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Review

Sex differences in innate and adaptive immunity impact fetal, placental, and maternal health†

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Abstract

The differences between males and females begin shortly after birth, continue throughout prenatal development, and eventually extend into childhood and adult life. Male embryos and fetuses prioritize proliferation and growth, often at the expense of the fetoplacental energy reserves. This singular focus on growth over adaptability leaves male fetuses and neonates vulnerable to adverse outcomes during pregnancy and birth and can have lasting impacts throughout life. Beyond this prioritization of growth, male placentas and fetuses also respond to infection and inflammation differently than female counterparts. Pregnancies carrying female fetuses have a more regulatory immune response, whereas pregnancies carrying male fetuses have a stronger inflammatory response. These differences can be seen as early as the innate immune response with differences in cytokine and chemokine signaling. The sexual dimorphism in immunity then continues into the adaptive immune response with differences in T-cell biology and antibody production and transfer. As it appears that these sex-specific differences are amplified in pathologic pregnancies, it stands to reason that differences in the placental, fetal, and maternal immune responses in pregnancy contribute to increased male perinatal morbidity and mortality. In this review, we will describe the genetic and hormonal contributions to the sexual dimorphism of fetal and placental immunity. We will also discuss current research efforts to describe the sex-specific differences of the maternal–fetal interface and how it impacts fetal and maternal health.

Summary Sentence
Fetal sex influences the fetal, placental, and maternal immune response during pregnancy.

Keywords: sexual dimorphism, innate immunity, adaptive immunity, placenta, pregnancy

Introduction

The phenotypic differences between males and females begin early in life, occurring shortly after fertilization when the zygote begins rapidly dividing. Male embryos reach the blastocyst stage faster and consist of more cells than female embryos [1, 2]. This increase in growth is complemented by increased metabolic activity, with male embryos taking up higher levels of pyruvate and glucose and producing higher levels of lactate [3]. Alternatively, female embryonic and fetal growth rate is more closely regulated and thought to modulate nutrient uptake in an adverse prenatal environment [4]. The trade-off of investing less in placental reserves and investing more in rapid growth, even in the event of compromised nutrition and placental function, leads male fetuses to "live dangerously in the womb" [5]. This strategy is considerably riskier and puts male fetuses at increased risk of adverse prenatal and neonatal outcomes [5].

The disparity between male and female births was first documented in 1788 by Dr Joseph Clarke who described a higher rate of stillbirth and mortality in male neonates [6]. Reports of increased rates of preterm birth, spontaneous abortion, small for gestational age (SGA), preeclampsia, and gestational diabetes in males followed [7–11]. Some of these outcomes are explained by the prioritization of feto-placental growth over the maintenance and supplementation of the placental energy reserve during male pregnancies [12]. However, there appear to be sex-specific differences in innate immune signaling and maternal–fetal antibody transfer as well [13, 14]. Collectively, these studies suggest fetal sex influences the placental and maternal immune response during pregnancy. However, while these studies provide insight into how fetal sex influences pregnancy outcomes, there is very little insight into the molecular mechanisms that leave male fetuses vulnerable to neonatal demise. In this review, we will outline how fetal sex influences the placental immune response and how those responses eventually influence pregnancy outcomes and fetal and maternal health (Figure 1).

Long-term effects of sex on adverse pregnancy outcomes

We will begin this review by outlining how the effects of the influence of fetal sex on adverse pregnancy outcomes can have lifelong consequences. The theory of developmental origins of health and diseases asserts that the in utero environment influences long-term health and risk of developing diseases later in life [15]. Associations have been made between maternal stress, obesity, infection, tobacco use, and diet and the development of cardiovascular, metabolic, and neurocognitive disorders later in life [16–21]. Development of negative postnatal outcomes in these cases is dependent on the type of in utero insult, the duration of fetal exposure to the compromised uterine environment, and the gestational timing of the insult in question. During pregnancy, male

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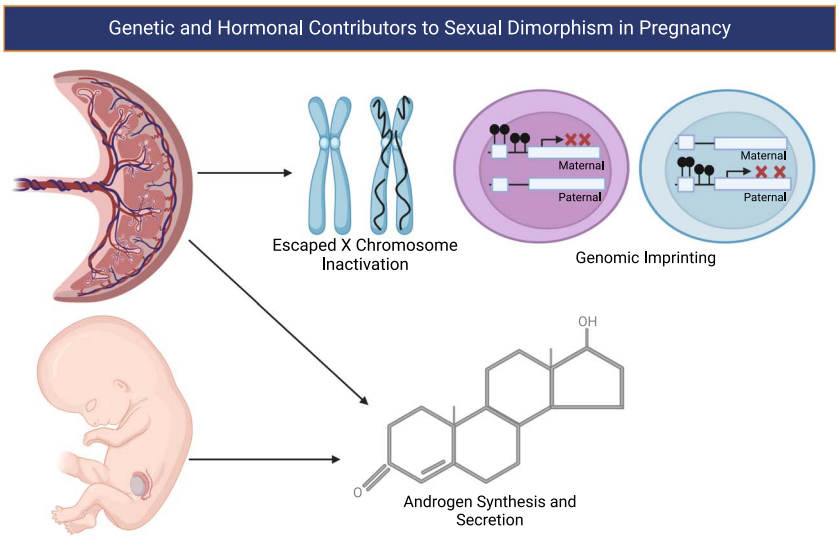

Figure 1. Summary of genetic and hormonal contributors to the sexual dimorphism of pregnancy.

fetuses are subject to increased severity of disease phenotypes and earlier onset thereof. However, female fetuses may be more susceptible to other complications during pregnancy (e.g., preterm birth) [22, 23]. Furthermore, a growing body of evidence has identified fetal sex as a factor in the eventual development of cardiovascular disease, kidney disease, and the incidence of neurodevelopmental delays in offspring with low birth weight [24–29].

