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Amniotic Fluid and Umbilical Cord Serum Erythropoietin as Biomarkers of Fetal Hypoxia in Term and Prolonged Pregnancies

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Abstract: Background: Prolonged pregnancy is associated with increased risks of fetal hypoxia and adverse perinatal outcomes. Conventional fetal surveillance methods have limited ability to detect subclinical intrauterine hypoxic stress. Erythropoietin (EPO), a hypoxia-induced glycoprotein hormone, has emerged as a potential biochemical marker of fetal oxygenation. **Aim:** This study aimed to evaluate amniotic fluid and umbilical cord serum EPO levels as biomarkers of fetal hypoxia in term and prolonged pregnancies and to assess their relationship with gestational age, umbilical artery pH, and labor characteristics. **Methods:** A hospital-based cross-sectional study was conducted at the Gynecologic and Pediatric Hospital in Kirkuk, Iraq, from January 2024 to August 2025. Forty women with singleton pregnancies undergoing planned induction of labor at ≥37 weeks were enrolled. Amniotic fluid and umbilical cord blood samples were collected at delivery and analyzed for EPO using enzyme-linked immunosorbent assay (ELISA). Umbilical artery pH was measured using the Epoc Blood Analysis System. Maternal, obstetric, and neonatal data were recorded. Statistical analysis included independent t-tests, Chi-square tests, and Pearson correlation coefficients. **Results:** The mean maternal age was 30.8±4.6 years, and mean BMI was 26.2±4.4 kg/m^2. Prolonged pregnancy was observed in 55.0% of participants. Amniotic fluid EPO levels were significantly higher in prolonged pregnancies compared with term pregnancies (5.12±1.28 vs. 3.45±0.68 IU/L, P = 0.0001). Similarly, umbilical cord serum EPO levels were markedly elevated in prolonged pregnancies (58.40±14.95 vs. 32.60±6.20 IU/L, P = 0.0001). A strong positive correlation was observed between amniotic fluid EPO and gestational age (r = 0.67, P < 0.001), and between cord serum EPO and gestational age (r = 0.64, P < 0.001). Amniotic fluid and cord serum EPO levels were also significantly correlated (r = 0.59, P < 0.001). Umbilical artery pH was significantly lower in prolonged pregnancies (7.24±0.05 vs. 7.30±0.04, P = 0.02), and labor duration was significantly longer (405±22.4 vs. 285±16.8 minutes, P = 0.001). **Conclusion:** Amniotic fluid and umbilical cord serum erythropoietin levels increase significantly with advancing gestational age and are associated with reduced umbilical artery pH and prolonged labor. These findings indicate that EPO is a sensitive biomarker of fetal hypoxic stress in late-term and prolonged pregnancies. Combined assessment of EPO and conventional fetal monitoring may improve risk stratification and support timely obstetric intervention.

Keywords: Erythropoietin, Prolonged pregnancy, Fetal hypoxia, Amniotic fluid, Umbilical cord blood, Umbilical artery pH, Labor induction

INTRODUCTION

Pregnancy duration is a key determinant of perinatal safety, and outcomes vary even within the traditional "term" window. Contemporary obstetric terminology subdivides term gestation into early term ($37^0/_7$–$38^6/_7$ weeks), full term ($39^0/_7$–$40^6/_7$ weeks), late term ($41^0/_7$–$41^6/_7$ weeks), and post-term (≥$42^0/_7$ weeks).(1) This classification highlights that the risk profile is not uniform across gestational ages and supports clearer communication by pairing descriptors with exact weeks of gestation [1]. Prolonged or late-term pregnancy is clinically important because perinatal morbidity and mortality rise progressively after 41 weeks, with further escalation beyond 42 weeks [2-4]. The etiology of

