

Increased Fetal Plasma and Amniotic Fluid Erythropoietin Concentrations: Markers of Intrauterine Hypoxia

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Key Words

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Abstract

Tissue hypoxia is the major stimulus of erythropoietin (EPO) synthesis in fetuses and adults. Since EPO does not cross the placenta and is not stored, fetal plasma and amniotic fluid levels indicate EPO synthesis and elimination. Acutely, the rate and magnitude of the increase in plasma EPO levels correlate with the intensity of hypoxia. Amniotic fluid EPO levels correlate with cord plasma levels in normal and abnormal pregnancies, with fetal plasma EPO levels in humans averaging 2.6 times higher than the corresponding amniotic fluid EPO levels. Recent experimental and clinical studies demonstrate that EPO has neuroprotective effects related to its anti-apoptotic and vascular growth-promoting properties. Although under basal conditions the fetal kidneys are the main site of EPO production, during hypoxia recent experimental data indicate an important role of the placenta. Amniotic fluid EPO levels have been shown to increase exponentially during fetal hypoxia in preeclamptic, diabetic and Rh-immunized pregnancies, to correlate inversely with cord blood pH, pO₂ and base excess and to predict neonatal morbidities and NICU admission. As an indicator of chronic intra-

uterine hypoxia, fetal EPO measurements have increased our knowledge about the pathogenesis and importance of intrauterine growth restriction, macrosomia, diabetic pregnancy, prolonged pregnancy, meconium staining, fetal hemorrhage, fetal anemia, maternal smoking and alcohol consumption, abnormal fetal heart rate and abnormal Doppler flow patterns. While the clinical utility of fetal amniotic fluid and plasma EPO measurements in the management of high-risk pregnancies and their offspring is promising, adequately powered clinical trials are urgently needed.

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Introduction

Hypoxia is the major stimulus of erythropoietin (EPO) synthesis in both the fetus and adult. In this review the importance of fetal EPO production in normal and abnormal pregnancies will be discussed based on experimental and clinical studies. Since recent studies have shown that EPO has neuroprotective properties in addition to its role in the regulation of erythropoiesis, the possible importance of the non-erythropoietic effects of EPO in the fetus will be reviewed.

Also included in this review is a discussion of the potential clinical use of fetal plasma and amniotic fluid EPO

measurements in evaluating the presence or absence of intrauterine hypoxia and subsequent neonatal outcome. This information is important because of a continuing urgent need to identify new methods for assessing ante- and peripartum fetal well-being. Limited data suggest that plasma and amniotic fluid EPO measurements may have advantages over other perinatal predictors of clinical outcomes.

Conditions Affecting Fetal Plasma EPO Concentrations

During early development of the embryo EPO is first synthesized in the yolk sac and later in the liver and finally in the peritubular interstitial cells of the kidneys [1, 2]. At term the main fetal production site of EPO is in the kidneys [3], but during hypoxia EPO is produced also in other sites of the fetus. Human fetuses with renal agenesis have normal or elevated cord plasma EPO levels and normal hematocrit levels at birth [4], indicating that the kidneys are not necessary for fetal EPO production.

The interpretation of EPO levels in the fetus is dependent on an understanding of processes that contribute to the kinetics of EPO in the fetus and its intravascular and amniotic fluid compartments. With the exception of murine species where less than 10% of maternal EPO traverses the placenta, EPO has not been shown to cross the placenta in either direction [5]. This observation along with the finding that EPO is not stored in tissues for later release indicate that fetal plasma EPO levels are indicative of acute fetal EPO production and elimination.

The half-life of EPO in the blood of human adults is 4–8 h [6] compared to 2–4 h in newborn infants [7]. Although the half-life of EPO in the human fetus is unknown, in the sheep a progressive shortening of EPO half-life is observed from adults to newborn lambs to late gestation fetuses [8].

In human adults plasma EPO levels start to increase approximately 90 min after the induction of acute hypoxia [9]. These human studies also demonstrated that the rate and magnitude of the increase in plasma EPO levels correlate with the severity of hypoxia [9]. Because experimental induction of fetal hypoxia in humans is precluded for obvious ethical reasons, experimental and *in vitro* studies have contributed considerably to our understanding of EPO production and elimination within the fetal compartment. Acute experiments in near-term rats and sheep have demonstrated that EPO levels in fetal plasma start to increase within 2–3 h after the beginning of mod-

erate to severe fetal hypoxia [1, 10, 11]. In contrast, the induction of chronic mild hypoxia in fetal sheep over a period of several days results in an initial increase in plasma EPO levels, after which levels decrease to a steady-state level slightly above that prior to the hypoxic period [12].

In uncomplicated term human pregnancies with vigorous newborn infants, EPO levels in umbilical cord blood at birth are higher after uncomplicated vaginal deliveries than after elective cesarean sections without labor contractions [13]. This suggests that even normal labor and vaginal delivery results in sufficient fetal tissue hypoxia to stimulate fetal EPO synthesis, but it can also be a physiologic process, which could improve the adaptation of the fetus to extrauterine life. Regular exercise among healthy women during the last weeks of pregnancy does not increase fetal EPO production as indicated by normal fetal amniotic fluid EPO levels [14]. This suggests that even strenuous exercise during normal pregnancy does not decrease oxygen delivery to the fetus.