Maternal nutrition

Rodent models demonstrate that undernutrition during pregnancy increases the risk of disease development in offspring in a sex-specific manner. Maternal undernutrition during preimplantation resulted in a greater incidence of hypertension in male offspring than in female littermates, along with earlier onset and greater severity of hypertension [30, 31]. Similarly, perinatal iron deficiency elicited defects in modulation of vascular tone and an increased risk of cardiovascular diseases exclusively in male offspring [32]. In contrast, fetal exposure to a maternal high fat diet induced endothelial dysfunction in male and female offspring but caused hypertension only in females [33]. Diet-induced obesity also resulted in increased insulin resistance in male rat pups [34].

Adult men born to obese mothers secrete more insulin compared with men born to lean mothers [35]. Additional nutritional information has been gleaned from studies assessing children and adults born during periods of famine. In multiple studies, female offspring born to mothers affected by famine during gestation were found to exhibit higher rates of obesity than their male counterparts [36, 37]. The diversity in long-term outcomes of maternal nutrition on offspring development appears to result predominantly from a combination of the nature of the insult (caloric restriction, nutrient restriction, high-fat diet, etc.) and the timing of the insult as it relates to critical periods of development for fetal tissues and systems [38].

Maternal stress

Maternal stress is another risk factor in the development of pregnancy complications, with the capacity to impact long-term developmental outcomes for the offspring. Stress is a physiological state that provokes the release of hormones and chemical mediators including glucocorticoids like cortisol, which function to decrease stress levels via immunosuppressive and anti-inflammatory properties; however, chronic exposure to stress can evoke pro-inflammatory responses [39]. In mice, early prenatal stress can increase the expression of cytokines and chemokines in male placentas (e.g., IL-6 and CXCL-10) [40]. However, female offspring from unstressed control animals had significantly increased serum corticosterone levels in comparison to males, a phenotype that was ablated by maternal stress during midgestation [41]. This work is corroborated by Alonso et al. [42], who demonstrated that stress in pregnant rats induces depressive symptoms in female, but not male, offspring in comparison to unstressed controls.

In cohort studies, mothers who reported higher psychosocial stress during pregnancy had children with an increased risk of developing wheeze past one year of age, and a rate of incidence in female offspring higher than in males [43]. Demographic research in times of increased stress and hardship like those during war demonstrated an increase in male infant mortality despite there being a significant increase in the number of male births [44–47]. To date there is no evolutionary explanation for the increased number of boys born during and immediately after wars.

To assess the impact of maternal stress on pregnancy, researchers quantified maternal cortisol levels and reported that increased cortisol levels were significantly associated with increased healthcare requirements for offspring, caused by decreased cognitive/motor development and increased rates of psychological and behavioral problems [48]. Birth cohort studies demonstrated that maternal stress increases the risk of developing schizophrenia- and autism spectrum disorders (ASD) in male offspring [49, 50]. Cortisol and other corticosteroids play key roles in activating anti-inflammatory and stress response pathways; however, chronic elevation of these stress hormones places mothers and fetuses at higher risk of pregnancy complications and postnatal developmental delays, seemingly in a sex-specific manner.

Maternal infection

Mounting evidence implicates inflammation resulting from maternal infection as a detrimental factor in central nervous system health of offspring, both acutely in the fetal context and chronically in the adult brain. The brain is especially vulnerable to insult during fetal life and infancy, and several neurodevelopmental disorders have been linked to inflammation (e.g., ASD, schizophrenia, epilepsy, and depression) [51]. While there is considerable phenotypic overlap among these disorders, their occurrence exhibits substantial sexual dimorphism in that males are more prone to developing neurodevelopmental disorders like ASD, whereas depression is more common in females [52–54].

It is possible that the observed sex differences in the developmental origins of adult diseases result from differences in male and female fetal capacity to respond to external stimuli or insults. However, since maternal stressors during pregnancy are mediated by maternal physiology and immunology, the effects of these stressors are not directly applied to the growing fetus but are instead translated via the placental interface. Bidirectional influence across this interface may elicit alterations in maternal physiology (e.g., genetic, epigenetic, or hormonal) in a manner contingent upon fetal sex, which may be exacerbated in instances of uterine insult during gestation.

Genetic influence of fetal sex and placental immunity

Until recently, the placenta was considered an asexual organ. However, as the early embryo gives rise to the placenta, it contains the same sex chromosomes as the fetus. Sex chromosomes influence placental function, signaling, and ability to respond to pathogens in the same way sex chromosomes influence fetal function and development. Similarly to the embryo, sex-specific differences related to metabolism and growth have been reported in the placenta. In term, human placentas, placental weight, and the fetal:placental weight ratio are greater in male compared with female placentas [55]. Additionally, gene expression experiments using first-trimester chorionic villous tissue samples revealed that male first-trimester placentas had several enriched genes related to metabolism and biosynthesis [56]. Further work analyzing the transcriptome of late first-trimester placentas described increased levels of regulators of fetal growth in male placentas, whereas female placentas had enhanced levels of genes related to immunity and cytokine-mediated signaling [57]. These findings are consistent with the idea that male fetuses and placentas prioritize growth at the expense of themselves and the mother. Interestingly, these data also suggest that female placental cells place a higher priority on response to surroundings and immune function.

X-chromosome inactivation

The X chromosome contains many genes related to immune regulation, ranging from pattern recognition receptors (PRRs) to transcription factors to cytokine receptors [58]. To prevent receiving a double dosage of X-chromosome genes, female somatic tissues must silence one X chromosome during embryogenesis [59, 60]. This phenomenon, known as X-chromosome inactivation (XCI), also occurs in extraembryonic and placental tissues. Contrary to the mouse, where the paternal placental X chromosome is preferentially silenced, XCI in the human placenta is random [61]. Furthermore, XCI is a highly variable process in the human with ~15% of the 1400 genes found on the X chromosome either partially or completely escaping XCI [62].

