A Multidisciplinary Prematurity Research Cohort Study

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25 **ABSTRACT**

26

27 **Background**

28 Worldwide, 10% of babies are born preterm, defined as a live birth before 37 weeks of gestation. Preterm
29 birth is the leading cause of neonatal death, and survivors face lifelong risks of adverse outcomes. New
30 approaches with large sample sizes are needed to identify strategies to predict and prevent preterm birth.
31 The primary aims of the Washington University Prematurity Research Cohort Study were to conduct three
32 prospective projects addressing possible causes of preterm birth and provide data and samples for future
33 research.

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35 **Study Design**

36 Pregnant patients were recruited into the cohort between January 2017 and January 2020. Consenting
37 patients were enrolled into the study before 20 weeks' gestation and followed through delivery. Participants
38 completed demographic and lifestyle surveys; provided maternal blood, placenta samples, and cord blood;
39 and participated in up to three projects focused on underlying physiology of preterm birth: cervical imaging
40 (Project 1), circadian rhythms (Project 2), and uterine magnetic resonance imaging and electromyometrial
41 imaging (Project 3).

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43 **Results**

44 A total of 1260 participants were enrolled and delivered during the study period. Of the participants, 706
45 (56%) were Black/African American, 494 (39%) were nulliparous, and 185 (15%) had a previous preterm
46 birth. Of the 1260 participants, 1220 (97%) delivered a live infant. Of the 1220 with a live birth, 163 (14.1%)
47 had preterm birth, of which 74 (6.1%) were spontaneous preterm birth. Of the 1220 participants with a live
48 birth, 841 participated in cervical imaging, 1047 contributed data and/or samples on circadian rhythms,
49 and 39 underwent uterine magnetic resonance imaging. Of the 39, 25 underwent electromyometrial
50 imaging.

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52 **Conclusion**

53 We demonstrate feasibility of recruiting and retaining a diverse cohort in a complex prospective,
54 longitudinal study throughout pregnancy. The extensive clinical, imaging, survey, and biologic data
55 obtained will be used to explore cervical, uterine, and endocrine physiology of preterm birth and can be
56 used to develop novel approaches to predict and prevent preterm birth.

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60 INTRODUCTION

61 Preterm birth, defined as delivery before 37 weeks' gestation, affects 1 in 10 babies worldwide and is the
62 leading cause of infant mortality (1). Neonates who survive are at increased risk of lifelong adverse health
63 outcomes (2-4). This problem is especially notable in St. Louis, Missouri, USA, where 13% of babies are
64 born preterm and racial disparities are pronounced; 11% of white women and over 17% of Black women
65 deliver preterm (5). Despite decades of research, we have limited understanding of the causes of preterm
66 birth and few strategies to predict or prevent this adverse pregnancy outcome.

67
68 In 2013, Lackritz and colleagues argued that preventing preterm birth would require rigorous research to
69 identify the underlying biological and social determinants. Additionally, they argued for development of new
70 tools to monitor pregnancy and identify those at highest risk of preterm birth (6). To that end, we formed
71 the Washington University in St. Louis Prematurity Research Center in 2014 with funding from the March
72 of Dimes Foundation, St. Louis Children's Hospital, Barnes-Jewish Hospital, and Washington University in
73 St. Louis. The Prematurity Research Center united a multidisciplinary group of investigators including
74 obstetricians, engineers, circadian biologists, and cardiac electrophysiologists to approach preterm birth
75 in novel ways.

76
77 The three primary projects of the Prematurity Research Center focused on identifying anatomic,
78 physiologic, and behavioral features that are associated with and can be used to predict preterm birth. The
79 first project, Cervical Imaging, used high-speed functional photoacoustic endoscopy to quantify anatomic
80 changes during cervical remodeling (7, 8). The second project, Circadian Rhythms, used actigraphy,
81 hormone secretion patterns, and surveys to determine whether disruption in circadian rhythms is a risk
82 factor for preterm birth (5). The third project, Uterine Electrical Activity, developed a novel imaging system
83 to noninvasively map electrical activity of the uterus during labor contractions (9-12).

84
85 To gather data for these projects, we initiated the Prematurity Research Cohort Study to longitudinally
86 follow over 1000 participants from early in pregnancy through delivery. The purpose of this report is to

describe the demographics of this cohort and the types of data and biospecimens obtained. In addition, we address the feasibility of conducting a multidisciplinary study in which a diverse cohort of pregnant patients are followed throughout pregnancy.

