the 2° sex ratio compared with a fertile comparison group (25), which may suggest a role for female factors, such as changes in the preovulatory follicular fluid affecting oocyte development and ensuing susceptibility to toxicants (26). Little is known about the effect of preconception stress on oocyte health, sex-specific fertilization patterns, or sex-specific implantation failures, but these mechanisms certainly deserve study.

We did not observe a significant relation between α -amylase and the 2° sex ratio across all assumptions regarding the unknown genders. Unlike cortisol, the relation was positive in that increasing α -amylase concentrations were associated with a higher sex ratio, indicative of a male excess. Cortisol and α -amylase are biomarkers of two independent stress pathways, and are reported to be markers of chronic and acute stress, respectively (27, 28). Correlations between these markers in the same individual are reported to be minimal, indicative of their distinct mechanistic pathways (29, 30). Similar opposing effects of the two stress biomarkers for female fecundity, as measured by the probability of pregnancy during the fertile window, have previously been reported for this study cohort, underscoring the importance of assessing both biomarkers when examining potential stress-related effects on human reproduction and development (21). The mechanisms that underlie these observations require careful study. It is well known that the hypothalamic–pituitary–adrenal and sympathetic–adrenal–medullary pathways have different response times and, thereby,

peripheral biomarkers may not correlate when drawn at the same time (27). However, little is known about how these biomarkers vary with each other relative to sensitive reproduction outcomes, or how best to interpret their opposing directions with regards to the 2° sex ratio. One possible explanation is that the hypothalamic–pituitary–adrenal axis is more sensitive to chronic stressors, whereas the sympathetic–adrenal–medullary pathway is more sensitive to acute stressors (31). Unfortunately, we did not have information on the type, timing, or persistency of stressors at baseline or while attempting to become pregnant.

In contrast to previous findings, we did not observe any relations between sociodemographic or reproductive history characteristics and the 2° sex ratio. Previous authors have reported a dose-dependent reduction in the 2° sex ratio for parental smoking (32), parental ages, and parity (33–35) although the effect size was relatively small. Possible additional explanations for the differences may reflect our relatively young (mean age 29.8 years) and homogeneous cohort with a low prevalence of smoking (12%). These characteristics differ somewhat from those reported for married women giving birth in the United Kingdom for whom the mean age at birth was 31.5 years and parity was 1.05 in 2008 (36). These observed differences may reflect selective factors that motivated women to participate in the Oxford Conception Study.

The present study has several strengths and the findings contribute to the current body of largely retrospective population studies that examine the 2° sex ratio. This study is the first to evaluate individual-level stress biomarkers in relation to the sex ratio. Cortisol and α -amylase have now been used extensively in stress research, correlate well with perceived psychosocial stress, and importantly, provide deeper insight into individual-level stress signaling and reactivity. Furthermore, the prospective, longitudinal design of women attempting pregnancy enables temporal ordering between events.

The present study is limited by its relatively small sample size and the results should be interpreted with caution, given its exploratory nature. The cortisol findings may be driven by the high proportion of males in the lowest quartile of cortisol concentration, and thus future research should focus on a larger-scale evaluation of the impact of stress biomarkers on the reproductive outcomes. We were able to further analyze the strength of our findings by testing their sensitivity to the additional ongoing pregnancies assuming these genders had no relation to maternal stress biomarkers. Furthermore, we performed the sensitivity analysis across four scenarios for the unknown genders, including the observed sex ratio, population sex ratio, all males and all females. When considered with the observed study cohort, the various assumptions resulted in overall theoretical sex ratios of 0.81, 0.84, 1.01, and 0.67, respectively. Findings were corroborated for the relation between the stress biomarkers and odds of a male birth save for assuming all were male. Under this scenario, the significant findings for the third quartile of cortisol (AOR = 0.267, CI = 0.095, 0.751) and the third quartile of α -amylase (AOR = 3.01, CI = 1.17, 7.72) were upheld. However, this assumption is not plausible because we know of no reason to support that all unknown genders in the database were systematically male.

The study findings may also be affected by potential selection bias given that participating couples were planning a pregnancy and responded to recruitment messages broadcast via

various media sources in the United Kingdom. In addition, the study is limited by the absence of information on the male partner, including semen quality, and other measures of female hormonal milieu. We were unable to assess the 1° sex ratio or the karyotype of pregnancy losses, which would be informative for determining if the female excess of births reflects fewer male conceptions or selective loss of males during pregnancy.

