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Longitudinal changes in hormonal and immune profiles associated with preterm birth among pregnant women in Zambia

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Abstract

Background

Spontaneous preterm birth (SPTB) remains a major cause of neonatal morbidity and mortality, particularly in sub-Saharan Africa. Although endocrine and immune pathways are implicated in the initiation of preterm labour, longitudinal evidence from African populations remains limited. This study investigated longitudinal changes in maternal hormonal and systemic immune profiles associated with SPTB in Zambia.

Methods

A prospective longitudinal cohort study was conducted under Preventing Preterm Births in Zambia (ZAPPs) among pregnant women attending the Women and New-born Hospital, Lusaka, Zambia, between January 2023 and December 2024. Women were enrolled during the first trimester and followed until delivery, with maternal serum collected during the first, second, and third trimesters. Concentrations of progesterone, estradiol, insulin-like growth factor-1 (IGF-1), corticotropin-releasing hormone (CRH), cortisol, and eight cytokines were measured using enzyme-linked immunosorbent assays and multiplex immunoassays, respectively. Participants were classified according to delivery outcome as SPTB (< 37 completed weeks) or term birth (≥ 37 weeks).

Results

Of 100 women enrolled, 65 with complete longitudinal data were included in the analysis, comprising 31 SPTB and 34 term births. Estradiol concentrations were significantly higher in the SPTB group during the second trimester (median 14,934.5 vs. 6,483.8 pg/mL; p = 0.042). IGF-1 concentrations were significantly lower among women with SPTB during the second (37.25 vs. 61.96 ng/mL; p = 0.006) and third trimesters (6.76 vs. 22.07 ng/mL; p = 0.018). Progesterone was significantly lower in the SPTB group during the third trimester (63.47 vs. 246.72 ng/mL; p = 0.010). CRH and cortisol did not differ significantly between groups. None of the measured cytokines showed statistically significant between-group differences at any trimester, although MIP-1α showed a trend toward higher concentrations in the SPTB group during the third trimester (p = 0.074).

Conclusions

SPTB was associated with distinct trimester-specific endocrine alterations, particularly increased second-trimester estradiol and reduced IGF-1 and late-pregnancy progesterone. In contrast, maternal circulating cytokines showed limited differentiation between SPTB and term pregnancies. Larger

longitudinal studies are warranted to validate these endocrine trajectories and determine their potential value for early identification of women at risk of SPTB.

Introduction

Preterm birth (PTB), defined as delivery before 37 completed weeks of gestation, remains the leading cause of neonatal mortality and morbidity; and long-term neurodevelopmental disability [1, 2]. Globally, approximately one in ten infants is born preterm each year, with sub-Saharan Africa and South Asia bearing the greatest burden [3, 4] and national estimates in Zambia indicating a PTB rate of approximately 13% [5]. Beyond the immediate risks of preterm births, premature infants face lifelong metabolic and cognitive vulnerabilities. This accounts for nearly one million deaths annually and remain the leading cause of mortality among children under five years of age [6, 7]. Despite advances in obstetric and neonatal care, PTB remains a substantial challenge, highlighting the need to understand the biological mechanisms underlying spontaneous preterm labour [8, 9]

Spontaneous PTB is a multifactorial syndrome resulting from complex interactions among endocrine, immunological and placental factors [10, 11]. It involves complex interactions among endocrine, immune and placental factors. Endocrine adaptations regulate placental development, fetal growth and parturition [12, 13]. Progesterone maintains uterine quiescence and cervical integrity, while estrogens prepare the uterus for labour. IGF-1, CRH and cortisol contribute to fetal growth, placental function and pathways regulating parturition [14, 15]. Disruption of these pathways has been implicated in spontaneous PTB, but hormonal trajectories preceding delivery remain poorly understood and Zambian cohorts are not an exception.

Successful pregnancy also requires precise regulation of the maternal immune system[16, 17]. Although labour is considered an inflammatory process, excessive or premature activation of inflammatory pathways has been associated with spontaneous preterm birth [18, 19]. Elevated concentrations of circulating pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor-α (TNF-α), have been reported in some women who subsequently deliver preterm. However, findings across studies remain inconsistent, reflecting differences in study design, timing of sample collection, biological specimens analysed and population characteristics. Consequently, the contribution of systemic immune responses to spontaneous preterm birth remains incompletely defined

Previous studies have frequently assessed hormonal and immune biomarkers separately using cross-sectional designs, limiting their ability to identify changes preceding spontaneous PTB [20, 21]. Evidence on combined longitudinal endocrine and immune trajectories is particularly scarce in sub-Saharan Africa, while much existing research comes from high-income settings [22, 23]. Differences in infectious disease burden, nutrition and environmental exposures may influence these trajectories, supporting the need for population-specific studies [24, 25].

Zambia has a substantial PTB burden, yet few longitudinal studies have examined maternal biomarkers associated with pregnancy outcomes (24,25). Identifying endocrine and immune changes preceding

spontaneous PTB may clarify its mechanisms and inform the development of biomarkers for earlier risk identification. These could facilitate targeted surveillance and timely interventions, ultimately improving maternal and neonatal outcomes in resource-limited settings.

This study investigated maternal endocrine and systemic immune trajectories across three trimesters among women attending the Women and New-born Hospital in Lusaka, Zambia. Serum progesterone, estradiol, IGF-1, CRH, cortisol and cytokines were compared between pregnancies ending preterm and at term. We hypothesized that spontaneous PTB would be associated with distinct longitudinal endocrine and immune trajectories.

Methodology

Study design and setting

This prospective longitudinal cohort study was conducted under Preventing Preterm Births in Zambia (ZAPPs) among pregnant women attending the Women and New-born Hospital, Lusaka, Zambia. Pregnant women were enrolled during early pregnancy and followed throughout gestation until delivery (11th January 2023-31st December 2024). Serial maternal blood samples were collected during each trimester to evaluate longitudinal changes in endocrine and immune biomarkers associated with pregnancy outcome. Participants were subsequently classified according to delivery outcome as either spontaneous preterm birth (< 37 completed weeks of gestation) or term birth (≥ 37 completed weeks of gestation) [26].

