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ORIGINAL ARTICLE

Interferon epsilon and preterm birth subtypes; a new piece of the type I interferon puzzle during pregnancy?

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Abstract

Problem: Interferon epsilon (IFNε) is a unique type I IFN that is expressed in response to sex steroids. Studies suggest that type I IFNs regulate inflammation-induced preterm birth (PTB), but no study has examined the role of IFNε in human pregnancy.

Method of Study: We used stored vaginal swabs between 8 and 26 weeks of gestation from the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) biobank and measured IFNε by enzyme-linked immunosorbent assay (ELISA). A total of 29 women with spontaneous preterm births, 34 women with medically indicated preterm births, and 134 women with term births were included. Secondary outcomes included a preterm birth with chorioamnionitis and preeclampsia with a preterm birth. Logistic regression calculated odds ratios (OR) and 95% confidence intervals (CI) adjusting for maternal age, race, body mass index, prior pregnancy complications, lower genital tract infections, chronic health conditions, and gestational age at blood draw.

Results and Conclusions: There was no significant association between IFNε and spontaneous preterm birth (OR_{adj} 1.0, 0.8–1.3) or chorioamnionitis (OR_{adj} 1.6, 0.7–3.5). A trend toward increased odds of medically indicated preterm birth (OR_{adj}. 1.3, 1.0–1.8) was observed. This was likely due to elevated IFNε among women with preterm preeclampsia (OR_{adj}. 2.0, 95% CI 1.3–3.2). While exploratory, our novel findings suggest that larger longitudinal studies of IFNε across human pregnancy may be warranted.

KEYWORDS
inflammation, interferons, preeclampsia, preterm birth

1 | INTRODUCTION

Preterm birth, delivery of a live born infant before 37 weeks gestation, is a serious public health concern as it is a leading cause of infant mortality.[1,2] Globally, preterm birth affects approximately 9% of pregnancies in high and middle-income countries and 12% in low-income countries.[2] The United States has the highest preterm birth rate of developed nations[2] with significant racial/ethnic disparities. For example, the rate in Black women is 14.4% compared to 9.8% in Hispanic women and 9.1% in White women. Unfortunately, little progress has been made in reducing rates of preterm birth.[3]

Preterm birth can be defined as spontaneous (preterm premature rupture of membranes or spontaneous labor) or medically indicated (iatrogenic intervention).[4] Various conditions result in medically indicated preterm birth, but disorders of the placenta, such preeclampsia, intrauterine growth restriction (IUGR), and placental abruption,

account for majority of cases.[5] Preeclampsia is a leading cause of maternal mortality and affects 3%–10% of pregnancies worldwide,[6] while IUGR and placental abruption affect 8%[7] and 1%[8] of pregnancies, respectively. These conditions share similar biological pathways with spontaneous preterm birth, such as disruption of pro- and anti-inflammatory cytokine balance and localized changes to the function of neutrophils, macrophages, T-cells, regulatory T-cells, and B-cells at the maternal-fetal interface.[9–12] Indeed, both sterile inflammation and infectious inflammation (due to polymicrobial bacterial infection) are major contributors to preterm births.[12–14] However, the field lacks a complete understanding of the biological pathways that explain these observations.[3] Additionally, treatments including antibiotics, tocolytics, and progesterone supplementation have been implemented with limited success.[15] Identification of new biomarkers that can predict women who may benefit from alternative therapeutics that block specific inflammatory pathways may be needed.[15] Thus, discovering novel immunological mechanisms leading to preterm birth is critical.

Type I interferons (IFNs) are stimulated by Toll-like receptors (TLR), NOD-like receptors, and RIG-I-like receptors on trophoblast cells to control maternal immune responses, create an antimicrobial state,[16] and promote tolerance to the fetus.[17] Challenging deficient type I IFN-receptor mice with lipopolysaccharide (LPS) leads to preterm birth, through elevated expression of interleukin (IL)-1β and tumor necrosis factor (TNF)-α.[18] However, type I IFNs can be both beneficial[19,20] and detrimental.[21–23] Mouse models have implicated the "double-hit hypothesis" in preterm birth.[24] Specifically, viral infection primes type I IFN responses to secondary challenge with LPS resulting in elevated IL-6, granulocyte colony stimulating factor (G-CSF), and monocyte chemoattractant protein-1 (MCP-1) in the placenta and decidua and IL-6, G-CSF, and IL-8 on trophoblast.[25] IFNβ priming also increases LPS-induced IL-6 and TNF in human decidual cells from women with preterm birth and chorioamnionitis.[9] These studies suggest that type I IFNs drive inflammation induced preterm birth, but most studies focus on IFNβ. In general, the roles of type I IFN's in human pregnancy are not completely elucidated.

