Published in final edited form as:

Physiol Behav. 2013 April 10; 0: 14–20. doi:10.1016/j.physbeh.2013.03.004.

Prenatal exposure to stressful life events is associated with masculinized anogenital distance (AGD) in female infants

Emily S. Barrett^a, Lauren E. Parlett^b, Sheela Sathyanarayana^c, Fan Liu^d, J. Bruce Redmon^e, Christina Wang^f, and Shanna H. Swan^g

Lauren E. Parlett: Lauren_parlett@urmc.rochester.edu; Sheela Sathyanarayana: Sheela.sathyanarayana@seattlechildrens.org; Fan Liu: fan.liu@mssm.edu; J. Bruce Redmon: redmo001@umn.edu; Christina Wang: wang@labiomed.org; Shanna H. Swan: shanna.swan@mssm.edu

^aDepartment of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

^bDepartment of Community and Preventive Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

^cDepartment of Pediatrics, Seattle Children's Research Institute, University of Washington, Seattle, WA 98101

^dDepartment of Preventive Medicine, Mount Sinai School of Medicine, New York, NY 10029

^eDepartments of Medicine and Urologic Surgery, University of Minnesota Medical School; Minneapolis, MN 55455

^fDivision of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA 90502

^gDepartment of Preventive Medicine, Mount Sinai School of Medicine, New York, NY 10029

Abstract

In animal models, prenatal stress programs reproductive development in the resulting offspring, however little is known about effects in humans. Anogenital distance (AGD) is a commonly used, sexually dimorphic biomarker of prenatal androgen exposure in many species. In rodents, prenatally stressed males have shorter AGD than controls (suggesting lower prenatal androgen exposure), whereas prenatally stressed females have longer AGD than controls (suggesting greater prenatal androgen exposure). Our objective was to investigate the relationship between stressful life events in pregnancy and infant AGD. In a prospective cohort study, pregnant women and their partners reported exposure to stressful life events during pregnancy. Pregnancies in which the couple reported 4+ life events were considered highly stressed. After birth (average 16.5 months), trained examiners measured AGD in the infants (137 males, 136 females). After adjusting for age, body size and other covariates, females born to couples reporting high stress had significantly longer (i.e. more masculine) AGD than females born to couples reporting low stress ($p=0.015$). Among males, high stress was weakly, but not significantly, associated with shorter AGD. Our results suggest prenatal stress may masculinize some aspects of female reproductive development

© 2013 Elsevier Inc. All rights reserved.

Corresponding author: Emily S. Barrett, PhD, Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box 668, Rochester, NY 14642 UNITED STATES, Phone: (585)275-9187; Fax: (585)276-2171, Emily_barrett@urmc.rochester.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

in humans. More sensitive measures of prenatal stress and additional measures of reproductive development are needed to better understand these relationships and clarify mechanisms.

1. Introduction

In humans, exposure to prenatal stress is associated with a wide range of postnatal outcomes, ranging from cognitive impairment to obesity to altered stress response [1–3]. A number of outcomes related to prenatal exposure to stress, such as autism spectrum disorders and schizophrenia, have well-documented sex differences in prevalence and presentation [4, 5]. Yet very little research in humans has directly examined whether there are sex differences in response to prenatal stressors. Prenatally stressed males may be at higher risk for attention deficit hyperactivity disorder (ADHD) and motor deficits [6, 7] than females, who are at greater risk for depression [8]. Even less is known about whether exposure to prenatal stress can affect reproductive development in humans, however data from natural experiments such as the Chernobyl disaster and the Dutch Hunger Winter suggest a need for further research [9, 10].

Animal models provide convincing evidence that prenatal stressors may, in fact, affect the developing reproductive system as well as reproductive behavior, and may do so in sex-dependent ways. It has long been known that exposing pregnant rats to stressors (such as crowding, immobilization, or cold) can produce male offspring who display feminized reproductive behaviors, such as lordosis, in adulthood [11–14]. In guinea pigs, prenatally stressed males show lower, less responsive, testosterone levels in the proximity of receptive females than do control animals [15]. Male reproductive physiology appears to be affected by exposure to prenatal stress as well, with lower testes weight and shorter anogenital distance (AGD) in males born to stressed dams compared to controls [16].

AGD, the distance from the anus to the genitals, is a reliable and sensitive biomarker of prenatal androgen exposure [17, 18]. It is highly sexually dimorphic across numerous animal species, with males typically having longer AGD (body size-adjusted) [19]. Thus the shorter AGD found in prenatally stressed male rodents suggests incomplete masculinization of reproductive development. In rats, testosterone production during a critical window is necessary for normal male reproductive development and administration of anti-androgens (such as phthalates) during this interval results in a shorter, more feminized AGD, as well as other indicators of impaired reproductive development [20]. Thus it has been proposed that in rats, prenatal stress may reduce AGD in male offspring by either suppressing the testosterone surge during the critical window or by altering the timing of that surge [21, 22].

