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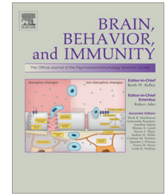

Short Communication

Fetal sex is associated with maternal stimulated cytokine production, but not serum cytokine levels, in human pregnancy

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ABSTRACT

Some studies suggest that fetal sex plays a role in maternal physiological processes during pregnancy including glycemic control, blood pressure, and cortisol regulation. However, data examining fetal sex-specific differences in maternal immune parameters is lacking. In the current study, serum levels of interleukin(IL)-6, IL-8, and tumor necrosis factor(TNF)-α as well as LPS-stimulated production of IL-6, IL-8, TNF-α, and IL-1β by PBMCs incubated for 24 h were assessed in early, mid, and late pregnancy among 80 women (46 with male and 34 with female fetuses). Linear mixed models showed that women carrying females versus males exhibited greater stimulated production of IL-6 at each timepoint ($ps \leqslant 0.03$), TNF-α in early pregnancy ($p = 0.04$), and IL-1β in mid- and late pregnancy ($ps \leqslant 0.05$). Despite changes in serum levels of IL-8 ($p = 0.002$) and TNF-α ($p < 0.0001$) across pregnancy, no differences in any serum cytokines were observed in relation to fetal sex ($ps > 0.85$). In conclusion, in pregnant women, those carrying female versus male fetuses exhibited greater stimulated cytokine production across pregnancy. Differential inflammatory responses could affect maternal health and fetal development. Fetal sex should be considered as a factor in studies of maternal inflammation. These findings have relevance both clinically and conceptually. For example, maternal asthma is exacerbated among women carrying female versus male fetuses. In addition, data on associations between fetal sex and maternal immune function among women with health conditions (e.g., preeclampsia) and adverse pregnancy outcomes (e.g., preterm birth) would be informative.

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1. Introduction

Pregnancy is characterized by substantial changes in maternal immune parameters. Pregnant women exhibit heightened serum levels of certain proinflammatory cytokines as well as greater lipopolysaccharide (LPS) stimulated proinflammatory cytokine production than non-pregnant adults (e.g., Brewster et al., 2008; Vassiliadis et al., 1998). Although limited, longitudinal examinations have demonstrated significant increases in some serum and LPS-stimulated cytokines across pregnancy, including serum TNF-α, stimulated IL-6, and stimulated IL-1β, while other markers exhibit a decline, such as serum IL-8 (Christian and Porter, 2014; Gillespie et al., 2016). Information regarding factors which may modify such adaptation is limited.

Bidirectional communication between the pregnant woman and fetus has been supported, with fetal sex shown to play a role (Glynn and Sandman, 2011). Specifically, though not found in all studies, some studies have shown that fetal sex is associated with differences in maternal physiology, including cortisol regulation, glycemic control, and blood pressure (DiPietro et al., 2011; Giesbrecht et al., 2015; Hocher et al., 2009; Petry et al., 2007). Studies examining associations between fetal sex and maternal immune parameters are limited. However, some studies have shown differences by fetal sex in immune-relevant gene expression as well as cytokine expression in the placenta, with women carrying female fetuses compared to males showing elevated levels (Clifton and Murphy, 2004; Scott et al., 2009). Data examining whether fetal sex is associated with distinct patterns in maternal inflammatory processes as measured in peripheral blood is lacking.

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Of clinical importance, differences in maternal physiological responses to health conditions (e.g., asthma) have been observed in relation to fetal sex. A substantial body of literature examining asthma in pregnant women shows exacerbated symptom severity in those carrying female fetuses versus male (Clifton, 2010; Clifton and Murphy, 2004; Scott et al., 2009). In addition, asthma is characterized by chronic inflammation, including increased expression of proinflammatory cytokines, such as TNF-α and IL-1β (Kips, 2001). Thus, data examining effects of fetal sex on healthy maternal immune adaptation would be informative.

