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Short title**: Erythropoietin in term pregnancy**

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Abstract

Objective. Erythropoietin - a hormone regulating erythropoiesis - is a biomarker of chronic fetal hypoxia. High erythropoietin levels in fetal plasma and amniotic fluid are associated with increased risk of adverse neonatal outcome. Since the risk of perinatal morbidity and mortality is increased in pregnancies beyond 41 gestational weeks, we evaluated erythropoietin levels in amniotic fluid and umbilical cord serum in apparently low-risk term ($\geq$37 gestational weeks) and prolonged pregnancies ($\geq$41 gestational weeks) with labor induction.

Study Design. This prospective cohort study comprised 93 singleton pregnancies at 37^{+0}-42^{+1} gestational weeks, of which prolonged pregnancies numbered 63 (67.7%). Amniotic fluid samples were collected at time of labor induction by amniotomy. Umbilical cord blood samples for evaluation of pH, base excess, and umbilical cord serum erythropoietin were collected at birth. Erythropoietin levels were measured by immunochemiluminometric assay. Normal value of amniotic fluid erythropoietin level was defined as $\leq$3 IU/L, and abnormal value as $\geq$27 IU/L. Normal umbilical cord serum erythropoietin was defined as <40 IU/L. Data on maternal pregnancy and delivery characteristics and short-term neonatal outcomes such as Apgar score were obtained from the hospital charts. Associations were calculated

using Spearman's rank correlation coefficient. The Chi-square test, Fisher's exact test and the Mann-Whitney U test were utilized to determine differences in the study groups.

Results. Amniotic fluid erythropoietin levels correlated with gestational age ($r=0.261$, $p=0.012$) and were higher among prolonged pregnancies as compared to term pregnancies ($p=0.005$). There were 78 (83.9%) vaginal deliveries, and among these erythropoietin levels in amniotic fluid correlated with the levels in umbilical cord serum ($r=0.513$, $p<0.000$). Umbilical cord serum erythropoietin levels correlated with gestational age among vaginal deliveries ($r=0.250$, $p=0.027$). Erythropoietin levels in amniotic fluid and umbilical cord serum did not correlate with umbilical artery pH or base excess, or other adverse pregnancy outcome.

Conclusions. In vaginal deliveries erythropoietin levels in amniotic fluid correlated with the levels in umbilical cord serum. Erythropoietin levels correlated with gestational age, probably due to weakening placental function and relative hypoxemia occurring in advanced gestation. However, in this relatively low-risk study population erythropoietin was not related to adverse delivery outcome.

Keywords. Prolonged pregnancy, fetal erythropoietin, amniotic fluid, intrauterine hypoxia, placental insufficiency

Introduction

The risk of perinatal morbidity and mortality increase in pregnancies beyond 41 gestational weeks [1,2]. The underlying mechanism is assumed to be aging of the placenta and subsequent relative placental insufficiency, thus predisposing the fetus to hypoxia. However, it is challenging to identify specific pregnancies at risk of intrapartum fetal compromise and adverse outcomes. Numerous methods, such as fetal Doppler parameters and computerized cardiotocography, are used to monitor placental function and fetal wellbeing. Nevertheless, these methods have not been demonstrated to be efficacious in predicting the outcomes of prolonged pregnancies [3-5].

Erythropoietin (EPO), a hormone regulating erythropoiesis, increases in response to fetal hypoxia [6]. EPO levels in amniotic fluid correlate well with levels in fetal plasma prior to the onset of labor contractions [7]. Increased EPO levels in amniotic fluid and umbilical cord serum occur in various complicated pregnancies [7]. High EPO levels have been associated with an increased risk of acute adverse neonatal outcomes, such as decreased umbilical artery pH and base excess (BE), and need for intensive care admission [7,8]. Thus, amniotic fluid EPO is used as a biomarker of chronic fetal hypoxia. We hypothesized that an evaluation of amniotic fluid EPO level prior to labor could be used to identify the individuals at risk of intrapartum distress, based on the assumption that the mechanism associated with adverse outcomes in prolonged pregnancies relates to hypoxia.