Study participants

Pregnant women attending antenatal care at the Women and New-born Hospital were recruited after providing written informed consent. Maternal demographic and obstetric information, including age, gestational age, previous pregnancies, previous miscarriages, previous preterm births, HIV status and pregnancy complications, were collected using standardized questionnaires and medical records[27].

A total 100 participants were recruited to the study; nonetheless, 65 women with complete longitudinal follow-up and biomarker measurements were included in the final analysis, comprising 31 women who subsequently experienced spontaneous preterm birth and 34 women who delivered at term. 35 participants fell off from the study.

Eligibility criteria

We recruited consenting pregnant women aged 18–40 years with confirmed singleton pregnancies at ≤ 12 weeks' gestation during routine antenatal visits. Eligible participants had no history of preterm birth, preeclampsia, or abortion and were able to comply with study procedures and follow-up requirements. We excluded women who had used antimicrobial agents, or vaginal douches within the preceding four

weeks, or if they had congenital or genetic fetal abnormalities, severe maternal medical conditions, active infections, or pre-existing comorbidities that could influence pregnancy outcomes, including diabetes mellitus, chronic hypertension, or autoimmune disorders.

Sample collection and processing

Samples were collected as part of the Zambian Preterm Birth Prevention Study (ZAPPS). Venous blood was collected by trained phlebotomists into serum separator tubes (SSTs) containing a clot activator and separating gel from each participant during the first, second, and third trimesters of pregnancy. Tubes were allowed to clot upright at room temperature for 30 minutes and were then centrifuged at 1,300 × g for 10 minutes to separate serum. Serum was aliquoted into cryovials and stored at − 80°C at the UNC Global Projects Zambia (UNC-GPZ) research laboratory, Lusaka, until analysis. All samples were handled under identical conditions to minimize pre-analytical variability prior to hormone and immune marker testing [27].

Hormone measurements

Maternal serum concentrations of progesterone, estradiol, insulin-like growth factor-1 (IGF-1), corticotropin-releasing hormone (CRH) and cortisol were analysed using commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' protocols. Samples and standards were analysed following the recommended assay procedures, and hormone concentrations were calculated from standard calibration curves generated for each assay. Quality control procedures were performed throughout the analytical process to ensure assay reliability and reproducibility.

Cytokine measurements

Maternal systemic immune responses were evaluated by quantifying serum cytokine concentrations using Milli-Plex Pro Human Cytokine Cytokine/Chemokine/ Growth 96-well plate Assay, catalogue no. HCYTA-60K, a magnetic bead-based multiplex immunoassay, on a Luminex 200. Assays were performed according to the manufacturer's instructions. Serum samples were analysed in duplicate alongside calibration standards and quality control materials on each plate. Median fluorescence intensity was acquired using xPONENT v4.3, and cytokine concentrations were interpolated from standard curves

Data collection

Clinical, demographic, obstetric, hormonal, and immune data were collected prospectively from 100 pregnant women attending antenatal care at the Women and New-born Hospital, University Teaching Hospitals in Lusaka, Zambia. Key variables, including maternal age, gestational age, previous pregnancies, previous miscarriages, previous preterm births, HIV status, pregnancy complications, and delivery outcome, were recorded using standardized questionnaires and medical records. Maternal

serum samples were collected during the first, second, and third trimesters and analysed for progesterone, estradiol, insulin-like growth factor-1 (IGF-1), corticotropin-releasing hormone (CRH), cortisol, and inflammatory and immune markers, including IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-10, IP-10, and MIP-1α. Participants were followed until delivery and classified as having spontaneous preterm birth (< 37 completed weeks) or term birth (≥ 37 completed weeks). Of the 100 enrolled participants, 65 women with complete longitudinal follow-up and biomarker measurements were included in the final analysis, while 35 were lost to follow-up or had incomplete data. Data were cleaned using excel and verified before statistical analysis, with inconsistencies and missing information assessed against the available clinical and laboratory records.

Study variables

The primary outcome of the study was spontaneous preterm birth, defined as spontaneous delivery before 37 completed weeks of gestation. Women who delivered at/beyond 37 completed weeks were classified as the term birth group and served as the comparison cohort. The independent variables included sociodemographic and obstetric characteristics, hormonal markers (progesterone, estradiol, IGF-1, CRH, and cortisol), and immune and inflammatory markers (IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-10, IP-10, and MIP-1α). Hormonal and immune/inflammatory markers were measured longitudinally across the first, second, and third trimesters to assess changes throughout pregnancy.

Data analysis

Data were exported to Microsoft Excel for cleaning and coding before analysis. Statistical analyses and data visualization were performed using Python (version 3.14.7 and R statistical software (version R 4.6.1). The distribution of continuous variables was assessed using the Shapiro–Wilk test and Q–Q plots. Biomarker concentrations reported in pg/mL and ng/mL were log-transformed where appropriate to improve normality and facilitate statistical analysis. Continuous variables were summarized using the mean and standard deviation (SD) for normally distributed data and the median and interquartile range (IQR) for non-normally distributed data, while categorical variables were presented as frequencies and percentages. For comparisons of biomarker levels across the three trimesters, repeated-measures analysis of variance (ANOVA) was used for normally distributed data, while the Friedman test was used for non-normally distributed data. Where significant differences were observed, pairwise comparisons between trimesters were performed using appropriate post-hoc tests, including Wilcoxon signed-rank tests for non-normally distributed data, with adjustment for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Table 1 presents the baseline demographic and clinical characteristics of the 65 study participants, comprising 31 women with preterm deliveries and 34 with term deliveries. The median age was 26.0

years (IQR: 22.0–32.0) overall, with no statistically significant difference between the preterm and term groups (26.0 vs. 27.0 years, respectively; $p = 0.567$). Gestational age at delivery was significantly lower among women with preterm delivery compared with those with term delivery (34.0 vs. 40.0 weeks; $p < 0.001$). The number of past pregnancies was similar between the groups (median: 1.0 vs. 1.5; $p = 0.792$). The number of previous miscarriages did not differ significantly between the preterm and term groups ($p = 0.063$). In contrast, a significant difference was observed in the history of previous preterm deliveries ($p = 0.017$), despite a median of zero in both groups. HIV reactivity was reported in 16 participants (24.6%) and did not differ significantly between women with preterm and term deliveries (29.0% vs. 20.6%; $p = 0.616$). None of the participants developed preeclampsia during the current pregnancy. Overall, the baseline characteristics were largely comparable between the two groups, with significant differences observed only in gestational age at delivery and history of previous preterm delivery. See Table 1 below. **Baseline demographic and Clinical Charecteristics of study participants**