The discovery and more recent characterization of interferon epsilon (IFNε) has led to interest regarding its biological functions, which are poorly understood. IFNε is immunomodulatory and signals via the IFNAR complex stimulating natural killer cells, T cells, and B cells, although to a lesser degree than IFNα and IFNβ.[26] Unlike other type I IFNs, IFNε is thought to be hormonally regulated and has been shown to protect against herpes simplex virus (HSV), *Chlamydia muridarum* and human immune deficiency virus (HIV), in mouse and experimental models.[27] Due to the unusual nature and expression in female reproductive mucosal tissue, IFNε has been suggested as a novel target for IFN-based therapeutics and possibly less likely to exacerbate disease than other type I IFNs.[17,26,28] As studies have not explored IFNε in relation to pregnancy outcomes, our objective was to determine if vaginal expression of IFNε in early- to mid-pregnancy differs among women with term and preterm pregnancies, while considering subtypes of the condition.

2 | PATIENTS AND METHODS

This investigation utilized data from the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) at the Seattle Children's Hospital. In total, stored vaginal swabs were available from 29 women with spontaneous preterm births, 34 women with medically indicated preterm births and 134 women with term births. Women had singleton pregnancies with a live born infant and no known fetal abnormalities. GAPPS trained staff approach women during their first prenatal visit to the University of Washington Medical Center, Yakima Valley Memorial Hospital, or the Swedish Medical Center. All women provided informed consent. All research was performed in accordance with relevant guidelines/regulations. GAPPS is approved by the Seattle Children's Institutional Review Board. This study was reviewed by the University of Texas Medical Branch Institutional Review Board and determined to be nonhuman subject research.

2.1 | Data collection

Data in GAPPS is collected using a web-based data management system called LabVantage that stores and tracks specimens and metadata. The GAPPS database included variables from medical records such as maternal age, self-reported race/ethnicity, body mass index (BMI), substance use, history of chronic diseases, history of prior pregnancy complications, use of fertility services, parity, gravidity, chronic health conditions, and mental health conditions. Infections including group B streptococcus and sexually transmitted infections (STI) were measured by standard hospital protocols.[29,30] There was only one case of chlamydia, one case of human papilloma virus, one case of bacterial vaginosis, and 28 cases of serological evidence of herpes simplex virus (supplementary methods and Supplementary Table 1). A composite lower genital tract infection variable was used in analyses. Labor and delivery characteristics, gestational age of the fetus at delivery, birthweight, fetal sex, Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score and other health measures (e.g., Neonatal Intensive Care Unit (NICU) stay), were also available from medical records (Supplementary Table 2).

Trained staff collected vaginal swabs at 8–26 weeks gestation from all women included in this study. As described,[31] biospecimen kits and protocols for collection, storage and distribution are standardized and appropriate for genital tract cytokine detection.[32,33] These protocols follow the International Society for Biological and Environmental Repositories (ISBER) guidelines[34] GAPPS developed in-house programs to further ensure proper monitoring to maintain specimens and data quality. While avoiding lubricant, midpoint specimens were collected using sterile polyester-tipped swabs (BD BBL CultureSwab EZII swabs, Becton, Dickerson and Company, Franklin Lakes, New Jersey). Within 15 min, swabs were placed in collection tubes with preloaded stabilizing solution in a microcentrifuge rack where specimens were centrifuged for 10 s to dislodge any material before removing swabs and placing them in 2 ml cryo-vial tubes. Tubes were frozen at a

minimum of −20°C for short term storage (<30 days) until shipped to the core repository for storage at −80°C within 5 min of transfer.