Incomplete XCI in the placenta contributes to sex differences in the placental innate immune response. Compared with all other chromosomes, the X chromosome holds the highest number of immune-related genes [63]. Several immune-related genes including cluster of differentiation 99 (*CD99*), interleukin 3 receptor subunit alpha (*IL3RA*), lysosomal-associated membrane protein 2 (*LAMP2*), ornithine transcarbamylase (*OTC*), X-linked inhibitor of apoptosis (*XIAP*), protein kinase cAMP-dependent X-linked catalytic subunit (*PRKX*), DEAD-box helicase 3 X-linked (*DDX3X*), dystrophin (*DMD*), thymosin beta 4 X-linked (*TMSB4X*), colony stimulating factor 2 receptor subunit alpha (*CSF2RA*), interleukin 1 receptor-associated kinase 1 (*IRAK1*), chromosome X open reading frame 21 (*CXorf21*), toll-like receptor 7 (*TLR7*), transmembrane protein 187 (*TMEM187*), CD40 ligand (*CD40L*), and CXC motif chemokine receptor 3 (*CXCR3*) at least partially escape XCI in adult immune cells [64–67]. Sexually dimorphic placental gene expression of several of these genes has also been reported. *DDX3X* is upregulated in female first-trimester and term placental cells compared with male [56, 57, 68]. Another study found that *TMSB4X* is significantly higher in female trophoblast cells of the first-trimester placenta [57]. Additionally, *TLR7* is hypermethylated in male term placental cells [69]. In term fetal-origin macrophage cells of the placenta, the Hofbauer cells, *IL3RA* is upregulated in female cells [70]. These findings all suggest that escape from XCI is prevalent in female placental cells and affects the placenta's ability to respond to infection and inflammation.

Interestingly, several of the above-mentioned genes are found on pseudoautosomal regions (PARs), which are short regions of homologous sequences of nucleotides between the X and Y chromosomes. PARs lie on the terminal ends of the X and Y chromosomes and the sequence homology allows for recombination between the chromosomes during meiosis, which is important for genetic diversity in reproduction [71]. PARs are often enriched with genes that code for immune signaling. *CD99* is found on a PAR and is significantly higher in the undifferentiated, multipotent progenitor cells of the placenta, the cytotrophoblast (CTB) cells, of males at all points in gestation [68, 72, 73]. An additional study observed clusters of X-linked genes biased toward male overexpression on placental PARs [74]. These data suggest that the placenta has a persistent bias of male PAR gene expression throughout development, potentially to help overcome the "leaky" XCI seen in female pregnancies.

Imprinted genes

Mammals use genomic imprinting to modulate fetal growth and assist placental development [75]. Imprinting prevents biallelic expression of autosomal genes by using epigenetic processes to suppress one allele of a certain gene. These silenced or "imprinted" genes become monoallelically expressed based on parent of origin (i.e., paternally or maternally imprinted). There are ~100 imprinted genes currently identified in the human genome, many of which are found in the placenta [76]. Some paternally expressed imprinted genes drive fetal growth, whereas maternally expressed imprinted genes regulate and suppress growth [77, 78]. This partially explains that the sex-specific differences in fetal growth and birth weights as male embryos, fetuses, and

neonates are larger, grow faster, and metabolize glucose more rapidly [29, 79–83]. Additionally, as maternally expressed, paternally imprinted genes are programmed to induce an adaptive response to preserve limited maternal resources upon insult [84], it provides context as to why male fetuses and neonates are more vulnerable to adverse pregnancy outcomes [85].

Genomic imprinting also influences the immune response. Imprinted genes regulate differentiation and activation of T and B lymphocytes, influence the expression of proinflammatory cytokines, and transactivate nuclear factor kappa B subunit 2 (NF-κB) [86–88]. Imprinted domains also contain clusters of microRNAs (miRNAs) [89]. The chromosome 19 MiRNA cluster (C19MC) found at Chr19q13 is a paternally-expressed, primate-specific miRNA cluster found exclusively in the placenta [89]. This cluster is most well-known for its regulation of migration and invasion in extravillous trophoblast (EVT) cells [90–95] but may also help protect the placenta against hypoxic stress [96]. Recent studies show that the C19MC cluster confers viral resistance against Zika virus, coxsackievirus B3, poliovirus, vesicular stomatitis virus (VSV), vaccinia virus, herpes simplex virus-1, and human cytomegalovirus in primary human trophoblast cells (PHT) [97, 98]. Exosomes collected from spent media of PHT contain C19MC miRNAs and treatment of cells with exosomes collected from spent PHT culture medium protected cells from VSV infection [97]. Transfection of cells with C19MC mimics induced autophagy and attenuated viral infection [98]. Further experiments indicated that C19MC miRNAs work independently of interferon pathways but induce autophagy through a currently unknown antiviral mechanism [97, 98]. These studies did not assess sex-specific differences in C19MC miRNA expression between male and female PHTs or determine a mechanism for miRNA viral resistance. Nonetheless, these studies provide an interesting insight into how imprinted genes regulate immunity during early placental development. Further research into how fetal sex and genomic imprinting influence antiviral activity during pregnancy is necessary to better understand how this mechanism may protect fetuses from insult.

Sex steroid hormones from the fetus and placenta

The fetal hypothalamus begins to develop as early as the 5th week of gestation and the presence of gonadotropin-releasing hormone can be found between weeks 14 and 16 [99]. By 18 weeks of gestation, the hypophyseal portal system has fully developed and begins releasing hormones to the anterior pituitary [99]. Completing the fetal hypothalamic–pituitary–gonadal axis, the bipotential gonad begins to differentiate toward either the testis or the ovary shortly after 7 weeks of gestation [100]. Secretion of testosterone from the Leydig cells of the fetal testis is critical for the formation of the male genital duct system and subsequent sex determination in males [101]. Prenatal testosterone is also critical for the defeminization and masculinization of male fetal brains [102].

The fetal testes begin synthesizing and secreting testosterone between 8 and 10 weeks of gestation [103]. Fetal circulating testosterone peaks at approximately week 16 and reaches concentrations analogous to circulating androgens in postpubertal males [72]. During this peak period, the testosterone concentrations of amniotic fluid are 2–5-fold higher in male compared with female amniotic fluid [104, 105]. Synthesis and secretion of testosterone and dihydrotestosterone (DHT) are critical for the formation of the internal duct system as well as development of male external genitalia [106]. However, by gestational week 24, testosterone levels stabilize and there are no discernable differences in sex hormone circulation between male and female fetuses [107]. While little is understood of how fetal testosterone might impact placental endocrine and developmental function, there is evidence to suggest that androgen production and function is different in male placentas.