Table 1
Baseline Demographic & Clinical Characteristics of study participants

Variable	**(N = 65)**	**Preterm (n = 31)**	**Term (n = 34)**	***p*-value**
Age, years	26.0 (22.0–32.0)	26.0 (22.0–32.0)	27.0 (22.0-32.5)	0.567
EGA at delivery, weeks	38.0 (34.0–40.0)	34.0 (33.0–35.0)	40.0 (39.0–41.0)	**< 0.001**
Past pregnancies	1.0 (0.0–2.0)	1.0 (0.0–3.0)	1.5 (0.0–2.0)	0.792
Miscarriages, < 20 wks.	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.063
Past preterm deliveries	**0.0 (0.0–0.0)**	**0.0 (0.0–0.0)**	**0.0 (0.0–0.0)**	**0.017**
HIV reactive	16 (24.6%)	9 (29.0%)	7 (20.6%)	0.616
Preeclampsia(this pregnancy)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.513

Table 2 presents the comparison of hormonal biomarker concentrations between women with preterm and term deliveries across the three trimesters. For Corticotropin-releasing hormone (CRH), median concentrations were comparable between preterm and term groups across the three sampling time points. Although the direction and magnitude of the differences varied across T1-T3, none reached statistical significance, and the corresponding effect sizes were small (Table 2). Similarly, cortisol concentration showed no evidence of group variation across the study period. The concentrations remained constant between groups at all three time points, with no measurable effect size, suggesting the lack of detectable differences in circulating cortisol between preterm and term pregnancies.

For estradiol, no significant difference was observed during the first trimester. However, a statistically significant difference emerged in the second trimester, with higher estradiol concentrations recorded in

women who delivered preterm than those who delivered at term ($P = 0.042$). This difference was associated with a small to moderate effect size. By the third trimester (T3), the between group difference was no longer statistically significant ($P > 0.05$), suggesting that the disparity in estradiol concentrations was most pronounced at the intermediate sampling point.

A distinct temporal pattern was observed for serum IGF-1. There was no significant difference between groups in the first trimester. In contrast, serum IGF-1 concentrations were significantly lower in the preterm group both during the second trimester ($P = 0.006$) and the third trimester ($P = 0.018$), accompanied by moderate effect sizes, with the strongest effect observed during the second trimester. The persistence of this difference across the latter two sampling points suggests a more consistent association between reduced IGF-1 concentrations and preterm pregnancy than was observed for other hormonal markers.

Progesterone concentrations did not vary significantly between groups in the first and second trimesters, however, in the third trimester, progesterone concentrations were significantly lower among women who subsequently experienced spontaneous preterm birth compared with women who delivered at term ($P = 0.010$), with a moderate effect size. Generally, the results demonstrate time-dependent differences in selected hormonal markers between preterm and term pregnancies. The most prominent differences were observed for estradiol at T2 and for IGF-1 and progesterone at near delivery sampling time point. In contrast, CRH and cortisol remained comparable between women with spontaneous preterm birth and those who delivered at term at any stage of pregnancy.

Table 2
Comparison of hormonal biomarker concentrations between women with preterm and term deliveries

Analyte	Time-point	Preterm Median (IQR)	Term Median (IQR)	nPT / TM	p-value	Effect r
Section A-Hormonal Fluctuations						
CRH (ng/ml)	T1	0.180 (0.090–0.315)	0.220 (0.090–0.480)	19 / 21	0.416	0.153
CRH (ng/ml)	T2	0.320 (0.145–0.380)	0.220 (0.100–0.390)	19 / 21	0.766	-0.058
CRH (ng/ml)	T3	0.200 (0.130–0.295)	0.180 (0.110–0.320)	19 / 21	0.336	-0.180
Cortisol (ug/dl)	T1	24.0 (24.0–24.0)	24.0 (24.0–24.0)	19 / 21	1.000	0.000
Cortisol (ug/dl)	T2	24.000 (24.0–24.0)	24.0 (24.0–24.0)	19 / 21	1.000	0.000
Cortisol (ug/dl)	T3	24.0 (24.0–24.0)	24.0 (24.0–24.0)	19 / 21	1.000	0.000
Estradiol (pg/ml)	T1	5,889.9 (3,742.8–10,491.8)	5,632.8 (3,340.4–11,716.5)	19 / 21	0.914	-0.023
Estradiol (pg/ml)	T2	14,934.5 (6,631.9–19,603.6)	6,483.8 (4,733.5-8,644.7)	19 / 21	0.042	-0.378
Estradiol (pg/ml)	T3	7,718.1 (2,841.6–16,464.5)	9,435.1 (4,442.9–12,263.5)	19 / 21	0.892	0.028
IGF-1 (ng/ml)	T1	46.910	54.350	19 / 21	0.766	0.058

Abbreviations: CRH, corticotropin-releasing hormone; IGF-1, insulin-like growth factor 1; T1, first trimester; T2, second trimester; T3, third trimester; PT, preterm; TM, term; IQR, interquartile range; p-value, probability value; r, effect size; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre; µg/dL, micrograms per decilitre.

Analyte	Time-point	Preterm Median (IQR)	Term Median (IQR)	nPT / TM	p-value	Effect r
		(30.025–86.120)	(30.370–78.240)			
IGF-1 (ng/ml)	T2	37.250 (22.270–56.200)	61.960 (40.060–85.850)	19 / 21	0.006	0.514
IGF-1 (ng/ml)	T3	6.760 (1.425–18.425)	22.070 (8.510–53.530)	19 / 21	0.018	0.441
Progesterone (ng/ml)	T1	49.300 (33.5–88.5)	41.8 (22.9-100.250)	19 / 19	0.579	-0.108
Progesterone (ng/ml)	T2	186.215 (118.075-277.555)	208.680 (112.060–276.9)	18 / 21	0.704	0.074
Progesterone (ng/ml)	T3	63.470 (39.430-179.480)	246.720 (97.330–428.9)	19 / 21	0.010	0.479

Abbreviations: CRH, corticotropin-releasing hormone; IGF-1, insulin-like growth factor 1; T1, first trimester; T2, second trimester; T3, third trimester; PT, preterm; TM, term; IQR, interquartile range; p-value, probability value; r, effect size; ng/mL, nanograms per millilitre; pg/mL, picograms per millilitre; µg/dL, micrograms per decilitre.

