



Research article

Defining a role for Interferon Epsilon in normal and complicated pregnancies



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ABSTRACT

Interferon epsilon (IFNe) is a recently described cytokine that is constitutively expressed in the female reproductive tract. However, the role of this hormonally regulated cytokine during human pregnancy is poorly understood. Moreover, whether IFNe participates in host immune response against bacteria-driven intra-amniotic infection or cervical human papillomavirus infection during pregnancy is unknown. Herein, using a unique set of human samples derived from multiple study cohorts, we aimed to uncover the role of IFNe in normal and complicated pregnancies. We showed that IFNe is expressed in the myometrium, cervix, and chorioamniotic membranes, and may therefore represent a constitutive element of host defense mechanisms in these tissues during pregnancy. The expression of IFNe in the myometrium and cervix appeared greater in late gestation than in mid-pregnancy, but did not seem to be impacted by labor. Notably, concentrations of IFNe in amniotic fluid, but not cervical fluid, were increased in a subset of women undergoing spontaneous preterm labor with intra-amniotic infection, indicating that IFNe could participate in anti-microbial responses in the amniotic cavity. However, stimulation with *Ureaplasma parvum* and/or lipopolysaccharide did not enhance IFNE expression by amnion epithelial or cervical cells *in vitro*, implicating alternative sources of this cytokine during intra-amniotic or cervical infection, respectively. Collectively, our results represent the first characterization of IFNe expression by human reproductive and gestational tissues during normal pregnancy and suggest a role for this cytokine in intra-amniotic infection leading to preterm birth.

1. Introduction

Interferon epsilon (IFNe) is a recently described member of the type I IFN family that is distinctively recognized for its constitutive expression in

the female reproductive tract [1, 2, 3, 4, 5]. As a prototypical type I IFN, IFNe signals through the IFNAR1 and IFNAR2 receptors and induces interferon-regulated genes (IRGs) [2]. By contrast with other IFNs, IFNe expression changes with the estrous cycle in animals [2, 6] and menstrual

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cycle in humans [5]. In pregnant mice, the uterine expression of IFN ϵ increases as gestation progresses; however, such levels are lower than in virgin mice [2]. Pseudopregnant females also showed changes in IFN ϵ expression that are similar to those in pregnant dams, further indicating the hormonal control of this interferon [2]. In non-pregnant women, IFN ϵ was shown to be expressed in the vagina [5], ectocervix [5], and endometrium [2, 5], and endometrial expression fluctuated with the menstrual cycle stage [5]. Moreover, mouse and human data have pointed to estrogen as a potential regulatory factor for IFN ϵ in the endometrial tissues, whereas progesterone shows inhibitory effects [2, 5]. Together, this accumulating evidence hinted at a unique hormone-regulated function for IFN ϵ in the female reproductive tract. A role in host defense against pathogens was proposed for IFN ϵ , since its gene deletion resulted in diminished anti-viral and anti-bacterial responses in non-pregnant mice [2]. However, the promoter for IFN ϵ lacks response elements for pattern recognition receptor (PRR) pathways [2], and thus the expression of this IFN seems to be minimally affected by specific microbes [2, 3, 4, 7]. Therefore, the role of IFN ϵ in host defense against pathogens in the female reproductive tract in the pregnant and non-pregnant states is still unclear.

Little is known about the expression of IFN ϵ in human pregnancy. A clinical study reported the levels of IFN ϵ in cervical and vaginal fluids throughout gestation, showing that the concentration of this cytokine increases as gestation progresses [8] and confirming similar findings in mice [2]. However, contrary to the proposed role for IFN ϵ in combating sexually transmitted infections (STIs) [2], the levels of this cytokine in vaginal fluids of pregnant women with herpes simplex virus (HSV) infection were lower than that of matched controls [8]. Hence, the involvement of IFN ϵ in host response against pathogens causing STIs requires further research using human samples. Furthermore, the role of IFN ϵ in pregnancy complications associated with intra-amniotic infection/inflammation leading to spontaneous preterm birth, the predominant cause of neonatal mortality and morbidity worldwide [9], also warrants investigation. A recent prospective study utilized vaginal swabs collected at 8–26 weeks of gestation to correlate vaginal IFN ϵ concentrations with pregnancy outcomes and did not indicate a relationship between IFN ϵ and preterm birth incidence or the presence of histological chorioamnionitis [10]. However, whether IFN ϵ is participating in the pathophysiology of preterm labor in the context of intra-amniotic inflammation has not been evaluated.

Herein, using a unique set of human samples derived from multiple study cohorts, we aimed to uncover the role of IFN ϵ in normal and complicated pregnancies. First, we explored the mRNA and/or protein expression of IFN ϵ in the myometrium and cervix of women in mid-gestation and late pregnancy in the presence or absence of labor. Next, we investigated the concentrations of IFN ϵ in cervical fluids of women who underwent spontaneous preterm birth with intra-amniotic inflammatory processes associated with the presence (i.e., intra-amniotic infection) or absence (i.e., sterile intra-amniotic inflammation) of detectable bacteria or human papillomavirus (HPV). In addition, we tested whether IFN ϵ expression by cervical cells increases in response to *in vitro* stimulation with *Ureaplasma parvum*, a common genital mycoplasma associated with intra-amniotic infection [11, 12, 13, 14, 15, 16, 17, 18, 19]. Moreover, we determined IFN ϵ concentrations in amniotic fluids of women with intra-amniotic infection or sterile intra-amniotic inflammation, and evaluated whether IFN ϵ expression by amnion epithelial cells increases in response to *Ureaplasma parvum* or lipopolysaccharide (LPS) *in vitro*. Lastly, we evaluated the mRNA and protein expression of IFN ϵ in the chorioamniotic membranes from women with intra-amniotic infection or sterile intra-amniotic inflammation who underwent spontaneous preterm labor and birth.

2. Methods

2.1. Human subjects and clinical specimens

Amniotic fluid, cervical fluid, and cervical tissues were retrospectively obtained from the biological bank at the University Hospital

Table 1. Clinical and demographic characteristics of women at term with and without labor from whom myometrium samples were collected.

	Term without labor (n = 13)	Term in labor (n = 11)	p-value
Maternal age (years; median [IQR]) ^a	32 (28–36)	29 (25.5–31.5)	0.2
Body mass index (kg/m ² ; median [IQR]) ^a	32.2 (31.2–36.4)	33.4 (29.4–49.9)	0.6
Primiparity ^b	0% (0/13)	18.2% (2/11)	0.2
Race/ethnicity ^b			1.0
African-American	76.9% (10/13)	90% (9/10) ^c	
White	7.7% (1/13)	0% (0/10) ^c	
Other	15.4 (2/13)	10% (1/10) ^c	
Gestational age at delivery (weeks; median [IQR]) ^a	39 (39–39.1)	39.4 (38.5–39.6)	0.4
Birthweight (grams; median [IQR]) ^a	3380 (3015–3850)	3360 (2998–3508)	0.6
Apgar score at 5 min (median [IQR]) ^a	9 (9–9)	9 (9–9)	1.0

Data are given as median (interquartile range, IQR) and percentage (n/N).

^a Mann-Whitney U test.

^b Fisher's exact test.

^c One missing datum.

Hradec Králové, Hradec Králové, Czech Republic. Chorioamniotic membranes and myometrial samples were retrospectively obtained from the biological bank of the Perinatology Research Branch, an intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services, Wayne State University (Detroit, MI, USA), and the Detroit Medical Center (Detroit, MI, USA). The collection and use of human materials for research purposes were approved by the Institutional Review Boards of the University Hospital Hradec Králové or Wayne State University and the NICHD. All participating women provided written informed consent prior to sample collection. The study

Table 2. Clinical and demographic characteristics of women who underwent hysterectomy and from whom cervical samples were collected.

	No Labor (n = 7)	Labor (n = 5)	p-value
Maternal age (years; median [IQR]) ^a	38 (36–39.5)	34 (31–36)	0.1
Body mass index (kg/m ² ; median [IQR]) ^a	34 (30–41)	26 (23–29)	0.05
Primiparity ^b	0% (0/7)	40% (2/5)	0.2
Race/ethnicity ^b			1.0
African-American	0% (0/7)	0% (0/5)	
White	100% (7/7)	100% (5/5)	
Other	0% (0/7)	0% (0/5)	
Cause of hysterectomy ^b			0.7
Abnormally invasive placenta	85.7% (6/7)	80% (4/5)	
Postpartum hemorrhage	0% (0/7)	20% (1/5)	
Placental abruption	14.3% (1/7)	0% (0/5)	
Gestational age at delivery (weeks; median [IQR]) ^a	36.4 (32.7–37.2)	39.4 (38.6–41)	0.07
Cesarean section ^b	100% (7/7)	100% (5/5)	1.0
Birthweight (grams; median [IQR]) ^a	2720 (2200–3075)	3100 (2790–3270)	0.5
Apgar score at 5 min (median [IQR]) ^a	10 (8.5–10)	10 (10–10)	0.4

Data are given as median (interquartile range, IQR) and percentage (n/N).