First- and third-trimester male placentas have de novo androgen synthesis abilities and testosterone precursors as well as testosterone and DHT are detectable as early as the first trimester [108, 109]. Additionally, as fetal DHT is synthesized by the testis, only male fetuses synthesize and secrete DHT [110]. Single-cell RNA-sequencing of the first-trimester placenta revealed that DHT regulates genes critical for trophoblast differentiation, migration, invasion, and immune modulation [57]. One target gene of interest is transforming growth factor beta 1 (*TGFB1)*, a cytokine critical for the maintenance of fetal-maternal immune tolerance [111]. Dysregulated TGFB1 is present in decidual tissue and plasma of patients suffering from recurrent spontaneous abortion and preeclampsia [111–113]. Differential regulation of TGFB1 production by DHT could potentially explain some of the differences in immune and trophoblast function between male and female placentas.

Several studies have documented abnormal androgen levels in pregnancies affected by adverse outcomes. Maternal serum testosterone levels were significantly higher in male and female preeclamptic pregnancies when compared with normotensive pregnancies [114]. Additionally, androgen levels in maternal serum from male-bearing preeclamptic pregnancies were significantly higher than female-bearing preeclamptic pregnancies [114]. Elevated maternal serum testosterone levels in the second and third trimester also correlated with lower birth weight and fetal length, although sex-specific differences were not found [115]. Further studies have confirmed that elevated circulating prenatal maternal serum testosterone because of polycystic ovarian syndrome is associated with intrauterine growth restriction and SGA babies [116]. Voegtline et al. assessed the levels of maternal salivary testosterone at 36 weeks of pregnancy and found that elevated salivary testosterone was associated with lower birth weight. Furthermore, in pregnancies with elevated maternal salivary testosterone, male birth weights were more negatively impacted than female [117]. Collectively, these descriptive studies suggest that fetal, placental, and maternal androgens do affect male prenatal development and growth in a sex-specific manner, yet the physiological mechanisms remain to be described.

While fetal hormones are critical for the masculinization of male fetuses, most sex steroids come from the placenta. Hormone production in pregnancy is tightly regulated and each compartment (maternal, fetal, and placental) has specific rate-limiting enzymes that tightly control the synthesis of each hormone [72]. All forms of estrogen (estriol, estrone, and estradiol) and progesterone are primarily synthesized by the placenta and are critical for the maintenance of pregnancy and prevention of parturition [118, 119]. While sex-specific differences in placental estrogen receptor 1 expression have been reported [56, 120], there is no evidence that placental sex affects synthesis or secretion of either estrogen or progesterone.

Effect of fetal sex on placental innate immunity

Cytokine signaling in normal pregnancies

Immune cells at the maternal–fetal interface encounter the complex challenge of adapting to the changing uterine environment throughout progression of pregnancy. The maternal immune system must fluidly change between pro- and anti-inflammatory profiles in response to developmental progress of the fetus [121]. Approximately 40% of the decidua is populated with immune cells [122]. Uterine natural killer cells (uNK) are the most abundant immune cell type, making up 70% of all decidual first-trimester leukocytes [123, 124]. Macrophages, dendritic cells, T cells, and B cells are also present, each with their own unique function to help with the establishment and maintenance of a successful pregnancy and mediate protection against pathogens [125, 126]. The other component of the maternal–fetal interface is the placenta, which provides a physical barrier against pathogens and interacts with the maternal environment to protect the fetus from the maternal immune system [127].

To detect pathogens, PRRs are found throughout the placenta and play key roles in innate immunity at the maternal–fetal interface [128–130]. Toll-like receptors (TLRs) are specific PRRs that recognize pathogens to regulate the induction of inflammation, triggering innate and adaptive immune responses. In healthy term placentas, *TLRs* 1–10 are expressed at the RNA level, and expression levels of all 10 human *TLRs* are found at varying levels within first-trimester primary trophoblasts, term primary CTBs, and several choriocarcinoma cell lines [131–133]. Work with mid-gestation mouse placentas illustrated significant differences in the expression of TLRs between the sexes [134]. Barke et al. [134] described higher levels of *TLR6* and *TLR8* in male placentas, whereas female placentas expressed significantly higher levels of *TLR5*, implicating sex-specific differences in the initial activation of the innate immune responses and susceptibility to infection. Once activated, TLRs employ various intracellular signaling cascades to initiate the production of pro-inflammatory cytokines or type I interferons (IFNs) [135].

IFNs are cytokines that facilitate communication between cells and induce pro- and anti-inflammatory pathways depending on the requirements for pathogen clearance. Binding of IFNs to their receptors results in the activation of the JAK/STAT pathway and induces the transcription of interferon stimulation genes (ISGs). Activation of the IFN signaling pathways results in the induction of hundreds to thousands of ISGs, which work to restrict viral replication, entry, spread, and modulate the immune system [136]. The placenta, especially during the first trimester, expresses abundant levels of type I and II IFNs. Microarray data of the villous parenchyma from term placentas revealed that several of immune and IFN-associated genes are expressed at higher levels in female placentas including Janus kinase 1 (*JAK1*), interleukin 2 receptor subunit beta (*IL2RB*), CXC motif chemokine ligand 1 (*CXCL1*), and interleukin 1 receptor like 1 (*IL1RL1*) [137]. Additional transcriptome analysis of first-trimester human placentas identified the increased expression of CC motif chemokine ligand 3 (*CCL3*), CC motif chemokine ligand 4, and CXC motif chemokine ligand 8 in female trophoblasts [57]. This study also assessed the transcriptome of the placental macrophages, Hofbauer cells, and found that *CCL13* was upregulated in female Hofbauer cells [57]. Overall, global gene expression analysis of male and female placentas determined that male CTB cells expressed an increased number of genes in comparison to female cells. Interestingly, a large portion of the differentially expressed genes were associated with the inflammation-associated transcription factor pathway NF-κB [138]. Collectively, these data suggest that even in uncomplicated, normal pregnancies there are sex-specific differences in placental cytokine signaling.