2.2 | Interferon epsilon measurement

All specimens were shipped to Texas A&M University and IFNε (pg/ml) was measured in triplicate using an Enzyme Linked ImmunoSorbent Assay (ELISA) following manufacturer instructions (MyBiosource, Vancouver). Samples had no prior thaws recorded. Prior to the assay all specimens were thawed before rocking gently (40 oscillations/min on horizontal platform rocker) at 4°C for 1 h in 300 μl of 1X PBS pH 7.4 (Thermo Fisher Scientific Ashville, NC). Samples were eluted and then centrifuged for 10 min at 14 000 x gravity (Thermo Fisher ST4R with TX750 rotor package, Thermo Fisher Scientific, Asheville NC) and then supernatants were stored at 4°C overnight as consistent with other studies of genital tract secretions.[33,35] Following a IFNε precoat, standards and samples were pipetted into the wells and a biotin-conjugated antibody was added. Avidin conjugated horseradish peroxidase (HRP) was added to the wells and a substrate solution was added while color was allowed to develop in proportion to the amount of IFNε bound in the initial step. The color development was stopped after exactly 20 min at 37°C and the intensity of the color measured at an optical density of 450 nm on a Biorad iMark microplate absorbance reader (Hercules CA). Each sample was standardized to average protein concentration (ug/ml) using Biorad Quick Start Bradford Assay (Hercules CA). Intra-assay precision was calculated by the manufacturer to have a CV% < 8%, interassay precision to be <10% and a range of detection from 15.6 to 1000 pg/mL. The r^2 value of our standard curves (triplicates of seven standard dilutions were run on each of 11 plates) ranged from 0.9924 to 0.9981. Using residual swabs, we attempted to measure IFNλ-1 (another hormonally driven IFN) using an ELISA following manufacturer instructions (MyBiosource, Vancouver) and the same procedures as IFNε. Sensitivity is reported as 3.9 pg/ml with <8% inter and intra-assay CV. However, we were unable to detect IFNλ-1 in residual swabs, thus results are not presented. No significant cross-reactivity for either IFN has been reported of the MyBiosource reagent with any IFN homolog.

2.3 | Outcome measurement

Preterm delivery is defined as delivery of a live born infant at <37 completed weeks of gestation (i.e., 36 + 6 weeks). Gestational age was determined by ultrasound using the American College of Obstetricians and Gynecologists (ACOG) criteria[36] if last menstrual period was not consistent. The mean gestational age of delivery for term pregnancies was 39.3 weeks and for preterm pregnancies it was 32.6 weeks. Preterm birth is a condition that can be characterized by clinical indication (spontaneous or medically indicated).[37] Separation of "subtypes" is common to consider distinct etiologies. Therefore, the primary analysis examined medically indicated preterm birth and spontaneous preterm birth separately. Spontaneous preterm birth included women with regular contractions and ≥2 cm dilatation indicative of cervical change. If cervical change was not present, then it was defined with the presence of spontaneous premature rupture of membranes (pPROM). Medically indicated preterm birth included women who had labor induction or Cesarean section before spontaneous labor or pPROM occurred. Reasons for medical indication vary[5] in our study 15 women had preeclampsia, which was defined using standard ACOG criteria (supplementary methods). We included preeclampsia as a secondary outcome. Another 7 had small for gestational age, 1 had placental abruption, 2 had fetal distress, and 3 had miscellaneous placental conditions. The remaining women had unknown indicators. Given the small sample sizes, we did not include these variables as secondary outcomes. We further stratified analyses by preterm birth with chorioamnionitis (n=15), inflammation of the fetal membranes (amnion and chorion) typically due ascending bacterial infections (e.g., Ureaplasma species and Mycoplasma hominis).

2.4 | Statistical analysis

Maternal characteristics (maternal age, race, pre-pregnancy body mass index), chronic diseases, pregnancy history (gravidity, prior complications), evidence of any lower genital tract infections, and group-B streptococcus (GBS) were compared between term and preterm pregnancies using logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI). Similar comparisons were made for labor status, delivery mode, and fetal characteristics (fetal sex, small for gestational age, APGAR, NICU stay) (Supplemntary Table 2). One woman reported drug use, one woman reported alcohol abuse, and one woman reported intimate partner violence. Only 8 women total smoked, where 3 had a preterm birth. Similarly, sample sizes for assisted reproductive technology and urinary tract infection were <4 in either term or preterm pregnancies. Due to the small sample size of those variables, they could not be included in the analysis.