^a Mann-Whitney U test.

^b Fisher's exact test.

Table 3. Clinical and demographic characteristics of women with preterm prelabor rupture of the membranes (PPROM) from whom cervical and amniotic fluids were sampled, classified according to the intra-amniotic inflammatory status.

	No SIAI/IAI (n = 46)	SIAI (n = 6)	IAI (n = 10)	p-value
Maternal age (years; median [IQR]) ^a	29 (27.3–33)	30.5 (27.5–33.5)	31 (28.5–35)	0.9
Body mass index (kg/m ² ; median [IQR]) ^a	22.1 (20.4–24.7)	22.8 (22.6–24.2)	20.4 (17.9–23.1)	0.3
Primiparity ^b	71.7% (33/46)	50% (3/6)	20% (2/10)	0.07
Race/ethnicity ^b				1.0
African-American	0% (0/46)	0% (0/6)	0% (0/10)	
White	100% (46/46)	100% (6/6)	100% (10/10)	
Other	0% (0/46)	0% (0/6)	0% (0/10)	
Gestational age at sampling (weeks; median [IQR]) ^a	34.9 (33.8–36)	30.9 (25.6–35.1)	31.1 (26.4–33.6)	0.04
IL-6 amniotic fluid concentration (ng/mL) ^a	0.2 (0.1–0.3)	3.9 (2.5–8.7)	10 (3.5–10)	<0.001
Gestational age at delivery (weeks; median [IQR]) ^a	35 (34–36.3)	32.2 (27.7–35.9)	31.5 (26.9–33.7)	0.03
Cesarean section ^b	19.6% (9/46)	33.3% (2/6)	40% (4/10)	0.3
Birthweight (grams; median [IQR]) ^a	2455 (2160–2717)	1810 (1048–2483)	1765 (752–2208)	0.008
Apgar score at 5 min (median [IQR]) ^a	10 (9–10)	9 (8–10)	10 (9–10)	0.3

Data are given as median (interquartile range, IQR) and percentage (n/N). IAI, intra-amniotic infection; SIAI, sterile intra-amniotic inflammation.

^a Kruskal-Wallis test.

^b Fisher's exact test.

included the following samples taken from four cohorts: (1) myometrial tissues from women with or without labor who underwent elective cesarean section at term (Cohort 1; Table 1); (2) cervical tissues from women in preterm or term gestations with or without labor who underwent obstetric hysterectomy at delivery (Cohort 2; Table 2); (3) amniotic and cervical fluids from women with preterm prelabor rupture of the membranes (PPROM) (Cohort 3; Table 3), which were grouped according to intra-amniotic inflammatory status as described below; and (4) chorioamniotic membranes from women who underwent spontaneous preterm labor with intact membranes (Cohort 4; Table 4), which were also grouped according to intra-amniotic inflammatory status. Myometrial and cervical samples were also obtained from a single case of obstetric hysterectomy due to cesarean scar ectopic pregnancy collected at 18 weeks of gestation. Maternal and neonatal data were obtained by retrospective clinical chart review.

2.2. Clinical definitions and sample collection

Gestational age was established based on the last menstrual period and confirmed by ultrasound examination. PPROM was defined as amniorrhexis confirmed by vaginal pooling, ferning, or a positive nitrazine test prior to the onset of labor before 37 weeks of gestation [20, 21,

22, 23]. Spontaneous preterm labor was defined as the presence of regular uterine contractions with a frequency of at least two every 10 min and cervical ripening between 20 and 36 6/7 weeks of gestation. Preterm delivery was defined as birth before 37 weeks of gestation.

The intra-amniotic inflammatory status of patients in Cohorts 3 and 4 was determined by combining the presence or absence of microbes, as determined by microbiological culture and/or molecular test, together with the evaluation of amniotic fluid interleukin (IL)-6 concentration, since this cytokine has been previously established as an indicator of intra-amniotic inflammation [24, 25]. Thus, a positive microbial signal (either by culture or molecular tests), together with an elevated IL-6 concentration [24, 26], indicates intra-amniotic infection [16, 27, 28, 29]; a negative microbial signal (indicated by both culture and molecular tests), together with an elevated IL-6 concentration, indicates sterile intra-amniotic inflammation [16, 27, 28, 29]; and a negative microbial signal (indicated by both culture and molecular tests), together with a low IL-6 concentration, indicates no intra-amniotic inflammation/infection.

2.2.1. Cohort 1

Myometrial samples were obtained from the lower uterine segment at the time of cesarean section. Biopsies were obtained from the midpoint of the superior edge of the uterine incision and transported to the laboratory

Table 4. Clinical and demographic characteristics of women with preterm labor and intact membranes from whom chorioamniotic membranes were collected, classified according to the intra-amniotic inflammatory status.

	No SIAI/IAI (n = 16)	SIAI (n = 18)	IAI (n = 17)	p-value
Maternal age (years; median [IQR]) ^a	23 (19.8–23.8)	26 (22.3–28.8)	23 (20–26)	0.1
Body mass index (kg/m ² ; median [IQR]) ^a	21.8 (20.1–27) ^c	29.9 (25.3–33.9) ^c	26.9 (22.2–31.9) ^c	0.02
Primiparity ^b	31.3% (5/16)	22.2% (4/18)	17.6% (3/17)	0.7
Race/ethnicity ^b				0.96
African-American	87.5% (14/16)	88.9% (16/18)	88.2% (15/17)	
White	12.5% (2/16)	11.1% (2/18)	5.9% (1/17)	
Other	0% (0/16)	0% (0/18)	5.9% (1/17)	
Gestational age at delivery (weeks; median [IQR]) ^a	34.3 (33.3–34.9)	26.3 (24.4–30.6)	28.1 (23.9–31.4)	<0.001
Birthweight (grams; median [IQR]) ^a	2230 (1808–2575)	871 (603–1456)	1120 (525–1525)	<0.001
Apgar score at 5 min (median [IQR]) ^a	9 (8–9)	6.5 (5.3–8.8)	5 (2–7)	0.008

Data are given as median (interquartile range, IQR) and percentage (n/N). IAI, intra-amniotic infection; SIAI, sterile intra-amniotic inflammation.

^a Kruskal-Wallis test.

^b Fisher's exact test.

^c One missing datum.

in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies Corporation, Grand Island, NY, USA) for the following preservation methods: (1) freezing in Tissue-Tek OCT Compound (Sakura Finetek USA, Torrance, CA, USA) for immunofluorescence staining; (2) fixation in 10% (v/v) neutral buffered formalin followed by paraffin embedding for immunohistochemistry; and (3) snap-freezing in liquid nitrogen and storage at -80 °C until use for RNA isolation.

2.2.2. Cohort 2

After obstetric hysterectomy, the uterine cervix was fixed in 10% (v/v) neutral buffered formalin. Tissue samples were obtained from the cervix and embedded in paraffin after processing.

2.2.3. Cohort 3

Cervical and amniotic fluids were collected at the time of diagnosis of PPROM. Ultrasound-guided transabdominal amniocentesis was performed upon admission, before the administration of corticosteroids and antibiotics; approximately 1–2 mL of amniotic fluid were obtained. A total of 100 µL of non-centrifuged amniotic fluid was used for the bedside assessment of IL-6 concentration. Two aliquots of non-centrifuged amniotic fluid were immediately transported to undergo PCR testing for *Ureaplasma* spp., *Mycoplasma hominis*, *Chlamydia trachomatis*, and the 16S rRNA gene, as well as for aerobic/anaerobic cultivation of amniotic fluid.

Cervical fluid samples were collected on a Dacron polyester swab placed in the cervical canal for 20 s to achieve saturation. Upon collection, the Dacron polyester swab was inserted into a polypropylene tube containing 1.5 mL of phosphate-buffered saline (PBS). The tube was shaken for 20 min, the Dacron swab was removed, and the tube was centrifuged for 15 min at 300 x g. The supernatant and pellets were aliquoted and stored at -80 °C until further analysis.

2.2.4. Cohort 4

Patients in Cohort 4 underwent amniocentesis due to suspected intra-amniotic infection, and the amniotic fluid was evaluated for the presence of microbes and IL-6 concentrations as described above. Chorioamniotic membrane samples were collected from the placenta within 30 min after delivery for formalin fixation/paraffin embedding for immunohistochemistry. A section of the chorioamniotic membranes that extended the entire length of the membrane was cut, rolled, and snap-frozen using liquid nitrogen. Snap-frozen chorioamniotic membrane samples were stored at -80 °C until use for RNA isolation.