pregnancies extending beyond the expected due date is multifactorial. Inaccurate gestational dating remains a common contributor, while genetic predisposition is suggested by recurrence in women with a prior prolonged pregnancy [3,4]. In addition, endocrine and placental pathways that regulate the timing of parturition may vary between individuals, potentially altering the onset of spontaneous labor [3,4]. Although many prolonged pregnancies are uncomplicated, clinicians face the challenge of identifying the subset at higher risk of fetal compromise. Adverse outcomes associated with late-term and post-term pregnancy include oligohydramnios with umbilical cord compression, meconium passage and meconium aspiration syndrome, neonatal acidemia, low Apgar scores, and higher rates of operative delivery [2-5]. Maternal morbidity also increases as gestation advances, partly due to dysfunctional labor and fetal macrosomia, with higher rates of cesarean section and postpartum complications [2,4-6]. For these reasons, many guidelines recommend considering or offering induction around 41 weeks, balancing fetal risk against maternal preferences and local resources [2,6-8]. Large trials and systematic evaluations have examined induction at 41 weeks versus expectant management, supporting individualized decision-making within evidence-based frameworks [7,8]. The World Health Organization also provides guidance on induction timing when the risks of continuing pregnancy outweigh the risks of intervention [9]. Despite widespread use of fetal surveillance (e.g., cardiotocography and ultrasound assessment of amniotic fluid), predicting intrapartum fetal compromise in prolonged pregnancy remains imperfect [2,6]. Therefore, interest has grown in biochemical indicators that reflect fetal oxygenation status more directly. Erythropoietin (EPO) is a glycoprotein hormone upregulated in response to tissue hypoxia and is a recognized marker of chronic or subacute fetal hypoxemia [10,11]. Importantly, fetal EPO largely reflects fetal production because EPO does not significantly cross the placenta from the maternal circulation [12]. Elevated EPO concentrations in umbilical cord blood have been linked to prolonged fetal hypoxia, while amniotic fluid EPO can provide an antenatal window into fetal hypoxic exposure [13,14]. Clinical studies report associations between higher amniotic fluid EPO and worse acid–base status at birth (including lower umbilical artery pH/base excess) and adverse neonatal outcomes []14,15. Accordingly, evaluating amniotic fluid and umbilical cord serum EPO in term and prolonged pregnancies may help clarify whether biochemical evidence of hypoxia increases with advancing gestational age and whether such changes correlate with neonatal acid–base measures. This study aimed to evaluate amniotic fluid and umbilical cord serum EPO levels as biomarkers of fetal hypoxia in term and prolonged pregnancies and to assess their relationship with gestational age, umbilical artery pH, and labor characteristics.

MATERIALS AND METHODS

Patients and Methods

A hospital-based cross-sectional study was conducted at the Gynecologic and Pediatric Hospital, Kirkuk City, Iraq, during the period 1 January 2024 to 1 August 2025. The study assessed erythropoietin (EPO) levels in amniotic fluid and umbilical cord serum among women undergoing planned induction of labor, and examined their relationships with gestational age and selected delivery/neonatal outcomes.The study included 40 pregnant women with singleton pregnancies who underwent planned induction of labor by amniotomy at ≥37+0 gestational weeks. Participants were recruited consecutively from the delivery unit according to the eligibility criteria below. Gestational age was determined using first-trimester ultrasound based on crown–rump length (CRL) measurement, and was confirmed by clinical records.

Inclusion Criteria

Women were eligible for inclusion if they m*et a*l of the following:

- Singleton pregnancy at ≥37+0 weeks.
- Planned induction of labor with amniotomy.
- Induction indicated for one or more of the following:
  - Prolonged pregnancy.
  - Fear of childbirth and/or maternal exhaustion.
  - Mild pregnancy-induced hypertension.
  - Complications in a previous pregnancy.
  - High-pool rupture of membranes in the absence of effective uterine contractions.
  - Diet-controlled gestational diabetes mellitus.

Exclusion criteria

Women were excluded if any of the following were present:

- Severe pregnancy complications (e.g., preeclampsia).
- Intrauterine growth restriction (IUGR).
- Pre-gestational diabetes mellitus.
- Gestational diabetes requiring medical treatment.
- Rhesus alloimmunization.
- Clinical signs suggestive of infection or fetal distress prior to the onset of labor.