Evidence of the effects of stress on AGD in females is less clear. The earliest studies finding effects in males did not find analogous effects on AGD in females; the AGD of prenatally stressed females was no different than that of control animals [23]. This apparent sex difference in the effect of stress was not entirely surprising given the dependence of AGD on testosterone and the lack of evidence showing effects of anti-androgens on female reproductive development (including AGD) to date. More recently, however, research on prenatal stress and reproductive development has considered intrauterine position effects, finding that female development may be affected as well. Under normal conditions in polytocous species, female mice positioned between two male siblings in the uterine horn (“2M females”) tend to be masculinized (including longer AGD), compared to females positioned between two female siblings (“0M females”) during gestation. This difference in AGD reflects the higher fetal androgen levels in 2M females as a result of their proximity to their androgen-producing male siblings [24]. When pregnant mice are exposed to intense stressors, however, all females (regardless of the sex of their neighboring littermates in utero) develop 2M-like characteristics, including longer AGD and higher circulating fetal

testosterone concentrations [25]. These elevated testosterone levels persist postnatally and affected females exhibit more masculinized play, courtship, parental, and social orientation behaviors than control females [11, 26]. Thus there is evidence that prenatal stress may masculinize female reproductive development and behavior, at least under certain conditions, in some species.

To our knowledge, no research in humans has examined these stress-related changes, particularly altered AGD. There is now a limited body of research on AGD in humans, demonstrating, that AGD varies in relation to body size and age [27], and shows the clear sexual dimorphism seen in other species [27–29]. It has been further documented that prenatal exposure to certain endocrine disrupting chemicals (including phthalates and dioxin) is related to AGD in human males [30, 31]. Yet it remains unclear whether exposure to prenatal stress affects AGD. Here, we examine the relationship between number of stressful life events (as reported by mothers and fathers during pregnancy) and AGD in the resulting infants, exploring stress-related differences between the sexes.

2. Methods

2.1 Study Population

Pregnant women and their partners from three U.S. cities (Los Angeles, CA; Minneapolis, MN; Columbia, MO) were recruited into Phase I of the Study for Future Families (SFFI) from 1999–2002. (Additional recruitment and study was conducted in Iowa City, IA, however due to methodological differences, those data are not included in the current analyses.) Eligibility criteria included: having a non-medically assisted pregnancy, being age 18 or over, and reading and speaking Spanish or English. As part of participation, both males and females in the couples completed questionnaires, and most subjects gave blood and urine samples as well. A follow-up study, SFFII, was conducted in 2001–2005 to examine the health and development of the offspring of the pregnancies from SFFI. Eligibility criteria for SFFII included: being an SFFI family who agreed to be recontacted, having a SFFI pregnancy ending in a live birth, living within 50 miles of a participating study center, and being willing to bring the infant for an in-person visit. Human subjects committees at all participating institutions approved both the main study and the follow-up and all participants signed informed consent forms prior to participation. Recruitment and study methods are described in greater detail elsewhere [32].

2.2 Questionnaire assessment of stress and covariates

In SFFI, pregnant women and their partners completed extensive questionnaires that included a section on stressful life events occurring during the previous three months of pregnancy. The items were derived from two standard questionnaires [33, 34], and included major life events chosen on the basis of principles outlined elsewhere [33, 35]. Participants reported on whether or not they had experienced: job loss or unemployment (self or partner); serious injury or illness (self or partner); death of a close family member (i.e. parent, child, sibling); divorce, separation, or serious relationship difficulties with one's partner; serious legal or financial problems (self or partner); or other major life events (write-in option). The questionnaires also included items on sociodemographic variables, race, age, current and former employment, smoking, alcohol, drugs, diet, and reproductive history, some of which are used as covariates in the current analyses.

2.3 Physical examination

Infants were brought to participating clinics at the SFF study centers to undergo brief physical examinations, according to protocols developed specifically for SFFII. Standard anthropometric measurements were made (including height, weight, head circumference,

and skin-fold thickness), after which genital exams were conducted by study staff under the supervision of pediatricians. Study staff was trained on standard examination methods and use of equipment at training sessions before and during the study. Using Vernier calipers, two measures of AGD were taken in male and female infants (Figure 1). In males, we measured: (1) the distance from the anus to the posterior base of the scrotum (**M-AGD-AS**); and (2) the distance from the anus to the cephalad insertion of the penis (**M-AGD-AP**). Analogous measurements were made in females, namely: (1) the distance from the anus to the posterior fourchette (**F-AGD-AF**); and (2) the distance from the anus to the clitoral hood (**F-AGD-AC**) [31]. M-AGD-AS and F-AGD-AF are “short measures” of AGD in males and females, respectively, whereas M-AGD-AP and F-AGD-AC are “long measures”.

2.4 Statistical analysis

After examining descriptive and summary statistics for relevant study variables, we fit several models for the relationship between prenatal stress and reproductive development outcomes. Based on findings from our previous work, we selected several covariates for *a priori* inclusion in models and these were included in the final models even if not significant [31]. These included infant race, infant age at AGD measurement, maternal age at birth, and infant weight percentile. Mother’s education was considered in initial models, but dropped from the final models as it was not significant and did not change effect estimates. For babies born prior to 38 weeks gestation, age at the time of the postnatal physical examination was calculated based on estimated date of conception rather than birth date. Because this was a multi-center study, we included a dummy variable for center in our models. Some of the infants had a second study visit as well, however for the current analyses, we limited our data set to only those measurements taken at the first postnatal visit. After initially examining maternal and paternal life events separately, we created a single summary variable that incorporated stressful life events reported by both parents during pregnancy. We first modeled stressful life events as an integer variable, however given that few families reported a large number of events, we elected to dichotomize this variable. Couples who collectively reported 4 or more life events stressors during pregnancy were considered “high stress” couples (representing 16% of the total cohort), whereas those who collectively reported fewer than four life events stressors (84%) were considered “low stress” couples.

