A study on the association between interleukin-1β, interleukin-6, and TNF-A levels and cervical morphological changes in the second trimester and preterm birth in twin pregnancies at Hai Phong Medical University Hospital

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ABSTRACT

Objective: To determine the correlation between maternal blood levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) measured at 16 weeks of gestation and second-trimester cervical morphological changes on ultrasound, as well as preterm birth outcomes in twin pregnancies. ***Methods:*** This study was conducted on 93 women with twin pregnancies at 14–24 weeks of gestation at Hai Phong Medical University Hospital between 2023 and 2025. Maternal serum levels of IL-1β, IL-6, and TNF-α were measured at 16 weeks of gestation. Transvaginal ultrasound examinations were performed every two weeks at 16, 18, 20, 22, and 24 weeks to assess cervical length and cervical angle. Comparisons were made between women who delivered preterm before 34 weeks, preterm before 37 weeks, and those who delivered at term. ***Results:*** The mean maternal age was 32.1 ± 4.7 years, and the mean body mass index (BMI) was 21.1 ± 2.2 kg/m². The mean serum concentrations of IL-1β, IL-6, and TNF-α were 208.98 ± 5.64, 26.59 ± 2.04, and 19.14 ± 1.05, respectively. No statistically significant differences were observed in serum IL-1β, IL-6, or TNF-α levels at 16 weeks among women who delivered preterm before 34 weeks, preterm before 37 weeks, and those who did not deliver preterm ($p > 0.05$). Furthermore, no statistically significant associations were found between maternal pro-inflammatory cytokine levels (IL-1β, IL-6, TNF-α) and cervical length or cervical angle measured by second-trimester ultrasound. ***Conclusion:*** This study did not demonstrate a statistically significant correlation between maternal serum IL-1β, IL-6, and TNF-α levels at 16 weeks of gestation and preterm birth, nor with changes in cervical length or cervical angle on second-trimester ultrasound in women with twin pregnancies.

Keywords: *preterm birth; cervical length; cervical angle; pro-inflammatory cytokines.*

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INTRODUCTION

Preterm birth (PTB), defined as delivery before 37 completed weeks of gestation, is a leading cause of perinatal mortality and neonatal morbidity worldwide, particularly when it occurs before 32 weeks of gestation [1].Each year, more than 15 million preterm births are reported globally, accounting for over one million deaths in young children and resulting in substantial lifelong sequelae among survivors [2, 3].

Currently, prediction of preterm birth risk relies primarily on obstetric history and sonographic assessment of cervical length. However, these tools have limited predictive performance, especially in high-risk pregnancies such as twin gestations [4]. Increasing evidence suggests that pro-inflammatory pathways play a crucial role in the initiation of preterm birth by inducing morphological and functional changes of the cervix [5].

Both infectious and non-infectious inflammatory processes can trigger the release of pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). These mediators stimulate prostaglandin synthesis and extracellular matrix-degrading enzymes, leading to cervical softening, cervical remodeling, and activation of uterine contractility [5]. Investigating inflammatory markers during pregnancy in conjunction with cervical morphology during the second trimester may not only improve understanding of the pathophysiology of preterm birth but also provide new opportunities for early screening and prevention [6].

This issue is particularly relevant in multiple pregnancies, especially twin gestations, which carry a substantially higher risk of preterm birth compared with singleton pregnancies. A longitudinal observational study by Silvano et al. (2024) assessed plasma concentrations of IL-1β, IL-6, IL-10, IL-12, and TNF-α in 82 women with twin pregnancies across gestational trimesters [7]. The authors reported a progressive increase in cytokine levels from the first to the third trimester ($p < 0.001$). Notably, TNF-α and IL-6 concentrations in the second and third trimesters were inversely correlated with gestational age at delivery ($p < 0.05$), suggesting that higher cytokine levels were associated with earlier delivery.

Although numerous studies have evaluated cervical morphological changes in twin pregnancies, data regarding the relationship between circulating pro-inflammatory markers and cervical morphology, as well as the prognostic value of these markers for preterm birth, remain limited [8]. To date, no such studies have been conducted in Vietnam. Moreover, there is currently no consensus regarding the optimal timing for inflammatory marker assessment or whether blood or cervical/vaginal samples provide greater predictive value, particularly in twin pregnancies.