Cytokine signaling in pathological pregnancies

More recent work has focused on dysregulated type I IFN signaling, leading to obstetric complications including preeclampsia, preterm birth, and neurocognitive impairments in the offspring [139–141]. Cappelletti et al. [139] demonstrated the effects of recombinant type I IFNs on induction of preterm birth in mice, an effect alleviated by knockout of interferon alpha and beta receptor, and additionally confirmed the significant upregulation of type I IFNs and proinflammatory cytokines in the chorion–amnion–decidua of human preterm births. These findings complement the known phenomenon that chronic upregulation of type I IFN, like in women with systemic lupus, can increase risk of preeclampsia or miscarriage [140, 142]. Therefore, while interferon signaling plays a role in the evolutionary advantage of immune protection, aberrant signaling can have detrimental effects on pregnancy outcomes.

There is growing evidence of a marked sex-specific difference in the interferon response and the severity of COVID-19 infection in male infants and children [143, 144]. These differences begin in utero, as female term placentas from mothers infected with SARS-CoV-2 had higher levels of placental transmembrane serine protease 2 (TMPRSS2) expression, whereas male placentas had reduced TMPRSS2 [145]. However, in noninfected mothers, TMPRSS2 is significantly higher in male placentas compared with female [145]. As TMPRSS2 is one of the host molecules necessary for SARS-CoV-2 viral entry, the increase of TMPRSS2 in male placentas offers a potential explanation for why there is sex-specific vulnerability to placental SARS-CoV-2 infection.

Bordt et al. noted significant increases in the expression of ISGs: interferon alpha inducible protein 6 (*IFI6*), *CXCL10*, 2′-5′-oligoadenylate synthetase 1, C-C motif chemokine ligand 2, and interleukin 10 (*IL-10*) and type I and II IFNs specifically in male placentas of women with SARS-CoV-2 infections compared with male placentas from uninfected pregnancies. However, these effects were not noted in their female placental cohorts [146]. Interestingly, only *IFI6* and *CXCL10* were significantly different between male and female placentas from pregnancies with a SARS-CoV-2 infection. However, control, noninfected female placentas had significantly higher expression of ISGs than those from males [146]. The male-specific increase in placental ISGs after SARS-Cov-2 infection is consistent with the heightened immune response in male children and adults infected with SARS-Cov-2 [144, 147]. This discrepancy in the baseline level of ISGs between male and female placentas could possibly explain the increased vulnerability of male fetuses to in utero insults from pathogens and in utero insults. In comparison to COVID-19, pregnant women carrying female fetuses were at a higher risk of developing a placental malaria infection [148]. While a mechanism for

this increased susceptibility to the malaria parasite remains unknown, it is speculated that hormones, immune modulation, as well as varying glucocorticoid receptor isoforms between sexes could play a role [148, 149].

Immune activating in vitro and in vivo studies

In vitro studies using Hofbauer cells treated with lipopolysaccharide (LPS) and polyinosine-polycytidylic acid (PolyI:C) mounted stronger immune responses when cells were isolated from female placentas [70]. Similarly, the use of LPS on human primary placental trophoblast cells from healthy pregnancies increased the secretion of tumor necrosis factor alpha (TNF-α) and IL-10 in male placental cells [150]. Yeganegi et al. [150] hypothesized that increased TLR-4 expression in male placentas could be driving increased secretion of TNF-α and IL-10, leading to sex-specific differences in the immune response after LPS stimulation.

In animal models, one study exposed pregnant rodents to inflammation noting increased expression of 17 inflammatory genes (*IRF*s and *STAT*s) in male placentas, whereas 45 genes were significantly altered in female placentas (*IRFs*, *STATs*, and *Ifnb1*). Pathway analysis also indicated female placentas had significant alterations in the insulin/IGF/MAPK pathways [134]. Female rat placental sections exposed to LPS and PolyI:C immune activation resulted in increased vulnerability to LPS significantly higher levels of TNF-α secretion in females [151]. Additionally, pregnant mice exposed to stress during pregnancy reported sex-specific changes in placental nutrient and oxygen transport gene expression. Male placentas had significantly elevated levels of peroxisome proliferator-activated receptor alpha (*PPARα),* insulin-like growth factor binding protein 1 *(IGFBP-1),* solute carrier family 2 member 4 *(SLC2A4/GLUT4),* and hypoxia inducible factor 3 subunit alpha, whereas female placentas had reduced *PPARα* and *IGFBP-1*. Additionally, while DNA methyltransferase 1 (*DNMT1)* was lower in control female placentas compared with male, maternal stress seemingly only increased *DNMT1* in female placentas, suggesting that female placentas have increased capacity for methylation during periods of maternal stress [152]. Follow-up work using stress models further demonstrated that prenatal stress induced upregulation of the pro-inflammatory cytokines IL-1β and IL-6 and chemokines CCL5 and chemokine ligand 10 [40]. In addition, prenatal stress increased levels of genes stimulated by inflammatory pathways. These genes include interleukin 2 receptor subunit alpha, prostaglandin-endoperoxide synthase 2, protein tyrosine phosphatase receptor type C, P-selectin, and endothelin 1 [40]. Collectively, these data indicate that prenatal stress induces an inflammatory state in male placentas.