Because AGD is sexually dimorphic we stratified our analyses by infant sex. We fit two linear regression models in male infants, exploring how exposure to stressful life events predicts: (1) **M-AGD-AP**; and (2) **M-AGD-AS**. Similarly, we fit two linear regression models in female infants exploring how exposure to prenatal stress predicts: (1) **F-AGD-AC**; and (2) **F-AGD-AF**. Lastly, in female infants, we fit two logistic regression models in which **F-AGD-AF** was modeled as (1) shortest quartile vs. longest quartile; and (2) shortest quartile vs. all other quartiles.

Children with missing data or AGD data deemed unreliable by pediatricians were excluded from analysis. For these reasons, 23 boys were excluded from **M-AGD-AS** analyses and 26 were excluded from **M-AGD-AP** analyses. In addition, 17 girls were excluded from both **F-AGD-AF** and **F-AGD-AC** analyses. Among twins, only the firstborn was included (n=4). Model assumptions of linearity between covariates and outcome and normally distributed error with constant variance were checked. For each model, we identified outliers (studentized residuals > |2|) and influential points (Cook’s D > 4/n) and reran the models excluding those subjects (n=28). Finally, a sensitivity analysis was conducted to examine the changes in effect estimates using weight z-scores rather than weight percentiles as a measure of body size. All analyses were conducted in SAS Version 9.2 (SAS Institute Inc., Cary, NC,

USA). Analyses were replicated by a second analyst and all p-values reported are two-tailed, with an alpha level of $p=0.05$.

3. Results

718 pregnant women and their partners completed prenatal questionnaires at the Missouri, Minnesota, and California SFF study sites. Of these, 477 also brought their children back for a physical examination after birth. In total, 137 boys and 136 girls had complete data and were included in these analyses. Parents who brought their children in for post-natal examinations in SFFII reported fewer life events stressors, on average, than parents who did not participate in SFFII (not shown). In most other ways, however, the SFFII participants are similar to those SFFI participants who did not participate in SFFII. Notably, there were fewer Hispanic participants among those who had physical examinations than in the larger cohort, primarily because fewer of the participants at the California study center (who were 46% Hispanic or Latino) participated in SFFII.

The subject population is described in greater detail in Table 1. The participating mothers were predominantly Caucasian and well-educated, and on average, their babies were examined at age 16 months. Among the mothers, nearly half (47%) reported no life events stressors during pregnancy, and an additional 29% reported just one life events stressor. The percentages were similar among fathers, with 49% reporting no stressors and 29% reporting one stressor. The number of life events stressors reported by mothers was strongly correlated with the number of life events stressors reported by their partners ($r=0.67$). Among couples, approximately 84% reported less than four life stressors total and were thus classified as “low stress” pregnancies. The remaining 16% reported four to nine life events total and were classified as “high stress” pregnancies. The number of life events stressors reported was not related to sex of the offspring (not shown). The distribution of both AGD measurements was approximately normal in male and female infants. On average, the longer AGD measurement (M-AGD-AP/F-AGD-AC) was 49% longer in males than in females and the shorter measurement (M-AGD-AS/F-AGD-AF) was 80% longer in males. The longer and shorter AGD measurements were correlated in both male ($r=0.53$) and female infants (0.54).

In linear regression models (Table 2), daughters born to couples with high life events stress had longer AGD than daughters born to couples with low life events stress ($\beta=2.61$, $p=0.015$) and ($\beta=2.15$, $p=0.14$) for F-AGD-AF and F-AGD-AC). M-AGD-AP and M-AGD-AS were both shorter in sons of couples with high life events stress, however the confidence intervals were wide ($p=0.90$ and $p=0.23$, respectively). The distribution of AGD measures by parental life events stress category is illustrated in Figure 2 for both infant boys and girls. Across the models, infant age and weight percentile were related to AGD measures and in sensitivity analyses examining weight z-scores, the results (not shown) were similar to those using weight percentiles.

4. Discussion

This is the first study to examine prenatal stress in relation to AGD in humans. Female infants exposed to more life events stressors *in utero* had significantly longer AGD than female infants exposed to fewer life events stressors *in utero*. By contrast, male infants exposed to more life events stressors *in utero* showed a non-significant trend towards shorter AGD than males exposed to fewer life events stressors *in utero*. Across species, longer female AGD indicates masculinization, thus these findings suggest that high levels of prenatal stress may be associated with masculinization of some aspects of female reproductive development, and possibly incomplete masculinization of some aspects of male reproductive development.