Figure 1 shows Hormone levels across pregnancy trimesters: Serum hormone concentrations (mean) at T1 (1st trimester), T2 (mid-pregnancy), and T3 (near delivery). Solid orange circles = preterm (n = 31); dashed green diamonds = term (n = 34). Panels: estradiol (pg/ml), progesterone (ng/ml), IGF-1 (ng/ml). Significance on panel titles: * p < 0.05; ** p < 0.01 (Mann–Whitney U). Cortisol (assay floor, invariant) and CRH (ns) excluded. Hormone subset n ≈ 40.** p < 0.05 ** p < 0.01 · Hormone subset n ≈ 40 · Cortisol was invariant across all samples (assay floor effect, interpret with caution) · No significant difference noted unless marked. Line colours: solid orange = preterm; dashed green = term. X-axis: pregnancy time points (T1, T2, and T3). Y-axis: mean hormone concentration (units as labelled per panel). Each panel represents one analyte; panels share the same x-axis scale.* Longitudinal analysis of maternal serum cytokine revealed no statistically significant differences between women who delivered preterm and those who delivered at term at any trimester of pregnancy (all $p > 0.05$) as illustrated in Table 3 and Fig. 2. However, several cytokines demonstrated moderate effect sizes and temporal changes between preterm and term differences suggesting potentially relevant variation in maternal inflammatory profiles across pregnancy trimesters. IL-1β showed a small positive effect ($p = 0.608$, $r = 0.165$), IL-6 ($p = 0.880$, $r = 0.059$), IL-8 ($p = 0.704$, $r = 0.129$), TNF-α ($p = 0.556$, $r = 0.188$), IP-10 ($p = 0.543$, $r = -0.200$) showed predominantly small

effects. Thus, the early maternal cytokine profile during the first trimester did not show a distinct inflammatory pattern differentiating pregnancies that subsequently resulted in preterm versus term delivery.

During the second trimester, IL-1β demonstrated a comparatively larger effect ($p = 0.141$, $r = 0.357$), representing one of the strongest between-group effects across the three trimesters, although the difference remained non-statistically significant ($p > 0.05$). IL-6 showed a small-to-moderate effect ($p = 0.378$, $r = 0.221$), whereas IL-8 ($p = 0.930$, $r = 0.029$), TNF-α ($p = 0.790$, $r = 0.071$), IP-10 ($p = 0.837$, $r = -0.057$), and MIP-1α ($p = 0.523$, $r = -0.143$) exhibited small effects. In the third trimester, several cytokines showed a shift in effect direction relative to earlier trimesters. IL-1β ($p = 0.274$, $r = -0.267$) IL-6 ($p = 0.231$, $r = -0.293$), TNF-α ($p = 0.301$, $r = -0.253$) demonstrated small-to-moderate effects favouring higher inflammatory activity women in preterm delivery. IL-8 ($p = 0.677$, $r = 0.107$), showed only a small effect, while IP-10 demonstrated a negligible effect ($p = 0.781$, $r = -0.073$).

The most notable finding during the third trimester was observed for MIP-1α, which showed the largest effect size across the cytokines examined ($p = 0.074$, $r = -387$). Generally, these findings indicate that systemic cytokine profiles remained largely stable throughout pregnancy irrespective of birth outcome. Nevertheless, the temporal pattern of effect sizes suggest that inflammatory differences may become more pronounced later in pregnancy, particularly for IL-1β, IL-6, TNF-α, and MIP-1α.

Table 3: **Longitudinal maternal serum cytokine profiles**

Table 3
Longitudinal maternal serum cytokine profiles between preterm and term

Analyte	Time-point	Preterm Median (IQR)	Term Median (IQR)	n PT / TM	p-value	Effect r
IL-1β (pg/ml)	**T1**	11.787 (0.549–32.390)	210.971 (2.234-336.488)	17 / 5	0.608	0.165
IL-1β (pg/ml)	**T2**	0.00 (0.00-4.009)	14.310 (0.549–18.288)	10 / 14	0.141	**0.357**
IL-1β (pg/ml)	**T3**	26.691 (14.117–87.974)	8.654 (0.0 -36.755)	10 / 15	0.274	-0.267
IL-6 (pg/ml)	**T1**	5.627 (2.511–10.500)	5.043 (4.070-12.066)	17 / 5	0.880	0.059
IL-6 (pg/ml)	**T2**	0.736 (0.031–1.823)	0.737 (0.536–3.237)	10 / 14	0.378	0.221
IL-6 (pg/ml)	**T3**	5.043 (2.020–9.525)	2.511 (0.368–5.773)	10 / 15	0.231	-0.293
IL-8 (pg/ml)	**T1**	1,702.7 (361.8-8,568.6)	4,001.3 (2,931.2–11,940.6)	17 / 5	0.704	0.129
IL-8 (pg/ml)	**T2**	538.6 (42.4-825.2)	293.9 (146.4-715.4)	10 / 14	0.930	0.029
IL-8 (pg/ml)	**T3**	1,687.8 (71.5-3,541.3)	1,455.2 (466.8-5,809.7)	10 / 15	0.677	0.107
TNF-α (pg/ml)	**T1**	1.445 (0.763–4.379)	2.474 (1.616–2.819)	17 / 5	0.556	0.188
TNF-α (pg/ml)	**T2**	0.234 (0.088–0.424)	0.256 (0.088–0.742)	10 / 14	0.790	0.071
TNF-α (pg/ml)	**T3**	0.510 (0.130–3.909)	0.256 (0.000-1.616)	10 / 15	0.301	-0.253
IP-10 (pg/ml)	**T1**	84.478	37.248	17 / 5	0.543	-0.200