The gestational age of 16 weeks represents a critical window for preventive interventions such as prophylactic cervical cerclage in women with cervical insufficiency [9]. Based on these considerations, we conducted this study among 93 women with twin pregnancies between 14 and 24 weeks of gestation at Hai Phong Medical University Hospital. The primary objective was to investigate the association between maternal serum IL-1β, IL-6, and TNF-α levels at 16 weeks of gestation, second-trimester cervical morphological changes, and the risk of preterm birth in twin pregnancies at Hai Phong Medical University Hospital.

MATERIALS AND METHODS

Study design

This was a prospective longitudinal study conducted between 09/2023 and 08/2025 at Hai Phong Medical University Hospital. The study included 93 pregnant women with twin gestations between 14 and 24 weeks of gestation. Transvaginal ultrasound examinations were performed every two weeks by a single certified sonographer to assess cervical morphology, including cervical length and uterocervical angle. Maternal blood samples were collected at 16 weeks of gestation to measure serum IL-1β, IL-6, and TNF-α concentrations.

Study population

All pregnant women with viable twin pregnancies between 14 and 24 weeks of gestation who were examined and managed at the Department of Obstetrics, Hai Phong Medical University Hospital, and who provided informed consent, were eligible for inclusion. Gestational age was determined based on the first day of the last menstrual period, first-trimester ultrasound, or embryo transfer date in pregnancies conceived by assisted reproductive technologies..

Inclusion criteria

- Twin pregnancies
- Gestational age ≥14 weeks
- Gestational age determined by last menstrual period or first-trimester ultrasound in spontaneous pregnancies, or by embryo transfer date in assisted reproduction
- Transvaginal ultrasound measurement of cervical length and uterocervical angle at 14, 16, 18, 20, 22, and 24 weeks
- Maternal blood sampling at 16 weeks for IL-1β, IL-6, and TNF-α quantification
- Written informed consent provided

Exclusion criteria

- At least one fetal demise
- Medically indicated termination of pregnancy during follow-up (e.g., placenta previa, preeclampsia)
- History of or current chronic inflammatory or autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis), severe infections, tuberculosis, or viral hepatitis
- Use of high-dose corticosteroids or immunosuppressive drugs during pregnancy
- Lower genital tract infection or antibiotic use within two weeks prior to blood sampling

Sample size calculation

Sample size:

The sample size was calculated using the formula for estimating a correlation coefficient:

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\frac{1}{2} \log_e \frac{1+r}{1-r}} \right)^2 + 3$$

Where:

- α: Type I error, α = 0.05 → $Z_{1-\alpha/2}$=1.96$Z_{1-\alpha/2}$=1.96
- β: Type II error, β = 0.20 (power = 80%) → $Z_{1-\beta}$=0.84$Z_{1-\beta}$=0.84
- r: correlation coefficient between cervical length and gestational age, based on Fujita (2002), r = 0.43 [10].

The calculated sample size was 41. To increase the study value, a design effect of 2 was applied; therefore, the minimum required sample size was 82.

In practice, the study was conducted on 93 pregnant women with twin pregnancies

Study procedures

Step 1: Clinical examination and selection of participants meeting eligibility criteria.

Step 2: Transvaginal ultrasound assessment of cervical morphology beginning at 14 weeks of gestation and repeated every two weeks until 24 weeks.

Step 3: Maternal blood sampling at 16 weeks of gestation for IL-1β, IL-6, and TNF-α quantification.

Step 4: Follow-up of pregnancy outcomes. Cases of medically indicated preterm birth, fetal demise, or missing ultrasound data at any scheduled assessment (14, 16, 18, 20, 22, or 24 weeks) were excluded from analysis.

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Step 5: Description of second-trimester cervical morphology and its association with pro-inflammatory cytokines.