Addressing gaps in the current literature, the current study examined serum levels of the proinflammatory cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α as well as LPS-stimulated production of IL-6, IL-8, TNFα, and IL-1β among 80 pregnant women assessed in early, mid, and late pregnancy. This study included 46 women carrying male fetuses, and 34 carrying females. The association between fetal sex and maternal levels of both serum and stimulated cytokine production was examined.

2. Materials and methods

2.1. Study design

Eighty-two pregnant women were recruited from the Ohio State University Wexner Medical Center (OSUWMC) Prenatal Clinic and surrounding community of Columbus, Ohio. Study visits were conducted during early (mean = 12.33, SD = 1.52), mid (mean = 20.61, SD = 1.29), and late pregnancy (mean = 29.22, SD = 1.41). A blood sample was collected at each visit. The broader study captured psychosocial functioning; these data were not utilized in the current analyses. In the current analyses, two women were excluded due to unavailable medical records following delivery resulting in a final sample of 80.

2.2. Participants

Women were ineligible if they had any major immunological conditions (e.g., rheumatoid arthritis), fetal anomaly, illicit drug use after pregnancy was known, or consumed more than two alcoholic drinks per week during pregnancy (per self-report or medical record). Women who described experiencing acute illness (e.g., flu-like symptoms) within 10 days of a study visit were rescheduled. Written informed consent was obtained at the first study visit, and participants received modest financial compensation at the completion of each visit. The study was approved by the OSU Biomedical Institutional Review Board.

2.3. Measures

2.3.1. Maternal demographics and fetal sex

Race/ethnicity, age, marital status, education level, annual household income, current cigarette use, and number of prior births (parity) were collected by self-report at the first study visit. Pre-pregnancy body mass index (BMI; kg/m2) was calculated using self-reported pre-pregnancy weight and measured height at the first visit. Gestational weight gain was calculated using measured weight prior to delivery (per medical record review) and self-reported pre-pregnancy weight. Adverse outcomes (i.e., gestational hypertension, preeclampsia, gestational diabetes, low birth weight, and preterm birth) and fetal sex were obtained per medical record review or self-report.

2.3.2. Serum cytokine levels

Whole blood was collected into vacutainer tubes while participants were in a seated position. Samples were immediately centrifuged, aliquoted, and placed in −80 °C freezer storage until analysis. Serum levels of IL-6, TNF-α, and IL-8 were assayed in duplicate on either single spot ultra-sensitive or multiplex V-Plex kits from Meso Scale Discovery (MSD, Meso Scale Discovery, 1601 Research Blvd, Rockville, MD). Plates were read by an MSD SECTOR Imager 2400 measuring electrochemiluminescence. Sample concentrations were extrapolated from a standard curve calculated using a four parameter logistic fit using MSD Workbench 3.0 software. The limits of detection were 0.31 pg/mL for IL-6, 0.17 pg/mL for TNF-α, and 0.27 pg/mL for IL-8. The inter- and intra- assay coefficients of variation were 8.69% and 5.89% for IL-6, 5.12% and 5.34% for TNF-α, and 5.27% and 3.71% for IL-8, respectively.

2.3.3. Stimulated cytokine production

PBMCs at a concentration of 1×10^6 cells/ml were stimulated with 1ug/ml LPS in RPMI-1640 supplemented with 10% human male serum for 24 h. A non-LPS media control was incubated simultaneously. After 24 h, samples were centrifuged and aliquots removed and frozen at −80 °C until assayed. Media samples were assayed neat, while LPS samples were diluted 1:6. Samples were assayed in duplicate for IL-6, TNF-α, IL-1β, and IL-8 (pg/ml) using human ProInflammatory II multiplex tissue culture kits from Meso Scale Discovery (MSD; 1601 Research Blvd., Rockville, MD). Plates were read by an MSD Sector Imager 2400 measuring electrochemiluminescence. The inter- and intra- assay coefficients of variation were 8.28% and 3.20% for IL-6, 6.02% and 2.36% for TNF-α, 8.59% and 1.91% for IL-1β, and 9.23% and 2.93% for IL-8, respectively.