METHODS

The Prematurity Research Cohort Study was a prospective, longitudinal cohort study performed at Washington University in St. Louis Medical Center between January 2017 and January 2020. Participants were enrolled in the first or early second trimester and followed through delivery. The study received ethical approval from the Washington University in St. Louis Institutional Review Board. All participants provided written informed consent for collection and use of clinical, biospecimen, imaging, or questionnaire data.

Inclusion and exclusion criteria

Women were approached for enrollment if they had a singleton pregnancy ≤ 20 weeks' gestation (determined by best obstetric estimate including last menstrual period or earliest ultrasound dating available) and met the following inclusion criteria: plan to deliver at Barnes-Jewish Hospital, 18 years of age or older, and English speaking. Patients were not eligible if they were incarcerated. Additionally, patients who conceived via *in vitro* fertilization were not enrolled. If a major fetal anomaly was diagnosed during pregnancy, it was reviewed by a maternal-fetal medicine attending physician. If the anomaly affected gestational age at delivery, the patient was withdrawn from the study.

Research staff

A large research staff was assembled to support all projects by enrolling participants, performing longitudinal follow-up, scheduling appointments, coordinating research study visits, contacting study participants who missed study visits, collecting data, and managing specimen collection. The research staff comprised one research coordinator, three registered nurses, one sonographer, three research associates, a research lab coordinator, and one research lab assistant. Additionally, eight staff members worked exclusively on the Labor and Delivery floor to support specimen collection and data acquisition at

delivery for multiple studies. This team was available 24 hours a day, seven days a week, 365 days a year, and each spent approximately 25% of their effort on the three Prematurity Research Center projects. In addition, two statisticians provided analytic support, and a scientific editor reviewed all scientific presentations and reports.

Participant recruitment and longitudinal follow-up

Patients were approached for enrollment at their initial prenatal appointment at two obstetric clinics on the Washington University Medical Campus. One clinic primarily serves patients with public health insurance, and the other primarily serves patients with private health insurance. All potential participants were offered enrollment into projects 1 and 2. Participants were seen at study visits longitudinally throughout pregnancy and at delivery. Study visits were scheduled to obtain data and samples in each of the three trimesters (**Figure 1**). All study visits were aligned as much as possible with routine obstetric care to minimize inconvenience to the participants. When additional visits were needed outside of routine medical care visits, appointments were scheduled during routine business hours at the participant's convenience. For follow-up and retention, participants were contacted by phone and/or text messages to remind them of study visits, and transportation was arranged, if needed, to facilitate study participation. Study personnel were available via phone and text during business hours to answer study-related questions.

Data and specimen collection

The timing and sources of data and specimens collected are illustrated in **Figure 1**.

Case report form. A comprehensive case report form was used to collect data from electronic medical records on the index pregnancy, previous pregnancies, maternal demographics and medical history, labor and delivery, neonatal outcomes until discharge from hospital, and maternal postpartum visits. Obstetric research personnel including research coordinators and research nurses were trained by a Research Nurse coordinator on abstracting clinical data. For quality assurance, the Research Nurse coordinator performed checks on these data. In cases in which the participant delivered at an outside institution,

141 medical record releases were requested to obtain delivery data. The case report form used to collect
142 pregnancy outcome data is shown in **Table 1**.

Table 1: Case report form for pregnancy outcomes

Date of delivery
Time of delivery
Preterm birth (<37 weeks)
Spontaneous preterm birth
If spontaneous preterm birth, presentation
Preterm premature rupture of membranes
Dilation
Preterm premature rupture of membranes + dilation
Other (specify)
Unknown/not available
Tocolytic medication during pregnancy
None
Magnesium
Indocin
Terbutaline
Nifedipine
Multiple
Unknown/not available
Gestational age at delivery
If term birth (≥37 weeks), presentation
Premature rupture of membranes
Dilation
Premature rupture of membranes + dilation
Induction
Other (specify)
Unknown/not available
Was magnesium sulfate administered?
Clinical chorioamnionitis
Intrapartum antibiotics
Preterm premature rupture of membranes prophylaxis
Group B streptococcus prophylaxis
Chorioamnionitis
Other
Unknown/not available
Type of delivery
Spontaneous vaginal
Operative vaginal
C-section
Unknown/not available

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144 Project 1—Cervical Imaging. A research nurse collected swabs from three areas of the vagina (posterior
145 fornix, mid-vagina, and introitus) via speculum exam immediately before performing cervical imaging.
146 Swabs were refrigerated at 4°C immediately after collection and transferred to -80°C within 8 hours. After
147 swab collection, novel cervical photoendoscopy devices were used to obtain cervical imaging data
148 transvaginally (7, 8). Additionally, standard transvaginal ultrasound images of the cervix were obtained and
149 used to measure cervical length at each imaging session. Vaginal swabs were collected and cervical

imaging was performed once in each trimester. A subset of participants underwent sampling and imaging up to three additional times, with a minimum of four weeks between sampling/imaging sessions.