2.3. Detection of microorganisms in amniotic fluid

2.3.1. Cohort 3

Amniotic fluid was cultivated for 6 days and checked daily for microbial growth. Microbial species identification was provided by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using MALDI Biotyper software (Bruker Daltonics; Bremen, Germany).

For molecular determinations, DNA was isolated from the amniotic fluid with the QIAamp DNA mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Real-time PCR was performed on a Rotor-Gene 6000 (QIAGEN) with the commercial kit AmpliSens *C. trachomatis/Ureaplasma/M. hominis-FRT* (Federal State Institution of Science, Central Research Institute of Epidemiology, Moscow, Russia) to detect the DNA from *Ureaplasma* spp., *M. hominis*, and *C. trachomatis*. Beta-actin was included as a control. The threshold cycle value for *Ureaplasma* spp. was used for the relative quantification of the microbial load of *Ureaplasma* spp. in amniotic fluid.

Bacterial DNA was identified by PCR targeting the 16S rRNA gene using the following primers: 5'-CCAGACTCCTACGGGAGGCAG-3' (V3 region) and 5'-ACATTTTCAACAACAC-GAGCTGACGA-3' (V6 region). Each individual reaction mixture contained 3 µL of target DNA, 500 nM of forward and reverse primers, and Q5 High Fidelity DNA polymerase (NEB; Hitchin, United Kingdom) in a total volume of 25 µL. The

amplification was performed in a 2720 Thermal Cycler (Applied Biosystems, Life Technologies Corporation; Foster City, CA, USA). The products were visualized on an agarose gel. Positive reactions yielded products of 950 bp, which were subsequently analyzed by sequencing. The 16S rRNA PCR products were purified before undergoing PCR utilizing specific primers and the BigDye Terminator kit version 3.1 (Applied Biosystems). The bacteria were then typed by aligning the sequences obtained in BLAST and SepsisTest BLAST.

2.3.2. Cohort 4

Amniotic fluid samples were collected by amniocentesis upon admission from women with spontaneous preterm labor due to suspected intra-amniotic infection. Standard clinical laboratory determinations included the microbiological culture of aerobic/anaerobic bacteria and genital mycoplasmas [11, 16]. Molecular detection of microbes was performed using the PCR/ESI-MS (Ibis Technology; Athogen, Carlsbad, CA, USA) platform, which includes a broad bacteria and *Candida* (BAC) detection assay that can identify 3,400 bacteria and 40 *Candida* spp., as described in the platform's signature database [30]. In addition, the platform includes a viral assay that was used to detect the following viruses: herpes simplex virus 1 (HHV-1), herpes simplex virus 2 (HHV-2), Varicella-Zoster virus (HHV-3), Epstein-Barr virus (HHV-4), cytomegalovirus (HHV-5), roseolovirus (HHV-6), Kaposi's sarcoma-associated herpesvirus (HHV-8), human adenoviruses, human enteroviruses, BK polyomavirus, JC polyomavirus, and parvovirus B19.

2.4. Determination of interleukin-6 concentrations in amniotic fluid

2.4.1. Cohort 3

IL-6 concentrations in amniotic fluid samples were assessed with a lateral flow immunoassay, Milenia QuickLine IL-6, using the Milenia POCScan Reader (Milenia Biotec, GmbH, Giessen, Germany) [25]. The inter- and intra-assay coefficients of variation for IL-6 were 15.5% and 12.1%, respectively. The detection limit of the assay was 50 pg/mL. A cut-off of 745 pg/mL was used to determine intra-amniotic inflammation, as previously established [26].

2.4.2. Cohort 4

IL-6 concentrations in amniotic fluid samples were measured with a sensitive and specific enzyme immunoassay from R&D Systems (Minneapolis, MN, USA), as previously established [24]. The IL-6 concentrations were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation for IL-6 were 8.7% and 4.6%, respectively. The detection limit of the assay was 0.09 pg/mL. A cut-off of 2.6 ng/mL was used to determine intra-amniotic inflammation, as previously established [24].

2.5. Determination of human papillomavirus infection in cervical fluids

Cervical specimens from Cohort 3 were taken from the endocervical canal using a cervical brush (Rovers Medical Devices; KV Oss, Netherlands) immediately after cervical fluid sampling and prior to amniocentesis. The cervical brush was detached and suspended in a liquid-based cytology vial (DiaPrep Fixative Gyn; Diapath S.P.A; Martignano, Italy) containing preservative fluid. DNA was immediately extracted from the cell suspensions using the MagCore Viral Nucleic Acid Extraction Kit (RBC Bioscience; New Taipei City, Taiwan), according to the manufacturer's protocol. HPV DNA detection and genotyping were performed using qualitative real-time PCR with the AmoyDx Human Papillomavirus Genotyping Detection Kit (Amoy Diagnostics; Xiamen, China). The test has been designed for specific amplification of the L1 gene in HPV DNA to detect and genotype 19 high-risk HPVs (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82) and two low-risk HPVs (HPV 6 and 11). The test was sensitive to a level of 100 copies of HPV DNA per reaction. An internal assay control was utilized to test for sample quality and the presence of inhibiting factors.

2.6. Hematoxylin and eosin staining

Formalin-fixed paraffin-embedded tissue sections of myometrium, cervix, and chorioamniotic membranes were cut in five- μm -thick sections and mounted on slides for hematoxylin and eosin (H&E) staining. All reagents were purchased from Thermo Fisher Scientific. Staining was performed using a Tissue-Tek DRS instrument (Sakura Finetek USA).

2.7. IFNe immunohistochemistry of the myometrium, cervix, and chorioamniotic membranes

Formalin-fixed paraffin-embedded myometrial ($n = 5$ per group), cervical ($n = 5$ – 7 per group), and chorioamniotic membrane ($n = 5$ per group) tissues were cut in five- μm -thick sections. Slides were deparaffinized in xylene and hydrated with decreasing concentrations of ethanol. Immunohistochemistry staining for IFNe (mouse anti-human IFNe, Clone 983338; R&D Systems) was performed using the Leica Bond Max automatic staining system (Leica Microsystems; Wetzlar, Germany). The Bond™ Polymer Refine Detection Kit (Leica Microsystems) was used to detect the chromogenic reaction of horseradish peroxidase upon oxidation of 3'3-Diaminobenzidine (DAB). Mouse IgG2B isotype (Cat. No. MAB004; R&D Systems) was used as the negative control. Brightfield images were taken using the Vectra Polaris Multispectral Imaging System and inForm software version 2.5.1. Representative images were taken at 200X magnification.

2.8. IFNe immunofluorescence of the myometrium

Ten-micrometer-thick sections of myometrial tissues frozen in Tissue-Tek OCT Compound from women who delivered at term with or without labor ($n = 5$ per group) were cryosectioned and mounted on microscope slides. For intracellular staining of myometrial tissues, slides were fixed with 4% paraformaldehyde (Electron Microscopy Sciences; Hatfield, PA, USA) in 1X PBS for 20 min at room temperature, then permeabilized using 0.25% Triton X-100 (EMD Millipore, Billerica, MA, USA) for 5 min at room temperature, rinsing the slides with 1X PBS after each step. Non-specific antibody binding was prevented by treating the slides with BlockAid blocking solution (Thermo Fisher Scientific) for 60 min at room temperature. Next, the slides were incubated with the primary antibody mouse anti-human IFNe or the mouse IgG2B isotype control (Clone 20116; R&D Systems) overnight at 4 °C. The slides were then washed three times with 0.1% Tween (MP Biomedicals, Solon, OH, USA) in PBS + 0.1% Tween 20 (MP Biomedicals, LLC., Solon, OH, USA) (PBS-T) for 10 min each wash. Next, the slides were incubated for 30 min at room temperature with the secondary antibody, goat anti-mouse IgG Alexa Fluor Plus 594 (Thermo Fisher Scientific). The slides were again washed three times with PBS-T for 10 min each, after which the slides were mounted using ProLong Gold Antifade Mountant with DAPI (Thermo Fisher Scientific). Immunofluorescence was visualized using the Vectra Polaris Multispectral Imaging System and inForm software version 2.5.1. Representative images were taken at 200X magnification.