In sum, our results suggest that chronic stress as measured by salivary cortisol prior to conception may be associated with a reduction in the 2° sex ratio resulting in a greater proportion of female to male births. Our findings are consistent with earlier research efforts that largely relied on proxy measures of stress. However, given the small sample size, our findings require cautious interpretation and await corroboration. The lack of consistent finding between the two salivary biomarkers suggests that the effect(s) may be mediated via the hypothalamic–pituitary–adrenal rather than the sympathetic–adrenal–medullary pathway and underscores the importance of assessing both stress pathways in assessing reproductive and developmental outcomes.

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References

1. United Nations DoEaSA, Population Division. World Population Prospects: The 2010 Revision. Vol. 2011. New York: 2011. (comprehensive Excel tables)
2. Mathews TJ, Hamilton BE. Trend analysis of the sex ratio at birth in the United States. *Natl Vital Stat Rep.* 2005; 53:1–17.
3. Davis DL, Webster P, Stainthorpe H, Chilton J, Jones L, Doi R. Declines in sex ratio at birth and fetal deaths in Japan, and in U.S. whites but not African Americans. *Environ Health Perspect.* 2007; 115:941–6. [PubMed: 17589604]
4. Cockburn, A.; Legge, S.; Double, M. Sex ratios, concepts and research methods. Cambridge: Cambridge University Press; 2001. Sex ratios in birds and mammals: can the hypotheses be disentangled?; p. 266–86.
5. James WH. The human sex ratio. Part 1: a review of the literature. *Hum Biol.* 1987; 59:721–52. [PubMed: 3319883]
6. Jacobsen R, Moller H, Mouritsen A. Natural variation in the human sex ratio. *Hum Reprod.* 1999; 14:3120–5. [PubMed: 10601107]
7. Catalano RA. Sex ratios in the two Germanies: a test of the economic stress hypothesis. *Hum Reprod.* 2003; 18:1972–5. [PubMed: 12923159]
8. Catalano R, Bruckner T, Gould J, Eskenazi B, Anderson E. Sex ratios in California following the terrorist attacks of September 11, 2001. *Hum Reprod.* 2005; 20:1221–7. [PubMed: 15734763]
9. Bruckner TA, Catalano R, Ahern J. Male fetal loss in the U.S. following the terrorist attacks of September 11, 2001. *BMC Public Health.* 2010; 10:273. [PubMed: 20500812]
10. Saadat M. Decline in sex ratio at birth after Bam (Kerman Province, Southern Iran) earthquake. *J Biosoc Sci.* 2008; 40:935–7. [PubMed: 18833587]
11. Bourque LB, Siegel JM, Shoaf KI. Psychological distress following urban earthquakes in California. *Prehosp Disaster Med.* 2002; 17:81–90. [PubMed: 12500731]