2.4. Statistical analyses

All analyses were conducted in SAS 9.4. Missing data were addressed utilizing the restricted maximum likelihood estimation method. Serum cytokines were log-transformed (base 10) to fit normality assumptions. Thirty-two data points of serum cytokines ($n = 21$) or LPS-stimulated cytokines ($n = 11$) were classified (±3 SD from mean) as outliers and excluded from respective analyses. To compare demographic characteristics between women who gave birth to girls vs boys, chi-square tests and t-tests were conducted, as appropriate. Mixed effects regression models were used to examine whether maternal serum cytokine levels and LPS-stimulated cytokine production by maternal PBMCs differed by fetal sex. All models included timepoint as an ordinal variable. A subject-level random effect was included in each of the models to account for the dependency between the repeated measures. A risk factor modeling approach was used to examine whether any covariates served as meaningful confounders in the relationship between fetal sex and maternal immune parameters.

3. Results

3.1. Maternal demographics by fetal sex

Participant characteristics for the total sample and by fetal sex are reported in Table 1. No significant differences in age, race, marital status, education, annual income, parity, BMI, gestational weight gain, cigarette use, or adverse outcomes were observed by fetal sex. Age, race, income, BMI, gestational weight gain, and cigarette use did not change coefficient estimate of fetal sex by greater than 15% and thus, consistent with a risk factor modeling approach (Bursac et al., 2008), these were not included in final analyses.

Table 1
Demographic and health characteristics.

	Total (n = 80)	Male Fetus (n = 46)	Female Fetus (n = 34)	Male v. Female Comparisons
Age [Mean (SD)]	25.4 (4.2)	24.9 (4.4)	26.0 (3.9)	p = 0.23
Race [n (%)]				
Black	39 (48.8)	22 (47.8)	17 (50.0)	p = 0.85
White	41 (51.2)	24 (52.2)	17 (50.0)	
Marital Status [n (%)]				p = 0.57
Married	33 (41.3)	17 (37.0)	16 (47.1)	
In a relationship	36 (45.0)	23 (50.0)	13 (38.2)	
Single	11 (13.8)	6 (13.0)	5 (14.7)	
Education [n (%)]				p = 0.47
High school graduate or less	22 (27.5)	15 (32.6)	7 (20.6)	
Some college	31 (38.8)	16 (34.8)	15 (44.1)	
College degree	27 (33.8)	15 (32.6)	12 (35.3)	
Income [n (%)]				p = 0.23
<$15,000	26 (32.5)	18 (39.1)	8 (23.5)	
$15,000–29,999	22 (27.5)	14 (30.4)	8 (23.5)	
$30,000–49,999	14 (17.5)	6 (13.0)	8 (23.5)	
>$50,000	18 (22.5)	8 (17.4)	10 (29.4)	
Parity (# of prev. births) [n (%)]				p = 0.47
0	27 (33.8)	13 (28.3)	14 (41.2)	
1	28 (35.0)	17 (37.0)	11 (32.4)	
2 or more	25 (31.3)	16 (34.8)	9 (26.5)	
BMI [Mean (SD)]	27.3 (6.0)	27.2 (6.6)	27.3 (5.4)	p = 0.98
Weight Gain [Mean (SD)]	32.5 (13.3)	33.9 (14.5)	30.7 (11.3)	p = 0.32
Cigarette Use [n (%)]				p = 0.59
Not Current	71 (88.8)	41 (89.1)	30 (88.2)	
Current	9 (11.3)	5 (10.9)	4 (11.8)	
Adverse Outcomes [n (%)]*	16 (20.0)	11 (23.9)	5 (14.7)	p = 0.31

Note: n = 9 are missing data on weight gain.
* Adverse outcomes defined as gestational hypertension, preeclampsia, gestational diabetes, low birth weight, and preterm birth.