Analyte	Time-point	Preterm Median (IQR)	Term Median (IQR)	n PT / TM	p-value	Effect r
		(30.524-402.932)	(24.623–97.141)			
IP-10 (pg/ml)	**T2**	11.355 (2.734–22.901)	12.150 (5.316–29.416)	10 / 14	0.837	0.057
IP-10 (pg/ml)	**T3**	21.079 (5.128-386.822)	34.184 (8.071–52.875)	10 / 15	0.781	-0.073
MIP-1α (pg/ml)	**T1**	27.137 (6.428–36.388)	25.410 (0.000-50.445)	17 / 5	0.906	-0.047
MIP-1α (pg/ml)	**T2**	0.000 (0.000-11.487)	0.000 (0.000-19.490)	10 / 14	0.523	0.143
MIP-1α (pg/ml)	**T3**	21.226 (0.000-82.378)	0.000 (0.000-2.075)	10 / 15	0.074	**-0.387**

Figure 2. Vaginal cytokine concentrations (pg/ml, median ± IQR) across three pregnancy time points in preterm (n = 31, solid orange circles) and term (n = 34, dashed green diamonds) births. Eight analytes shown: IFN-γ, IL-1β, IL-6, IL-8, IL-10, TNF-α, MIP-1α (CCL3), IP-10 (CXCL10). Cytokine subset n ≈ 22–25 per time point. X-axis: T1 = 1st trimester, T2 = mid-pregnancy, T3 = late pregnancy/near delivery. Y-axis: pg/ml (linear scale; note differing scales per panel). Error bars = IQR. Significance markers above data points: L* p < 0.05; † p < 0.10 (Mann–Whitney U, two-sided, uncorrected). *No cytokine reached statistical significance at any time point (all p > 0.05 after FDR). MIP-1α showed a trend toward elevation in preterm at T3 (p = 0.074). At T1, IL-8 and TNF-α were negatively correlated with Lactobacillus abundance (Spearman r = − 0.549 and − 0.433, p < 0.05). IL-1β at T3 positively correlated with Gardnerella (r = 0.504, p = 0.010) in preterm.*

Discussion

This prospective longitudinal cohort study investigated maternal endocrine and systemic immune changes associated with spontaneous preterm birth among pregnant women in Zambia. The study findings highlight that women who subsequently delivered preterm exhibited a distinct pattern of trimester-specific endocrine alterations, comprising higher second-trimester estradiol concentrations, lower second- and third-trimester IGF-1 concentrations, and lower third-trimester progesterone concentrations than women who delivered at term. In contrast, no consistent outcome-related differences were detected in maternal serum cytokine concentrations. Collectively, these findings indicate that endocrine trajectories differentiated the two delivery groups more clearly than the

measured systemic immune mediators. However, the findings are not to be interpreted as evidence that endocrine dysfunction is more important than inflammation in the pathogenesis of spontaneous preterm birth. Rather, they suggest that endocrine alterations were more readily detectable in maternal circulation at the gestational intervals examined in this cohort.

Progesterone concentrations increased across pregnancy in both groups, consistent with its central role in maintaining uterine quiescence, limiting myometrial excitability, and regulating cervical and fetal-membrane function. Nevertheless, women who subsequently delivered preterm had significantly lower progesterone concentrations during the third trimester. In humans, labour usually occurs without the pronounced systemic decline in progesterone has been observed. Consequently, the initiation of labour has been attributed principally to functional progesterone withdrawal, involving changes in progesterone-receptor isoform expression, receptor activity and downstream inflammatory signalling rather than complete withdrawal of circulating progesterone [28]. Nadeem et al. demonstrated that suppression of progesterone-receptor function contributes to both term and preterm labour, providing molecular support for the concept that diminished progesterone responsiveness can activate parturition pathways even while circulating concentrations remain relatively high [29, 30].

The lower third-trimester progesterone concentrations observed in the present study are therefore compatible with premature endocrine transition towards parturition. Nevertheless, circulating progesterone does not directly measure progesterone activity within the myometrium, cervix or fetal membranes. The present results cannot establish whether the observed difference represents reduced placental synthesis, altered metabolism, differences in gestational timing at sample collection, or functional withdrawal at the tissue level. The finding is better interpreted as evidence of altered late-gestational progesterone physiology than as proof that an absolute progesterone deficiency caused preterm labour. This distinction is particularly important because spontaneous preterm birth is a heterogeneous syndrome and progesterone-related pathways may be prominent in only a subset of cases.

Estrogens contribute to placental development and fetal growth but also promote the transition of the myometrium from a quiescent to a contractile phenotype through increased expression of contraction-associated proteins, gap-junction proteins, prostaglandin-synthesis enzymes and oxytocin receptors[31, 32]. An earlier-than-expected increase in estrogenic activity could therefore shift the functional balance between progesterone-mediated quiescence and estrogen-mediated uterine activation. Supporting this interpretation, Parisi et al. reported that elevated mid-trimester maternal serum unconjugated estradiol was independently associated with a higher risk of spontaneous preterm birth [33, 34].Estriol and estradiol are not interchangeable analytes, but both findings support the broader hypothesis that premature alteration of estrogenic signalling may accompany pregnancies destined for early delivery [35, 36].

The second-trimester timing of the estradiol difference is noteworthy because it preceded the significant third-trimester progesterone difference. Considered together, the two findings may indicate a progressive

alteration in the estrogen–progesterone balance rather than two isolated hormonal abnormalities. This interpretation is biologically plausible because increasing estrogenic activity can promote expression of labour-associated genes, while reduced progesterone abundance or responsiveness diminishes opposition to these effects. Nevertheless, the estradiol-to-progesterone ratio was not explicitly evaluated in the present analysis. Future studies should investigate whether within-person changes in this ratio provide better discrimination of spontaneous preterm birth than either hormone alone.

Another notable finding was the lower IGF-1 concentrations observed among women who subsequently experienced preterm delivery during both the second and third trimesters. The consistency of this difference across two successive stages of pregnancy suggests that it may reflect a sustained alteration rather than a temporary fluctuation. The maternal and placental IGF systems play essential roles in trophoblast development, placental nutrient transport, vascular adaptation, and fetal growth and development [37, 38]. Disturbance of this system has been associated with impaired placental function and adverse pregnancy outcomes. Evidence that preterm labour involves altered IGF-system regulation has also been reported in analyses of maternal and fetal biological compartments, although individual studies have assessed different components of the pathway, including IGF-1, IGF receptors and IGF-binding proteins [39, 40]. Genetic variation in maternal IGF1 and IGF1R has similarly been associated with spontaneous preterm-birth susceptibility, further supporting the potential relevance of this pathway [41].