12. Schuster MA, Stein BD, Jaycox L, Collins RL, Marshall GN, Elliott MN, et al. A national survey of stress reactions after the September 11, 2001, terrorist attacks. *N Engl J Med*. 2001; 345:1507–12. [PubMed: 11794216]
13. Nepomnaschy PA, Welch K, McConnell D, Strassmann BI, England BG. Stress and female reproductive function: a study of daily variations in cortisol, gonadotrophins, and gonadal steroids in a rural Mayan population. *Am J Hum Biol*. 2004; 16:523–32. [PubMed: 15368600]
14. Mastorakos G, Pavlatou MG, Mizamtsidi M. The hypothalamic-pituitary-adrenal and the hypothalamic-pituitary-gonadal axes interplay. *Pediatr Endocrinol Rev*. 2006; 3(Suppl 1):172–81. [PubMed: 16641855]
15. Berga SL, Daniels TL, Giles DE. Women with functional hypothalamic amenorrhea but not other forms of anovulation display amplified cortisol concentrations. *Fertil Steril*. 1997; 67:1024–30. [PubMed: 9176439]
16. Subbaraman MS, Goldman-Mellor SJ, Anderson ES, Lewinn KZ, Saxton KB, Shumway M, et al. An exploration of secondary sex ratios among women diagnosed with anxiety disorders. *Hum Reprod*. 2010; 25:2084–91. [PubMed: 20570972]
17. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*. 2006; 31:137–41. [PubMed: 16046076]
18. Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol*. 2005; 55:333–42. [PubMed: 15708646]
19. Kahn JP, Rubinow DR, Davis CL, Kling M, Post RM. Salivary cortisol: a practical method for evaluation of adrenal function. *Biol Psychiatry*. 1988; 23:335–49. [PubMed: 3257706]
20. Pyper C, Bromhall L, Dummett S, Altman DG, Brownbill P, Murphy M. The Oxford Conception Study design and recruitment experience. *Paediatr Perinat Epidemiol*. 2006; 20(Suppl 1):51–9. [PubMed: 17061974]
21. Louis GM, Lum KJ, Sundaram R, Chen Z, Kim S, Lynch CD, et al. Stress reduces conception probabilities across the fertile window: evidence in support of relaxation. *Fertil Steril*. 2011; 95:2184–9. [PubMed: 20688324]
22. Byrne J, Warburton D. Male excess among anatomically normal fetuses in spontaneous abortions. *Am J Med Genet*. 1987; 26:605–11. [PubMed: 3565477]
23. Fukuda M, Fukuda K, Shimizu T, Moller H. Decline in sex ratio at birth after Kobe earthquake. *Hum Reprod*. 1998; 13:2321–2. [PubMed: 9756319]
24. Fukuda M, Fukuda K, Shimizu T, Yomura W, Shimizu S. Kobe earthquake and reduced sperm motility. *Hum Reprod*. 1996; 11:1244–6. [PubMed: 8671433]
25. Eisenberg ML, Schembri M, Croughan MS, Walsh TJ. Fecundity and sex ratio of offspring in an infertile cohort. *Fertil Steril*. 2011; 96:833–6. [PubMed: 21864839]
26. Grant VJ, Irwin RJ. A simple model for adaptive variation in the sex ratios of mammalian offspring. *J Theor Biol*. 2009; 258:38–42. [PubMed: 19490877]
27. Engert V, Vogel S, Efanov SI, Duchesne A, Corbo V, Ali N, et al. Investigation into the cross-correlation of salivary cortisol and alpha-amylase responses to psychological stress. *Psychoneuroendocrinology*. 2011; 36:1294–302. [PubMed: 21470780]
28. Out D, Bakermans-Kranenburg MJ, Granger DA, Cobbaert CM, van Ijzendoorn MH. State and trait variance in salivary alpha-amylase: a behavioral genetic study. *Biol Psychol*. 2011; 88:147–54. [PubMed: 21827821]
29. Granger DA, Kivlighan KT, el-Sheikh M, Gordis EB, Stroud LR. Salivary alpha-amylase in biobehavioral research: recent developments and applications. *Ann N Y Acad Sci*. 2007; 1098:122–44. [PubMed: 17332070]
30. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*. 2009; 34:486–96. [PubMed: 19249160]
31. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *J Am Med Assoc*. 1992; 267:1244–52.

32. Fukuda M, Fukuda K, Shimizu T, Andersen CY, Byskov AG. Parental periconceptional smoking and male:female ratio of newborn infants. *Lancet*. 2002; 359:1407–8. [PubMed: 11978342]
33. Feitosa MF, Krieger H. Some factors affecting the secondary sex ratio in a Latin American sample. *Hum Biol*. 1993; 65:273–8. [PubMed: 8449486]
34. Ulizzi L, Zonta LA. Factors affecting the sex ratio in humans: multivariate analysis of the Italian population. *Hum Biol*. 1995; 67:59–67. [PubMed: 7721279]
35. Ruder A. Paternal-age and birth-order effect on the human secondary sex ratio. *Am J Hum Genet*. 1985; 37:362–72. [PubMed: 3985011]
36. UK Office for National Statistics. Birth statistics, England and Wales (Series FM1). 2008.

TABLE 1

Sociodemographic and lifestyle characteristics by infant gender.