Median or mean cord plasma EPO levels from uncomplicated term pregnancies before the onset of labor range between 20 and 35 mU/ml with the upper limit of normal ranging between 50 and 60 mU/ml [15–18]. Most studies have reported that during the latter half of pregnancy during normal stable conditions fetal plasma EPO levels do not change [13, 16, 18, 19]. The large study by Forestier et al. [18] is particularly important in this regard. These investigators measured plasma EPO levels from cord blood obtained by percutaneous sampling between 18 and 37 weeks of gestation in 163 healthy fetuses. Plasma EPO levels did not correlate with gestational age. In some studies fetal plasma EPO levels have been observed to increase 2- to 4-fold between midgestation and term [17, 20–22]. This apparent rise in plasma EPO as pregnancy progresses could be explained by inclusion of cord EPO levels obtained after the onset of labor or from high-risk pregnancies with hypoxic fetuses.

Finne [23] was the first to show increased EPO levels in cord blood and amniotic fluid in pregnancies complicated by preeclampsia or diabetes. He measured EPO concentrations with an *in vivo* mouse bioassay, which required several milliliters of sample for even a single measurement. Subsequently, a radioimmunoassay method was developed for EPO measurements, which required sample volumes of 0.2 ml but took a full day to assay [24]. More recently, results of EPO measurements can be obtained reliably within 4 h, e.g., by a chemiluminescent enzyme-labeled immunometric assay.

Conditions Affecting Amniotic Fluid EPO Concentration

In humans, EPO is found in the extra-embryonic coelomic fluid and in the amniotic fluid as early as 7 weeks of gestation [25]. Amniotic fluid EPO concentrations correlate well with simultaneously obtained cord plasma EPO levels before uterine labor contractions both in normal and abnormal human pregnancies [16, 26–28]. It is therefore possible to follow fetal plasma EPO levels antenatally by repeated measurements of EPO in the amniotic fluid. Amniotic fluid EPO levels obtained 1 or 2 days before labor by cesarean section also correlate well with fetal cord plasma EPO levels at birth in pregnancies complicated by hypertension or diabetes [28, 29].

In human pregnancy, fetal plasma EPO levels are approximately 2.6 times higher than the corresponding concentration in the amniotic fluid. This is true both during normal steady-state conditions when amniotic fluid levels are <20 mU/ml and during severe fetal hypoxia when amniotic fluid levels are >50 mU/ml [16], but not when the amniotic fluid EPO levels are intermediate to those values (fig. 1) [30]. This lack of correlation is likely due to the delay in the increase in EPO concentration in the amniotic fluid compared with the increase in fetal plasma. In contrast, in ovine pregnancy amniotic fluid and fetal plasma EPO levels correlate only weakly during stable non-hypoxic conditions [31]. During severe fetal hypoxia in the sheep, amniotic fluid EPO concentrations doubled after 12 h, although fetal plasma EPO levels increased by a factor of nearly 30 during the same time period [31]. Thus, it appears that the kinetic behavior of EPO into the amniotic fluid is different in sheep compared to humans. Alternatively, this could also indicate that the synthesis and function of EPO in the placenta, fetal membranes and amniotic fluid are different in humans and sheep. In normal human pregnancies without signs of fetal hypoxia, amniotic fluid EPO levels remain unchanged between midgestation and term [16, 26]. Amniotic fluid EPO levels obtained at delivery by elective cesarean section from healthy vigorous term fetuses range from 2 to 19 (median 6.3–7.5) mU/ml [16, 28].

It is unclear how EPO reaches the amniotic fluid and whether EPO has any functional effects in the amniotic fluid. EPO along with its receptor and EPO mRNA are all present in both ovine and human fetal membranes in the latter half of pregnancy [32–35]. Hence, the chorion or amnion, or both, are likely the source of amniotic fluid EPO. Since EPO is present in urine, it too is a contributor to amniotic fluid EPO. Based on available data, uri-