We calculated the geometric mean and standard deviation of IFNe for term and preterm pregnancies, which was log-transformed. The proportion of values below the limit of detection were also calculated (9.1%, n=18). Those values could represent a true zero or anywhere between zero and the limit of detection. For this analysis, multiple imputation with fully conditional specification, which is an accepted method for values below the limit of detection,[38] was utilized. This approach was also used for missing covariate data. The primary analysis examined the association between IFNe and spontaneous and medically indicated preterm birth using multivariable logistic regression. We adjusted for maternal age (continuous), race (White, Hispanic, Other), gestational age at blood draw (continuous), body mass index (continuous), chronic health conditions (any condition: yes, no), lower genital tract infections (any STI or GBS: yes, no), and prior pregnancy complications (any complication: yes, no). Secondary analyses were conducted examining chorioamnionitis and preterm preeclampsia using the same models as above. For all analyses, to account for small sample size, the penalized likelihood approach was used, when necessary, as this approach addresses issues of separability and reduces bias.

Samples available for analysis spanned two trimesters, thus, we conducted sensitivity analyses by stratifying primary analyses by those sampled at 8-12 weeks (N=123) and those sampled at 13-26 weeks gestation (N=74). For secondary analyses the sample size was too small to further stratify. SAS version 9.4 (Cary, NC) was used.

3 | RESULTS

Women with term and preterm pregnancies were similar in age [median 31.0 yrs. (interquartile range 7.0) vs. 30.0 (11.5), BMI [26.0 (7.2) vs. 26.5(8.2)] and gravidity where a majority had two or more prior pregnancies [(43.6% and 42.2%) Table 1]. Women with preterm birth were more likely to be of Hispanic ethnicity (OR 2.3, 95% CI 1.0–5.0) or of nonwhite "other" races (OR 2.2, 95% CI 1.0–4.7) and more likely to have a chronic health condition (OR 3.3, 95% CI 1.6–6.8). Only two women with term birth (1.5%) had prior preterm birth compared to 25 (39.1%) women with a current preterm birth. Results were similar for prior stillbirth (2.3% vs. 6.3%). Women with preterm birth were more likely to have GBS (OR 4.5, 95% CI 1.4–13.7) but other lower genital tract infections were not significantly different. Lastly, women with preterm birth were more likely to have a cesarean section (OR 2.2, 95% CI 1.2–4.1) than women with term births.

3.1 | Primary analyses

Our primary analysis examined the association between IFNε and preterm birth subtypes (Table 2). After adjustments, there was no significant association between IFNε and spontaneous preterm birth [geometric mean (SD): 3.9 (1.6) vs. 3.7 (1.5); OR_{adj} 1.0, 95% CI 0.8–1.3]. In contrast, there was an association between IFNε and medically indicated preterm birth [3.9 (1.6) vs. 4.7 (1.6); OR_{adj} 1.3, 95% CI 1.0–1.8] (Table 3).

3.2 | Stratification by trimester

Sensitivity analyses revealed similar results for spontaneous preterm birth when IFNε was stratified by first (OR_{adj} 1.0, 95% CI 0.8–1.4) or second (OR_{adj} 0.9, 95% CI 0.6–1.1) trimester measurements (Tables 2 and 3). This was also true of medically indicated preterm birth where effect estimates were similar for first (OR_{adj} 1.2, 95% CI 0.8–1.8) and second trimester measurements (OR_{adj} 1.2, 95% CI 0.9–1.6).

3.3 | Secondary analyses

There was no significant association with chorioamnionitis, although odds were slightly elevated [3.9 (1.6) vs. 5.1 (1.4); OR_{adj} 1.6, 95% CI 0.9–2.7] (Table 4). We found a significant association between IFNε and preterm preeclampsia [3.9 (1.6) vs. 5.5 (1.4); OR_{adj} 2.0, 95% CI 1.3–3.2] after adjustments. The sample sizes were too small to separate first and second trimester measurements.

As biomarkers are often reported as tertiles in medical research, we examined the percentage of women with term pregnancies and preterm birth subtypes that fall within low (<19.7 pg/ml), mid (19.7–98.7pg/ml) and high (>98.8 pg/ml) levels of vaginal IFNε (Figure 1). Most women with term births had IFNε levels that fell within the low to midtertile range (low 33.8%; mid 35.4%; high 30.8%). Results were similar for spontaneous preterm birth (low 37.9%; mid 31.0%; high 31.0%). Most women with medically indicated preterm births (low 26.5%; mid 29.4%; high 44.1%) and chorioamnionitis (low 13.3%; mid 26.7%; high 60.0%) fell within the high tertile category. Results were similar when we examined preterm preeclampsia (low 20.0%; mid 26.7%; high 53.3%). Because few participants were in the lowest tertile for some outcomes, we examined associations between the highest tertile and preterm birth subtypes with low to mid-levels as the reference. We found no association between high tertile levels and spontaneous preterm birth (OR_{adj} 1.2, 95% CI 0.5–3.5). or medically indicated preterm birth (OR_{adj} 1.8, 95% CI 0.7–4.7). Women with vaginal IFNε in the highest tertile did have trends toward increased odds of chorioamnionitis (OR_{adj} 3.3, 95% CI 1.1–9.5) and preterm preeclampsia (OR_{adj} 2.4, 95% CI 0.9–6.9).