3.2. Changes in serum cytokines and stimulated cytokine production across pregnancy

In the overall sample, significant changes in serum TNF-α ($p < 0.0001$) and IL-8 ($p = 0.004$) were observed over time. Specifically, TNF-α significantly increased between early and mid-pregnancy ($p < 0.0001$), with no change from mid- to late pregnancy ($p = 0.67$). IL-8 declined from early to mid-pregnancy ($p = 0.01$) and exhibited no change from mid- to late pregnancy ($p = 0.67$). No significant changes across pregnancy were observed for IL-6 ($p = 0.15$).

In relation to LPS-stimulated cytokine production, we have previously reported longitudinal changes across time in this study sample (Gillespie et al., 2016). Our prior report showed IL-6 and TNF-α production increased from early to mid-pregnancy, as well as mid- to late pregnancy. In addition, an increase from early to mid-pregnancy was observed in IL-1β production. No significant changes were found in IL-8 production across pregnancy (Gillespie et al., 2016).

3.3. Immune parameters across pregnancy by fetus sex

Mixed effects regression models showed no significant main effects of fetal sex on serum levels of IL-6 ($\beta = 0.02$, $SE = 0.12$, $p = 0.86$), TNF-α ($\beta = -0.01$, $SE = 0.04$, $p = 0.85$), or IL-8 ($\beta = 0.01$, $SE = 0.08$, $p = 0.87$) (Table 2). In contrast, as shown in Fig 1 and

Table 2
Models with immune parameters by fetal sex.

	F	df	p-value
IL-6			
Time	1.14	2143	0.32
Fetal Sex	0.03	1143	0.86
Time X Fetal Sex	1.17	2143	0.31
IL-8			
Time	4.48	2144	0.01
Fetal Sex	0.03	1144	0.87
Time X Fetal Sex	0.95	2144	0.39
TNF-α			
Time	11.71	2141	<0.001
Fetal Sex	0.04	1141	0.85
Time X Fetal Sex	00.17	2141	0.85
IL-6 production			
Time	20.70	2146	<0.001
Fetal Sex	15.37	1146	0.0001
Time X Fetal Sex	0.29	2146	0.75
IL-8 production			
Time	0.69	2130	0.50
Fetal Sex	0.29	1130	0.59
Time X Fetal Sex	0.34	2130	0.71
TNF-α production			
Time	22.38	2146	<0.0001
Fetal Sex	5.83	1146	0.02
Time X Fetal Sex	0.25	2146	0.78
IL-1β production			
Time	8.33	2144	0.0004
Fetal Sex	11.36	1144	0.001
Time X Fetal Sex	0.40	2144	0.67

Table 2, women carrying female versus male fetuses exhibited heightened levels of LPS-stimulated cytokine production of IL-6 ($\beta = -5027.72$, $SE = 1282.24$, $p = 0.0001$), TNF-α ($\beta = -608.55$, $SE = 252.02$, $p = 0.02$), and IL-1β ($\beta = -1403.54$, $SE = 416.41$, $p = 0.001$). Post-hoc simple effects at each timepoint showed significant differences in early ($\beta = -4259.17$, $SE = 1967.82$, $p = 0.03$), mid ($\beta = -6149.71$, $SE = 1959.20$, $p = 0.002$), and late ($\beta = -4674.28$, $SE = 2003.87$, $p = 0.02$) pregnancy for IL-6, early ($\beta = -750.77$, $SE = 356.65$, $p = 0.04$) pregnancy for TNF-α, and mid ($\beta = -1826.33$, $SE = 629.99$, $p = 0.004$) and late ($\beta = -1250.11$, $SE = 644.54$, $p = 0.05$) pregnancy for IL-1β. No differences emerged in stimulated production of IL-8 based on fetal sex ($\beta = -1681.45$, $SE = 3107.95$, $p = 0.59$). The observed differences in LPS-stimulated cytokine production by fetal sex remained when women with adverse outcomes (i.e., gestational hypertension, preeclampsia, gestational diabetes, low birth weight, or preterm birth; n = 16) were excluded from analyses.