The mechanisms underlying stress-related differences in AGD are unknown. Given evidence from animal models suggesting that androgens play an important role in determining AGD, the most obvious possible mechanistic explanation is that high levels of stress are associated with differences in androgen production or activity, possibly via corticosteroids [36, 37]. Maternal stress during gestation may increase fetal exposure to cortisol by elevating maternal cortisol (which crosses the placenta) and/or affecting regulation of placental enzymes implicated in **glucocorticoid** metabolism, such as 11 β -hydroxysteroid dehydrogenase type 2 [38, 39]. Fetal cortisol exposure, in turn, may affect fetal testosterone exposure. Testosterone and cortisol levels in amniotic fluid are positively correlated, with stronger associations in females ($r=0.46$) than in males ($r=0.30$) [40]. Because amniotic fluid **derives** primarily from the fetus, it is an ideal medium for examining the fetal hormonal milieu [41, 42]. Unfortunately, it was impossible to obtain amniotic fluid in our study. Nevertheless, if stress-related increases in fetal cortisol were associated with increased testosterone (produced by the placenta, or fetus) as well, it would provide a plausible mechanism for our findings of masculinization of AGD in girls exposed to high life events stress *in utero*. In one rodent study, maternal stress increased fetal testosterone in female offspring, however AGD was only lengthened in those females situated next to no males or one male *in utero* [25]. Further research is needed to investigate how putative stress-induced endocrine changes (and associated outcomes, like AGD) vary by context in humans, for instance whether the relationship differs in multiple pregnancies and if so, whether the sex of the fetuses matters.

Our findings are clinically relevant for several reasons. If the longer AGD seen here in females exposed to a greater number of stressful life events is indicative of more extensive prenatal androgen exposure, then there may be other reproductive outcomes that are masculinized. Indeed, a recent study found that among adult females, longer AGD was associated with having more ovarian follicles [43], which may be of clinical importance [44, 45]. It has been hypothesized, furthermore, that in humans, polycystic ovary syndrome is caused, at least in part, by excess prenatal androgen exposure [46] and may be linked to biomarkers of prenatal androgen exposure [47].

Here, we focus on infants, but it is plausible that additional reproductive outcomes associated with prenatal exposure to stress could emerge at reproductive maturity, including HPG axis dysregulation, altered timing of puberty, and even impaired fertility [48]. Indeed, in prenatally stressed female rats, changes in dopamine and catecholamine concentrations have been observed compared to controls and these changes in neurotransmitter concentrations appear to dysregulate the HPG axis, lengthening the estrous cycle in affected animals [49, 50]. Ultimately, female rats who experienced stress *in utero* have fewer conceptions, more miscarriages, and fewer surviving offspring at birth and day 10 than control females [48]. In one study, testosterone levels were significantly higher in female adolescents whose mothers lived through the Chernobyl explosion during pregnancy than in age-matched controls, suggesting possible effects of prenatal stress on the developing HPA and/or HPG axis [9]. However few differences in reproductive development and function have been noted in women who were gestated during the Dutch Hunger Winter and were presumably exposed to high *in utero* stress (in conjunction with severe undernutrition) [10]. Clearly additional research is needed to understand these relationships in females.

In contrast to our findings in females, there was a weak, non-significant trend towards shorter AGD in males exposed to 4+life events stressors in our population compared to males exposed to <4 stressors.. Studies in several species have shown results in the same direction; maternal stress during gestation is associated with shorter AGD and other less masculine outcomes [13, 14, 16, 19, 51]. This shorter, less masculine AGD can also be elicited by administering hydrocortisone or ACTH to the mother during gestation, although

corticosterone failed to elicit similar effects on AGD [19, 23, 52]. Naltrexone administered to stressed, pregnant females blocks de-masculinization of AGD and brain development in sons, suggesting that opioids may also play an important role in the pathway between prenatal stress and de-masculinization of AGD in males [53, 54]. A larger sample size or more sensitive measures of stress in humans are needed to clarify the relationship between prenatal stress and AGD.

That the direction of the effects of prenatal stress is opposite in males and females appears contradictory, however previous work has suggested that perinatal androgen exposure may produce both masculinizing and de-masculinizing effects depending on the concentrations and timing of exposure [55]. It is also plausible that prenatal stress exerts different effects on androgen production by the fetal testes and the fetal adrenals. Whereas nearly all male androgen production is testicular in origin, the ovaries and adrenals contribute approximately equally in females [56], thus if fetal adrenal androgen secretion were stimulated by prenatal stress, but fetal testicular androgen secretion were suppressed, sex differences in the effects of prenatal stress on AGD might be evident. Indeed there is evidence to suggest that maternal stress during gestation may suppress fetal testosterone secretion in male rodents, thereby interfering with typical masculinization of the genitals [16].