4 | DISCUSSION

Numerous circulating and vaginal inflammatory cytokines and chemokines (e.g., IL-6, IL-1β, TNFα) have been associated with preterm birth, chorioamnionitis and preeclampsia.[39–41] Past studies focused on general inflammatory markers, but many have not investigated associated mechanisms that may modulate proinflammatory responses and none have identified an unambiguous biomarker. Often overlooked are type I IFNs, despite growing evidence of significant immune modulation during pregnancy[17,42] and influences on maternal health and disease severity.[18] For the first time, we report an association between vaginal type I IFNε in early- to mid-pregnancy and preterm preeclampsia

The role of the type I IFN axis in pregnancy is most notably related to the "double-hit" hypothesis.[17] Specifically, viral infections, such as murid herpesvirus 4, can reduce levels of type I IFNβ and dull secondary responses to insults through Interferon Regulatory Factor 3 (IRF3).[43] Studies demonstrate that viral infection exacerbates bacterial induced preterm birth by increasing IL-6, IFN-β, and TNF-α through TLR4. Furthermore, the microbiota has been implicated in regulating type I IFN expression by promoting tolerance to the fetus, suggesting that the relationship between commensal bacteria and IFNs is critical for pregnancy.[42] Despite these observations, we did not find any association between vaginal IFNε and spontaneous preterm birth.

Levels of IFNε were elevated among women with medically indicated preterm birth but this was likely driven by preterm preeclampsia. Few studies have examined type I IFNs in preeclampsia. Type II IFN-γ was found to be associated with preeclampsia in a meta-analysis[44] and first trimester plasma IFN-γ is associated with preterm preeclampsia.[45]

TABLE 1 Maternal characteristics and clinical variables among term and preterm births

Characteristics and clinical variables	Term births (≥37 Weeks) *N* = 134	Preterm births (<37 Weeks) *N* = 63	Odds ratio (95% CI)
Maternal age			
<25 years	16 (12.0)	117 (87.9)	Ref
≥25 years	9 (14.1)	55 (85.9)	1.0 (0.9–1.1)
Race/Ethnicity, *n* (%)			
Non-Hispanic-White	89 (68.5)	31 (49.2)	Ref.
Hispanic	20 (15.4)	16 (25.4)	2.3 (1.0–5.0)
Other	21 (16.2)	16 (25.4)	2.2 (1.0–4.7)
Overweight/Obese			
No	108 (81.2)	25 (18.8)	Ref
Yes	48 (75.0)	16 (25.0)	1.4 (0.7–2.9)
§ History of chronic diseases *n* (%)			
Yes	18 (13.5)	22 (34.4)	3.3 (1.6–6.8)
No	115 (86.5)	42 (65.6)	Ref.
Gravidity, *n* (%)			
No prior pregnancies	38 (28.6)	13 (20.3)	Ref.
One prior pregnancy	37 (27.8)	24 (37.5)	1.9 (0.8–4.2)
2+ prior pregnancies	58 (43.6)	27 (42.2)	1.3 (0.6–2.9)
Prior preterm birth, *n* (%)			
Yes	2 (1.5)	25 (39.1)	†
No	131 (98.5)	39 (60.9)	Ref.
Prior spontaneous abortion, *n* (%)			
Yes	36 (27.1)	28 (43.8)	2.1 (1.1–3.9)
No	97 (72.9)	36 (56.3)	Ref.
Prior stillbirth, *n* (%)			
Yes	3 (2.3)	4 (6.3)	†
No	130 (97.7)	60 (93.8)	Ref.
§ Lower genital infection, *n* (%)			
Yes	24 (17.9)	7 (10.9)	0.6 (0.2–1.4)
No	109 (81.3)	57 (89.1)	Ref.
Group B Streptococcus			
Yes	5 (3.8)	10 (15.6)	4.5 (1.5–13.7)
No	128 (96.2)	54 (84.4)	Ref.
Urinary tract infection			
Yes	2 (1.5)	5 (7.8)	†
No	131 (98.5)	59 (92.2)	Ref.
Type of delivery, *n* (%)			
Vaginal	98 (73.7)	36 (56.3)	Ref.
Cesarean	35 (26.3)	28 (43.8)	2.2 (1.2–4.1)

Odds ratios and 95% confidence intervals were calculated with logistic regression.