Project 2—Circadian Rhythms. All participants completed validated questionnaires about sleep habits and other lifestyle measures (**Figure 1 and Supplemental Table 1**). Participants were given the option of completing surveys via an online link or taking the questionnaires home and returning them to research staff at their next obstetric or research study visit. Research staff called or texted participants to remind them to bring completed surveys to subsequent study visits. Patients with incomplete third trimester surveys were offered the opportunity to complete the surveys on an iPad during admission for delivery.

Participants provided salivary samples every four hours during one 24-hour period each trimester, with collection starting at 18:00 hours. Participants received supplies to collect saliva (Salimetrics, United Kingdom) at home. Research staff called or texted participants to remind them of instructions for collecting saliva and to bring their samples to their next visit. Participants were instructed to place samples in the freezer until bringing them to the research staff. Once received, the samples were timestamped, stored at -80 °C, and processed to measure melatonin and cortisol concentrations by ELISA (Salimetrics Melatonin ELISA kit and Salimetrics Cortisol ELISA kit) in a research laboratory.

Participants wore wrist actigraphy devices (Motionwatch8, CamNTEch, United Kingdom) for two-week time periods during their first, second, and third trimesters, as outlined by Martin-Fairey et al. (5). The actigraphy devices captured minute-level movement and light exposure and remained charged for approximately 90 days, ensuring continuous data collection. Research staff called, texted, or emailed participants to remind them to bring the devices back to the next study visit after the two-week data capture period, or they arranged a courier service for retrieval. If the device was not returned, self-addressed, stamped envelopes were mailed to participants' addresses with a letter requesting return of the device and offering a \$20 gift card if they did so.

177 Project 3—Uterine Electrical Activity. Sixty-three participants from the total cohort were enrolled to
178 participate in Project 3. Participants included those at low risk for preterm birth (defined as a normal cervical
179 length at anatomy screen and no history of spontaneous preterm birth) and those at high risk for preterm
180 birth (defined as a previous spontaneous preterm birth less than 35 weeks or a cervical length less than 2
181 cm during the index pregnancy). Those in the low-risk group underwent uterine magnetic resonance
182 imaging (MRI) at 37 weeks' gestation, and those in the high-risk group underwent MRI at 24, 28, and 32
183 weeks' gestation. Once patients presented for induction or in spontaneous labor and were in active labor
184 (defined as greater than 4 cm dilation and regular contractions), body surface potential mapping was
185 performed for approximately one hour. The combined uterine MRI and body surface potential mapping
186 resulted in data used for electromyometrial imaging (EMMI), which has been described elsewhere (9-12).
187 MRI was performed in a 3T Siemens Prisma/Vida whole-body MRI Scanner with a radial volume
188 interpolated breath-hold examination fast T1-weighted sequence. Patients who also consented to be a part
189 of the cervical imaging project had MRI performed on the same day.

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191 **Biological specimens**

192 Maternal blood serum and plasma samples were collected throughout pregnancy during routine clinical
193 lab visits during business hours (or drawn by research staff if labs were done elsewhere or missed),
194 refrigerated at 4 °C, and centrifuged at 1620 x g for 5 minutes at 4 °C within 12 hours of collection. Aliquots
195 (1 mL) were stored at -80 °C. Cord blood serum and plasma were collected at delivery and processed in
196 the same manner as the maternal blood. In cases in which cord blood was not collected, infant buccal
197 swabs were collected with the mother's consent. At least 30 minutes after the infant was fed, a swab was
198 rubbed firmly against the inside cheek and lower and upper lip for one minute and stored at room
199 temperature. Four sets of placenta specimens (1x3 cm) were collected at delivery from each of four sites:
200 chorionic amnion, basal plate, villous tissue, and subchorion. All placental samples were snap frozen in
201 liquid nitrogen and stored at -80 °C. Amniotic fluid was collected at the time of delivery from 56 patients
202 who underwent unlabored, intact cesarean section. Fluid was centrifuged at 1620 x g for 5 minutes at 4 °C
203 and then stored at -80 °C.