	Male infant (n = 58)	Female infant (n = 72)	P-value ^a
Female age (y)			
<30	26 (45)	37 (51)	.46
30	33 (55)	35 (49)	
Mean (±SD)	30.4 (4.2)	29.2 (3.5)	.08
Male age (y)			
<30	15 (26)	27 (38)	.16
30	43 (74)	45 (62)	
Mean (±SD)	32.9 (5.1)	31.4 (4.2)	.07
Gravidity			
Nulligravid	13 (22)	24 (34)	.20
1	19 (31)	24 (33)	
2	14 (24)	8 (11)	
3+	13 (23)	16 (22)	
Mean (±SD)	1.7 (1.5)	1.3 (1.3)	.15
Parity			
Nulliparous	27 (47)	44 (61)	.24
1	22 (38)	21 (29)	
2+	9 (15)	7 (10)	
Mean (±SD)	0.8 (1.0)	0.5 (0.8)	.11
Number of cigarettes ^b			
0	49 (84)	65 (90)	.37
1–9	5 (9)	3 (4)	
10–19	4 (7)	4 (6)	
Mean (±SD)	1.7 (5.0)	0.8 (2.9)	.22
Acts of intercourse ^b			
1–9	32 (55)	48 (67)	.42
10–19	21 (36)	20 (28)	
20+	5 (9)	4 (6)	
Mean (±SD)	10.2 (6.0)	9.4 (5.1)	.43
Acts of intercourse in fertile window ^c			
Mean (±SD)	3.4 (2.2)	3.4 (1.9)	.99
Season of conception			
Winter	8 (14)	12 (17)	.55
Spring	6 (11)	13 (18)	
Summer	19 (33)	22 (30)	
Fall	25 (42)	25 (35)	

Note: Values are n (%), unless otherwise noted.

^a Cochran–Armitage trend test for distribution among categories or Student's *t*-test for comparison of means.

^b Standardized to a 28-day menstrual cycle.

^c Within the conception cycle.

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TABLE 2

Distribution of stress biomarkers by infant gender.

	Male Infant	Female Infant	P-value
Cortisol ($\mu\text{g/dL}$)			
Q1 [0.02, 0.29]	17 (29)	8 (11)	.022 ^a
Q2 [0.30, 0.41]	12 (21)	16 (22)	
Q3 [0.42, 0.55]	15 (26)	24 (33)	
Q4 [0.56, 1.36]	14 (24)	24 (33)	
Median (IQR)	0.41 (0.27–0.54)	0.48 (0.37–0.59)	.044 ^b
α -Amylase (U/mL)			
Q1 [0.4, 2.9]	12 (21)	24 (33)	.026 ^a
Q2 [3.0, 5.3]	8 (14)	19 (27)	
Q3 [5.4, 10.3]	21 (36)	13 (18)	
Q4 [10.4, 42]	17 (29)	16 (22)	
Median (IQR)	7.5 (4.1–11.0)	3.7 (2.3–8.8)	.019 ^b

Note: Values are n (%), unless otherwise noted.

^a Distribution across quartiles calculated by the Cochran–Armitage trend test.

^b Kruskal–Wallis test.

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TABLE 3

Unadjusted and adjusted odds of a male birth by stress biomarker quartile.

	Unadjusted analysis		Adjusted analysis ^a	
	OR	95% CI	OR	95% CI
Cortisol ($\mu\text{g/dL}$)				
Q1 [0.02, 0.29]	Referent	Referent	Referent	Referent
Q2 [0.30, 0.41]	0.35	0.12, 1.09	0.36	0.11, 1.17
Q3 [0.42, 0.55]	0.29	0.10, 0.85	0.32	0.11, 0.95
Q4 [0.56, 1.36]	0.28	0.09, 0.80	0.26	0.09, 0.79
α -Amylase (U/mL)				
Q1 [0.2, 2.9]	Referent	Referent	Referent	Referent
Q2 [3.0, 5.3]	0.84	0.29, 2.48	0.89	0.30, 2.69
Q3 [5.4, 10.3]	3.23	1.21, 8.60	2.98	1.10, 8.09
Q4 [10.4, 159.7]	2.13	0.80, 5.62	2.19	0.81, 5.91

Note: Referent group is the bottom quartile of the stress levels [Q1].

AOR = adjusted odds ratio; CI = confidence interval; OR = odds ratio.

^a Adjusted for female age, partner age and gravidity.

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