2.9. IFNE expression in the myometrium and chorioamniotic membranes

The expression of *IFNE* was determined in myometrial tissues collected from women who underwent cesarean section at term with or without labor (Cohort 1) and in chorioamniotic membranes collected from women who underwent spontaneous preterm labor with intact membranes (Cohort 4). Total RNA was isolated from chorioamniotic membranes and myometrial tissues using QIAshredder columns and the RNeasy mini kit (both from QIAGEN), following the manufacturer's instructions. The resulting RNA concentrations and integrity were assessed using the Agilent Bioanalyzer System and RNA 6000 Nano kit (both from Agilent; Santa Clara, CA, USA). Reverse transcription of RNA to complementary (c)DNA was performed in a 20 μL reaction, consisting of 500 ng RNA and 4 μL SuperScript IV VIL0 Master Mix (Invitrogen, Thermo

Fisher Scientific Baltics UAB, Vilnius, Lithuania), within a Veriti 96-well Thermal Cycler (Thermo Fisher Scientific; Wilmington, DE, USA) or a T100 Thermal Cycler (BIO-RAD, Hercules, California USA). As a negative control, the same reaction was prepared using SuperScript IV VIL0 Master Mix lacking reverse transcriptase (Invitrogen). The thermal cycler conditions were as follows: 25 °C for 10 min, 50 °C for 10 min, and 85 °C for 5 min. Next, gene expression was assessed by performing quantitative real-time PCR (qRT-PCR) using the ABI 7500 FAST Real-Time PCR System (Applied Biosystems). As internal controls, *GAPDH*, *ACTB*, and *RPLPO* (chorioamniotic membranes) or *GAPDH* (myometrium) were also detected. The 20 μL PCR reaction contained 10 μL TaqMan™ Fast Advanced Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 1 μL 20X *IFNE* TaqMan gene expression assay (Thermo Fisher Scientific; Assay ID: Hs00703565_s1), 1 μL 20X *GAPDH* primer (Thermo Fisher Scientific; Assay ID: Hs02786624_g1), and 2 μL cDNA. The thermal cycler profile included holding at 50 °C for 2 min prior to initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. Each sample was analyzed in duplicate.

2.10. IFNe detection in amniotic and cervical fluids

IFNe concentrations were determined in human amniotic and cervical fluids from Cohort 3 using the Human Interferon Epsilon (IFNe) ELISA kit (Cusabio Technology LLC; Wuhan, China), according to the manufacturer's instructions. Plates were read using the SpectraMax iD5 (Molecular Devices; San Jose, CA, USA), and analyte concentrations were calculated with the SoftMax Pro 7 (Molecular Devices). The detection limit of the assay was 3.9 pg/mL.

2.11. IFNE expression in human amnion epithelial cells and cervical cells

Primary amnion epithelial cells were isolated from chorioamniotic membranes collected from women who underwent normal delivery at term ($n = 3$), as previously described [19]. Non-immortalized primary cervical stromal cells were previously isolated at our laboratory from cervical biopsies collected for clinical purposes. Ectocervical epithelial cells (Ect1/E6E7) and endocervical epithelial cells (End1/E6E7) were obtained from ATCC (CRL-2614 and CRL-2615, respectively). Amnion epithelial, cervical stromal, ectocervical, and endocervical cells were plated in six-well tissue culture plates (Corning, Inc.; Corning, NY, USA) at 1.5×10^5 cells/well and cultured at 37 °C with 5% CO₂. The cells were then incubated with *Ureaplasma parvum* isolated from amniotic fluid of women with intra-amniotic infection (1.3×10^5 cells/well with serum-free Opti-MEM [Life Technologies]) as previously described [19], SP4 broth with serum-free Opti-MEM (control media), lipopolysaccharide (LPS; 1 $\mu\text{g}/\text{mL}$; *Escherichia coli* O 55:B5; Sigma-Aldrich, St. Louis, MO, USA) (amnion epithelial cells only), or sterile 1X PBS with serum-free Opti-MEM (control media) (amnion epithelial cells only). After 24 h of treatment, cell pellets were collected for determination of *IFNE* expression. Total RNA was isolated from cell pellets by using the RNeasy Micro kits according to the manufacturer's instructions. RNA concentrations were assessed with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). RNA integrity was assessed with an Agilent Bioanalyzer System and RNA 6000 Nano kit. Next, cDNA was synthesized using SuperScript IV VIL0 Master Mix, according to the manufacturer's instructions. As a negative control, the same reaction was prepared using SuperScript VIL0 IV Master Mix lacking reverse transcriptase. The thermal cycler conditions were as follows: 25 °C for 10 min, 50 °C for 10 min, and 85 °C for 5 min. Messenger RNA expression was determined by qPCR utilizing a BioMark high-throughput qRT-PCR system (Fluidigm) and the *IFNE* TaqMan gene expression assay.

2.12. Statistics

Statistical analyses were conducted using GraphPad Prism version 8.0.1 for Windows (GraphPad Software, San Diego, California, USA,

www.graphpad.com). For patient demographics, the Mann-Whitney *U*-test or the Kruskal-Wallis test was used to compare continuous variables and the Fisher's exact test was used for nominal variables. For gene expression analysis, negative delta threshold cycle ($-\Delta C_T$) values were calculated according to the reference genes listed above. Gene expression data were compared using the unpaired student's *t*-test or one-way ANOVA with Tukey's correction for tissues and the paired student's *t*-test for cell cultures. IFNe concentrations in amniotic and cervical fluids were compared using the Mann-Whitney *U*-test or Kruskal-Wallis test with Dunn's correction. A *p*-value <0.05 was considered statistically significant for all tests.

3. Results

3.1. IFNe is expressed in the human myometrium

First, we determined the mRNA and protein expression of IFNe in the myometrium (Figure 1A). To establish the baseline expression patterns of IFNe in mid-pregnancy, we utilized a myometrial sample from a hysterectomy case collected at 18 weeks of gestation (Figure 1B). IFNe was modestly expressed in mid-gestation myometrial tissues (Figure 1B). Next, we determined the mRNA and protein expression of IFNe in the

myometrial tissues from women who delivered at term with or without spontaneous labor. Myometrial tissues at term expressed IFNe but this did not differ between the term labor and no labor groups (Figure 1C). Consistently, immunohistochemistry and immunofluorescence staining both revealed strong IFNe expression by smooth muscle cells at term that did not differ with labor status (Figure 1D). Yet, protein expression of IFNe in the myometrium was more apparent at term than in mid-gestation (Figure 1D vs. 1B), which is consistent with murine findings [2]. Together, these results show that IFNe is gradually expressed in the myometrial tissues from mid to late gestation; yet, this cytokine does not increase with the physiological inflammatory process of labor at term.

3.2. IFNe is expressed in the human cervix and is present in cervical fluid

Next, we investigated whether IFNe was expressed by the cervical tissues in mid and late pregnancy (Figure 2A). Similar to the myometrium, modest IFNe expression was noted in cervical tissues from mid-gestation (Figure 2B). Such expression was more pronounced in late gestation and appeared to be primarily localized in the smooth muscle cells and fibroblasts surrounding the blood vessels of the cervix (Figure 2C). Yet, IFNe was largely unaltered between samples from preterm and term gestations, suggesting consistent basal expression of