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Participant incentives

All participants received gift cards for completing study visits. Participants received \$25 gift cards each trimester for completing the combination of surveys, wrist-worn actigraphy, and 24-hour saliva collection. Participants also received \$25 gift cards for each completed transvaginal imaging exam, \$50 gift cards for each MRI, and a \$50 gift card at delivery if the majority of study procedures were completed. Participants also received a small non-monetary gift (e.g., pen, reusable bag, children's book) at the completion of each study visit and at delivery. For participants without reliable transportation, taxis or Uber Health cars were arranged for transport to and from study appointments at no cost to the participant. For prolonged study visits that spanned a mealtime (typically 3+ hours; combining clinical appointment with transvaginal imaging and MRI), a meal was provided to the participant. Granola bars and other small snacks were available to participants for shorter study visits. Crayons and coloring pages were offered to the children of participants who attended study visits.

RESULTS

Participant attrition and demographics

A flow diagram of participant enrollment and outcomes is provided in **Figure 2**. We screened 7478 patients for potential enrollment; 2718 (36.3%) met inclusion criteria (<20 weeks' gestation, age >18 years, English speaking) and were approached. Among those approached, 1523 (56.0%) gave consent and enrolled. Of the enrolled participants, 190 (12.5%) were lost to follow-up and 73 (4.8%) withdrew and were not included in the final analyses. We have complete clinical and outcome data on 1260 (82.7%) participants.

Demographic characteristics of the 1260 participants are described in **Table 2**. The majority of participants reported being employed (70.8%), Black/African American (56.0%), and single (61.2%). More than one-third of participants (36.9%) reported an annual income <\$25,000, 44.8% had public insurance or were uninsured, and 53.9% had private insurance.

Table 2: Demographic data of study participants

Total enrolled with outcome data	N=1260
Education	
Less than 12 th grade	77 (6.1%)
High school degree/GED	583 (46.3%)
College degree (4 years)	179 (14.2%)
Graduate degree	250 (19.8%)
Missing/Unknown	171 (13.6%)
Marital status	
Single	771 (61.2%)
Married	460 (36.5%)
Other	29 (2.3%)
Employment	
Yes	892 (70.8%)
No	273 (21.7%)
Student	39 (3.1%)
Missing/Unknown	56 (4.4%)
Annual income (T1)	
Government Assistance	92 (7.3%)
<\$25,000	465 (36.9%)
\$25,000-\$74,999	265 (21.0%)
\$75,000-\$124,999	155 (12.3%)
≥\$125,000	160 (12.7%)
Missing/Unknown	123 (12.7%)
Insurance	
Medicaid	410 (32.5%)
Medicare	21 (1.7%)
Individual/Group Health Insurance	679 (53.9%)
VA/Military	12 (1.0%)
Uninsured	134 (10.6%)
Missing/Unknown	4 (0.3%)
Race	
Black or African-American	706 (56.0%)
White	501 (39.8%)
Other	53 (4.2%)
Ethnicity	
Non-Hispanic	1213 (96.3%)
Hispanic	36 (2.9%)
Unknown	11 (0.9%)
English first language	1251 (99.3%)

Pregnancy characteristics and outcomes

Table 3 demonstrates maternal and pregnancy characteristics in our cohort. The majority (n=766, 60.8%) were multiparous, and 185 (14.7%) had a history of preterm birth. The most common medical complications among these participants was asthma and chronic hypertension. The most common previous pregnancy complications were gestational hypertension/preeclampsia and intrauterine growth restriction (**Table 3**).

Table 4 describes pregnancy and neonatal outcomes in the 1220 participants who delivered a live birth. Induction of labor was performed in 529 (43.3%) participants, and 368 (30.2 %) were delivered via

cesarean section. In the cohort, there were 1220 (96.8%) live births, 23 stillbirths $\geq$ 20 weeks, and 17 pregnancy losses <20 weeks. Among the 1220 participants with a live birth, 1057 (86.6%) delivered at term and 163 (13.4%) delivered preterm (< 37 weeks). Of the live-born neonates, 145 (11.9%) required neonatal intensive care unit admission, 145 (11.9%) had low birthweight (<2500 g), and 153 (13.1%) were small for gestational age, defined as birthweight <10th percentile for gestational age.