4. Discussion

The current data demonstrate significant differences in maternal inflammatory immune function across pregnancy by fetal sex. Although no differences in serum cytokine levels emerged in relation to fetal sex, women carrying females versus males exhibited greater stimulated production of proinflammatory cytokines at early, mid, and late pregnancy. Specifically, after an immune challenge (i.e., LPS stimulation), women carrying females exhibited greater production of IL-6 at each timepoint, TNF-α in early pregnancy, and IL-1β in mid and late pregnancy. These data provide novel evidence on associations between fetal sex and maternal immune parameters.

Our finding of greater inflammatory cytokine production among women carrying females is consistent with prior data. As described earlier, prior data from pregnancies complicated by asthma show

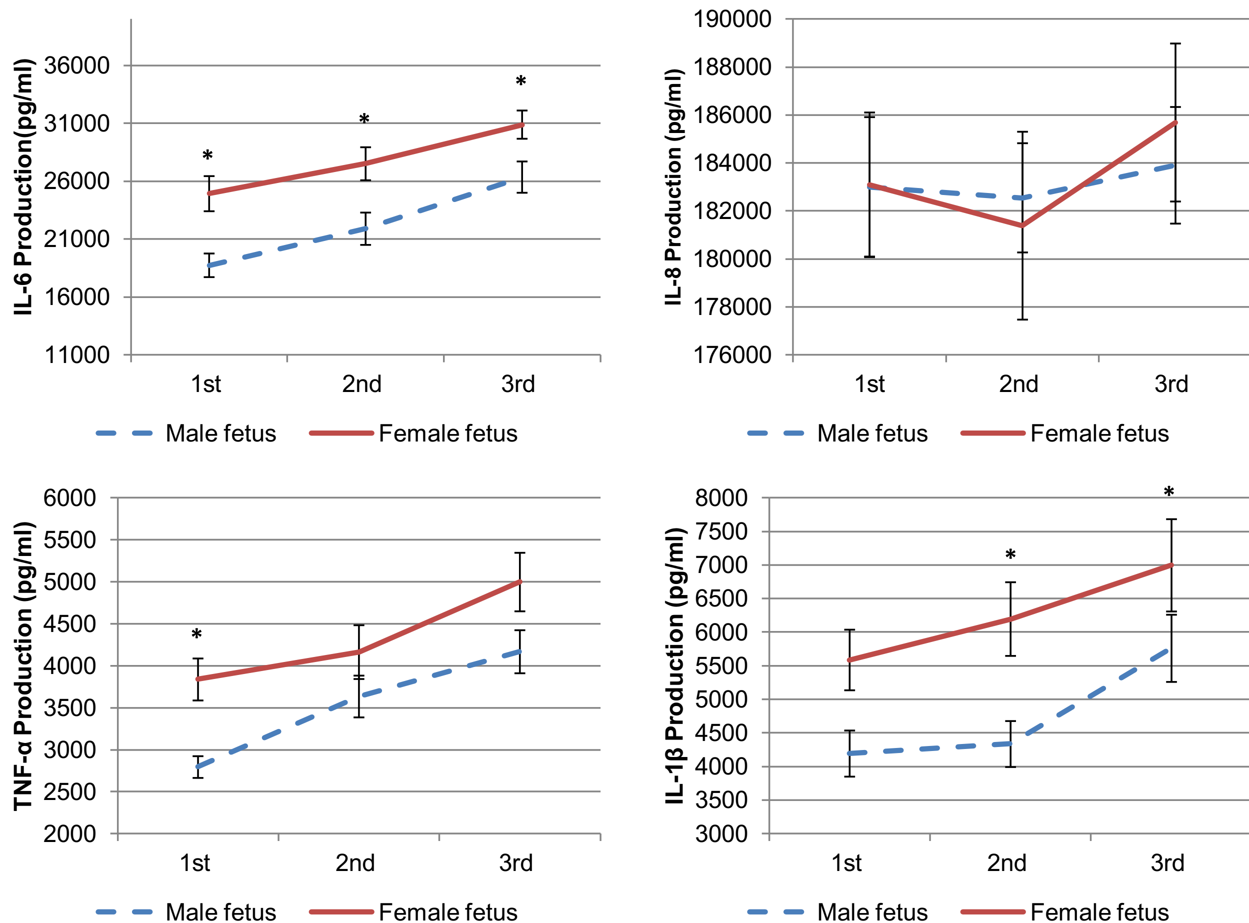

Fig. 1. LPS-stimulated proinflammatory cytokine production among women during pregnancy based on fetal sex. Numerical values on X-axis refer to early, mid, and late pregnancy. Asterisks signify significant differences by fetal sex at that timepoint. Linear mixed models showed that women carrying females versus males exhibited higher stimulated production of IL-6 at each timepoint ($ps \leqslant 0.03$), TNF-α in early pregnancy ($p = 0.04$), and IL-1β in mid- and late pregnancy ($ps \leqslant 0.054$).

heightened placenta cytokine expression among women carrying female fetuses compared to males (Scott et al., 2009). Similarly, elevated TNF-α and IL-1β responses have been found in women compared to men after PBMCs were pre-treated with interferon-γ or cigarette smoke extract and then stimulated with LPS (Moscovis et al., 2014). The current data extend the literature by showing that stimulated cytokine production by peripheral maternal PBMCs differs by fetal sex.

While data consistently show heightened asthma severity among women carrying females versus males, adverse outcomes, such as preterm birth and infant mortality, affect male fetuses at higher rates than females (Gregory and MacDorman, 2015). In addition, some data show heightened amniotic fluid cytokine levels in women carrying male fetuses compared to females (Chow et al., 2008). This could be due to sex-specific differences in survival strategies employed by the fetus (Clifton, 2010). For example, data indicate that female fetuses exhibit