Lower maternal IGF-1 may reflect impaired placental development, altered maternal metabolic adaptation, or reduced capacity to support fetal placental growth. However, it should not yet be described as a clinically useful biomarker [42]. Biomarker status requires evidence of reproducibility, incremental predictive performance, calibration, clinically relevant thresholds and external validation. The present study instead identifies IGF-1 as a promising candidate for further investigation. Its sustained separation between groups may be particularly valuable because a repeated trajectory could be more robust than a single concentration [43, 44]. Future modelling should test whether longitudinal IGF-1 improves prediction beyond established maternal characteristics and whether its association with preterm birth is independent of fetal growth, maternal nutritional status, body mass index, HIV status and placental complications.

In contrast to estradiol, progesterone and IGF-1, maternal serum CRH and cortisol concentrations did not differ significantly by delivery outcome. This result differs from studies reporting accelerated activation of the placental–fetal adrenal axis in pregnancies ending preterm. Some studies observed higher CRH concentrations at 24 and 32 weeks among women with recurrent preterm birth [45]. However, CRH alone showed only modest discrimination, and its performance improved when combined with obstetric history. Earlier longitudinal work also indicated that elevated maternal cortisol in early pregnancy could be associated with a later rise in placental CRH and shorter gestation [46].

Several factors could explain the divergence between these studies and the present findings. CRH associations may be stronger in high-risk women with recurrent preterm birth than in a more

heterogeneous cohort. CRH measurement is also particularly sensitive to specimen preparation, extraction and assay platform because much of the circulating hormone is protein-bound. Herrera et al. found that associations detected using extracted radioimmunoassay were not reproduced using an unextracted ELISA, illustrating how analytical methods can affect conclusions. Furthermore, cortisol varies with time of day, stress, medication exposure and sampling conditions. Thus, the absence of significant CRH and cortisol differences does not exclude activation of the maternal–placental–fetal hypothalamic–pituitary–adrenal axis. It indicates that the circulating measurements obtained under the present sampling and assay conditions did not distinguish delivery groups reliably.

No significant outcome-related differences were observed across the maternal serum cytokine panel, although MIP-1α showed a nonsignificant tendency towards higher late-pregnancy concentrations among women who subsequently delivered preterm. This finding is not necessarily inconsistent with the established role of inflammation in spontaneous preterm labour. A systematic review found that increased IL-6 in cervicovaginal and amniotic fluid was more consistently associated with spontaneous preterm birth than IL-6 measured in maternal plasma, supporting the view that inflammation at the maternal–fetal interface may not be mirrored proportionately in peripheral circulation [47]. More recently, Hornaday et al. found that second- and early third-trimester maternal circulating cytokine profiles had limited ability to predict preterm birth, with substantial heterogeneity among spontaneous preterm labour, preterm prelabour rupture of membranes and medically indicated preterm birth [48]. Turra et al. likewise reported limited evidence that individual second-trimester circulating cytokines could reliably identify asymptomatic women who would subsequently deliver preterm [49].

The present cytokine findings may therefore reflect biological compartmentalization rather than absence of immune involvement. Inflammation associated with preterm labour may originate in the decidua, placenta, fetal membranes, amniotic cavity or cervicovaginal environment and remain locally concentrated until close to the onset of labour. Maternal blood measurements may dilute these tissue-specific signals. Cytokines also follow mediator-specific gestational trajectories; some decrease while others increase across normal pregnancy, complicating interpretation of isolated concentrations [50]. Furthermore, transient inflammatory episodes could have occurred between scheduled trimester visits. Repeated trimester sampling improves temporal resolution relative to single-time-point studies but may still miss short-lived immune changes immediately preceding preterm labour [51, 52].

The MIP-1α trend should consequently be interpreted as hypothesis-generating. MIP-1α is a chemokine involved in leukocyte recruitment and amplification of inflammatory responses, making its late-pregnancy elevation biologically plausible. However, the probability value of 0.074 does not meet the pre-specified threshold for statistical significance, and multiple cytokine testing increases the possibility of chance findings. Its potential relevance should therefore be examined in a larger independent cohort and, ideally, in paired maternal serum and local reproductive-tract or placental specimens. This would determine whether the weak systemic signal corresponds to stronger compartment-specific chemokine activity.

Strengths

Despite the sample size limitations, a key strength of this study is its prospective, longitudinal design starting early in the first trimester (up to 12 weeks' gestation). Recruiting participants early on made it possible to follow women before any clinical complications arose, avoiding recall bias and ensuring a clear timeline between baseline factors and pregnancy outcomes. Additionally, our strict eligibility criteria enrolling only singleton pregnancies and excluding women with a history of preterm birth, preeclampsia, prior pregnancy loss, or chronic medical conditions helped minimize major confounding factors and strengthened the reliability of our findings.

Limitation

A major limitation of this study was the high rate of participant attrition (35%), which resulted in a final analytical sample of 65 women out of the 100 initially targeted. This sample size reduction may have limited the statistical power of the analysis to detect subtle effect sizes or less pronounced differences in risk factors associated with preterm birth. Although baseline demographic and clinical characteristics were compared between retainees and dropouts to evaluate potential attrition bias, the reduced sample size highlights the need for larger prospective cohorts to validate these findings

Conclusion

This longitudinal cohort study demonstrated that pregnancies resulting in spontaneous preterm birth were characterized by distinct maternal endocrine changes, particularly higher second-trimester estradiol and lower second and third-trimester IGF-1 and third-trimester progesterone concentrations. In contrast, maternal systemic cytokine profiles remained largely unchanged throughout pregnancy, suggesting that peripheral inflammatory markers may have limited utility for identifying women at risk of spontaneous preterm birth in this cohort. These findings underscore the importance of endocrine adaptations during pregnancy and support further investigation of longitudinal hormonal trajectories as potential biomarkers of spontaneous preterm birth. Larger multicentre studies incorporating more diverse populations, more frequent sampling, and integrated assessment of endocrine, placental, immunological and molecular pathways are needed to validate these observations and improve early prediction of spontaneous preterm birth.