Table 3: Maternal and pregnancy characteristics

Gravidity	
Gravida, median (IQR)	2 (1,4)
Parity	
Nulliparous	494 (39.2%)
Multiparous	766 (60.8%)
History of preterm birth	
Indicated	10 (0.8%)
Spontaneous	175 (13.9%)
24-33 6/7 weeks	107 (8.5%)
34-36 6/7 weeks	68 (5.4%)
History of pregnancy and medical complications	
Asthma	248 (19.7%)
Gestational hypertension/Preeclampsia	214 (17.0%)
Chronic hypertension	144 (11.4%)
Diabetes	71 (5.6%)
Intrauterine growth restriction	60 (4.8%)
Thyroid disease	57 (4.5%)
Anomaly	47 (3.7%)
Heart disease	37 (2.9%)
Renal disease	29 (2.3%)
Polyhydramnios	22 (1.7%)
Oligohydramnios	10 (0.8%)
Lupus	6 (0.5%)

Except where noted, data are presented as n (%); IQR, interquartile range

Table 4: Pregnancy and neonatal outcomes

Birth outcome	
Live birth	1220 (96.8%)
Loss, <20 weeks	17 (1.3%)
Loss, 20+ weeks	23 (1.8%)
Gestational age at birth	
<24 weeks	3 (0.2%)
24 weeks-31 weeks 6 days	32 (2.6%)
32 weeks-36 weeks 6 days	128 (10.5%)
37+ weeks	1057 (86.6%)
Preterm Birth	
Induced	89 (7.3%)
Spontaneous	74 (6.8%)
<24 weeks	2 (0.02%)
24-33 weeks 6 days	22 (1.8%)
34-36 weeks 6 days	50 (4.1%)
Delivery method	
Vaginal	806 (66.0%)
Operative vaginal	46 (3.8%)
Cesarean section	368 (30.2%)
Reason for induction (N=529)*	
Oligohydramnios	6 (1.1%)
PROM	8 (1.5%)
PPROM	7 (1.3%)
Preeclampsia/eclampsia	59 (11.2%)
Comorbidity at 39 weeks	35 (6.6%)
Elective	236 (44.6%)
Non-reassuring antenatal testing	40 (7.6%)
Gestational Diabetes	16 (3.0%)
Postdates	64 (12.1%)
Intrauterine growth restriction	46 (8.7%)
Macrosomia	2 (0.4%)
polyhydramnios	4 (0.8%)
Other	76 (14.4%)
Neonatal Sex	
Female	581 (47.6%)
Male	639 (52.4%)
Apgar score at 1 minute	
0-3	59 (4.8%)
4-6	83 (6.8%)
7-10	1053 (86.3%)
Apgar score at 5 minutes	
0-3	8 (0.7%)
4-6	40 (3.3%)
7-10	1163 (95.3%)
NICU information	
NICU admission	145 (11.9%)
Length of NICU stay in days	8 (4, 21)
Neonatal health outcomes	
Low birth weight (<2500 grams)	145 (11.9%)
Small, for gestational age (<10 th percentile)	153 (13.1%)
Arterial umbilical cord pH	7.26 (7.22, 7.31)
Newborn death within 28 days	8 (0.7%)

*The percentage of reason for induction is calculated according to the total number of inductions. Some participants had more than one reason for induction. Data represent n (%) or median (interquartile range).

Data and specimen collection associated with study procedures

Collectively, study participants attended 6135 study visits, which included 1892 cervical imaging exams, 39 uterine MRIs, 2239 actigraphy recordings, 28,240 lifestyle surveys, and over 12,000 biological specimens (see **Table 5**). Fewer participants provided saliva samples in the first trimester than in the second and third trimesters. Conversely, more participants completed lifestyle surveys and provided actigraphy data in the first trimester than in the second and third trimesters. More patients underwent transvaginal imaging, which required additional scheduling after consent, in the first trimester than in the second and third trimesters.