Study flowchart:

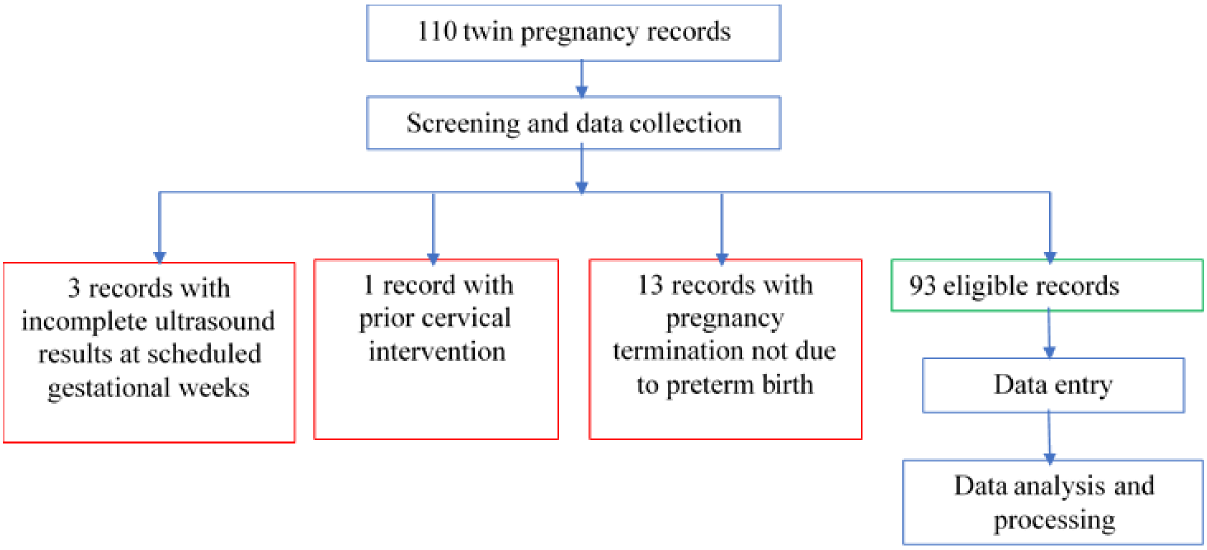

Figure 1. *Study flowchart*

Assessment of cervical length, uterocervical angle, and pro-inflammatory cytokines

Cervical length and uterocervical angle measurement

Cervical length and uterocervical angle were measured via transvaginal ultrasound by a single physician certified and supervised by the Fetal Medicine Foundation, using a Voluson S8 ultrasound system with a 7.5–10 MHz transvaginal probe [11].

Participants were examined in the lithotomy position with an empty bladder. The probe was gently placed in the anterior vaginal fornix and adjusted to visualize the cervical canal without exerting excessive pressure. The cervix occupied approximately 50–75% of the ultrasound image and was measured along its longitudinal axis. Cervical length was defined as the linear distance between the internal os and external os. Three measurements were obtained, and the shortest value was recorded (Figure 2a).

The uterocervical angle was defined as the angle between two lines following the method described by Dziadosz et al.: the first line extended from the external os to the internal os, and the second line was drawn tangentially along the anterior wall of the lower uterine segment passing through the internal os [12]. Three measurements were obtained, and the largest value was used for analysis (Figure 2b).