†Cell size too small for analysis.

§History of chronic health conditions includes asthma, autoimmune diseases, thyroid disease, chronic hypertension, cardiovascular disease, diabetes, endometriosis, gastrointestinal disorders (e.g., celiac disease) and mental illness (anxiety and depression).

§Lower genital tract infections included serology for HSV, diagnosis of HPV, chlamydia, bacterial vaginosis, or indication of another STI.

TABLE 2 Associations between vaginal IFNε and spontaneous preterm birth

	Term births geometric mean (SD)	Preterm births geometric mean (SD)	† OR, 95% CI
Vaginal IFNε, pg/ml, 8–26 weeks	3.9 (1.6)	3.7 (1.5)	1.0, 0.8–1.3
Vaginal IFNε, pg/ml, 8–12 weeks	3.9 (1.6)	3.9 (1.5)	1.0, 0.8–1.4
Vaginal IFNε, pg/ml, 13–26 weeks	3.9 (1.7)	3.4 (2.0)	0.9, 0.6–1.1

†Odds ratios (OR) and 95% confidence intervals (CI) were calculated with logistic regression. Models were adjusted for maternal age, race, gestational age at blood draw, body mass index, chronic health conditions, lower genital tract infections, and prior pregnancy complications.

TABLE 3 Associations between vaginal IFNε and medically indicated preterm birth

	Term births geometric mean (SD)	Preterm births geometric mean (SD)	† OR, 95% CI
Vaginal IFNε, pg/ml, 8–26 weeks	3.9 (1.6)	4.7 (1.6)	**1.3, 1.0–1.8**
Vaginal IFNε, pg/ml, 8–12 weeks	3.9 (1.6)	4.9 (1.4)	1.2, 0.8–1.8
Vaginal IFNε, pg/ml, 13–26 weeks	3.9 (1.7)	4.5 (1.9)	1.2, 0.9–1.6

†Odds ratios (OR) and 95% confidence intervals (CI) were calculated with logistic regression. Models were adjusted for maternal age, race, gestational age at blood draw, body mass index, chronic health conditions, lower genital tract infections, and prior pregnancy complications.

TABLE 4 Associations between vaginal IFNε and chorioamnionitis and preterm preeclampsia

	Term births geometric mean (SD)	Preterm preeclampsia geometric mean (SD)	† OR, 95% CI
Vaginal IFNε, pg/ml	3.9 (1.6)	5.5 (1.4)	2.0, 1.3–3.2
	Term births geometric mean (SD)	**Chorio-amnionitis geometric mean (SD)**	
Vaginal IFNε, pg/ml	3.9 (1.6)	5.1 (1.4)	1.6, 0.9–2.7

†Odds ratios (OR) and 95% confidence intervals (CI) were calculated with logistic regression. Models were adjusted for maternal age, race, gestational age at blood draw, body mass index, lower genital tract infections, chronic health conditions, and prior pregnancy complications.

However, most human studies of the immunology of preeclampsia are focused on circulating cytokines and the role of vaginal immune markers is unclear. Type I IFNs play a critical role in pregnancy success, consistent with what is known about preeclampsia pathogenesis.[11] Preeclampsia has several phenotypes[46] with preterm preeclampsia being driven by an inappropriate maternal/fetal immune interaction and abnormal placentation.[11] It is also accepted that the maternal response to such stimuli may drive the hallmark of preeclampsia, which is systemic inflammation and endothelial dysfunction.[6] Given our investigation is the first to examine vaginal IFNε in preterm preeclampsia, additional research is needed to understand a possible mechanistic link.