Notably, in both sexes, exposure to life events stress was more strongly associated with the shorter of the two AGD measurements (**M**-AGD-AS in males, **F**-AGD-AF in females). Previous work in humans supports the greater clinical importance of the shorter AGD measure. Among young men, **M**-AGD-AS (but not the longer measure, **M**-AGD-AP) was positively associated with semen quality [57], while in adult women, **F**-AGD-AF was more strongly related to ovarian follicle number than the longer measure, **F**-AGD-AC [43]. However, data also suggests that in **male** infants, the longer of the two measures (**M**-AGD-AP) is associated with exposure to phthalates, the anti-androgenic environmental chemicals [31]. Further research is needed to understand the differences between these two biomarkers and how they might reflect different aspects of the prenatal hormonal milieu.

vom Saal has proposed that in rodents, modulation of gender-specific traits in response to prenatal stressors may be adaptive. For instance, 0M females tend to be most attractive to males and have greater reproductive success than more masculine 2M females under typical, low-density, low-stress conditions. However under more high-population density, stressful circumstances, the more aggressive tendencies characteristic of 2M females might confer an adaptive advantage, thus there could be selection for increased masculinization of all females in the litter under certain, specific conditions [58]. In complex, crowded social environments, for example, 2M females have more surviving offspring than 0M females (due to greater infant death among the 0M mothers), however when housed singly with a male, no such differences were apparent [58]. The fetal origins hypothesis of adaptation and disease suggests that fetal development is programmed in accordance with the intrauterine environment, which may provide the best proxy for the environmental and ecological conditions that the individual will face after birth. Following that logic, under conditions of high prenatal stress, it may be advantageous for both males and females to adapt their sex-specific reproductive strategies to better compete in a stressful postnatal environment. Thus it is possible that the masculinized AGD observed in females born to high stress couples may be associated with other features conferring greater reproductive success in high stress environments. Alternatively, it is possible that any reproductive changes are simply non-adaptive by-products of stress-related dysregulation of the prenatal hormonal environment.

One possible limitation of our study is use of our questionnaire-based inventory to assess stress during pregnancy. Life events stress has been associated with numerous outcomes,

however it is possible that subjects' self-reporting was inaccurate or did not appropriately account for variation in perceptions of severity of the stressors. Our inventory, moreover, only assessed major life events at one time point during pregnancy and did not examine lower-level acute daily stressors. Because so little is known about the relationship between prenatal stress and reproductive development, we cannot rule out the possibility that daily, cumulative stress or short-term acute stressors may be also associated with reproductive development. Similarly, we cannot address whether social support or personality might attenuate the prenatal effects of life events stressors on reproductive development.

In addition, we elected to combine maternal and paternal self-reported stress into a single measure of couples' stress. We examined associations between AGD and maternal stress alone (not shown), and although the relationships were similar, they were stronger when we considered stress reported by both partners. It is unclear why this is the case. One possibility is that the physiological effects of prenatal stress are more profound when mothers experience not only their own stress, but also indirectly experience "bystander stress" through their partners. In rats, pregnant females who were not themselves stressed, but were housed with stressed cage-mates, gave birth to offspring who showed profound changes in gene expression, DNA methylation, and behavior. Thus it may be the case that exposure to a partner's stress (in addition to one's own) intensifies any biological effects on the offspring's developing reproductive system [59]. There is evidence from rodent models, furthermore, suggesting that reproductive outcomes associated with prenatal exposure to stress may be passed through paternal lineage as well, thus future work in this field may further consider stress in fathers as well as mothers, as we have here [60].

Our protocol for measuring AGD had some limitations. First, due to logistic and funding constraints, we were unable to examine the children at birth and instead allowed for examination at a range of ages to maximize the size of the cohort. Although we adjusted for the child's age in our analyses, ideally we would standardize the age at which the measurement was made. Given that it is still uncertain to what extent AGD is stable within individuals as they age, conducting these measurements at birth and again in infancy would be optimal. Second, only one AGD measurement was made for each child at the examination and although data from our training session suggests that the measurements are reliable within and across examiners (coefficient of variation of 7.2%; not shown), ideally, several measurements would be made during the same visit.

To our knowledge, this is the first study to examine the association between prenatal parental stress and AGD in the subsequent offspring and additional work will be needed to confirm this relationship. Future follow-up in this cohort is needed, moreover, to examine whether the stress-related differences in AGD described here are associated with differences in other aspects of gender-specific reproductive anatomy, physiology, or behavior over time.

Acknowledgments

We wish to acknowledge the SFF Study team as well as the families who participated in the study. Funding for the Study for Future Families was provided by the following grants from the National Institutes of Health and Environmental Protection Agency: R01ES09916, M01-RR00400, M01RR0425. Funding for the current analyses was provided by K12 ES019852-01 and UL1TR000124.

Works Cited

1. Dancause KN, Laplante DP, Fraser S, Brunet A, Ciampi A, Schmitz N, et al. Prenatal exposure to a natural disaster increases risk for obesity in 5(1/2)-year-old children. *Pediatr Res*. 2012; 71:126–31. [PubMed: 22289861]