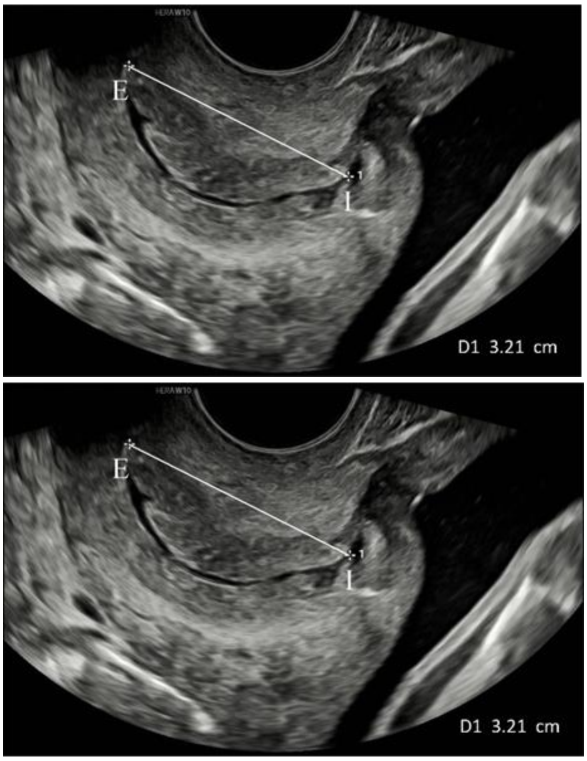

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Figure 2. *(a) Transvaginal measurement of cervical length from the external os (E) to the internal os (I). (b) Transvaginal measurement of the uterocervical angle between the cervical canal and the lower uterine segment; external os (bold arrow), internal os (thin arrow) [12, 13].*

Pro-inflammatory cytokine analysis

- Sample collection, storage, and transport

At 16 weeks of gestation, 5 mL of venous blood was collected into tubes without anticoagulant. After clotting at room temperature for 30 minutes, samples were centrifuged at 3000 rpm for 10 minutes to separate serum. Serum aliquots were transferred into sterile cryotubes and stored at −80°C until analysis. Samples were transported in insulated containers with dry ice to maintain the cold chain. Samples not meeting storage criteria were excluded [14-16].

- Cytokine quantification

Serum concentrations of IL-1β, IL-6, and TNF-α were quantified using sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Concentrations were determined based on optical density values and standardized calibration curves. All samples were analyzed in duplicate alongside negative controls, positive controls, and internal standards to ensure accuracy and reproducibility [17].

Ethical approval

The study was approved by the Ethics Committee in Biomedical Research of Hai Phong University of Medicine and Pharmacy (Decision No. 206/QĐ-ĐHYDHP dated October 21, 2023) and the Scientific Council of Hai Phong Medical University Hospital.

RESULTS

General Characteristics of the study population

Table 1. *General characteristics of the study population (n= 93)*

Characteristics	n (%)
Maternal age (mean ±SD) (years)	32.1± 4.7 (min-max : 18-47)
BMI (mean ±SD) (kg/m^2)	21.1 ± 2.2 (min-max: 16.8-29.4)
History of pregnancy loss	
Yes	70 (75.3)
No	23 (24.7)
Gynecological history	
Healthy	85 (91.4)
Fibroids/Polyp/PCOS	8 (8.6)
Current pregnancy status	
Healthy	74 (79.6)
Complicated/Disease	19 (20.4)

Comments: The mean age was 32.1 ± 4.7 years, and the mean BMI was 21.1 ± 2.2. Approximately 75.3% of the pregnant women had a history of pregnancy loss, which is triple the rate of those without such a history. Only 8 subjects (8.6%) presented with a history of gynecological pathologies such as uterine fibroids, polyps, or Polycystic Ovary Syndrome (PCOS). The majority of twin pregnancies were achieved through Assisted Reproductive

Technology (ART), specifically IVF or IUI (72%). Most subjects had a healthy clinical course during the current pregnancy (79.6%). Dichorionic diamniotic twin pregnancies accounted for 83.9% of the cases.

Characteristics of Pro-inflammatory Factor Concentrations

Table 2. *Characteristics of Genetic and Pro-inflammatory Factor Concentrations*

Characteristics	Mean (SE)	Median (IQR)
IL-1β (pg/mL)	208.98 (5.64)	200.78 (50.63)
IL-6 (pg/mL)	26.59 (2.04)	20.63 (19.29)
TNF-α (pg/mL)	19.14 (1,05)	16.94 (7.91)

Comments: The mean concentrations of the pro-inflammatory factors IL-1β, IL-6, and TNF-α were 208.98 ± 5.64, 26.59 ± 2.04, and 19.14 ± 1.05, respectively. The distribution of these indicators showed relatively wide variability, as reflected by the interquartile ranges (IQR).