We found a trend toward elevated IFNε in preterm birth with chorioamnionitis. Confidence intervals included one, but this may be due to the small sample size. When we examined women in the highest tertile of IFNε levels, there was a significant association with chorioamnionitis. Elevated IFN-β is present in the chorioamnion and decidua in women with chorioamnionitis.[9] Given that chorioamnionitis is polymicrobial and IFNε has immune modulatory effects similar to IFNβ, our results may suggest that higher levels of vaginal IFNε at early- to mid-trimester are indicative of aberrant type I IFN responses and subsequent bacterial ascension and inflammation of the maternal-fetal unit. However, it should be noted that following infection, TLRs are thought to be responsible for inappropriate type I IFN responses. This makes our observations somewhat curious as IFN-ε may be hormonally regulated.[27] Fung et al, report that expression of IFNε is highest during the estrous cycle of mice, undetected in the early stages of pregnancy and protective against genital tract infections.[27] Additionally, IFNε is 10-fold higher in the uterine epithelial cells in the secretory phase rather than the proliferative phase which is associated with high levels of estrogen. The progesterone driven secretory phase correlates with numerous immunological changes to ensure proper fertilization and implantation.[47–49] This may come with increased risk of genital tract infections.[50] Results from a study using a ZIKA mouse model found that estradiol treatment protected against infection in mice with type I IFN signaling deficiencies suggesting that IFNλ is responsible for hormonally driven antiviral effects in the female genital tract rather than IFNε.[51] However, IFNλ was not detected in our study.

The biological role and clinical relevance of IFNε in humans remains a mystery. IFNε expression in 33 nonpregnant women has been confirmed in the lower and upper female genital tract, but with increased endometrial levels in the secretory phase (progesterone dominant) rather than the proliferative stage of the menstrual cycle.[26] We have found in a study of 30 women with term pregnancies that IFNε is expressed in the genital tract and increases across the first, second, and third trimesters, albeit reduced in women with serological evidence of herpes simplex virus (HSV).[31] It is not known if lower IFNε increases risk of HSV or if HSV reduced levels of IFNε, similar to effects of murid herpesvirus 4 on IFNβ.[43] Serological evidence for HSV is common and in our study we found that a majority seropositive women delivered at term. Adjustment for HSV did not alter our results nor did exclusion of HSV infected women; therefore, we do not