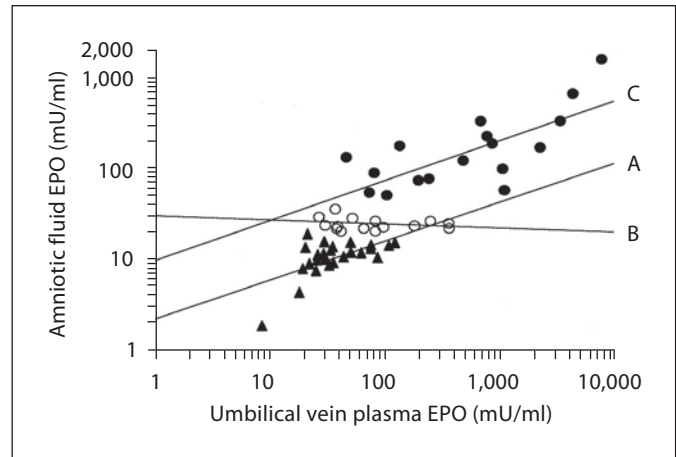


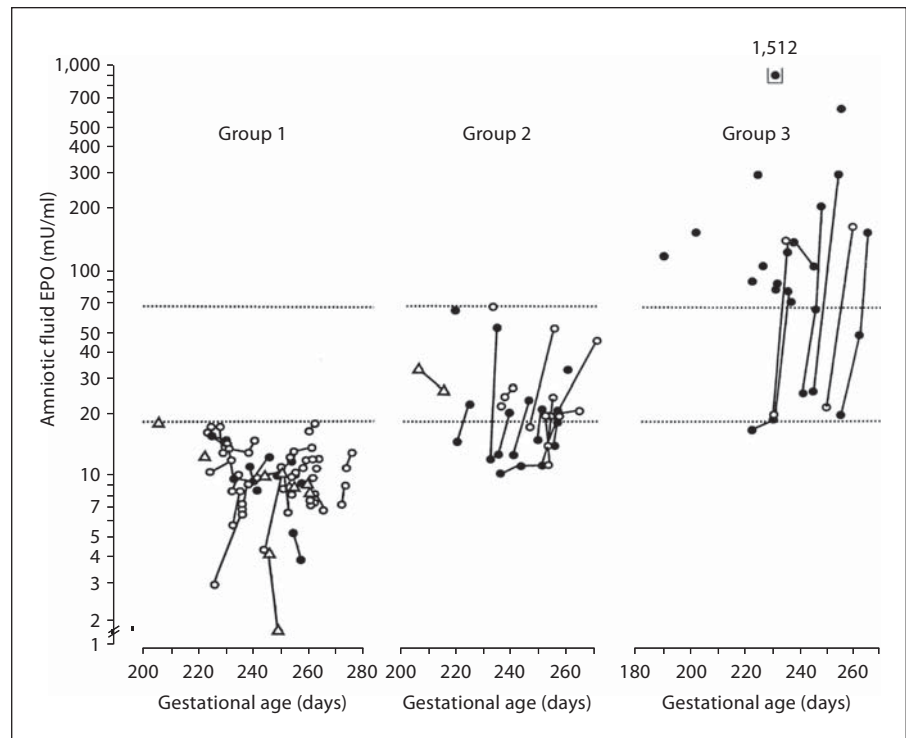
Fig. 1. Fifty-eight pregnancies complicated by preeclampsia or hypertension grouped into low (<20 mU/ml, regression line A, $r = 0.87$, $p < 0.0001$, $n = 28$) and high (>50 mU/ml, regression line C, $r = 0.86$, $p < 0.0001$, $n = 17$) amniotic fluid EPO levels correlate with cord plasma EPO levels obtained simultaneously at cesarean section. Slightly elevated amniotic fluid EPO levels (20–50 mU/ml, regression line B, $r = -0.10$, $n = 13$) do not correlate with cord plasma EPO levels. Reproduced with permission from Teramo [30].

nary EPO excretion does not appear to be of sufficient magnitude to explain the high amniotic fluid EPO levels measured during fetal hypoxia in either human [28, 29] (fig. 2) or sheep pregnancy [31]. Sequential amniotic fluid EPO measurements in preeclamptic and diabetic pregnancies have revealed that EPO levels can increase exponentially during fetal hypoxia (fig. 2) [28, 29]. Similar exponential increases in amniotic fluid levels have been reported in pregnancies complicated by Rh immunization in fetuses with severe hypoxia [26]. Among high-risk pregnancies, daily increases in amniotic fluid EPO levels can be as much as 22–25 mU/ml [28, 29].

During acute hypoxia in the rat, amniotic fluid EPO levels start to increase 6 h after fetal serum levels [11]. In this study fetal serum EPO levels returned to normal 12–48 h after ending of hypoxia, while amniotic fluid EPO levels remained elevated during the same time period [11]. During vaginal delivery in humans, amniotic fluid EPO levels start to increase 6 h after the fetal membranes have been ruptured [14]. These studies indicate that fetal EPO levels start to increase several hours later in the amniotic fluid compared to plasma.

Amniotic fluid EPO levels in humans remain elevated after the fetus dies from a chronic cause [36]. If the fetus dies from an acute cause, e.g., acute total placental abruption or severe cord entanglement, amniotic fluid EPO

Fig. 2. Seventy-five pregnancies complicated by hypertension grouped into low amniotic fluid (<20 mU/ml, n = 38, group 1), intermediate amniotic fluid (20.0–71.2 mU/ml, n = 19, group 2) and high amniotic fluid EPO levels (>71.2 mU/ml, n = 18, group 3). The lower horizontal dotted line represents the upper range of healthy control pregnancies (19.1 mU/ml) and the upper horizontal dotted line represents 8× the median of controls (71.2 mU/ml). Serial amniotic fluid samples from the same patient are connected by lines. Fetal and neonatal data of group 1 (normal EPO) and group 3 (high EPO) are given in table 1 under Hypertensive pregnancies. Reproduced with permission from Teramo et al. [29].



levels are not increased. This suggests that the fetal demise was so acute that amniotic fluid EPO did not have sufficient time to increase. Thus, by measuring the amniotic fluid EPO level after fetal death, it is possible to determine whether the fetus died from an acute or a chronic cause [36]. While a decline in amniotic fluid EPO levels following fetal demise may occur as a result of metabolic EPO degradation, *in vitro* experiments conducted at 37°C have demonstrated that endogenous EPO levels in amniotic fluid decrease slowly and linearly (i.e., only by one third from its initial concentration in 3 weeks) [37]. It can therefore be assumed that the amniotic fluid EPO levels were higher at the time of the fetal death than at the time of the subsequent amniotic fluid sampling.