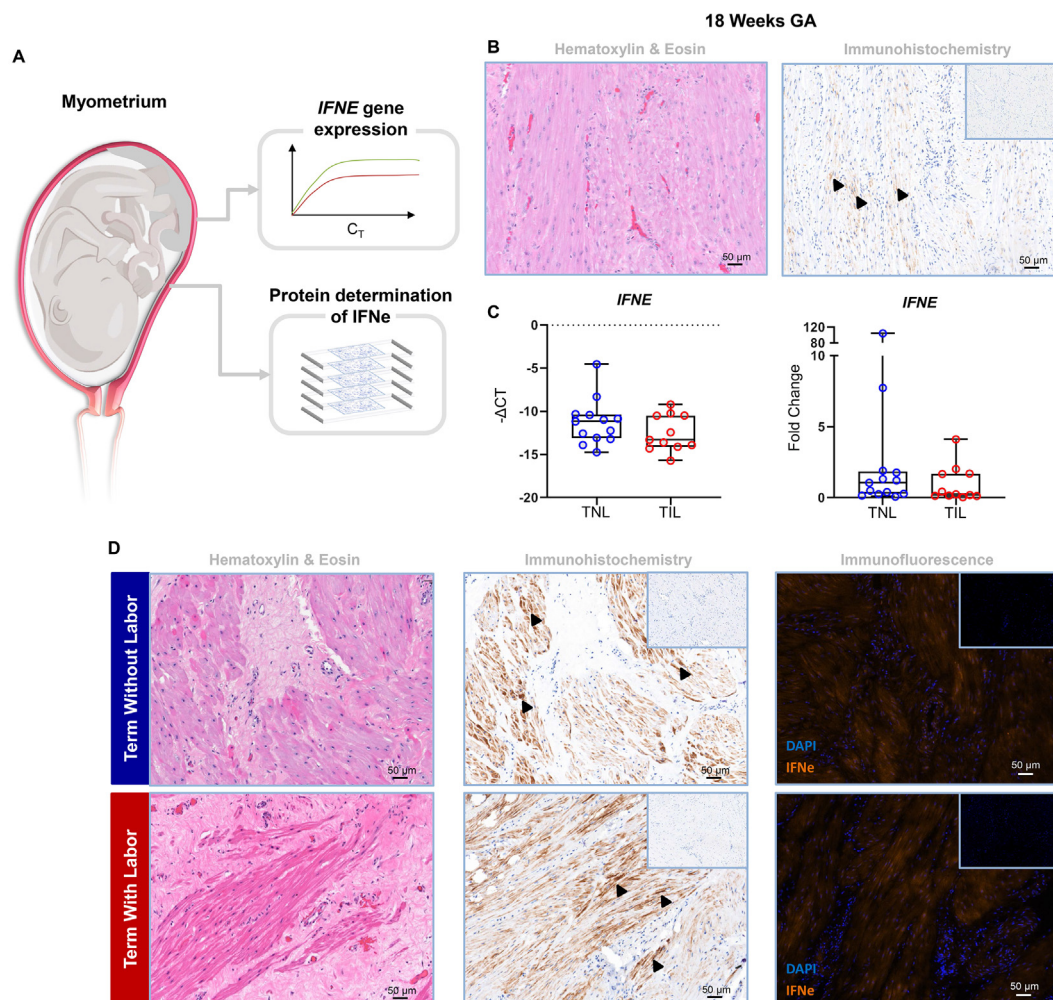


Figure 1. IFNe expression in the myometrium in mid-pregnancy and at term. (A) Experimental design for the determination of mRNA and protein expression of IFNe in the myometrium. (B) Hematoxylin & Eosin staining and immunohistochemistry staining for IFNe in myometrial tissues from a single case collected at 18 weeks of gestation. (C) IFNe expression in the myometrial tissues collected from women who delivered at term without (TNL, $n = 13$) or with (TIL, $n = 11$) labor. $-\Delta C_T$ values and fold changes are shown. (D) Representative Hematoxylin & Eosin staining, immunohistochemistry staining for IFNe, and immunofluorescence staining for IFNe (orange) and nuclei (DAPI, blue) in myometrial tissues from women who delivered at term without or with labor ($n = 5$ per group). All images were taken at 200X magnification. Inset images show isotype control staining from the same case. Black arrowheads indicate areas of positive staining for IFNe. Scale bars represent 50 μ m. GA, gestational age; TIL, term with labor; TNL, term without labor.

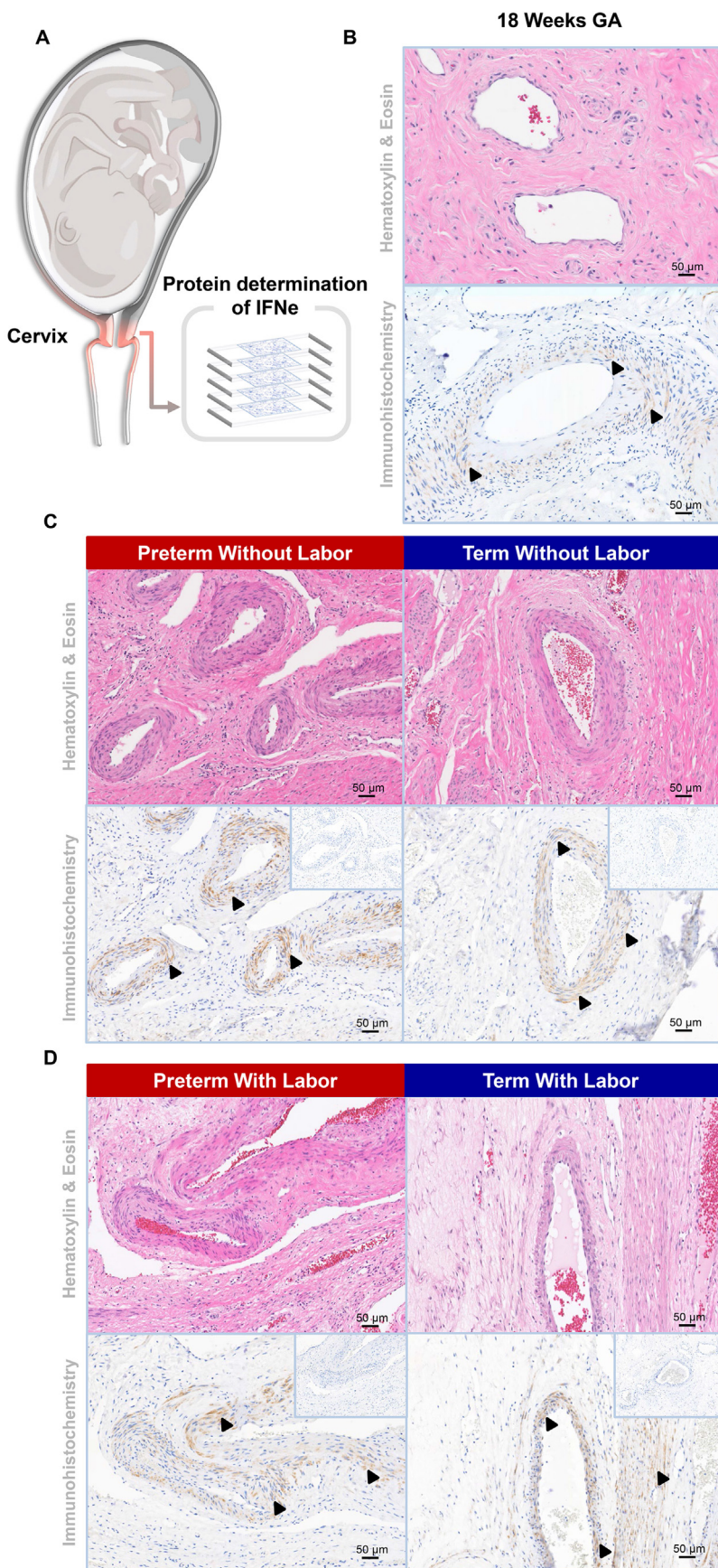


Figure 2. IFNε expression in the cervix in mid-pregnancy and late gestation. (A) Experimental design for the determination of IFNε protein expression in the cervix. (B) Hematoxylin & Eosin staining and immunohistochemistry staining for IFNε in cervical tissues from a single case collected at 18 weeks of gestation. Representative Hematoxylin & Eosin staining and immunohistochemistry staining for IFNε in myometrial tissues from women who delivered preterm or at term (C) without (n = 7) or (D) with (n = 5) labor. All images were taken at 200X magnification. Inset images show isotype control staining from the same case. Black arrowheads indicate cells with positive staining for IFNε. Scale bars represent 50 μm. GA, gestational age.