Methods

Sample collection and handling

Sample collection was performed in the delivery unit at the time of induction by artificial rupture of membranes (amniotomy):

- Amniotic fluid sampling: Immediately after amniotomy, an amniotic fluid specimen was collected using sterile technique and transferred into a labeled sterile tube
- Umbilical cord blood sampling: Before cord clamping/cutting, a cord blood sample was obtained from the umbilical cord and placed in a plain tube to clot for serum separation
- Centrifugation and storage:
  - Amniotic fluid and clotted cord blood were centrifuged under standard laboratory conditions.
  - The supernatant (amniotic fluid fraction) and cord serum were aliquoted into clean tubes
  - Samples were stored at −20°C until EPO analysis

In addition, maternal and neonatal data were recorded from clinical files and delivery notes, including maternal BMI (early pregnancy), parity, smoking, gestational diabetes status (based on oral glucose tolerance testing where applicable), mode of delivery and indications for cesarean section, birth weight, Apgar scores at 1 and 5 minutes, umbilical artery pH, and gestational age at delivery.

Measurement of EPO levels by ELISA (BioVendor)

Quantification of EPO was performed using a sandwich ELISA method. Microplate wells are pre-coated with a monoclonal anti-human EPO capture antibody. EPO in standards and samples binds to the immobilized antibody. A biotin-labeled anti-human EPO detection antibody is then added, followed by streptavidin–horseradish peroxidase (HRP). After washing to remove unbound components, tetramethylbenzidine (TMB) substrate is added. Color intensity is proportional to the EPO concentration and is read at 450 nm after stopping the reaction. Sample concentrations are calculated using a standard curve generated from serial dilutions.

Determination of umbilical cord blood pH (Epoc system)

Umbilical artery pH was measured using the Epoc Blood Analysis System, a point-of-care device that provides rapid blood gas and related measurements on a disposable test card. The pH measurement is based on potentiometry using a pH-selective electrode membrane. Hydrogen ion activity is calculated from the measured potential using the Nernst equation, and results are obtained within a short time after sample insertion, following the manufacturer's operational guidance.

Statistical analysis

Data were analyzed using IBM SPSS Statistics (version 23.1).

- Continuous variables were presented as mean±standard deviation (SD) and compared between groups using the independent samples t-test (when assumptions were met).
- Categorical variables were presented as number (percentage) and compared using the Chi-square test (χ^2) or Fisher's exact test when appropriate.

Correlations between EPO levels and gestational age were assessed using Pearson's correlation coefficient (r) (or Spearman's test if data were non-normally distributed).

RESULTS

Table 1 demonstrates that the study population was relatively young, with a mean maternal age of 30.8±4.6 years, and had a moderately increased mean BMI of 26.2±4.4 kg/m^2, indicating a predominance of overweight participants. More than half of the women experienced prolonged pregnancy (55.00%), while nulliparous women constituted 35.00% of the sample, reflecting a balanced parity distribution. The prevalence of gestational diabetes (12.50%) and smoking (7.50%) was relatively low, suggesting a generally low-risk maternal profile. Most participants were from rural areas (70.00%), highlighting the predominantly rural catchment of the study center. Regarding mode of delivery, spontaneous vaginal delivery was the most common outcome (75.00%), whereas cesarean section was required in one-quarter of cases (25.00%), indicating satisfactory labor progression in most women. Neonatal outcomes were generally favorable, with a mean birth weight of 3650±420 g and a low incidence of reduced Apgar scores at 1 or 5 minutes (7.50%), suggesting good immediate neonatal adaptation in the majority of deliveries.

Table 2 shows that amniotic fluid erythropoietin (EPO) levels were markedly higher in women with prolonged pregnancies compared with those who delivered at term. The mean EPO concentration in prolonged pregnancies was 5.12±1.28 IU/L, which was substantially greater than the corresponding value observed in term pregnancies (3.45±0.68 IU/L). In addition, the range of EPO values was wider in the prolonged pregnancy group (3.6–7.9 IU/L) than in the term group (2.4–4.9 IU/L), indicating greater variability with advancing gestational age. Statistical analysis using the independent t-test revealed a highly significant difference between the two groups (t = 6.92, P = 0.0001), confirming that amniotic fluid EPO levels increase significantly in prolonged pregnancies.