Fetal Adaptation to Chronic Hypoxia

Oxygenation of fetal tissues depends on normal maternal oxygenation and the ability of the placenta to deliver an adequate supply of oxygen from the maternal to the fetal circulation. Fetal circulation adapts to decreased oxygen delivery by redistributing cardiac output. Hypoxia also stimulates EPO synthesis in the fetus resulting in an increase in fetal erythrocyte production [38] and in an

increase in the oxygen-carrying capacity. For additional details describing the role of EPO in regulating fetal erythropoiesis, see the reviews by Vora and Gruslin [39] and Widness and Teramo [40].

An increase in the number of nucleated red blood cells (NRBCs) is an indicator of increased red cell production. The NRBC level in cord blood at birth is used clinically to evaluate hypoxic events *in utero* [41, 42]. Because the increase in the hematocrit from acute or chronic hypoxic events is a relatively slow process, a high NRBC level in cord blood at birth is considered indicative of hypoxic events that have occurred before the onset of labor [43]. EPO is the trigger for the increased erythropoiesis and, as expected, the NRBC level in cord blood has been shown to correlate directly with cord plasma EPO levels at birth [44].

Regulation of Fetal EPO Synthesis during Hypoxia

Only a few years ago it became clear that tissue oxygenation is regulated locally by the transcription factor hypoxia-inducible factor (HIF-1) [45]. It is well established that HIF-1 α is involved in several key processes necessary for maintaining adequate tissue oxygenation

[45, 46]. Unlike during normoxia when HIF-1 α is inactivated by the ubiquitin system, during hypoxia HIF-1 α becomes activated, stimulating both EPO and vascular endothelial growth factor production [47].

By measuring renal venous and arterial plasma EPO differences and renal blood flow in fetal sheep near term, Davis et al. [48] showed that the kidneys are the main site of EPO synthesis during normoxia, during which no EPO production could be observed in the placenta. During hypoxia, however, these investigators observed a marked switch of the primary site of fetal EPO synthesis from the kidneys to the placenta. During moderate to severe fetal hypoxia the placenta was estimated to produce EPO at 1.1×10^6 mU/h, a production rate 15 times greater than that of the fetal kidneys [48].

Fetal Erythropoietin Levels in Pregnancies at High Risk of Fetal Hypoxia

Correlation with Umbilical Artery pH, Base Excess, Lactate, and Blood Gases

Fetal plasma and amniotic fluid EPO levels increase regardless of the etiology of fetal hypoxia. Several studies have shown that both umbilical plasma and amniotic fluid EPO levels at birth correlate inversely with umbilical artery pH, base excess and pO₂ and directly with pCO₂ and lactate [16, 28, 49–52]. In the fetal sheep when the oxygen content falls below 60% of basal levels in arterial blood, plasma EPO levels increase exponentially [53]. Similarly, in human pregnancies complicated by type-1 diabetes, amniotic fluid EPO levels increase exponentially when the umbilical artery pO₂ levels decrease below 2.0 kPa (fig. 3) [28].

Correlation with Fetal Iron Status

Intrauterine chronic hypoxemia, commonly observed among growth-restricted fetuses and among fetuses of insulin-treated diabetic mothers, stimulates fetal EPO production which in turn increases red blood cell production. Because the accelerated hemoglobin synthesis requires increased amounts of iron, fetal iron is preferentially diverted away from fetal tissue stores to red blood cell production [54, 55]. The zinc protoporphyrin/heme ratio, an indicator of fetal iron status, has been correlated directly with fetal plasma EPO levels at birth [56]. This suggests that fetal chronic hypoxemia associated with pregnancies of women with diabetes and those carrying growth-retarded fetuses may be the cause of fetal tissue iron deficiency, and possibly of subsequent abnormal

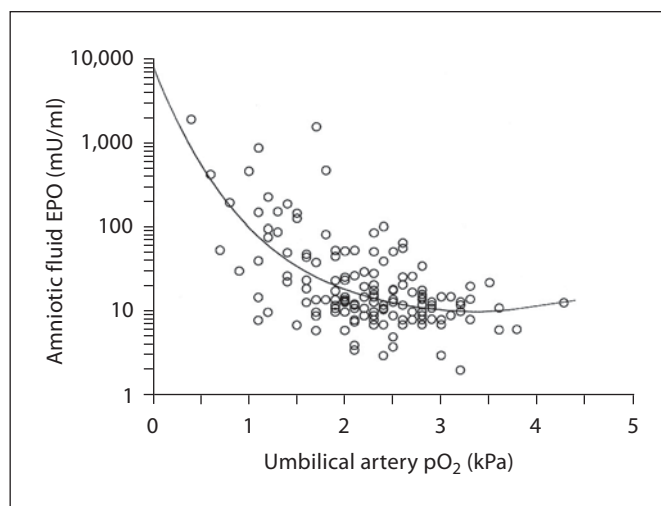


Fig. 3. Logarithmically transformed last amniotic fluid EPO concentration obtained 1 day (median) before delivery by cesarean section correlates negatively with umbilical artery pO₂ in type-1 diabetic pregnancies. Reproduced with permission from Teramo et al. [28].

neurodevelopmental outcomes reported in their offspring [57].