The objective of the current study was to evaluate a correlation between EPO levels in amniotic fluid and those in the umbilical cord serum in apparently low-risk term and prolonged pregnancies, and to investigate whether EPO level in amniotic fluid was predictive of the capacity of the fetus to tolerate the stress caused by delivery.

Materials and methods

The study was conducted at the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland. Women undergoing singleton pregnancies with planned induction of labor by amniotomy at $\geq 37^{+0}$ gestational weeks between September 2012 and December 2014 were recruited. Indications for induction were a prolonged pregnancy ($\geq 41^{+0}$ gestational weeks), fear of childbirth, maternal exhaustion, mild pregnancy-induced hypertension, complications in a previous pregnancy, high-pool rupture of membranes in the absence of delivery contractions, diet-controlled gestational diabetes, suspicion of large-for-gestational age fetus in a non-diabetic mother, unstable presentation, polyhydramnios, or intrahepatic cholestasis of pregnancy. Women with severe pregnancy complications – such as pre-eclampsia, intrauterine growth restriction, pre-gestational diabetes, medically treated gestational diabetes, Rhesus alloimmunization, and signs of infection or fetal distress before labor – were excluded owing to the increased risk of fetal hypoxia and associated elevated EPO levels [7].

Amniotic fluid samples were collected from 121 women at induction of labor by amniotomy (Figure 1). Two pregnancies were excluded owing to neonatal diagnoses of VACTERL syndrome and nonketotic hyperglycinemia. The deliveries with both amniotic fluid and umbilical cord serum EPO samples available (n=93) were included in the final analysis. The deliveries lacking umbilical cord serum EPO measurements (n=26) were also analysed to avoid selection bias. They did not differ from the study group in terms of amniotic fluid EPO levels (p=0.887), number of cesarean section deliveries (p=1.000), umbilical artery pH or BE (p=0.066 and p=0.703, respectively), or low Apgar score ($\leq$7 at one or five minutes) (p=1.000 for both). Women with vaginal deliveries were divided into two groups, term ($\geq 37^{+0}$- 40^{+6} gestational weeks) and prolonged pregnancies ($\geq 41^{+0}$ gestational weeks), for data analysis (Figure 1). Composite adverse outcomes were documented if at least one of the following

criteria applied: umbilical artery pH ≤7.15, umbilical artery BE ≤ -12meq/L, one or five-minute Apgar <7, or emergency cesarean section for fetal distress [9].

Gestational age was defined by the fetal crown-rump length measurement at the first trimester ultrasound screening. Data on maternal pregnancy and delivery characteristics (body mass index [BMI] in early pregnancy, in vitro fertilization, parity, gestational diabetes diagnosed by using oral glucose tolerance test, smoking, the main indication for cesarean section) and short-term neonatal outcomes (Apgar score at one and five minutes, umbilical artery pH and BE, birthweight, and gestational age) were obtained from the hospital charts.

The EPO levels were measured using a solid-phase immunochemiluminometric assay (Immulite® 2000 XPi, Siemens, Tarrytown, USA). The intra-assay coefficient of variation (CV) was 3.6-6.8% and the total CV was 6.4-10.3% for the concentration range 4-615 IU/L. The detection limit of the assay was 1.0 IU/L, with functional sensitivity (CV of 20%) of 1.5 IU/L. The classification of amniotic fluid EPO levels into three categories (normal-intermediate-abnormal) has been described previously [6,10]. A normal value of amniotic fluid EPO level is defined as < 3 IU/L, and a value of > 27 IU/L is defined as abnormal [6,10]. A normal umbilical cord serum EPO is defined as < 40 IU/L [7]. The amniotic fluid EPO values were analyzed after birth and they were not used in the clinical management of the deliveries. The umbilical cord serum EPO values were measured from blood samples collected from the umbilical cord at birth. Measurements of umbilical artery pH and BE were routinely performed with Radiometer ABL800 Flex blood gas analyzer (Radiometer, Copenhagen, Denmark).