There is overwhelming evidence that the placental immune response is sex-dependent. Based on the studies we described earlier assessing differentially expressed genes in normal placentas, there are baseline differences in the placenta's ability to interact with the decidua even in the absence of pathogens. For example, as we mentioned earlier Sun et al. described an increase in *CCL3* in female first-trimester placentas. CCL3 works through its decidua-specific receptor, CC motif chemokine receptor 1, to recruit monocytes to the decidua [153]. This finding is complemented by the increase of *CCL13* in female first-trimester Hofbauer cells, as CCL13 is also implicated in the recruitment and attraction of immune cells to the decidua [57]. These data imply that more immune cells are recruited to the female maternal–fetal interface and suggest a more regulatory role for immune cells during pregnancies carrying a female fetus. Other genes described in the above paragraphs include *JAK1, IL2RB, CXCl1, and IL1R1*. These are all genes critical for regulation of the immune response. JAK1 is an activator of the STAT signaling pathway and activates the transcription of many cytokines. Additionally, loss of JAK1 in a mouse model led to a deficiency in the innate immune response [154]. CXCL1 modulates the early innate immune response by recruiting neutrophils [155] but also is implicated in the stimulation of decidual angiogenesis during early pregnancy [156]. These differences in the expression of genes involved in the recruitment and regulation of immune cells suggest that the female placenta enjoys a more tightly regulated immune response, making it able to respond more effectively to insults during pregnancy. There is a clear sexual dimorphism of the infection rate and incidence of pregnancy complications leading to preterm birth and other adverse pregnancy outcomes. Better understanding mechanistically how fetal sex influences the regulation of the immune milieu of the maternal–fetal interface will have important implications in the prevention and treatment of infections and inflammation during pregnancy [157] (Figure 2).

Effect of fetal sex on adaptive immunity at the maternal–fetal interface

T cells

During pregnancy, the uterine milieu is dynamic and immune cell populations adapt to meet the needs of the fetus and the mother. Uterine NK cells are the most abundant lymphocytes within the decidua immediately after implantation. However, as pregnancy progresses, T cells comprise ~10–20% of the decidual leukocyte population during the first trimester and expand in number throughout gestation until birth [158, 159]. Many subsets of T cells have been described within the uterus over the course of pregnancy, but effector T cells account for ~60% of decidual lymphocytes by the third trimester [122, 159–161].

During the second trimester, CD8+ T cells are among the most common lymphocytes in the uterus because of their rapid expansion in number and concurrent decline in uNK cell abundance [159]. EVT cells express human leukocyte antigens (HLA) C, E, and G that are recognized by uNK and T cells to regulate trophoblast invasion, spiral artery remodeling, and promote immune tolerance [162, 163]. As T-cell populations grow during pregnancy, so does the expression of HLA molecules on EVTs, specifically HLA-G [163]. CD4+ T cells express the HLA-G receptor, leukocyte immunoglobulin (IgG)-like receptor B1, and HLA-G assists in transforming T cells into Tregs [164]. Interestingly, HLA-G expression in term EVT is higher in placentas from a male fetus [165]. This finding is in line with previous reports that found increased levels of soluble HLA-G in the amniotic fluid of male fetuses [166]. As sex hormones influence soluble HLA-G expression, HLA-G is potentially stimulated by fetal testosterone and increased HLA-G could protect against an increased maternal immune response because of the presence of Y chromosome-related antigens like H-Y [167, 168]. Finally, a Finnish study assessing the sex ratios and *HLA-G* haplotypes in preeclampsia and stillbirth documented that the highest risk for preeclampsia

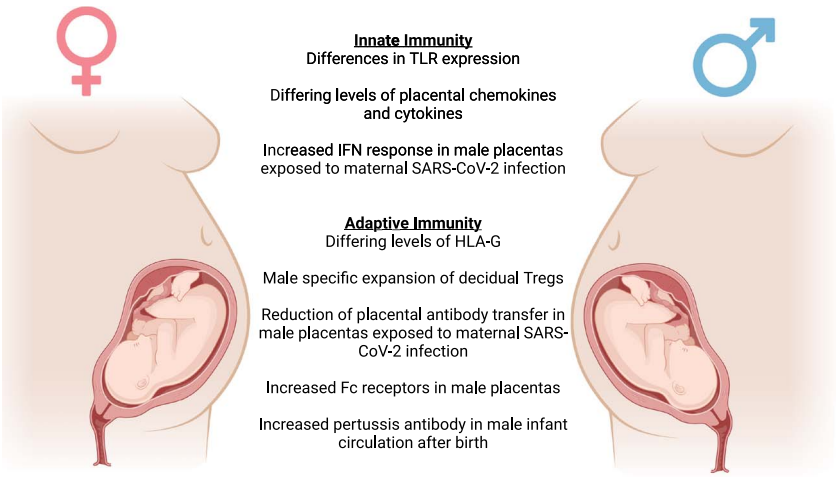

Figure 2. Highlights of sex-specific differences in the immune response during pregnancy.

in first time pregnancies was a male fetus homozygous for an *HLA-G* 3'UTR polymorphism that causes decreased HLA-G expression [169]. These data suggest that elevated HLA-G is critical for the protection against excess inflammation during pregnancies carrying male fetuses.

An immunosuppressive subpopulation of T cells termed regulatory T cells (Tregs) can be observed in maternal serum and within the decidua throughout gestation [170, 171]. Mouse models of pregnancy indicate that Tregs are critical for the transition from a pro-inflammatory to anti-inflammatory state during gestation [172]. In the absence of Tregs, fetuses with mismatched major histocompatibility complexes (MHC) like HLA are rejected from the uterus, whereas those with matching maternal MHC molecules are not [173, 174]. Similarly, mismatching HLA-C between maternal and fetal cells is associated with elevated numbers of activated Tregs in the decidua [175]. Higher numbers of Tregs are found at the maternal–fetal interface following allogeneic pregnancy than syngeneic, which implicates the expansion of Tregs is because of the presence of mismatched fetal antigens [176, 177]. Male trophoblast cells specifically express H-Y antigens, which are recognized by CD4+ T cells and lead to male-pregnancy-specific expansion of Tregs within the decidua. Depletion of Tregs in pregnant mice led to the rejection of male fetuses in utero [173]. Treg-depleted pregnant mice had a significant reduction in the number of male pups born compared with saline-treated controls with the weight of the remaining male fetuses significantly reduced [173]. This suggests that sufficient numbers of Tregs are critical for immune suppression in proportion to fetal antigen present during pregnancy and that Tregs are necessary for prenatal male survival.