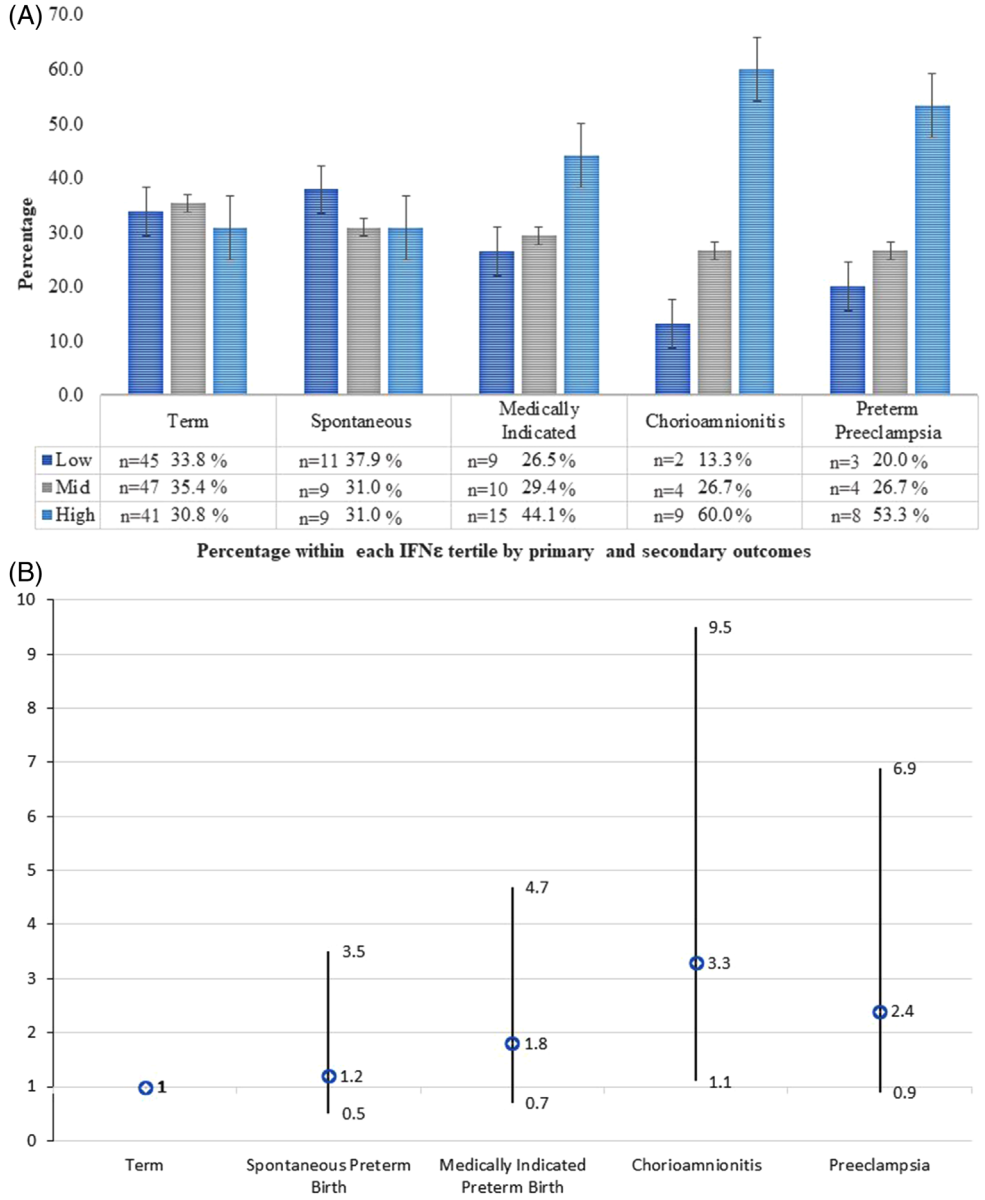

FIGURE 1 (A, B) Vaginal levels of IFNε were separated into equal tertiles to create three groups: Tertile 1 "low levels": 0.49–18.74 pg/ml; Tertile 2 "mid levels": 19.74–95.86 pg/ml; Tertile 3 "high levels": 98.72–929.47 pg/ml. For each outcome, we calculated the frequency and percentage for each IFNε group. (B) Displays associations between high levels and each outcome (low and mid levels as the reference) as determined by logistic regression with penalized likelihood approach adjusting for maternal age, race, gestational age of blood draw, body mass index, chronic diseases, lower genital tract infections, and prior pregnancy complications. Odds ratios and 95% confidence intervals are presented

believe that HSV seroprevalence significantly influenced our findings. We, of course, cannot rule out the possibility of other infections. In addition to serological evidence of HSV, we did have data on GBS. The prevalence was low in the cohort (7.6%). While GBS has been associated with chorioamnionitis, other infections are predominant such as *Mycoplasma* and *Ureaplasma* species. Therefore, a limitation of this study is that we could not fully explore the interplay between IFNε, maternal infection and subsequent adverse outcomes. This certainly should be explored in future investigations.

We also cannot rule out misclassification of preterm birth subtypes despite that a strength of our study was the use of a database that has high-quality control. In this case, misclassification of outcome could bias toward the null. While one strength of our study was access to numerous potential confounders and detailed high-quality data on birth outcomes, unmeasured confounding is always a possibility. We did not have extensive data on education and insurance, although there is no evidence that these factors would directly affect IFNs. Still, we calculated E-values to assess unmeasured confounding.[52] We found no association where the E-value for the point estimate and CI neared one to strongly indicate unmeasured confounding. E-values were 2.15 (1.43 for confidence interval closest to the null) for the association with medically indicated preterm birth and 3.91 (1.92 for the confidence interval

closest to the null) for the association with preterm preeclampsia. This suggest that moderate unmeasured confounding may have biased our results as the variable would have to have a magnitude of association of at least 3.91 with both exposure and outcome to bias results. Lastly, we were unable to measure other Type I IFNs, general markers of inflammation, or hormones, which should be examined in future studies.

Our novel results warrant further investigation of IFNε in human pregnancy. Investigations are needed to improve understanding of the longitudinal relationship between multiple type I IFNs and reproductive success. This type of research would be significant. Indeed, maternal morbidity is associated with both intrauterine infection (chorioamnionitis) and preeclampsia resulting in a preterm birth. Increasing our understanding of type I IFNs during pregnancy, beyond that of IFNβ, would advance the field and shed light on the immunological mechanisms that drive morbid complications.

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CONFLICTS OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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