2. Dunkel Schetter C, Tanner L. Anxiety, depression and stress in pregnancy: implications for mothers, children, research, and practice. *Current opinion in psychiatry*. 2012; 25:141–8. [PubMed: 22262028]
3. O'Connor TG, Heron J, Golding J, Beveridge M, Glover V. Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. *Br J Psychiatry*. 2002; 180:502–8. [PubMed: 12042228]
4. Khashan AS, Abel KM, McNamee R, Pedersen MG, Webb RT, Baker PN, et al. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. *Arch Gen Psychiatry*. 2008; 65:146–52. [PubMed: 18250252]
5. Kinney DK, Munir KM, Crowley DJ, Miller AM. Prenatal stress and risk for autism. *Neurosci Biobehav Rev*. 2008; 32:1519–32. [PubMed: 18598714]
6. Gerardin P, Wendland J, Bodeau N, Galin A, Bialobos S, Tordjman S, et al. Depression during pregnancy: is the developmental impact earlier in boys? A prospective case-control study. *J Clin Psychiatry*. 2011; 72:378–87. [PubMed: 21208585]
7. Rodriguez A, Bohlin G. Are maternal smoking and stress during pregnancy related to ADHD symptoms in children? *J Child Psychol Psychiatry*. 2005; 46:246–54. [PubMed: 15755301]
8. Van den Bergh BR, Van Calster B, Smits T, Van Huffel S, Lagae L. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology*. 2008; 33:536–45. [PubMed: 17507916]
9. Huizink AC, Bartels M, Rose RJ, Pulkkinen L, Eriksson CJ, Kaprio J. Chernobyl exposure as stressor during pregnancy and hormone levels in adolescent offspring. *J Epidemiol Community Health*. 2008; 62:e5. [PubMed: 18365332]
10. Lumey LH. Reproductive outcomes in women prenatally exposed to undernutrition: a review of findings from the Dutch famine birth cohort. *Proc Nutr Soc*. 1998; 57:129–35. [PubMed: 9571718]
11. Kinsley CH, Bridges RS. Prenatal stress and maternal behavior in intact virgin rats: response latencies are decreased in males and increased in females. *Horm Behav*. 1988; 22:76–89. [PubMed: 3350479]
12. Ward IL. Prenatal stress feminizes and demasculinizes the behavior of males. *Science*. 1972; 175:82–4. [PubMed: 5008583]
13. Williams MT, Hennessy MB, Davis HN. Stress during pregnancy alters rat offspring morphology and ultrasonic vocalizations. *Physiol Behav*. 1998; 63:337–43. [PubMed: 9469724]
14. Dahlof LG, Hard E, Larsson K. Influence of maternal stress on offspring sexual behaviour. *Anim Behav*. 1977; 25:958–68. [PubMed: 564150]
15. Kemme K, Kaiser S, Sachser N. Prenatal maternal programming determines testosterone response during social challenge. *Horm Behav*. 2007; 51:387–94. [PubMed: 17303135]
16. Dahlof LG, Hard E, Larsson K. Influence of maternal stress on the development of the fetal genital system. *Physiol Behav*. 1978; 20:193–5. [PubMed: 662940]
17. vom Saal FS, Bronson FH. In utero proximity of female mouse fetuses to males: effect on reproductive performance during later life. *Biol Reprod*. 1978; 19:842–53. [PubMed: 743525]
18. Clemens, LG. Neurohormonal control of male sexual behavior. In: Montagna, W.; Sadler, W., editors. *Reproductive Behavior*. New York: Plenum Press; 1974. p. 23–53.
19. Lay DC Jr, Kattesh HG, Cunnick JE, Daniels MJ, McMunn KA, Toscano MJ, et al. Prenatal stress effects on pig development and response to weaning. *J Anim Sci*. 2008; 86:1316–24. [PubMed: 18272847]
20. Moore RW, Rudy TA, Lin TM, Ko K, Peterson RE. Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. *Environ Health Perspect*. 2001; 109:229–37. [PubMed: 11333183]
21. Ward IL, Weisz J. Maternal stress alters plasma testosterone in fetal males. *Science*. 1980; 207:328–9. [PubMed: 7188648]
22. Ward IL, Weisz J. Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. *Endocrinology*. 1984; 114:1635–44. [PubMed: 6714159]