Prolonged Pregnancy and Meconium Staining of the Amniotic Fluid

Cord plasma EPO levels at birth increase progressively after 40 weeks of gestation in pregnancies with spontaneous onset of labor [58]. This is in agreement with the concept that in some pregnancies beyond 40 weeks gestation placental function starts to deteriorate, thereby favoring close fetal surveillance in prolonged post-term pregnancies.

Meconium staining of amniotic fluid has been a controversial marker of intrauterine hypoxia. In support of the association of meconium and fetal hypoxemia are studies showing that fetal plasma EPO levels are increased at birth independent of gestational age in pregnancies with meconium-stained amniotic fluid [59–61].

Intrauterine Growth Restriction

Cord plasma and amniotic fluid EPO levels are frequently elevated in growth-restricted fetuses [29, 62–64]. In pregnancies complicated by preeclampsia, intrauterine growth restriction is often a result of placental insufficiency and presumed fetal hypoxia. Thus, it is not surprising that amniotic fluid EPO levels correlate inversely with the birth weight adjusted for gestational age in preg-

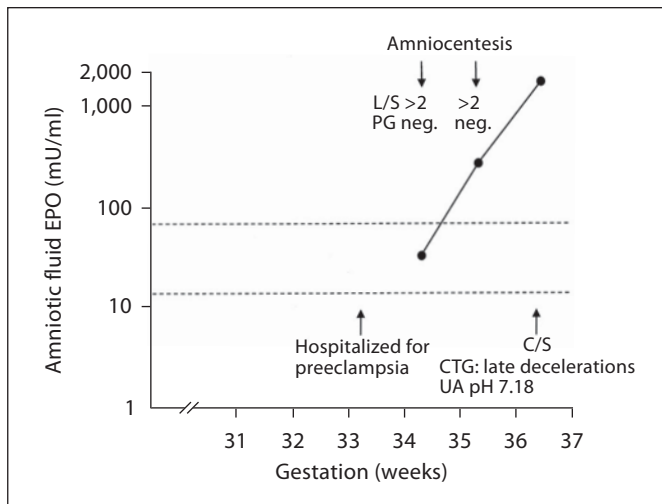


Fig. 4. Exponentially increasing amniotic fluid EPO level in a White's class C diabetic with poor glycemic control during the last trimester (HbA1c ranged from 8.4 to 8.8%). The patient was hospitalized for preeclampsia during the 34th gestational week. She had two amniocenteses done for fetal lung maturity evaluation at 34²/₇ and 35²/₇ gestational weeks. Amniotic fluid EPO levels were not known to the clinicians. An emergency cesarean section was performed at 36³/₇ weeks of gestation because of late decelerations in the fetal heart rate recording. Umbilical artery pH was 7.18 at birth. L/S = Lecithin/sphingomyelin ratio; PG = phosphatidylglycerol; C/S = cesarean section; CTG = cardiotocography.

nancies with growth-restricted fetuses [29, 65]. In this regard it is noteworthy that, in twins with discordant growth, the smaller, and presumably more hypoxic, twin has higher EPO levels [66, 67]. Monochorionic twin fetuses more often have elevated serum EPO levels compared to dizygotic twins [67].

As is well recognized from high-altitude pregnancies, chronic hypoxia results in decreased fetal growth. In a recent study, maternal circulating EPO concentrations and EPO receptor density in the syncytial microvilli were both increased, but the density of the glucose transporter GLUT-1 in the syncytial microvilli was decreased in high-altitude pregnancies [68]. Thus, chronic hypoxia seems to reduce fetal growth by reducing both oxygen and nutrient delivery to the fetus.

Abnormal Fetal Heart Rate Recordings

Abnormal fetal heart rate recordings during labor have been correlated with cord plasma EPO levels at birth in both appropriately grown and growth-restricted fetuses [64, 69, 70]. The highest fetal EPO levels occur in cases with the most abnormal fetal heart rate patterns during

the last 4 h of labor [69]. Cord plasma and amniotic fluid EPO levels at delivery are higher in those delivered by emergency cesarean section for abnormal fetal heart rate recordings before the onset of labor than in those with normal fetal heart rate recordings and elective cesarean section delivery [70]. Serial amniotic fluid EPO measurements have demonstrated that in individual high-risk pregnancies EPO levels start to increase several days before abnormal fetal heart rate changes are observed (fig. 4) [28, 29].

Nuchal Cord

Cord entanglement is observed in up to 35% of newborn infants at birth and it is a potential risk factor for the subsequent development of cerebral palsy and delayed neurodevelopment [71]. In a recent study, Hashimoto and Clapp [72] observed that amniotic fluid EPO levels obtained either at amniotomy for induction of labor or during labor via a uterine catheter were higher in pregnancies in which the umbilical cord was around the neck of the fetus at birth compared with pregnancies without a nuchal cord. Cord plasma EPO levels did not differ between the groups at birth. The authors suggested that nuchal cord is a mild, but independent risk factor for chronic fetal hypoxia before labor. The elevated amniotic fluid EPO levels in pregnancies with a nuchal cord could indicate that these fetuses had experienced one or more chronic antepartum hypoxic episodes, since it is known that amniotic fluid EPO levels remain elevated [11] or decrease only slowly [37] while fetal plasma EPO levels decrease within hours [11] after recovery from transient hypoxic episodes.