Abbreviations

PTB — Preterm Birth

sPTB — Spontaneous Preterm Birth

SSA — Sub-Saharan Africa

IGF-1 — Insulin-Like Growth Factor 1

CRH — Corticotropin-Releasing Hormone

HPA — Hypothalamic–Pituitary–Adrenal

IL-1β — Interleukin-1 Beta

IL-6 — Interleukin-6

IL-8 — Interleukin-8

IL-10 — Interleukin-10

TNF-α — Tumour Necrosis Factor-Alpha

IFN-γ — Interferon-Gamma

IP-10 — Interferon Gamma-Induced Protein 10

CXCL10 — C-X-C Motif Chemokine Ligand 10

MIP-1α — Macrophage Inflammatory Protein-1 Alpha

CCL3 — C-C Motif Chemokine Ligand 3

ELISA — Enzyme-Linked Immunosorbent Assay

EGA — Estimated Gestational Age

T1 — First Trimester

T2 — Second Trimester

T3 — Third Trimester

PT — Preterm

TM — Term

IQR — Interquartile Range

SD — Standard Deviation

ANOVA — Analysis of Variance

HIV — Human Immunodeficiency Virus

UTH — University Teaching Hospitals

SMHS — School of Medicine and Health Sciences

NIHR — National Institute for Health and Care Research

EDCTP2 — European and Developing Countries Clinical Trials Partnership 2

UK Aid — United Kingdom Aid

BMI — Body Mass Index

ART — Antiretroviral Therapy

FDR — False Discovery Rate

Declarations

Ethical considerations and consent to participants

Ethical approval for this study was obtained from the Mulungushi University School of Medicine and Health Sciences (SMHS) Research Ethics Committee (Approval No.: SMHS-MU2-2024-113) on **[insert approval date]**. The study was conducted in accordance with approved ethical standards, the principles of the Declaration of Helsinki, and applicable national ethical guidelines governing research involving human participants. Written informed consent was obtained from all participants prior to enrolment. To ensure confidentiality and protect participants' privacy, all collected data were de-identified and handled with strict confidentiality. Personal identifiers were not included in the analytical dataset, and data were accessed only by authorized members of the research team and used solely for research purposes.

Clinical Trial number

Not applicable (N/A).

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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Author's contributions

MS conceptualized the study, developed the methodology, conducted the investigation, curated and formally analysed the data, performed visualization, and wrote the original draft. SM, MKL, and NS provided supervision, while SM and MKL contributed to methodology and validation. MKL also provided resources. BH and LS conducted data analysis. M contributed to formal analysis, software, and validation. PK, EM, and ZT conducted sample analysis. LM contributed to methodology. KA and SM contributed to review and editing. All authors contributed to the article edits and approved the final manuscript.