As regulators of CD8+ T cells, Tregs secrete IL-10 and transforming growth factor beta (TGF-β), which alternatively activate macrophages and dendritic cells [161, 178]. Male human primary placental trophoblasts exhibit increased production of IL-10 in response to LPS [150]. In the absence of infection, maternal serum levels of IL-10 are significantly higher in those carrying male fetuses [150, 179]. Similarly, in cases of spontaneous preterm birth, male placentas displayed significant decreases in the presence of *TGF-β*, which was not observed in female placentas [180]. The altered secretion of IL-10 and TGF-β observed between male and female placentas may result from a difference in the number of Tregs at the maternal–fetal interface between male and female pregnancies.

CD4+ and CD8+ T cells express androgen receptors and are influenced by androgens [181]. As DHT levels vary between fetal sexes during gestation, it is possible that androgens either directly or indirectly influence the function of T cells in a sex dependent manner [109, 182]. In vitro studies using human male- and female-derived T cells found treatment with DHT induced significantly higher levels of the Treg transcription factor forkhead box P3 (FOXP3) in male-derived cells [183]. FOXP3 is essential for the development of Tregs and decreased levels of FOXP3 are observed in women with recurrent spontaneous abortions [184]. Other in vitro experiments revealed that CD8+ T cells promote proliferation of epithelial cells under low-DHT conditions [185]. Overall, Tregs play an important role in controlling the inflammatory landscape during pregnancy in order to maintain a tolerant uterine environment. Since Tregs exert different effects based on fetal sex, it is possible that they enable and maintain pregnancy in a sexually dimorphic manner.

B cells

The role and regulation of B cells during pregnancy is less understood. However, as pregnant women have higher serum levels of IgG compared with nonpregnant women, B cells clearly have an important role in pregnancy [186]. Within the decidua, B cells are found at low numbers compared with other leukocytes. However, because of the increase in number as gestation progresses, it is proposed that they assist in regulation of both placental development and the immune response at the maternal–fetal interface [159, 187].

Clustering analysis of decidual leukocytes demonstrated the presence of IL-10 secreting B cells [188]. These regulatory B cells, Bregs, use IL-10 and other cytokines to suppress pro-inflammatory events. Maternal serum samples contained elevated numbers of Bregs during pregnancy compared with nonpregnant women [189]. In a murine model, Bregs were present at lower numbers in animals with inflammation-induced spontaneous abortion [190]. Comparatively, a B-cell deficient mouse model demonstrated increased susceptibility to LPS-induced fetal loss because of inhibition of dendritic cell and Treg activity [191]. Busse et al. [191] further demonstrated that the transfer of B cells from IL-10 deficient mice

was not enough to rescue LPS-induced fetal loss, and fetal loss was only rescued by recombinant IL-10 or transfer of IL-10 producing Breg cells. As B cells co-localize with T cells, it is hypothesized that there is a delicate balance between B and T cells at the maternal–fetal interface. T cells produce cytokines to facilitate the induction of B cells, and B cells produce IL-10 to contribute to the induction and maintenance of Tregs, which are required for a successful pregnancy [188].

Antibody production

Recent research indicates that infection with SARS-CoV-2 during pregnancy is associated with a decrease in the production of virus-specific IgG in women carrying male fetuses. Additionally, male placentas had a reduction in placental antibody transfer [146]. Reduced transfer of SARS-CoV-2 antibodies observed with male pregnancies may result in compensatory mechanisms for immune function, with sex-specific changes in Fc receptor expression on the placenta [146]. Fc receptors function to mediate the transfer of IgG across the placenta, and while displayed by placental cells, they can also be observed on placental macrophages [192–194]. Bordt et al. [146] observed significant increases in the size of placental macrophages from male pregnancies following maternal SARS-CoV-2 infection compared with those from females. This phenomenon may contribute to the upregulation of Fc receptors observed in male placentas [146]. Similar reductions in transplacental IgG transfer in male pregnancies are observed in a stress-induced inflammation model of pregnancy in nonhuman primates [14].

Research using human cord blood found, that for several respiratory infections acquired during pregnancy, only rubella infection was associated with increased IgG levels in the cord blood of male fetuses [195]. Following vaccination for SARS-CoV-2, no significant differences exist in maternal serum IgG or cord blood IgG [196, 197]. However, work with the tetanus, diphtheria, acellular pertussis (Tdap) vaccine indicates that fetal sex plays a role in the transfer of antibodies, with increased levels of pertussis toxin antibody in male infant circulation following birth [198]. There currently is no known mechanism determining the physiological basis for the sex-specific differences in prenatal antibody production. However, at least one study in adult mice has documented the role of sex steroids in the immune response to vaccination [199]. Potluri et al. vaccinated adult mice against the 2009 H1N1 vaccine and assessed the impact of sex on vaccine efficacy. Males with the highest serum testosterone levels also had the lowest H1N1-specific antibody response [199]. Furman et al. [200] corroborated this finding in human males and again demonstrated an inverse relationship between androgen levels and antibody responses against an inactivated seasonal influenza vaccine. While the impact of sex hormones on antibody production has not been replicated in a pregnant mouse model, these studies do provide some insight into how sex hormones and sex might influence antibody production after vaccination.

Despite growing evidence that maternal antibody transfer is essential for protection of the offspring during neonatal life, there is limited reporting on the success of vaccine-related antibody production and transfer comparing fetal sexes (Figure 2). As vaccinations during pregnancy become more commonplace, more emphasis on fetal sex as a variable should be taken during experimental design examining vaccine efficacy. This is especially true for COVID-19, influenza, and Tdap vaccines as all are recommended for pregnant women.