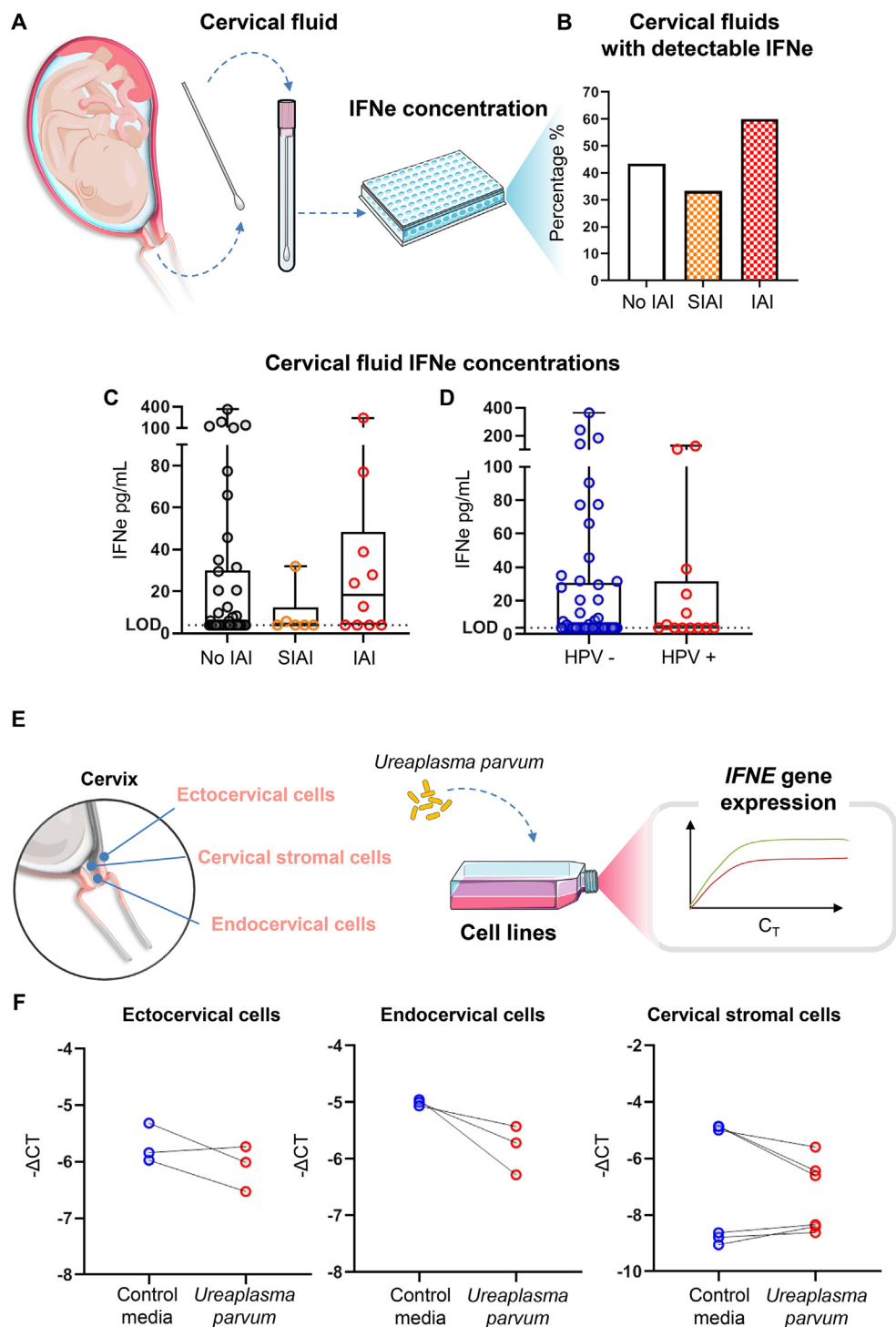


Figure 3. Intra-amniotic infection does not alter IFN ϵ expression in cervical fluid or cervical cells. (A) Experimental design for the determination of IFN ϵ concentrations in cervical fluid. (B) Percentage of cervical fluid samples with detectable IFN ϵ from women who underwent spontaneous preterm birth without intra-amniotic inflammation (No IAI, n = 46), with sterile intra-amniotic inflammation (SIAI, n = 6), or with intra-amniotic infection (IAI, n = 10). (C) Concentrations of IFN ϵ in cervical fluid samples collected from women who underwent spontaneous preterm birth with No IAI, SIAI, or IAI. (D) Concentrations of IFN ϵ in cervical fluid samples grouped according to HPV negative (HPV-) or positive (HPV+) status. (E) Experimental design for the determination of IFN ϵ expression by cervical cell lines. (F) IFN ϵ expression (shown as $-\Delta C_T$) in (left to right) ectocervical epithelial (n = 3 technical replicates), endocervical epithelial (n = 3 technical replicates), and cervical stromal cells (n = 2 cases with 3 technical replicates each) co-cultured with *Ureaplasma parvum* or control media. HPV, human papillomavirus.

this cytokine in the third trimester (Figure 2C). In line with observations in the myometrium, the process of labor did not seem to alter IFN ϵ expression in the cervical tissues, regardless of preterm or term gestation (Figure 2C vs. 2D). Thus, IFN ϵ is a component of the cytokine repertoire present in the cervical tissues throughout gestation, but its protein levels do not change with the process of labor.

We next determined whether IFN ϵ was detectable in cervical fluids from women who underwent spontaneous preterm birth with sterile intra-amniotic inflammation or intra-amniotic infection (Figure 3A). Women who underwent spontaneous preterm birth without intra-amniotic inflammation or infection were considered as controls. IFN ϵ

was detectable in a proportion of cervical fluid samples (Figure 3B); however, its concentrations did not differ among study groups (Figure 3C). To determine whether HPV infection could impact cervical IFN ϵ levels, we then grouped all cervical fluids according to the presence or absence of this viral infection. Consistently, cervical fluid IFN ϵ concentrations did not differ based on HPV status (Figure 3D). Therefore, cervical fluid concentrations of IFN ϵ do not vary based on the inflammatory status of the amniotic cavity or the presence of HPV in the cervix.

Following this result, we investigated the direct effect of *Ureaplasma parvum*, a genital mycoplasma commonly found in the amniotic cavity of women with intra-amniotic infection [11, 12, 13, 14, 15, 16, 17, 18], on IFN ϵ

expression by cervical cell lines (Figure 3E). *Ureaplasma parvum* did not induce notable changes in IFNe expression in ectocervical, endocervical, or cervical stromal cells (Figure 3F), suggesting that this cytokine is not implicated in the local host response against genital mycoplasma in the cervix.

3.3. IFNe is increased in amniotic fluid of women with intra-amniotic infection

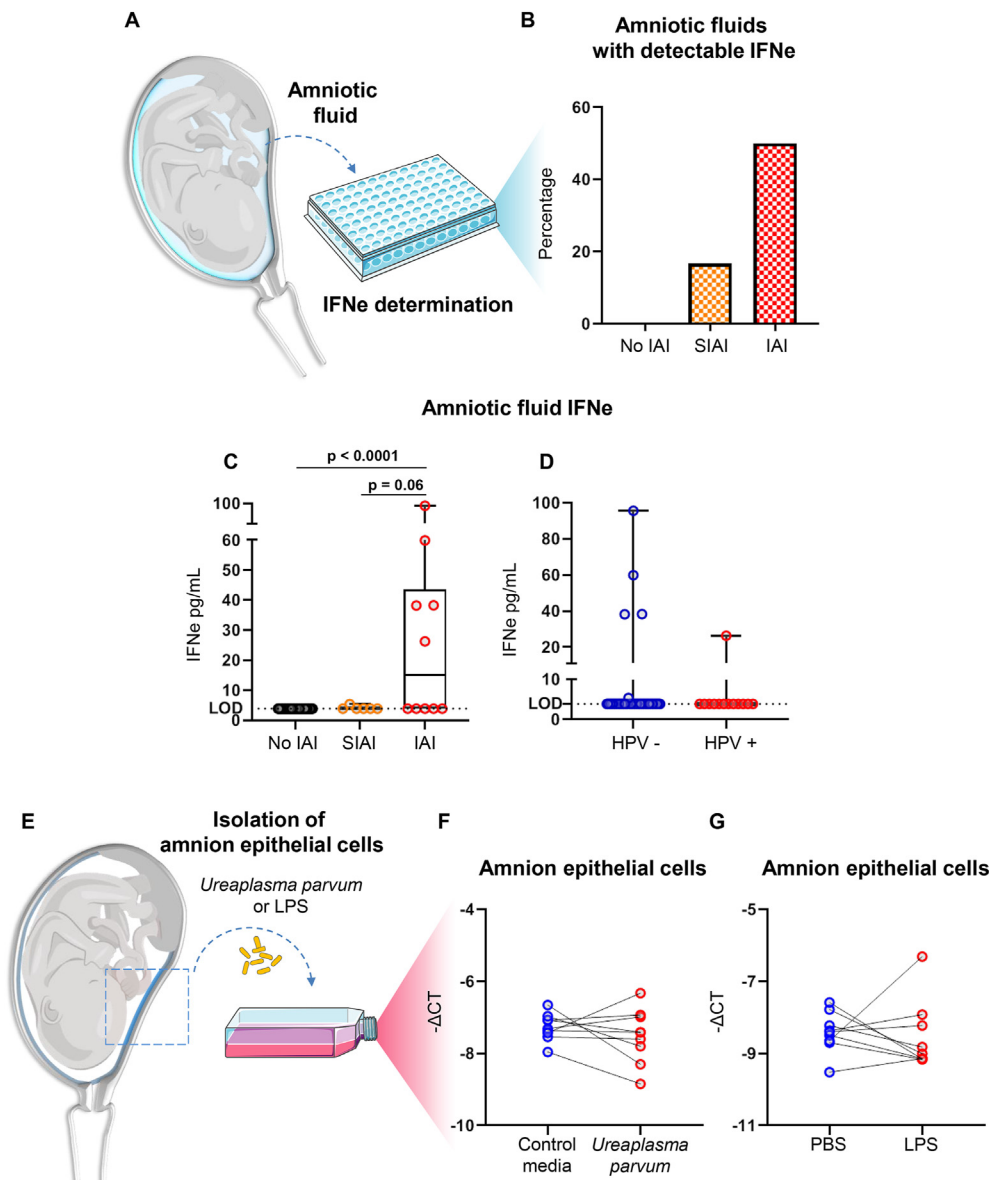
Specific bacteria from the lower genital tract can invade the amniotic cavity through the cervix, resulting in an inflammatory process termed intra-amniotic infection [31, 32, 33, 34, 35, 36]. Therefore, we next investigated whether IFNe was present in amniotic fluid samples derived from the same patient cohort in which cervical fluid sampling was performed (Figure 4A). Notably, IFNe was not detected in any of the control cases, but was detectable in a small proportion of amniotic fluid samples from women with sterile intra-amniotic inflammation and in one-half of those from women with intra-amniotic infection (Figure 4B). Furthermore, amniotic fluid concentrations of IFNe were increased in cases of intra-amniotic infection compared to those of controls or of women with sterile intra-amniotic inflammation (Figure 4C). When amniotic fluid samples were classified according to the presence or absence of HPV in cervical fluid, no difference