Table 3. *Mean Pro-inflammatory Factor Concentrations in Preterm Birth Groups (Before 34 and 37 Weeks)*

Factor ($\overline{X}$ ± SD)	**Preterm < 34 weeks**			**Preterm < 37 weeks**		
	Preterm	**Non - preterm**	**p**	**Preterm**	**Non - preterm**	**p**
IL-1β (pg/mL)	212.79 ± 76.13	198.42 ± 51.52	0,4	198.86 ± 61.47	201.39 ± 49.9	0.83
IL6 (pg/mL)	29.11 ± 20.12	25.85 ± 18.86	0.61	27.14 ± 18.49	25.59 ± 19.45	0.7
TNF-α (pg/mL)	15.86 ± 6.57	18.3 ± 9.82	0.28	16.45 ± 6.37	19.2 ± 11.25	0.14

Comments: No statistically significant differences were observed in IL-1β, IL-6, or TNF-α concentrations between the preterm birth groups (<34 weeks and <37 weeks) compared to the non-preterm groups (p > 0.05).

Table 4. *Mean Cervical Length (CL) in Preterm and Non-preterm Groups*

CL (mm) $\overline{X}$ ± SD (mm)	**Preterm < 34 weeks**			**Preterm < 37 weeks**		
	Preterm	**Non - preterm**	**p**	**Non - preterm**	**Non - preterm**	**p**
Weeks 16	36.26 ± 5.61	34.02 ± 4.87	0.17	37.16 ± 5.75	34.67 ± 5.03	**0.03**
Weeks 18	37.7 ± 4.55	33.46 ± 3.81	**<0.01**	38.01 ± 4.73	35.95 ± 4.36	**0.03**
Weeks 20	37.2 ± 4.83	30.97 ± 6.29	**<0.01**	37.84 ± 4.78	34.56 ± 5.69	**<0.01**
Weeks 22	37.74 ± 5.44	28.83 ± 7.61	**<0.01**	38.73 ± 5.26	33.88 ± 6.86	**<0.01**
Weeks 24	37.47 ± 7.02	29.03 ± 7.69	**<0.01**	39.19 ± 6.08	32.83 ± 7.94	**<0.01**

Comments: At 16 weeks, there was no statistically significant difference in cervical length (CL) between the preterm and non-preterm groups for birth before 34 weeks ($p > 0.05$); however, a significant difference was observed for birth before 37 weeks ($p < 0.05$). From weeks 18 through 24, statistically significant differences in CL were observed between the preterm and non-preterm groups for both 34-week and 37-week thresholds ($p < 0.05$), indicating a correlation between cervical length and preterm birth outcomes.

Table 5. *Mean concentrations of pro-inflammatory factors across weeks and changes in Cervical Length (CL)*

CL		**IL-1β** (pg/mL) ($\bar{X} \pm$ **SD**)	**IL6** (pg/mL) ($\bar{X} \pm$ **SD**)	**TNF-α** (pg/mL) ($\bar{X} \pm$ **SD**)
Weeks 16 - 18	Decrease	205.15 ± 56.49	24.4 ± 18.85	16.33 ± 6.33
	Increase or Stable	196.43 ± 54.01	27.75 ± 19.07	19.29 ± 11.24
	p*	0.45	0.4	0.14
Weeks 18 - 20	Decrease	205.79 ± 52.01	27.97 ± 20.19	17.23 ± 6.89
	Increase or Stable	193.28 ± 58.46	24.12 ± 17.25	18.95 ± 12
	p*	0.28	0.33	0.39
Weeks 20 - 22	Decrease	201.31 ± 59.91	26.29 ± 16.51	17.67 ± 6.34
	Increase or Stable	198.96 ± 48.76	26.25 ± 21.86	18.39 ± 12.44
	p*	0.84	0.99	0.72
Weeks 22 - 24	Decrease	197.31 ± 56.44	26.61 ± 19.37	18.03 ± 7.98
	Increase or Stable	202.71 ± 54.22	25.99 ± 18.78	17.95 ± 10.62
	p*	0.64	0.88	0.97

**t-test*

Comments: No statistically significant association was observed between changes in cervical length and the concentrations of pro-inflammatory factors at any gestational time point[2]. Although slight variations in mean values were noted at certain points, none reached statistical significance ($p > 0.05$).