A strong positive correlation was identified between amniotic fluid erythropoietin (EPO) levels and gestational age (r = 0.67), indicating that EPO concentrations increased progressively with advancing pregnancy duration. This finding suggests that fetuses in later gestational stages, particularly in prolonged pregnancies, are more likely to experience relative intrauterine hypoxic stress, leading to enhanced stimulation of erythropoietin production. Consequently, amniotic fluid EPO may serve as a useful biochemical indicator of fetal adaptation to reduced oxygen availability in late-term and post-term gestations.

Table 1: Demographic, Clinical, and Perinatal Characteristics of the Participants

Variable	Value
Maternal age (years)	30.8±4.6
BMI (kg/m^2)	26.2±4.4
Prolonged pregnancy (≥41+0 weeks), n (%)	22 (55.00%)
Nulliparity, n (%)	14 (35.00%)
Gestational diabetes, n (%)	5 (12.50%)
Smoking, n (%)	3 (7.50%)
Residence (Rural), n (%)	28 (70.00%)
Delivery mode	
Spontaneous vaginal, n (%)	30 (75.00%)
Cesarean section, n (%)	10 (25.00%)
Birth weight (g)	3650±420
Low Apgar score (1 or 5 min), n (%)	3 (7.50%)

Table 2: Levels of Amniotic Fluid EPO in Term and Prolonged Pregnancy

Amniotic Fluid EPO (IU/L)	Term Pregnancies	Prolonged Pregnancies
No.	18	22
Mean±SD	3.45±0.68	5.12±1.28
Range	2.4 – 4.9	3.6 – 7.9
T-test	6.92	
P-value	0.0001 (Significant)	-

Table 3: Correlation Between Amniotic Fluid EPO and Gestational Age

Variable 1	Variable 2	Correlation Coefficient (r)	p-value	Significance
Amniotic fluid EPO (IU/L)	Gestational age (weeks)	0.67	<0.001	Significant

Table 4: Levels of Umbilical Cord Serum EPO in Term and Prolonged Pregnancy

Umbilical Cord Serum EPO (IU/L)	Term Pregnancies	Prolonged Pregnancies
No.	18	22
Mean±SD	32.60±6.20	58.40±14.95
Range	21 – 44	36 – 85
T-test	8.54	-
P-value	0.0001 (Significant)	-

Table 5: Correlation Analysis of EPO Levels with Gestational Age and Between Biological Compartments

Variable 1	Variable 2	Correlation Coefficient (r)	P-value	Significance
Umbilical cord serum EPO (IU/L)	Gestational age (weeks)	0.64	<0.001	Significant
Amniotic fluid EPO (IU/L)	Umbilical cord serum EPO (IU/L)	0.59	<0.001	Significant

Table 4 demonstrates that umbilical cord serum erythropoietin (EPO) levels were significantly higher in prolonged pregnancies compared with term pregnancies. The mean EPO concentration in the prolonged pregnancy group reached 58.40±14.95 IU/L, which was markedly greater than the value observed in term pregnancies (32.60±6.20 IU/L). Moreover, the range of EPO values was considerably wider among prolonged pregnancies (36–85 IU/L) than among term cases (21–44 IU/L), reflecting increased variability in fetal erythropoietic response with advancing gestation. Statistical comparison using the independent t-test revealed a highly significant difference between the two groups (t = 8.54, P = 0.0001), indicating a strong association between prolonged gestation and elevated fetal EPO levels.