was observed between groups (Figure 4D); indeed, 4/5 of the samples from women with intra-amniotic infection that showed elevated IFNe were HPV-negative. Next, we evaluated the direct effect of *Ureaplasma parvum* or LPS on IFNe expression by amnion epithelial cells, the cell layer that is in direct contact with amniotic fluid [37] (Figure 4E). Neither *Ureaplasma parvum* nor LPS upregulated the expression of IFNe in amnion epithelial cells (Figure 4F-G). These results suggest that IFNe is implicated in the host defense mechanisms against microbial invasion of the amniotic cavity in women who ultimately undergo spontaneous preterm birth; yet, amnion epithelial cells are not the main source of this cytokine.

3.4. IFNe is expressed in the human chorioamniotic membranes

The amniotic cavity is enclosed by the chorioamniotic membranes [37], which may serve as a source of the soluble products found in this compartment, particularly in the context of preterm labor and birth [38, 39, 40, 41, 42, 43, 44]. Therefore, we lastly determined the expression of IFNe in the chorioamniotic membranes from women who underwent spontaneous preterm labor with sterile intra-amniotic inflammation or intra-amniotic infection (Figure 5A). Women without intra-amniotic inflammation or infection who underwent spontaneous preterm labor

Figure 4. Intra-amniotic infection increases IFNe concentrations in amniotic fluids from a subset of preterm birth cases. (A) Experimental design for the determination of IFNe concentrations in amniotic fluid. (B) Percentage of amniotic fluid samples with detectable IFNe from women who underwent spontaneous preterm birth without intra-amniotic inflammation (No IAI, n = 46), with sterile intra-amniotic inflammation (SIAI, n = 6), or with intra-amniotic infection (IAI, n = 10). (C) Concentrations of IFNe in amniotic fluid samples collected from women who underwent spontaneous preterm birth with No IAI, SIAI, or IAI. P-values were determined using the Kruskal-Wallis test followed by Dunn's correction for multiple comparisons. (D) Concentrations of IFNe in amniotic fluid samples grouped according to HPV negative (HPV-) or positive (HPV+) status. (E) Experimental design for the co-culture of primary amnion epithelial cells with *Ureaplasma parvum* or lipopolysaccharide (LPS) to determine IFNe expression. (F) IFNe expression (shown as $-\Delta C_T$) in primary amnion epithelial cells co-cultured with *Ureaplasma parvum* or control media. N = 3 cases with 3 technical replicates each. (G) IFNe expression (shown as $-\Delta C_T$) in primary amnion epithelial cells co-cultured with LPS or PBS control. N = 3 cases with 3 technical replicates each. HPV, human papillomavirus.



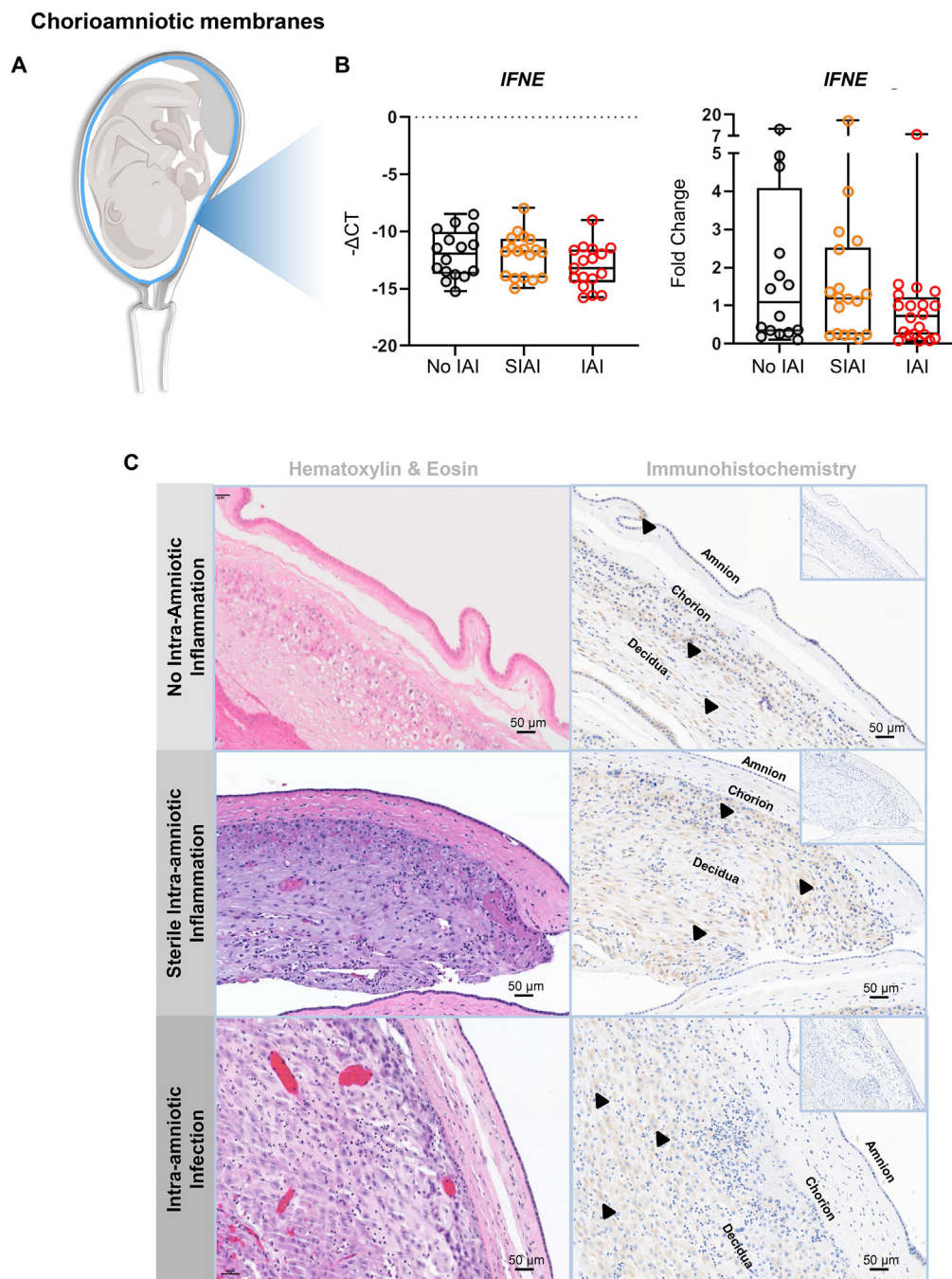


Figure 5. IFNe expression in the chorioamniotic membranes is unaltered by intra-amniotic infection. (A) Experimental design showing the collection of the chorioamniotic membranes to determine *IFNE* expression. (B) *IFNE* expression in the chorioamniotic membranes collected from women who underwent spontaneous preterm labor without intra-amniotic inflammation (No IAI, $n = 16$), with sterile intra-amniotic inflammation (SIAI, $n = 18$), or with intra-amniotic infection (IAI, $n = 17$). $-\Delta C_T$ values and fold changes are shown. (C) Representative Hematoxylin & Eosin staining and immunohistochemistry staining for IFNe in chorioamniotic membranes from women who underwent spontaneous preterm labor with No IAI, SIAI, or IAI ($n = 5$ per group). All images were taken at 200X magnification. Inset images show isotype control staining from the same case. Black arrowheads indicate cells with positive staining for IFNe. Scale bars represent 50 μm .

were considered as controls. The expression of *IFNE* in the chorioamniotic membranes did not differ among study groups (Figure 5B). Immunohistochemistry staining for IFNe demonstrated apparent and widespread expression of this protein in the chorion and decidua, which did not noticeably differ among study groups (Figure 5C). Together, these findings point to IFNe as being constitutively expressed by the chorioamniotic membranes; yet, this cytokine does not participate in the tissular inflammatory milieu that accompanies the intra-amniotic inflammatory response observed in women with microbial invasion of the amniotic cavity and spontaneous preterm labor leading to preterm birth.