Table 5: Sample and survey numbers from participants with live births

Samples and Data Collected	First Study Visit	Second Study Visit	Third Study Visit	Delivery
Biologic Samples				
Maternal blood	795	855	864	1023
Saliva	453	822	778	-
Vaginal swabs	212	791	588	-
Maternal buffy coat	790	855	863	430
Placenta	-	-	-	1047
Cord blood	-	-	-	870
Cord blood buffy	-	-	-	864
Amniotic fluid	-	-	-	56
Infant buccal swab	-	-	-	211
Cervical Imaging	673	629	590	n/a
Actigraphy	737	806	696	n/a
Surveys				
Perceived Stress Scale	1136	786	825	n/a
Munich Chronotype Questionnaire	1047	784	823	n/a
Pittsburgh Sleep Quality Index	1039	783	822	n/a
Berlin Questionnaire	984	781	823	n/a
Women's Health Initiative Insomnia Rating Scale	983	782	821	n/a
Epworth Sleepiness Scale	1035	783	821	n/a
Intern. Restless Legs	1032	783	821	n/a
Kaiser Physical Activity	1030	781	821	n/a
Edinburgh Postnatal Depression	1212	874	866	n/a
Demographic/Med. Hist.	1111	801	845	n/a
Difficult Life Circumstances	n/a	n/a	n/a	654
NIH Diet Questionnaire	n/a	n/a	n/a	751

A total of 1220 participants had live births.

A total of 63 participants provided consent to undergo uterine MRI and electrical mapping of the uterus (electromyometrial imaging [EMMI]) at labor (**Table 6**). A total of 24 (17 low-risk, 7 high-risk) participants

withdrew before the MRI for reasons such as delivery before MRI (8), lost contact (6), and patient/family request to withdraw (6) (**Table 6**). A total of 25 participants in the low-risk group and 14 in the high-risk group underwent MRI at least once during pregnancy (**Table 7**). In addition, 20 participants in the low-risk group and 5 in the high-risk group underwent EMMI at labor (**Table 6, 7**). Reasons for missed EMMI included: not notified by Labor and Delivery staff (3), delivered at offsite hospital (3), COVID-19 research shut-down (2), and emergent cesarean section (2). Among those who underwent both MRI and EMMI, none of the low-risk participants and two of the high-risk participants delivered preterm (**Table 6**).

Table 6: Project 3 Participants

Cohort	Consented	Withdrew	Reasons for Withdrawal	Underwent MRI	Missed EMMI	Reasons for Missed EMMI	Underwent MRI and EMMI
Low Risk	42	17	Delivered before MRI (7) Per request (5) Lost contact (4) Medical issue (1)	25	5	Missed by L&D staff (3) Delivered precipitously (2)	20 (0 preterm, 20 term)
High Risk	21	7	Lost contact (2) Delivered before MRI (1) Lethal anomaly (1) Spontaneous abortion (1) Per request (1) Social issues (1)	14	9	Delivered at offsite hospital (3) COVID (2) Emergency cesarean (2) Intrauterine Fetal Demise (1) Delivered precipitously (1)	5 (2 preterm, 3 term)

EMMI, electromyometrial imaging; L&D, Labor and Delivery; MRI, magnetic resonance imaging

Table 7. Project 3 procedures performed

Group and procedure	Timing				
	24 weeks	28 weeks	32 weeks	37 weeks	Labor
Low Risk - Uterine MRI	-	-	-	25	
High Risk - Uterine MRI	14	9	9	-	
Low Risk - EMMI	-	-	-	-	20
High Risk - EMMI	-	-	-	-	5

EMMI, electromyometrial imaging; MRI, magnetic resonance imaging

DISCUSSION

The Prematurity Research Cohort Study was a multi-faceted study aimed at identifying mechanisms underlying preterm birth. This report demonstrates the feasibility of conducting a longitudinal study in pregnant participants and maintaining high consent and retention rates. Moreover, we describe the rich data and specimen source now available for longitudinal studies of pregnancy.

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283 The primary intent of establishing this cohort was to identify causes of, and develop novel diagnostics to
284 predict, preterm birth. Analyses of data from surveys, swabs, specimens, and imaging are ongoing for the
285 three projects. Specimens are also banked for future research to identify both risk factors and potential
286 biomarkers. The data and specimens we collected will be useful for addressing maternal and neonatal
287 health disparities. This is because over 50% of the participants were Black, and all lived in the St. Louis,
288 Missouri, area, where Black women have a 50% higher risk of preterm birth than white women (5).

289

290 **Feasibility**

291 Within three years, we enrolled 1260 participants who delivered, and we performed 6135 study visits
292 spanning all trimesters. Notably, 977 women attended three or more visits over the course of pregnancy.
293 We collected over 12,000 biological specimens with linked clinical and imaging data. Among the 1260
294 enrolled participants, 859 (68.1%) participated in Project 1, Project 2, or both, the two projects open to all
295 participants.