Retrograde impact of fetal sex on maternal immunity

The maternal immune system adapts throughout pregnancy to simultaneously protect mother and fetus from pathogens while also suppressing the maternal immune response against potential rejection of an allogeneic fetus. These adaptations alter the maternal immune milieu and modulate the maternal immune response during pregnancy [201, 202]. This is made most evident by how pregnancy affects women diagnosed with autoimmune disorders. Pregnant women diagnosed with Th1 mediated autoimmune disorders like rheumatoid arthritis and multiple sclerosis often enjoy a period of remission during pregnancy [201, 203]. Alternatively, Th2 mediated autoimmune disorders like neuromyelitis optica spectrum disorders often worsen in symptoms during pregnancy [204].

Fetal sex also influences the stimulation and modulation of the maternal immune system during pregnancy [13, 205]. Serum cytokine profiling of pregnant women revealed that carrying a female fetus was associated with increased levels of serum cytokines in the first trimester [206]. Of the tested cytokines, the anti-inflammatory cytokine IL-9 was the most strongly increased [206]. Enninga et al. reported similar maternal serum cytokine profiles with women carrying female fetuses measuring higher levels of the regulatory cytokines IL-5, IL-9, IL-17, and IL-25. Conversely, male fetuses stimulate the production of more proinflammatory cytokines including granulocyte colony-stimulating factor, interleukin 12 heterodimer (IL-12p70), IL-21, and IL-33 [205]. These data suggest that female fetuses can trigger a more regulatory Th2 like maternal immune response, whereas male fetuses bias the maternal immune response toward a Th1 inflammatory response [205].

Alternatively, a study performed by Mitchell et al. [207] reported no differences in maternal serum between women carrying male or female fetuses. However, upon LPS stimulation, peripheral blood mononuclear cells (PBMCs) isolated from pregnant women had varying levels of cytokine production based on fetal sex. LPS-stimulated PBMCs isolated from women carrying female fetuses in the first, second, and third trimester had increased levels of IL-6 at all three time points. TNF-α production was increased in PBMCs isolated during the first trimester, and IL-1β was increased in PBMCs isolated during the second and third trimesters [207].

Greater inflammatory cytokine production in pregnant women carrying female fetuses has also been documented elsewhere. The placentas of female fetuses with mothers who had mild asthma had increased mRNA levels of *TNF-α, IL-1β, IL-6, IL-8*, and *IL-5*, whereas the placentas of male fetuses had unchanged cytokine levels [208]. Furthermore, cord blood cortisol levels seemingly had an inverse correlation with *TNF-α, IL-1β, IL-6, IL-8,* and *IL-5* in females only. These cortisol levels were found to negatively correlate with birth weight in females born to mothers with asthma. Women with moderate to severe asthma had higher cortisol concentrations and lower mRNA levels of *TNF-α, IL-1β, IL-6,* and *IL-8* relative to female fetal cortisol levels of mild asthmatic subjects [208]. These adaptations in the female placenta can alter placental function, specifically by reducing 11β-HSD2 activity and increasing cortisol concentrations. Without inhaled

glucocorticoid administration, increased maternal inflammation because of maternal asthma causes reduced fetal growth in female fetuses. Additionally, women prescribed inhaled glucocorticoids gave birth to female neonates with birth weights comparable to neonates born from nonasthmatic women. Women who did not use inhaled glucocorticoids had significantly altered placental function and a reduction in female fetal growth [209]. These data suggest that maternal asthma can alter placental inflammatory cytokine production in a sex-specific manner and that this sexually dimorphic cytokine production is influenced by fetal cortisol.

The severity of maternal asthma is also affected by fetal sex. Asthmatic women carrying female fetuses have increasing asthma severity and require more steroid intervention as pregnancy progresses [210, 211]. Women who did not use inhaled steroids during pregnancy had a significant rise in circulating monocytes when carrying female fetuses, suggesting increased maternal inflammation [209]. This finding, in conjunction with no change in circulating eosinophils, suggests that the increase in asthma severity may be driven by noneosinophilic pathways. Sex hormones are implicated in the sex differences behind worsening maternal asthma. Testosterone derived from the fetal testis is thought to have anti-inflammatory effects and promote bronchodilation [212]. Whereas, female sex steroids are considered to increase inflammation and sex-specific placental proteins [213]. However, these studies are currently descriptive and future mechanistic studies would assist our understanding of how fetal sex influences fetomaternal tolerance.

Conclusion

It is well understood that male fetuses and placentas grow faster and metabolize nutrients faster than female fetuses. Male fetuses and placentas prioritize feto-placental growth throughout gestation, whereas female fetuses and placentas prioritize biosensing and adaptability, especially in the event of maternal complications. The inability to adapt to a compromised uterine environment leaves male fetuses increasingly vulnerable to adverse pregnancy outcomes.

However, if we move beyond the focus of prioritization of growth, male fetuses also respond to and elicit sexually dimorphic immune responses. In this review, we have summarized a large body of work that has clearly demonstrated a sex-specific difference in the prenatal immune response. In normal pregnancies, male fetuses elicit higher levels of inflammatory cytokines and proangiogenic cytokines, whereas female fetuses elicit a more regulatory cytokine pattern. This divergence only widens when pathological pregnancies are assessed. Male fetuses are associated with significantly different innate and adaptive immune responses including altered interferon and interferon stimulated gene expression, the expansion of Tregs and presentation of HLAs, and antibody production and transfer.

Fetal sex also influences both the maternal immune milieu as well as the developmental trajectory of the fetus far beyond prenatal life. Sex differentially stimulates the production of maternal circulatory cytokines and worsens the effects of maternal asthma in pregnant women. On the fetal side, sex can modulate the response to maternal nutrition, stress, and infection and these responses often have lifelong effects on cardiac, metabolic, and mental health.

Unfortunately, most of the reviewed studies are descriptive and correlative. They have deepened our understanding of the clear sexual dimorphism of the immune response during pregnancy but leave a need to elucidate the molecular mechanisms driving the sex-specific differences in increased male vulnerability. Better understanding of these mechanisms will be essential for the development of better diagnostic and treatment strategies to prevent adverse pregnancy outcomes in male neonates.

Author contributions

KJB wrote the manuscript. RCW wrote the manuscript, prepared the figures, and critically revised the manuscript. All authors read and approved the final version of the manuscript.

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