Abnormal Fetal Doppler Flow

Umbilical artery and fetal ductus venosus Doppler flow measurements are widely used clinically to evaluate hemodynamic function especially in growth-restricted fetuses. In a recent study on growth-restricted fetuses with birth weight z-scores below -2.0 SD, cord serum EPO levels at birth were increased in direct association with the severity of the fetal hemodynamic compromise [73]. Cord serum EPO levels were significantly higher in fetuses with an abnormal ductus venosus blood velocity waveform pattern than in fetuses with either a normal or an abnormal umbilical artery blood velocity waveform pattern but a normal ductus venosus flow pattern.

Diabetic Pregnancies

Intrauterine hypoxic complications are clearly increased and 'unexplained' fetal deaths still occur during

the last weeks of diabetic pregnancies [28, 74, 75]. The finding that iron stores in the liver and the brain are completely depleted in most fetuses who die in utero indicates that the majority of stillbirths of diabetic mothers is preceded by a period of chronic fetal hypoxia in which iron is diverted away from tissues in favor of red blood cell production [54, 55]. Although the exact mechanisms of fetal hypoxia in diabetic pregnancies are not fully understood, several factors which occur in combination or alone can result in decreased delivery of oxygen to the fetus of diabetic mothers [76]. Experimental and human studies have shown that both fetal hyperglycemia and hyperinsulinemia can independently result in fetal hypoxemia [53, 77–79]. In type-1 diabetic mothers HbA1c levels during the last month of pregnancy correlate positively with concurrent fetal plasma and amniotic fluid EPO levels [28, 79]. These observations emphasize the importance of maintaining adequate glycemic control throughout pregnancy.

Amniotic fluid EPO measurements in type-1 diabetic pregnancies have revealed a U-shaped correlation between the birth weight z-score and the amniotic fluid EPO levels [28]. Above +1.0 SD units the correlation is positive, but below -0.6 SD units it is clearly negative. Thus, it appears that the optimal birth weight of fetuses of diabetic mothers is quite narrow (i.e., from -0.6 to +1.0 SD units). This new observation suggests that fetuses of type-1 diabetic mothers with a birth weight z-score below -0.6 SD units should be considered as 'growth-restricted' and possibly suffering from chronic hypoxia. If this observation can be confirmed by other studies, it has important clinical consequences in the management of diabetic pregnancies.

Acute Fetal Hemorrhage

In the study by Kim et al. [80], plasma EPO concentrations increased 3–4 h after an acute massive hemorrhage in fetal sheep. This is similar to the increase in fetal plasma EPO levels observed after induction of severe fetal hypoxia by reducing oxygen delivery to fetal tissues [10]. Maximal fetal EPO concentrations that were 30–40 times above the basal levels were reached 16 h after the removal of 40% of the estimated fetal blood volume over 2 h. Subsequent fetal plasma EPO levels decreased rapidly despite persistent fetal anemia [80]. In this study the plasma EPO concentration correlated inversely with fetal hematocrit, but not with pO₂ 6 h after the acute hemorrhage despite a decrease in mean pO₂ from 20 to 14.5 mm Hg. In a stillbirth case due to massive fetomaternal hemorrhage, the amniotic fluid EPO level was not increased 3

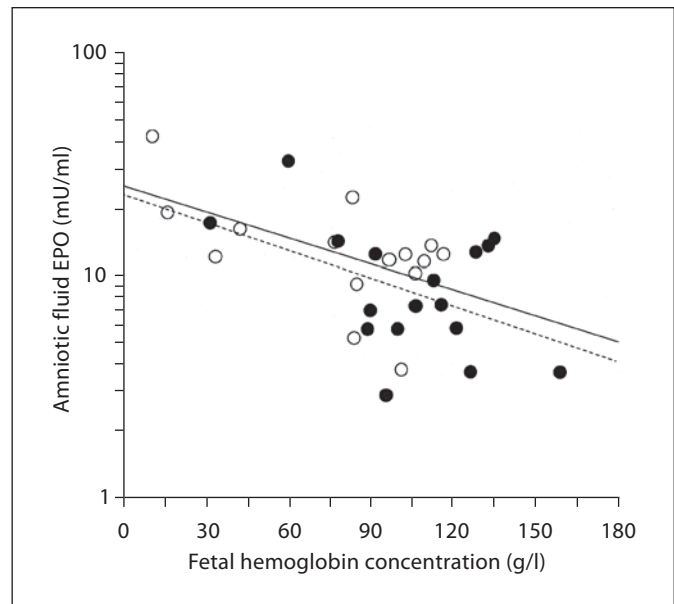


Fig. 5. Negative correlation between fetal hemoglobin concentration and logarithmically transformed amniotic fluid EPO level is the same in fetuses with a gestational age of <27 weeks (○; $r = -0.57$, $p = 0.027$, $n = 15$) and in fetuses with a gestational age above 27 weeks (●; $r = -0.49$, $p = 0.039$, $n = 17$). The regression slopes do not differ from each other. The fetal blood samples were obtained by cordocentesis before the first intrauterine red cell transfusion done for maternal Rh immunization. Amniotic fluid samples were obtained at the same time as the first cordocentesis.

days after the fetal death suggesting that the fetal demise was acute with insufficient time for the EPO level to increase [36].