placental changes and reduced growth in response to maternal asthma (Murphy et al., 2003). In contrast, for male fetuses, typical growth is observed in the context of maternal asthma, but exacerbation of the condition has been associated with increased vulnerability for preterm birth (Murphy et al., 2005). Thus, male fetuses may be at risk for elevated inflammatory markers and adverse birth outcomes after exposure to multiple stressors (Clifton, 2010).

It is notable that in the current study, differences in maternal stimulated cytokine production, but not serum cytokine levels were seen based on fetal sex. Thus, such associations may only be observed in the context of an immune challenge. Of note, as is common practice, the LPS stimulation protocol in this study utilized 10% human male serum. Although it is perhaps unlikely given the magnitude of response elicited by LPS, it is possible that exposure to male serum affected our results. Thus, specific methodology should be carefully considered and alternatives compared in future studies.

Of note, while we observed no differences in serum levels of IL-6, IL-8, TNF-α by fetal sex, this is in contrast to Enninga et al. (2015) who reported higher TNF-α during mid to late pregnancy in women carrying males vs females, as well as differences in other markers not examined in the current study (e.g., IL-12p70). Thus, replication and extension of the current findings is warranted. In addition, in the current study, the latest assessment occurred at ~30 weeks gestation. This late pregnancy timepoint was selected to prevent systematic exclusion of women experiencing preterm birth. However, it is possible that inflammatory processes occurring in closer proximity to the initiation of labor could differ by fetal sex.

We observed a significant increase in serum TNF-α, decline in IL-8, and no significant change in IL-6 across pregnancy. Patterns of change in these markers replicate our prior findings from a different cohort of 60 women (Christian and Porter, 2014). Similarly, other groups have shown increases in serum TNF-α (Coussons-Read et al., 2007; Winkler et al., 2002). Some data show significant increases in serum IL-6 (Coussons-Read et al., 2007); a trend was observed for a similar effect in the current dataset ($p = 0.15$) as well as our prior cohort. Thus, the current data likely reflect typical serum cytokine patterns across pregnancy.