296

297 Study participants were more likely to comply with study procedures that could be timed with clinical
298 appointments or labs (e.g., blood draws, surveys done in waiting room or exam room). Fewer data and
299 specimens were collected in the third trimester than in the first and second trimesters. In part, this was
300 because some participants delivered before their third trimester study visit.

301

302 **Scientific Implications**

303 The Prematurity Research Cohort Study has generated a rich set of data and specimens that can be used
304 to test hypotheses pertaining to mechanisms of preterm birth and preventive targets. This dataset and
305 specimen bank will also allow investigators to explore new questions regarding preterm birth, use imaging
306 and biomarkers to assess preterm birth risk in the first trimester, and identify modifiable lifestyle factors
307 that increase risk of preterm birth (6). Our cohort was predominantly those with the highest risk of preterm
308 birth: women of color and women with socioeconomic stressors. Our cohort had a higher percentage of

African Americans (56%) than the percentage in the US population (13.4%) and had high rates of several chronic conditions, which likely reflects the fact that the study was conducted at a tertiary care hospital.

Our experience reveals that pregnant patients are willing to participate in studies that require multiple research visits, undergo serial transvaginal ultrasounds or MRI, answer extensive surveys, wear actigraphy monitors, and collect timed saliva samples and other biological specimens beyond those required for routine clinical care. Thus, other researchers should recognize that pregnant patients both can and should be included in studies for obstetric and non-obstetric outcomes.

Cost and Resource Utilization

Conducting this study required extensive investments of time and financial resources. Full or partial salaries were required for the 80 staff members, 31 trainees, and 23 faculty members who participated in various aspects of the study. In addition, the study required abundant supplies (e.g., blood and placenta sample collection tubes), dedicated freezer space, gift cards, and transportation arrangements. Such costs and resources are important to consider in planning any longitudinal study in pregnancy. To reduce costs, future studies could maximize use of web-based and social media tools to optimize recruitment and retention (13, 14). Although we did not formally assess patient-reported acceptability of participating in the cohort study, future work should investigate barriers and facilitators to patient adherence to multiple research visits during pregnancy.

In summary, we report successful enrollment and follow-up of a large longitudinal cohort of pregnant patients. Additionally, we describe the substantial investments made by participants and research personnel to collect data and specimens. We are optimistic that their contributions will lead to new discoveries to improve the health of pregnant patients and their babies.

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Supplemental Table 1: Sleep and lifestyle data surveys collected from participants

Survey/Questionnaire	Number of questions	Example question	When implemented
Perceived Stress Scale (15)	10	In the last month, how often have you been upset because of something that happened unexpectedly?	Once per trimester
Munich Chronotype Questionnaire (16)	4	I have a regular work schedule (this includes being a house wife or a house husband). Y/N	Once per trimester
Pittsburgh Sleep Quality Index (17)	9	During the past month, how often have you taken medicine to help you sleep (prescribed or “over the counter”)?	Once per trimester
Berlin Questionnaire (18)	10	Have you ever nodded off or fallen asleep while driving a vehicle?	Once per trimester
Women’s Health Initiative Insomnia Rating Scale (19)	5	In the past 4 weeks, did you wake up several times at night?	Once per trimester
Epworth Sleepiness Scale (20)	8	How sleepy are you while sitting and reading?	Once per trimester
International Restless Legs Syndrome Rating Scale (21)	5	Do you experience an urge to move or unpleasant sensations that begin or worsen during periods of rest or inactivity such as laying or sitting?	Once per trimester
Kaiser Physical Activity Survey (22)	29	How many minutes a day do you usually walk and/or bicycle to and from work, school or errands?	Once per trimester
Edinburgh Postnatal Depression Scale (23)	10	I have been able to laugh and see the funny side of things. (As much as always, not quite so much now, definitely not so much, not at all)	Once per trimester
Difficult Life Circumstances (24)	30	Are you having regular arguments or conflicts with your present partner?	Second trimester
National Institutes of Health Diet History Questionnaire II (25)	153	Over the past 12 months, how often did you drink carrot juice?	Third trimester or within three months of delivery
Demographic and medical history questions	11	At what age did you have your first menstrual period?	First trimester
Lifestyle questions	12	Do you currently smoke cigarettes?	Once per trimester