Fetal Chronic Anemia

It has been suggested that fetuses near term have a greater capacity to synthesize EPO during hemolytic anemia than fetuses under 24 weeks of gestation [21]. Our observations differ from those of Moya et al. [21]. We found that the hemoglobin concentration in the fetus and amniotic fluid EPO levels correlated inversely in a similar fashion in fetuses under and above 27 weeks of gestation (fig. 5). The explanation for the difference between our results and those of Moya et al. [21] could be that the cord plasma EPO level was measured only once near term (at birth) in their study. Thus, since some of the older fetuses in the study by Moya et al. [21] may have been chronically hypoxic, this could have increased the fetal EPO synthesis beyond that of fetal anemia alone. Although most of the fetuses in our study were severely anemic (the

median hemoglobin concentration was 9.8 g/dl with a range from 1.1 to 15.9 g/dl), the median of amniotic fluid EPO levels was 11.7 (range 2.8–42.0) mU/ml. These relatively low levels may be the result of the gradual progressive onset of fetal anemia. Under these chronic conditions, adaptive cardiovascular changes may mitigate the effects of tissue hypoxia. It is also possible that the low EPO levels may be the result of accelerated EPO metabolism in the erythroid progenitor cell pool with high EPO receptor density [81]. Nonetheless, it has been shown that fetuses younger than 30 weeks gestation from pregnancies with severe Rh immunization can markedly increase their amniotic fluid EPO levels when fetal hypoxia acutely worsens [26].

Maternal Smoking and Alcohol Consumption

In addition to decreasing fetal growth, maternal tobacco smoking increases fetal plasma EPO levels [82–84]. This suggests that maternal smoking results in chronic fetal hypoxemia. Heavy alcohol use (more than 300 g ethanol/week) during pregnancy results frequently in fetal damage (fetal alcohol syndrome). This amount of maternal alcohol consumption also results in increased cord plasma EPO levels at birth compared to the offspring of mothers with moderate or abstinent alcohol use [85]. Because ethanol infusions into fetal Rhesus monkeys have been shown to result in fetal hypoxemia [86], it seems likely that heavy maternal alcohol use causes chronic fetal hypoxia.

Does the Fetus Increase EPO Production to Protect Its Brain during Hypoxia?

It was originally thought that the only reason for the increase in EPO synthesis during hypoxia was to increase erythropoiesis for augmenting the oxygen-carrying capacity in the circulation [3]. However, during the last decade, a large number of experimental [87–92] and some clinical studies [93, 94] have shown that exogenously administered EPO has also neuroprotective effects related to its anti-apoptotic and vascular growth-promoting properties. EPO treatment has also been shown to reduce vasoconstriction and infarction volume in the brain after hypoxic injury [47]. Since EPO and its receptor are both expressed in glial cells, neurons and endothelial cells of the brain [47], increased endogenous EPO production by the brain is another adaptive mechanism in response to the detrimental effects of hypoxia. EPO also seems to exert a protective role in attenuating the effects of hypoxic

insults in other tissues, including peripheral nerves [95], the heart [96, 97] and the retina [98, 99].

EPO crosses the blood-brain barrier within 2–4 h after exogenous administration of rHuEPO in the fetal sheep [100, 101]. This observation is in accordance with both experimental and clinical studies that show a beneficial effect of exogenous rHuEPO on brain tissue after hypoxic insults [92, 102, 103]. When high-dose rHuEPO was administered parenterally to adult humans after stroke, neurologic recovery was better compared to those receiving placebo [93]. In a recent double-masked, randomized controlled study of preterm infants with birth weights of 1,000 g or less, parenterally administered rHuEPO during the neonatal period resulted in a high serum EPO concentration (500 mU/ml) [94]. The EPO-treated group of preterm infants had a significantly higher mental developmental index at 18–22 months corrected age than those receiving placebo. Multicenter clinical trials have recently been initiated to answer the question whether rHuEPO treatment immediately after birth can improve neurodevelopmental outcomes of very preterm infants [104, 105]. The results of these studies will determine whether exogenous EPO can be used in the future as a first-line therapeutic agent in fetuses with severe hypoxia.

Based on these considerations, it is plausible that the exponential increase in amniotic fluid EPO concentration observed in hypoxic human fetuses [26, 28, 29] is the result of a marked increase in placental EPO production, the purpose of which may be to protect the fetal brain from hypoxic damage. More studies are urgently needed to delineate the role and magnitude of placental EPO production.