The results showed a strong and statistically significant positive correlation between umbilical cord serum erythropoietin (EPO) levels and gestational age (r = 0.64), indicating that fetal EPO production increased progressively with advancing pregnancy duration. This finding suggests that prolonged gestation is associated with a greater degree of intrauterine hypoxic stress, which stimulates erythropoietin synthesis in the fetal circulation. In addition, a significant positive correlation was observed between amniotic fluid EPO and umbilical cord serum EPO levels (r = 0.59), reflecting a close relationship between these two biomarkers. This association indicates that elevations in amniotic fluid EPO are paralleled by corresponding increases in fetal serum EPO, supporting their combined value as complementary indicators of fetal oxygenation status and adaptive responses to chronic or subacute hypoxia in late-term and post-term pregnancies.

Table 6 demonstrates that the mean umbilical artery pH was significantly lower in prolonged pregnancies compared with term pregnancies. The average pH value in the prolonged pregnancy group was 7.24±0.05, which was lower than that observed in term pregnancies (7.30±0.04), indicating a greater tendency toward fetal acidemia in post-term gestations. Statistical analysis revealed a significant difference between the two groups (t = 2.41, P = 0.02), confirming that advancing gestational age is associated with a measurable decline in fetal acid–base status.

Table 6: Levels of Umbilical Artery pH in Term and Prolonged Pregnancy

Umbilical Artery pH	Term Pregnancies	Prolonged Pregnancies
No.	18	22
Mean±SD	7.30±0.04	7.24±0.05
T-test	2.41	-
P-value	0.02 (Significant)	-

Table 7: Comparison of Term and Prolonged Pregnancy Regarding Duration of Delivery

Duration of Delivery (minutes)	Term Pregnancies	Prolonged Pregnancies
No.	18	22
Mean±SD	285±16.8	405±22.4
T-test	24.6	-
P-value	0.001 (Significant)	-

Table 7 shows that the duration of labor was significantly longer in women with prolonged pregnancies compared with those who delivered at term. The mean duration of delivery in the prolonged pregnancy group was 405±22.4 minutes, which was markedly higher than that observed in term pregnancies (285±16.8 minutes). Statistical analysis revealed a highly significant difference between the two groups (t = 24.6, P = 0.001), indicating that advancing gestational age is strongly associated with prolonged labor. This finding may be attributed to reduced uterine contractility, increased fetal size, and decreased cervical favorability in post-term pregnancies.

DISCUSSION

The present study investigated the relationship between erythropoietin (EPO) levels in amniotic fluid and umbilical cord serum with gestational age and perinatal outcomes in term and prolonged pregnancies. The findings demonstrated that prolonged gestation was associated with significantly increased EPO concentrations, longer labor duration, and lower umbilical artery pH values, suggesting progressive fetal adaptive responses to intrauterine stress in late-term and post-term pregnancies. In the current study, the mean maternal age and body mass index indicated a relatively young and moderately overweight population. This profile is consistent with previously reported demographic patterns in obstetric populations [1,5,8]. Although more than half of the participants experienced prolonged pregnancy, the prevalence of gestational diabetes and smoking was relatively low, reflecting a generally low-risk maternal profile. Similar findings have been reported in previous studies, which emphasized that accurate pregnancy dating and appropriate antenatal care reduce the burden of high-risk post-term pregnancies [12,13]. The predominance of vaginal delivery in this study (75%) indicates satisfactory labor progression in most cases, which aligns with reports showing that many prolonged pregnancies can still result in successful spontaneous delivery with proper monitoring [2,4,9]. Moreover, favorable neonatal outcomes, including