Table 6. *Mean concentrations of pro-inflammatory factors across weeks and changes in Uterocervical Angle (UCA)*

UCA		**IL-1β** ($\bar{X} \pm$ **SD**)	**IL6** ($\bar{X} \pm$ **SD**)	**TNF-α** ($\bar{X} \pm$ **SD**)
Weeks 16 - 18	Decrease	200.91 ± 49.42	25.07 ± 15.52	19.57 ± 11.09
	Increase or Stable	199.62 ± 60.71	27.5 ± 22.01	16.37 ± 7.23
	p*	0.91	0.54	0.1

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Weeks 18 - 20	Decrease	203.16 ± 55.43	24.87 ± 19.73	17.47 ± 10.78
	Increase or Stable	196.77 ± 54.93	27.98 ± 18.03	18.61 ± 7.66
	p*	0.58	0.43	0.57
Weeks 20 - 22	Decrease	199.02 ± 59.21	27.28 ± 19.62	18.48 ± 11.04
	Increase or Stable	202.18 ± 48.65	24.75 ± 18.04	17.24 ± 6.49
	p*	0.79	0.53	0.54
Weeks 22 - 24	Decrease	194.11 ± 53.37	23.46 ± 15.93	18.41 ± 11.4
	Increase or Stable	207.45 ± 56.61	29.54 ± 21.68	17.5 ± 6.66
	p*	0.25	0.12	0.65

**t-test*

Comments: There was no statistically significant relationship between changes in the uterocervical angle and pro-inflammatory factor levels at the 16, 18, 20, 22, and 24-week markers[5]. Observed differences in mean values remained statistically non-significant ($p > 0.05$).

DISCUSSION

General Characteristics

In our study, the cohort consisted of 93 women with twin pregnancies, with a mean age of 32.1 ± 4.7 years, falling within the common reproductive age range. The mean BMI was 21.1 ± 2.2, which is within the normal range, suggesting that most participants did not have overt nutritional issues prior to pregnancy. Notably, 72% of the cases resulted from Assisted Reproductive Technology (ART), specifically IVF/IUI (Table 1).

Compared to international publications, the maternal age and BMI in this study are consistent with a previous report by Conde-Agudelo et al. (2010), which reviewed 16 studies on asymptomatic twin pregnancies and found mean maternal ages between 30–34 and BMIs ranging from 22–25 [4].However, the proportion of pregnancies achieved through ART in our study is significantly higher than most domestic and international reports. For instance, the US Centers for Disease Control and Prevention (CDC) records that ART-assisted twin pregnancies account for only 12% of all twin births [9].

This markedly higher ART rate is attributed to the recruitment of the study sample in Hai Phong city. A primary factor is the relatively high mean maternal age, as advanced age is closely linked to a higher demand for and frequency of ART due to age-related fertility decline. Furthermore, the urban nature of the setting characterized by better access to healthcare services, higher income levels, and increased awareness along with the hospital being a primary destination for patients following ART cycles at private clinics, contributed to the elevated rate at the study site. These factors largely explain the differences observed compared to general population cohorts.

The incidence of gestational pathologies was 20.4%, while the majority of pregnancies remained healthy (79.6%). This relatively low frequency of underlying medical conditions, combined with the 8.6% rate of pre-existing gynecological disorders, suggests that while clinical comorbidities played a limited role, they must still be considered as potential confounding factors in the analytical models (Table 1).