These data extend prior examinations of differences in maternal physiological adaptation across pregnancy by fetal sex (DiPietro et al., 2011; Giesbrecht et al., 2015; Hocher et al., 2009; Petry et al., 2007). For example, in cortisol, healthy pregnant women carrying female fetuses have exhibited flatter diurnal cortisol rhythms than women carrying males (Giesbrecht et al., 2015). However, the current findings demonstrate that maternal stimulated cytokine production is important to consider when examining bidirectional communication between pregnant women and fetuses.

Findings from the current study remained when women with pregnancy complications and adverse outcomes of preeclampsia, gestational hypertension, gestational diabetes, low birth weight, and preterm birth were excluded from analyses. As such, these data reflect typical fetal sex differences in maternal inflammation within the context of a healthy pregnancy. This study was not comprised of a large enough cohort to examine fetal sex differences among women experiencing these pregnancy complications. The potential role of differences in maternal immune adaptation associated with fetal sex in such complications should be considered in future studies.

In addition, infectious illnesses (e.g., influenza) and health conditions (e.g., Multiple Sclerosis) offer unique maternal immune milieus during pregnancy to continue examinations of fetal sex-specific differences. For example, animal models show greater adverse immune reactions to the influenza infection in females compared to males (Larcombe et al., 2011), and human studies show that pregnant women are at greater risk than the general population for complications associated with influenza (Mak et al., 2008). Thus, it is possible fetal sex plays a moderating role in these complex relationships.

The role of fetal signals in influencing maternal physiological patterns during pregnancy is receiving increasing attention (e.g., Glynn and Sandman, 2011). However, the specific mechanisms remain largely unknown. Although mixed, some data suggest differences in maternal levels of progesterone and estradiol by fetal sex (Haning et al., 1989; Toriola et al., 2011; Wuu et al., 2002). In addition, lower maternal testosterone levels have been found in women carrying females versus males in mid- and late pregnancy (Meulenberg and Hofman, 1991) and testosterone has been shown to suppress cytokine expression (Fish, 2008). Finally, some studies have shown women carrying females versus males exhibit flatter cortisol rhythms as well as elevated levels of human chorionic gonadotropin (hCG) and leptin (Al Atawi et al., 2005; Giesbrecht et al., 2015; Gol et al., 2005); these could in turn affect immune functioning. Continued examination of potential mechanisms will address remaining questions.

Conversely, we cannot exclude the possibility that the maternal immune milieu selects for fetal sex. Maternal exposure to stressors, including war or natural disasters, has been associated with differences in offspring sex ratios (Grant, 2009). However, this relationship is complex. On one hand, stress-induced testosterone secretion proximal to *conception* as well as high circulating maternal glucose may favor increased male births (Grant and Chamley, 2010). However, maternal stress in *early pregnancy* has been linked with risk for spontaneous abortion, which disproportionately affects males (e.g., Catalano et al., 2006). Thus, studies initiated prior to conception would help to elucidate the directionality of the observed relationship between fetal sex and maternal immune function.

In sum, these data provide novel evidence of associations between fetal sex and maternal immune parameters across pregnancy, with greater inflammatory responses after an immune challenge in women carrying females versus males. These effects remained when women experiencing adverse outcomes were excluded, indicating that these patterns are present in the context of healthy pregnancy. From an empirical perspective, these data suggest that in studies of maternal immune parameters in pregnancy, the role of fetal sex should be considered within statistical models. These findings have clinical and conceptual relevance for understanding effects of fetal sex on maternal asthma during pregnancy. In addition, examination of the potential moderating role of fetal sex differences among women with health conditions (e.g., preeclampsia) and adverse pregnancy outcomes (e.g., preterm birth) in future studies would be informative.

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Conflict of interest

The authors report no potential conflicts of interest.

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