4. Discussion

In the current study, we demonstrated that IFNe is expressed in the myometrium, cervix, and chorioamniotic membranes, and may therefore

represent a constitutive element of host defense mechanisms in these tissues during human pregnancy. The expression of IFNe in the myometrium and cervix appeared greater in late gestation than in mid-pregnancy but did not seem to be impacted by labor. Notably, concentrations of IFNe in amniotic fluid, but not cervical fluid, were increased in a subset of women who underwent spontaneous preterm birth with intra-amniotic infection, indicating that IFNe could participate in antimicrobial responses in the amniotic cavity. Yet, *in vitro* stimulation of amnion epithelial cells with *Ureaplasma parvum* or LPS did not increase *IFNE* expression, suggesting that these cells are not the primary source of this cytokine during intra-amniotic infection. Together, these findings provide an overview of local IFNe expression during normal pregnancy and its complications.

Previous studies in humans and animal models have proposed a role for IFNe as a constitutively expressed cytokine that participates in host

immunity, particularly in mucosal tissues [45]. The concept of constitutive type I IFN expression has been well-documented for better characterized members of this family, such as IFN β [46]. Early reports suggested that type I IFNs were continuously induced by mild insults such as pathogens or tissue damage [47], with subsequent studies demonstrating constitutive type I IFN expression under steady-state conditions or after physical exercise [48, 49, 50]. The regulation of constitutive type I IFN expression has not been fully elucidated; yet, several essential transcriptional regulators have been identified, including AP-1 and NF-KB [46]. At the cellular level, constitutive type I IFN expression serves important homeostatic functions including maintenance of hematopoietic stem cells, promoting macrophage functions, and regulation of natural killer (NK) cell populations [46]. The above roles for constitutively expressed IFNs suggest that IFNe may carry out similar functions; indeed, recent reports investigated the properties of this cytokine and demonstrated activation of T, B, and NK cells together with anti-viral and anti-bacterial activity [45, 51]. Thus, IFNe may similarly serve as a homeostatic regulator and first line of defense against infection at barrier sites.

Herein, we used immunohistochemistry to show that IFNe expression in the myometrium and cervix was stronger in late gestation compared to mid-pregnancy, suggesting a gestational age-dependent increase that is consistent with a prior human study [8]. *IFNE* expression was observed to fluctuate based on the menstrual cycle in both non-pregnant mice and humans, which led to the proposed hormonal regulation of IFNe expression in the reproductive tract, particularly in the endometrium [2, 5]. Estrogen administration to ovariectomized mice induced *Ifne* expression in the uterine tissues, and analysis of endometrial epithelial cells derived from women in different stages of the menstrual cycle demonstrated highest *IFNE* expression in the proliferative phase [2]. A negative correlation between endometrial progesterone receptor expression and IFNe has also been demonstrated, and the direct treatment of an endometrial epithelial cell line with progesterone resulted in decreased IFNe expression [5]. Thus, deeper investigation is required to determine the relationship between endometrial/myometrial IFNe expression and sex hormone signaling, particularly during pregnancy. By contrast with the endometrium, hormonal regulation of IFNe was not reported in the non-pregnant cervix [5]. However, other regulatory mechanisms for this cytokine have also been proposed, as one study noted that IFNe could be induced in the cervical tissues in response to stimuli such as semen [52], which may result in protection against potential viral transmission [53]. Taken together, these studies provide an overview of the changes in IFNe signaling that take place in the reproductive tissues in the pregnant and non-pregnant states. However, additional investigation into the mechanisms that regulate the expression of IFNe is needed.

We reported herein that IFNe concentrations in cervical and amniotic fluids were unaffected by patient HPV infection status. This observation indicates that constitutive basal IFNe expression in the reproductive tissues may provide sufficient defense against potential infections. Indeed, IFNe displays protective properties against multiple viral and bacterial STIs. *In vitro* investigations using human or primary cell lines have established a role for IFNe in the restriction of HIV replication [51, 54], and this cytokine induces an anti-viral state in human macrophages that protects against HIV [55]. Mice deficient for *Ifne* display increased susceptibility to HSV-2 and *Chlamydia* infections [2], and treatment of murine epithelial cells with recombinant mouse (rm) IFNe reduced *Chlamydia* inclusion *in vitro* [51]. Of note, the protective effects of rmIFNe reported in the latter study were less potent than those of rmIFN β [51], suggesting that additional type I IFN responses beyond those provided by constitutive IFNe expression are necessary for maximum protection. Reduced or impaired expression of IFNe may also increase disease susceptibility in humans, as reduced vaginal IFNe was observed in HSV-infected pregnant women compared to healthy pregnant women [8]. A study in rhesus macaques showed that the expression of IFNe in the rectal mucosal tissue was not altered by simian immunodeficiency virus (SIV) infection [4], which is consistent with the results of the current

study demonstrating that the presence of viruses such as HPV does not alter cervical expression of IFNe. Thus, the physiological basal IFNe expression in the reproductive and mucosal tissues may provide a sufficient initial defense against local viral and bacterial exposure.

A notable finding of the current study indicates that the diagnosis of intra-amniotic infection was associated with increased amniotic fluid concentrations of IFNe in a subset of cases. However, the unaltered *IFNE* expression observed in the chorioamniotic membranes or amnion epithelial cells in the context of intra-amniotic infection suggests that this tissue may not be the source of increased IFNe in amniotic fluid. The majority of the abovementioned literature on IFNe supports the relatively stable expression of this cytokine in the reproductive tissues, even in the context of infection [56]; thus, our current findings highlight a necessity to delineate the pathological conditions that can result in the upregulation of IFNe, as well as its putative sources. The severity of the intra-amniotic inflammatory response may play a role in the induction of IFNe in the context of intra-amniotic infection, given that TNF, a key component of the cytokine milieu in the amniotic cavity of women with this clinical condition [57, 58, 59], has been shown to function upstream of IFNe in an *in vitro* setting [60]. Given the current lack of a demonstrable connection between classical PRR signaling and IFNe expression [2], it remains unclear how bacteria present in the amniotic cavity can drive the enhanced release of this interferon as observed herein. Bacteria such as *Ureaplasma parvum* may be able to influence IFNe expression through PRRs or alternative unknown pathways; however, additional investigation is required to explore the source of IFNe in the amniotic cavity, as well as the underlying mechanisms of its enhanced expression in the context of intra-amniotic infection.

The current study has some limitations. First, the sample sizes from our clinical cohorts are small; yet, given the rarity of obtaining well-characterized samples from cases of confirmed intra-amniotic inflammation, we consider that our findings provide a useful representation of IFNe expression in different compartments throughout pregnancy. Moreover, the use of samples collected from different patient cohorts throughout pregnancy represents an additional study limitation, which future investigations may address by obtaining biopsies of different tissues from the same patient. Last, the use of *in vitro* culture conditions has a limited capacity to mimic the scenario of intra-amniotic infection, given the complexity of the *in vivo* microenvironment. However, the use of relevant cell types (e.g., amnion epithelial cells) together with *Ureaplasma parvum*, the bacterium most frequently detected in women with intra-amniotic infection [11, 12, 13, 14, 15, 16, 17, 18], provides translational value to our findings.

Collectively, the current study provides an overview of IFNe expression in the myometrium and cervix during mid and late pregnancy as well as in the cervical fluid, amniotic fluid, and chorioamniotic membranes in women who underwent spontaneous preterm birth with proven intra-amniotic infection. Our findings emphasize the constitutive nature of IFNe expression in these tissues, which represents a first line of defense against viral or bacterial infections during pregnancy. Importantly, this study underscores the heterogeneity of the intra-amniotic inflammatory response by revealing enhanced amniotic fluid IFNe expression in a subset of women with spontaneous preterm labor and intra-amniotic infection. Future research may focus on the regulation of IFNe, including the sources of this cytokine in the amniotic cavity, and evaluate the degree of protection conferred by basal IFNe expression against viral and microbial infection. Overall, our results contribute to a better understanding of IFNe expression by the reproductive tissues during normal pregnancy and in the context of spontaneous preterm labor and birth.

Declarations

Author contribution statement

Derek Miller; Roberto Romero: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Marian Kacerovsky; Ivana Musilova: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jose Galaz; Valeria Garcia-Flores; Yi Xu; Errile Pusod; Catherine Demery-Poulos: Performed the experiments; Analyzed and interpreted the data.

Pedro Gutierrez-Contreras; Tzu Ning Liu; Eunjung Jung; Kevin R. Theis; Lanetta A. Coleman: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Nardhy Gomez-Lopez: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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