Correlation Between Interleukin 1β, 6, and TNF-α Assays and Second-Trimester Cervical Morphological Changes

Our study results indicated mean concentrations for IL-1β, IL-6, and TNF-α 208.98 ± 5.64; 26.59 ± 2.04 và 19.14 ± 1.05, respectively (Table 2). No statistically significant associations were found between cervical morphology at intervals of 16–18, 18–20, 20–22, and 22–24 weeks and the levels of IL-1β, IL-6, or TNF-α (Table 3, 5, 7). These findings are consistent with research by Manju Chandiramani, which demonstrated no correlation between blood-derived pro-inflammatory cytokines at 16 weeks gestation and cervical morphology [18]. Although pro-inflammatory cytokines are potentially linked to preterm birth risk, the relationship between these systemic blood markers and cervical morphological changes during this stage is often indistinct. Cervical remodeling at this phase is primarily influenced by the gravitational pressure of the fetus and adnexa on an inherently weak cervix, which alters the lower uterine segment and subsequently the uterocervical angle. In contrast, serum cytokine concentrations reflect systemic inflammatory status and do not necessarily synchronize with histological transformations at the cervix. However, some studies have reported significantly higher levels of vaginal pro-inflammatory cytokines in pregnant women with short cervices, such as the work by Ida Vogel (2007) [19]. Additionally, Silvano et al. (2024) evaluated plasma levels of IL-1β, IL-6, IL-10, IL-12, and TNF-α across each trimester in 82 twin pregnancies, finding that these cytokines increased from the first to the third trimester ($p < 0.001$) [7]. Specifically, second and third-trimester TNF-α and IL-6 levels showed an inverse relationship with gestational age at birth ($p < 0.05$), suggesting that higher concentrations are associated with earlier delivery.

The discrepancies in research outcomes may stem from our study's focus on the 16-week mark, a time when inflammation-related processes linked to preterm birth may not yet be evident in systemic circulation . Alternatively, the difference could arise from utilizing serum samples instead of vaginal fluid, as seen in other studies. Pro-inflammatory cytokine elevation is often more pronounced in late pregnancy or upon the appearance of threatened preterm labor signs (Ida Vogel 2007) [19]. Consequently, our lack of observed correlation may reflect the distinct biological progression of the inflammatory response versus cervical morphological changes. Our results further suggest that at 16 weeks, cervical changes are minimally affected by pro-inflammatory factors, which typically exert their influence at later stages of pregnancy.

Predictive Value of Pro-inflammatory Factors for Preterm Birth in Twin Pregnancies

In our sample of 93 twin pregnancies, no statistically significant differences in any cytokine levels were found when comparing the preterm birth groups (before 34 and 37 weeks) with the non-preterm group ($p > 0.05$). The correlation coefficients between pro-inflammatory factors and preterm birth outcomes were very low $r \approx 0.02–0.03$). The absence of a significant link between 16-week serum cytokines and preterm birth risk aligns with recent reports suggesting that single-point mid-gestation serum cytokine measurements often fail to predict preterm birth [20, 21].

Pathophysiologically, preterm birth is more closely associated with localized inflammatory processes (involving the endometrium, amniotic membranes, cervical/vaginal fluid, or placenta)20. These localized markers have demonstrated superior predictive value compared to serum cytokines across numerous analyses [22, 23]. Furthermore, the mechanism of preterm birth may involve acute inflammatory episodes or short-term fluctuations occurring near the onset of labor; thus, a single 16-week serum sample might miss the inflammatory peak. While some studies indicate that mid-gestation systemic markers like TNF-α may relate to early preterm birth, they often require combined sampling or consideration of metabolic/lipid factors to yield a clear signal [24].

Additional limitations include population characteristics (twin pregnancies, high ART/IVF/IUI rates) and high inter-individual variability in baseline cytokine levels, which may mask correlations found in singleton studies. Sampling techniques, test kits, and the timing of measurements are also critical constraints [25].

Our study indicates that at 16 weeks, pro-inflammatory factors have not yet significantly impacted preterm birth, as evidenced by the minimal cervical morphological changes at this stage. However, the relatively small sample size may have limited the statistical power of the study and increased the risk of type II error, potentially obscuring subtle associations. Future research should consider later sampling time points and comparative analysis of blood versus cervical fluid to better define the relationship between pro-inflammatory factors and preterm birth.

CONCLUSION

An analysis of 93 twin-pregnant women (14–24 weeks) in this study found no association between 16-week serum pro-inflammatory factors and cervical morphological changes or preterm birth . However, the study suggests these factors may influence cervical morphology and preterm birth at later stages of gestation.

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