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Figures

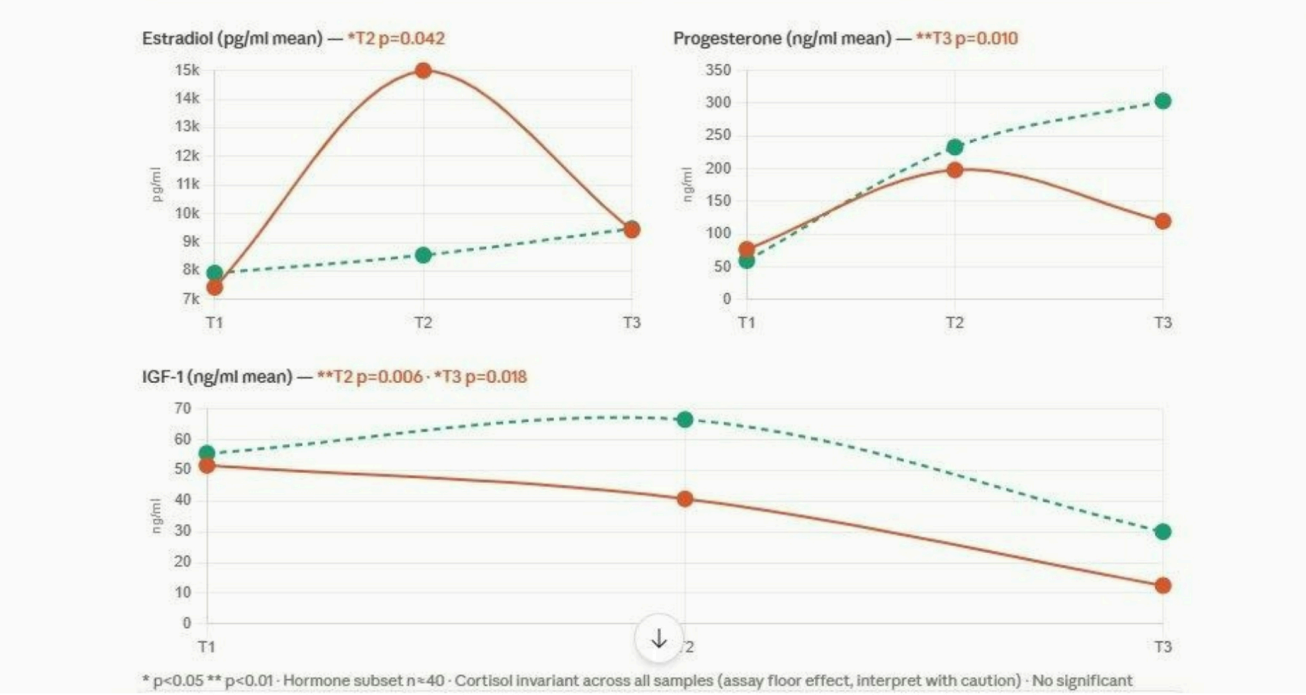


Figure 1

Figure 1 shows Hormone levels across pregnancy trimesters: Serum hormone concentrations (mean) at T1 (1st trimester), T2 (mid-pregnancy), and T3 (near delivery). Solid orange circles = preterm (n = 31); dashed green diamonds = term (n = 34). Panels: estradiol (pg/ml), progesterone (ng/ml), IGF-1 (ng/ml). Significance on panel titles: * p < 0.05; ** p < 0.01 (Mann–Whitney U). Cortisol (assay floor, invariant) and CRH (ns) excluded. Hormone subset n ≈40.* *p<0.05 ** p<0.01 · Hormone subset n≈40 · Cortisol was invariant across all samples (assay floor effect, interpret with caution) · No significant difference noted unless marked. Line colours: solid orange = preterm; dashed green = term. X-axis: pregnancy time points (T1, T2, and T3). Y-axis: mean hormone concentration (units as labelled per panel). Each panel represents one analyte; panels share the same x-axis scale.* Longitudinal analysis of maternal serum cytokine revealed no statistically significant differences between women who delivered preterm and those who delivered at term at any trimester of pregnancy (all *p*> 0.05) as illustrated in **Table 3** and **Figure 2**. However, several cytokines demonstrated moderate effect sizes and temporal changes between preterm and term differences suggesting potentially relevant variation in maternal inflammatory profiles across pregnancy trimesters. IL-1β showed a small positive effect (*p* = 0.608, *r* = 0.165), IL-6 (*p* = 0.880, *r* = 0.059), IL-8 (*p* = 0.704, *r* = 0.129), TNF-α (*p*= 0.556, *r* = 0.188), IP-10 (*p* = 0.543, *r* = -0.200) showed predominantly small effects. Thus, the early maternal cytokine profile during the first trimester did not show a distinct inflammatory pattern differentiating pregnancies that subsequently resulted in preterm versus term delivery.

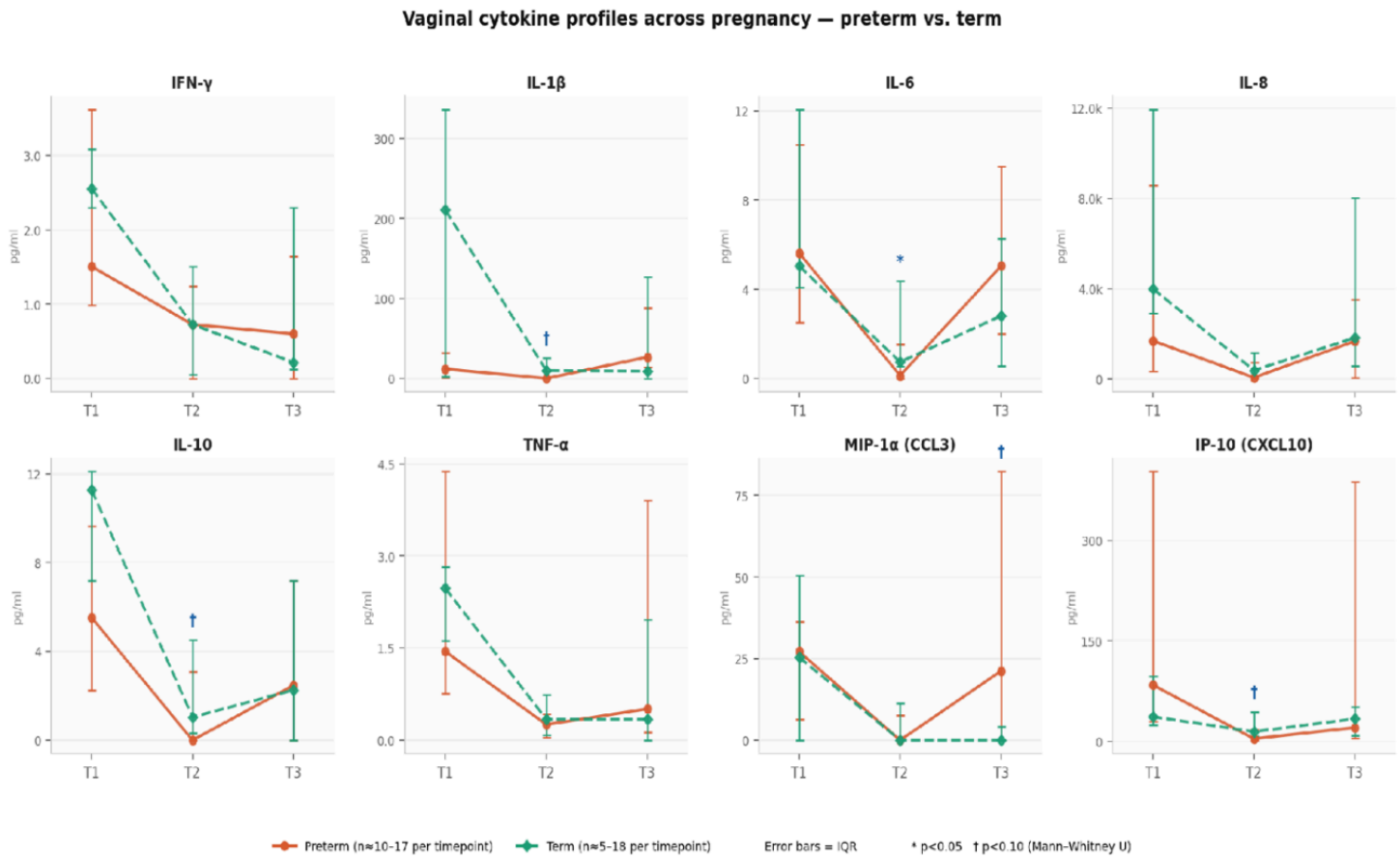


Figure 2

Vaginal cytokine concentrations (pg/ml, median ± IQR) across three pregnancy time points in preterm (n = 31, solid orange circles) and term (n = 34, dashed green diamonds) births. Eight analytes shown: IFN-γ, IL-1β, IL-6, IL-8, IL-10, TNF-α, MIP-1α (CCL3), IP-10 (CXCL10). Cytokine subset n ≈22–25 per time point. X-axis: T1 = 1st trimester, T2 = mid-pregnancy, T3 = late pregnancy/near delivery. Y-axis: pg/ml (linear scale; note differing scales per panel). Error bars = IQR. Significance markers above data points: L* $p < 0.05$; † $p < 0.10$ (Mann–Whitney U, two-sided, uncorrected). *No cytokine reached statistical significance at any time point (all $p > 0.05$ after FDR). MIP-1α showed a trend toward elevation in preterm at T3 ($p = 0.074$). At T1, IL-8 and TNF-α were negatively correlated with Lactobacillus abundance (Spearman $r = -0.549$ and -0.433, $p < 0.05$). IL-1β at T3 positively correlated with Gardnerella ($r = 0.504$, $p = 0.010$) in preterm.*