23. Dahlof LG, Hard E, Larsson K. Sexual differentiation of offspring of mothers treated with cortisone during pregnancy. *Physiol Behav.* 1978; 21:673–4. [PubMed: 740788]
24. Ryan BC, Vandenberg JG. Intrauterine position effects. *Neurosci Biobehav Rev.* 2002; 26:665–78. [PubMed: 12479841]
25. vom Saal FS, Quadagno DM, Even MD, Keisler LW, Keisler DH, Khan S. Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. *Biol Reprod.* 1990; 43:751–61. [PubMed: 2291911]
26. Kaiser S, Sachser N. The social environment during pregnancy and lactation affects the female offsprings' endocrine status and behaviour in guinea pigs. *Physiol Behav.* 1998; 63:361–6. [PubMed: 9469727]
27. Sathyanarayana S, Beard L, Zhou C, Grady R. Measurement and correlates of ano-genital distance in healthy, newborn infants. *Int J Androl.* 2010; 33:317–23. [PubMed: 20132349]
28. Salazar-Martinez E, Romano-Riquer P, Yanez-Marquez E, Longnecker MP, Hernandez-Avila M. Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. *Environmental health : a global access science source.* 2004; 3:8. [PubMed: 15363098]
29. Thankamony A, Ong KK, Dunger DB, Acerini CL, Hughes IA. Anogenital distance from birth to 2 years: a population study. *Environ Health Perspect.* 2009; 117:1786–90. [PubMed: 20049133]
30. Vafeiadi M, Agramunt S, Papadopoulou E, Besselink H, Mathianaki K, Karakosta P, et al. In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. *Environ Health Perspect.* 2013; 121:125–30. [PubMed: 23171674]
31. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect.* 2005; 113:1056–61. [PubMed: 16079079]
32. Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, et al. Geographic differences in semen quality of fertile U. S. males. *Environ Health Perspect.* 2003; 111:414–20. [PubMed: 12676592]
33. Dohrenwend BS, Krasnoff L, Askenasy AR, Dohrenwend BP. Exemplification of a method for scaling life events: the Peri Life Events Scale. *J Health Soc Behav.* 1978; 19:205–29. [PubMed: 681735]
34. Holmes TH, Rahe RH. The Social Readjustment Rating Scale. *J Psychosom Res.* 1967; 11:213–8. [PubMed: 6059863]
35. Dohrenwend BS, Dohrenwend BP. Some issues in research on stressful life events. *J Nerv Ment Dis.* 1978; 166:7–15. [PubMed: 619005]
36. Macleod DJ, Sharpe RM, Welsh M, Fiskens M, Scott HM, Hutchison GR, et al. Androgen action in the masculinization programming window and development of male reproductive organs. *Int J Androl.* 2010; 33:279–87. [PubMed: 20002220]
37. van den Driesche S, Scott HM, MacLeod DJ, Fiskens M, Walker M, Sharpe RM. Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. *Int J Androl.* 2011; 34:e578–86. [PubMed: 21631528]
38. O'Donnell KJ, Bugge Jensen A, Freeman L, Khalife N, O'Connor TG, Glover V. Maternal prenatal anxiety and downregulation of placental 11beta-HSD2. *Psychoneuroendocrinology.* 2012; 37:818–26. [PubMed: 22001010]
39. Mairesse J, Lesage J, Breton C, Breant B, Hahn T, Darnaudery M, et al. Maternal stress alters endocrine function of the feto-placental unit in rats. *American journal of physiology Endocrinology and metabolism.* 2007; 292:E1526–33. [PubMed: 17264224]
40. Bergman K, Glover V, Sarkar P, Abbott DH, O'Connor TG. In utero cortisol and testosterone exposure and fear reactivity in infancy. *Horm Behav.* 2010; 57:306–12. [PubMed: 20060000]
41. van de Beek C, Thijssen JH, Cohen-Kettenis PT, van Goozen SH, Buitelaar JK. Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: what is the best source of information to investigate the effects of fetal hormonal exposure? *Horm. Behav.* 2004; 46:663–9.
42. Sarkar P, Bergman K, O'Connor TG, Glover V. Maternal antenatal anxiety and amniotic fluid cortisol and testosterone: possible implications for foetal programming. *J Neuroendocrinol.* 2008; 20:489–96. [PubMed: 18266948]

43. Mendiola J, Roca M, Minguez-Alarcon L, Mira-Escolano M-P, Lopez-Espin JJ, Barrett ES, et al. Anogenital distance is related to ovarian follicular number in young Spanish women: a cross-sectional study. *Environmental Health*. 2012 In press.
44. Adams J, Franks S, Polson DW, Mason HD, Abdulwahid N, Tucker M, et al. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. *Lancet*. 1985; 2:1375–9. [PubMed: 2867389]
45. Galli-Tsinopoulou A, Moudiou T, Mamopoulos A, Karamouzis M, Nousia-Arvanitakis S. Multifollicular ovaries in female adolescents with cystic fibrosis. *Fertil Steril*. 2006; 85:1484–7. [PubMed: 16600228]
46. Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome - a hypothesis. *J Endocrinol*. 2002; 174:1–5. [PubMed: 12098657]
47. Cattrall FR, Vollenhoven BJ, Weston GC. Anatomical evidence for in utero androgen exposure in women with polycystic ovary syndrome. *Fertil Steril*. 2005; 84:1689–92. [PubMed: 16359966]
48. Herrenkohl LR. Prenatal stress reduces fertility and fecundity in female offspring. *Science*. 1979; 206:1097–9. [PubMed: 573923]
49. Herrenkohl LR, Politch JA. Effects of prenatal stress on the estrous cycle of female offspring as adults. *Experientia*. 1978; 34:1240–1. [PubMed: 569073]
50. Moyer JA, Herrenkohl LR, Jacobowitz DM. Stress during pregnancy: effect on catecholamines in discrete brain regions of offspring as adults. *Brain Res*. 1978; 144:173–8. [PubMed: 638759]
51. Williams MT, Hennessy MB, Davis HN. CRF administered to pregnant rats alters offspring behavior and morphology. *Pharmacol Biochem Behav*. 1995; 52:161–7. [PubMed: 7501659]
52. Zagron G, Weinstock M. Maternal adrenal hormone secretion mediates behavioural alterations induced by prenatal stress in male and female rats. *Behav Brain Res*. 2006; 175:323–8. [PubMed: 17023059]
53. Keshet GI, Weinstock M. Maternal naltrexone prevents morphological and behavioral alterations induced in rats by prenatal stress. *Pharmacol Biochem Behav*. 1995; 50:413–9. [PubMed: 7617680]
54. Reznikov AG, Nosenko ND, Tarasenko LV. Opioids are responsible for neurochemical feminization of the brain in prenatally stressed male rats. *Neuro Endocrinol Lett*. 2005; 26:35–8. [PubMed: 15726017]
55. Gorski, RA. Sexual differentiation of the brain. In: Krieger, DT.; Hughes, JC., editors. *Neuroendocrinology*. Sunderland, MA: Sindauer Associates; 1980.
56. Longcope C. Adrenal and gonadal androgen secretion in normal females. *Clin Endocrinol Metab*. 1986; 15:213–28. [PubMed: 3013468]
57. Mendiola J, Stahlhut RW, Jorgensen N, Liu F, Swan SH. Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. *Environ Health Perspect*. 2011; 119:958–63. [PubMed: 21377950]
58. vom Saal FS. Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. *J Reprod Fertil*. 1981; 62:633–50. [PubMed: 7252935]
59. Mychasiuk R, Schmold N, Illytskyy S, Kovalchuk O, Kolb B, Gibb R. Prenatal bystander stress alters brain, behavior, and the epigenome of developing rat offspring. *Dev Neurosci*. 2011; 33:159–69. [PubMed: 21893948]
60. Morgan CP, Bale TL. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. *J Neurosci*. 2011; 31:11748–55. [PubMed: 21849535]