Clinical Use of Amniotic Fluid and Fetal Plasma EPO Measurements in High-Risk Obstetrics and Neonatology

Since fetal plasma and amniotic fluid EPO levels increase regardless of the etiology of fetal hypoxia and since high amniotic fluid EPO levels in humans are highly correlated with fetal plasma EPO levels, the presence of chronic or subchronic fetal hypoxia may be assessed by measuring the amniotic fluid EPO concentration. In our experience, the exponential increase observed in amniotic fluid EPO levels in abnormal pregnancies is an alarming signal of imminent serious fetal hypoxia and compromise [26, 29]. When comparing fetuses with normal (<20 mU/ml) to those with high (>60 mU/ml) amniotic fluid

Table 1. Umbilical artery pH and blood gas values, birth weight z-score and neonatal complications in cases with normal (<20 mU/ml) and high (>60 mU/ml) amniotic fluid EPO levels in pregnancies complicated by type-1 diabetes mellitus [28] or hypertension [29]

	Type-1 diabetic pregnancies		Hypertensive pregnancies	
	normal EPO (n = 76)	high EPO (n = 21)	normal EPO (n = 38)	high EPO (n = 18)
Amniotic fluid EPO, mU/ml	10.0 (2.0–13.4)	153.0 (66.0–1,975)	10.4 (1.8–19.1)	144.5 (74.1–1,512)
Gestational age, days	259 (212–275)	254 (212–271)	253 (205–275)	233 ^a (188–263)
Birth weight z-score (SD units)	+1.3 (–2.5 to +6.8)	+2.8 ^c (–1.4 to +6.5)	–1.6 (–4.1 to +2.1)	–3.3 ^b (–4.8 to –0.3)
Umbilical artery pH	7.26 (7.12–7.32)	7.19 ^d (7.06–7.26)	7.31 (7.19–7.39)	7.23 ^d (7.09–7.28)
Umbilical artery pO ₂ , kPa	2.4 (1.1–4.2)	1.3 ^d (0.4–3.5)	2.2 (1.3–4.1)	1.6 ^c (0.5–3.3)
Umbilical artery base excess, mEq/l	–1.4 (–8.3 to +4.4)	–2.5 ^d (–9.6 to 0.0)	–1.8 (–9.4 to +3.4)	–4.0 ^c (–8.9 to –1.9)
Lowest blood glucose, mmol/l	2.6 (1.0–4.2)	1.8 ^d (0.4–3.2)	2.3 (0.5–3.7)	1.0 ^d (0.2–2.5)
Cardiomyopathy, %	2.6	28.6 ^d	–	–
Admitted to NICU, %	10.5	28.6 ^a	7.9	50.0 ^c

Figure 2 shows amniotic fluid EPO concentrations of the hypertensive pregnancies. ‘Normal EPO’ is group 1 and ‘high EPO’ is group 3 in figure 2. All deliveries were by cesarean section before labor contractions. Results are expressed as median (range) or frequency (%).

^a p = 0.03; ^b p < 0.01; ^c p < 0.001; ^d p < 0.0001.

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EPO levels, umbilical artery pH, pO₂ and neonatal blood glucose are significantly decreased and umbilical artery base excess increased in those with high amniotic fluid EPO levels (table 1). An amniotic fluid EPO cutoff level of 36 mU/ml had 56% sensitivity and 92% specificity in predicting low umbilical artery pH (<7.26) at birth and 53% sensitivity and 82% specificity for predicting low pO₂ (<12 mm Hg) in pregnancies complicated by pre-eclampsia or pregnancy-induced hypertension [29]. In type-1 diabetic pregnancies an amniotic fluid EPO cutoff of 37 mU/ml had a 49% sensitivity and 86% specificity in predicting low umbilical artery pH (<7.21) at birth and a 53% sensitivity and 91% specificity for predicting low pO₂ (<15 mm Hg) [28].

We have previously shown that admission to the neonatal intensive care unit was 2.8 times higher in type-1 diabetic pregnancies [28] and 6.3 times higher in hypertensive pregnancies [29] when the amniotic fluid EPO levels obtained within 2 days of delivery were >60 mU/ml compared with admissions from pregnancies with normal EPO levels (table 1). Thus, a normal amniotic fluid EPO level may be equally important as a high EPO level in managing high-risk pregnancies as pregnancy may be continued with less concern and concomitant less need for frequent fetal surveillance. While currently available amniotic fluid EPO data may be useful in assessing pregnancies complicated by diabetes, alloimmunization, and fetal growth restriction, similar data from pregnancies at

risk of fetal hypoxia from other maternal and fetal causes are needed before considering their clinical application.

To our knowledge, only one follow-up study exists on the clinical significance of high fetal plasma EPO levels at birth on the subsequent outcome of the infant [106]. In this study infants with high cord plasma EPO levels were shown to have poorer outcomes (cerebral palsy or delayed mental development) at 2 years of age compared to infants with low cord plasma EPO levels [106]. In contrast, similar correlations were not found with elevated cord plasma vasopressin or hypoxanthine levels or with metabolic acidosis and low Apgar scores [107]. Clearly more follow-up studies on the long-term outcomes are needed to assess the clinical significance of high amniotic fluid and cord plasma EPO levels in the peripartum period in determining whether EPO is a better indicator of long-term outcomes than cord blood pH, base excess, lactate or low Apgar scores. Amniotic fluid EPO measurements have the advantage that they can be made prior to birth during the time when clinical decision making is ongoing.

Based on available studies, we speculate that amniotic fluid and cord plasma EPO be measured as an adjunct to other clinical and laboratory data whenever amniotic fluid is already being sampled for other purposes, e.g., fetal assessment of lung maturity, chorioamnionitis, etc. To be of clinical value, available EPO assay systems should provide results during the same day of sampling. The defin-

itive answer of the clinical utility of amniotic fluid and cord plasma EPO measurements in the management of high-risk pregnancies and their offspring must await the results of future sufficiently large controlled studies. Available data indicate that such studies are not only well justified, but are urgently needed.

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