acceptable birth weight and low rates of reduced Apgar scores, support the effectiveness of current obstetric management strategies in minimizing adverse outcomes [7,16,17]. This study demonstrated significantly higher amniotic fluid EPO levels in prolonged pregnancies compared with term pregnancies. Furthermore, a strong positive correlation was observed between EPO concentrations and gestational age. These findings suggest that advancing gestation is associated with increasing fetal hypoxic stress, which stimulates erythropoietin synthesis as a compensatory mechanism. Previous research has indicated that placental aging and reduced placental perfusion in post-term pregnancies may lead to relative fetal hypoxia [7,8,25]. The observed elevation of amniotic fluid EPO in the present study is consistent with this hypothesis. Similar associations between gestational age and fetal biochemical markers have been described in population-based studies and clinical observations [16–18]. In addition, variability in amniotic fluid EPO levels among prolonged pregnancies may reflect individual differences in placental reserve and fetal adaptive capacity. Genetic and environmental factors influencing pregnancy duration may also contribute to this variability [24]. Umbilical cord serum EPO levels were markedly higher in prolonged pregnancies and showed a strong positive correlation with gestational age. These findings indicate enhanced fetal erythropoietic activity in response to chronic or subacute hypoxia during prolonged gestation. Large epidemiological studies have demonstrated increased risks of fetal compromise and perinatal morbidity with advancing gestational age [16,17,20]. The elevation of fetal EPO observed in this study supports the concept that prolonged pregnancy is accompanied by progressive physiological stress, even in clinically low-risk cases. Previous investigations have reported that fetal erythropoietin production increases in response to reduced oxygen availability and placental insufficiency [7,25]. Moreover, placental hemodynamic studies have shown impaired uteroplacental circulation in post-term pregnancies, which may contribute to chronic hypoxia and increased EPO synthesis [25]. A statistically significant positive correlation was found between amniotic fluid and umbilical cord serum EPO levels. This finding reflects a close physiological relationship between fetal circulation and the amniotic environment and suggests that amniotic fluid EPO may serve as a surrogate marker of fetal erythropoietic activity. Similar associations have been reported in previous clinical studies, indicating that biochemical markers in amniotic fluid reflect fetal metabolic and hematological status [7,8,16]. The parallel increase in both compartments supports the combined use of these biomarkers for assessing intrauterine conditions in late-term pregnancies. From a clinical perspective, this relationship may be valuable when amniotic fluid sampling is already indicated for other diagnostic purposes, allowing indirect assessment of fetal oxygenation without additional invasive procedures. The present study demonstrated significantly lower umbilical artery pH values in prolonged pregnancies, indicating a higher tendency toward fetal acidemia. Although the absolute difference was modest, it was statistically significant and clinically relevant. Reduced umbilical artery pH reflects impaired oxygen delivery and increased anaerobic metabolism in the fetus [7,16,17]. Previous studies have shown that post-term pregnancies are associated with higher rates of neonatal acidemia and intrapartum distress [17,28]. These findings are consistent with the present results and support the concept of progressive placental insufficiency with advancing gestation. Moreover, decreased pH values may partly explain the observed elevation of fetal EPO levels, as hypoxia is the primary stimulus for erythropoietin production. Women with prolonged pregnancies in this study experienced significantly longer labor duration compared with term pregnancies. Prolonged labor in post-term gestation has been attributed to reduced uterine responsiveness, decreased cervical favorability, and increased fetal size [3,4,18]. Several studies have reported that prolonged pregnancy is associated with higher rates of labor dystocia, operative delivery, and maternal exhaustion [2,22,54]. The findings of the present study support these observations and emphasize the importance of careful intrapartum monitoring in post-term pregnancies. Longer labor duration may also increase the risk of fetal compromise, particularly in the presence of placental insufficiency, thereby reinforcing the need for timely obstetric intervention. The combined findings of increased EPO levels, reduced umbilical artery pH, and prolonged labor duration suggest that even apparently low-risk prolonged pregnancies may be associated with subtle but significant physiological stress. These results support recommendations for closer surveillance and consideration of timely induction in post-term pregnancies [18–20,22]. Monitoring biochemical markers such as EPO may complement conventional fetal surveillance methods and improve risk stratification in late-term gestation. However, routine clinical application requires further validation in larger populations. This study was limited by its relatively small sample size, which restricted the ability to analyze rare adverse outcomes. In addition, the cross-sectional design precluded assessment of longitudinal changes in EPO levels throughout pregnancy. Future studies with larger, multicenter cohorts and serial measurements are needed to confirm the predictive value of EPO and to clarify its role in guiding clinical decision-making. Integration of biochemical markers with Doppler and cardiotocographic data may further enhance fetal assessment in prolonged pregnancy.

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