Highlights

- Anogenital distance (AGD) is a marker of prenatal androgen exposure.
- Here, we examine prenatal exposure to life events stress and AGD in humans.
- Prenatal exposure to stressful life events was associated with longer AGD in females.
- Prenatal stress was weakly associated with shorter AGD in male infants.
- Further research is needed to understand underlying mechanisms.

Figure 1. Measurements of anogenital distance in females (a) and males (b). Adapted from Sathyanarayana *et al.* (2010) [27].

Figure 2. Distribution of age- and weight-percentile-adjusted (a) AGD-AS (in mm) in infant boys (n=137), and (b) AGD-AF (in mm) in infant girls (n=136); in relation to prenatal life events stress reported by their parents. Mean AGD for high and low life events stress groups indicated by vertical dotted lines. **Solid lines indicate smoothed Gaussian kernel densities.**

Table 1

Demographic characteristics and anthropometrics of the SFF subjects by parental life events stress category[†] (n=273).

Characteristic	Low Stress (n=238)	High Stress (n=45)	All (n=273)
	N(%) [*]	N(%) [*]	N(%) [*]
<i>Maternal Life Stressors</i>			
0	125 (56)	.	125 (46)
1	81 (36)	5 (11)	86 (32)
2 or more	19 (8)	40 (89)	59 (22)
<i>Paternal Life Stressors</i>			
0	133 (59)	.	133 (49)
1	76 (34)	4 (9)	80 (30)
2 or more	16 (7)	41 (91)	57 (21)
<i>Study Center</i>			
California	43 (19)	18 (40)	61 (22)
Minnesota	108 (47)	12 (27)	120 (44)
Missouri	77 (34)	15 (33)	92 (34)
<i>Baby Race</i>			
White	188 (82)	29 (64)	217 (80)
Other	40 (18)	16 (36)	56 (20)
<i>Maternal education</i>			
Less than College	18 (8)	10 (22)	28 (10)
College or more	210 (92)	35 (78)	245 (90)
	Mean (Min-Max)	Mean (Min-Max)	Mean (Min-Max)
Baby Age (months)	16.4 (2–36)	16.6 (2–37)	16.4 (2–37)
Height (cm)	78.6 (57–97)	78.1 (58–95)	78.5 (57–97)
Weight (kg)	10.2 (4–17)	10.7 (5–19)	10.3 (4–19)
Maternal Age at Birth	30.1 (18–40)	28.6 (19–41)	29.9 (18–41)
Paternal Age at Birth	32.0 (18–53)	31.9 (21–48)	32.0 (18–53)
Boys: M-AGD-AS (mm)	37.3 (21–56)	35.7 (22–51)	37.0 (21–56)
Boys: M-AGD-AP (mm)	69.9 (42–104)	70.4 (48–98)	70.0 (42–104)
Girls: F-AGD-AF (mm)	19.9 (9–37)	22.8 (16–30)	20.3 (9–37)
Girls: F-AGD-AC (mm)	46.7 (27–66)	50.4 (41–65)	47.2 (27–66)

[†] High stress= parents collectively report 4 or more life events stressors during pregnancy. Low stress=parents collectively report fewer than 4 life events stressors during pregnancy.

^{*} Percentages may not add up to 100 due to rounding.

Table 2

Associations between prenatal life events stress and AGD in multivariate models.*

AGD measurement	Female				Male			
	β coefficient [†]	Lower 95%	Upper 95%	p-value	β coefficient [†]	Lower 95%	Upper 95%	p-value
Shorter AGD measurement (F-AGD-AF M-AGD-AS in mm)	2.61	0.51	4.70	0.015	-2.01	-5.30	1.28	0.23
Longer AGD measurement (F-AGD-AC M-AGD-AP in mm)	2.15	-0.74	5.05	0.14	-0.28	-4.41	3.86	0.90

* Models also include the following covariates: infant age, mother's age, infant race, infant weight centile, and study center.
[†] For infants born to couples reporting high life events stress (4+) versus infants born to couples reporting low life events stress (<4).