To determine a correlation between vaginally obtained amniotic fluid samples and amniotic fluid samples obtained by amniocentesis, a comparison of EPO levels was made in five

patients undergoing amniocentesis for fetal lung maturation assessment followed by an induction of labor by amniotomy (r=0.9, p=0.037).

Statistics. Statistical analysis was performed using Statistical Package for Social Sciences® version 22.0 (SPSS Inc., Chicago, USA). Associations between the pH, BE, and EPO values were calculated using Spearman's rank correlation coefficient. The Chi-square test, Fisher's exact test and the Mann-Whitney U test were utilized to determine significant differences in the study populations, when appropriate. A probability (p) value of <0.05 was considered statistically significant.

Ethical approval. Approval to conduct the study was obtained from the local research ethics committee (Ref no: 105/13/03/03/2012), Department of Obstetrics and Gynaecology, Helsinki University Hospital, Finland. All participants provided informed written consent to participate in the study.

Results

The study population comprised 93 women for whom both amniotic fluid and umbilical cord blood samples were available. The demographic and clinical characteristics of the study population are shown in Table 1.

The median level of amniotic fluid EPO was 5.9 IU/L (range 0.5-89.8 IU/L) and that of umbilical cord serum EPO 32.9 IU/L (range 8.0-664.0 IU/L). The amniotic fluid EPO levels correlated with the EPO levels in umbilical cord serum (r=0.480, p<0.000) and with gestational age (r=0.261, p=0.012). Among the 63 (68%) prolonged pregnancies amniotic fluid EPO levels were higher than among the 30 (32%) term pregnancies (median 7.1 and 3.9

IU/L, respectively) (p=0.005). The amniotic fluid EPO level was abnormal in three of the pregnancies (one term and two prolonged pregnancies) (3%); with levels of 58.2 IU/L, 46.5 IU/L and 89.8 IU/L, respectively.

Composite adverse outcomes were reported for 18 of the deliveries (19%) (Table 1). Amniotic fluid EPO levels were not higher among these pregnancies as compared with the pregnancies without adverse outcomes (p=0.903). No difference in adverse outcomes was observed between the term and prolonged pregnancies (p=0.162). Amniotic fluid EPO levels did not correlate with umbilical artery pH or BE (r=0.092, p=0.381, and r=0.051, p=0.626, respectively).

Vaginal delivery occurred in 78 (84%) pregnancies. EPO levels in amniotic fluid and umbilical cord serum did not differ between vaginal delivery and cesarean section patients (amniotic fluid EPO median 6.0 IU/L [range 0.5-58.2 IU/L] and 5.8 [range 1.6-89.8 IU/L] respectively, p=0.350; umbilical cord serum EPO median 32.7 [range 8.0-178.0 IU/L] and 32.9 [range 13.0-664.0 IU/L] respectively, p=0.222). Indications for an emergency cesarean section were fetal distress (n=3), labor dystocia (n=7), chorioamnionitis (n=4), and fetal malpresentation (n=1) (Figure 1). Both the amniotic fluid EPO level (89.8 IU/L) and the umbilical cord serum EPO level (340.0 IU/L) were clearly abnormal in one patient undergoing a cesarean section for intrapartum fetal distress. In the other two cases with fetal distress umbilical cord serum EPO levels were abnormal (89.2 and 664.0 IU/L). None of these newborns had a pH ≤ 7.15 or BE ≤ -12 meq/L.

Umbilical cord serum EPO levels correlated with gestational age among vaginal deliveries (r=0.250, p=0.027), but the levels were not higher in prolonged pregnancies than in term pregnancies (p=0.057, Table 2). Umbilical cord serum EPO levels were abnormal in 28 (36%) of the vaginal deliveries, comprising eight term and 20 prolonged pregnancies.

Umbilical cord serum EPO levels did not correlate with umbilical artery pH, BE, duration of the delivery, or duration of the second stage of the delivery (p=0.897, p=0.390, p=0.287, and p=0.783, respectively).

Comment

The current study demonstrates that amniotic fluid EPO levels could be measured from samples obtained at induction of labor by amniotomy. EPO levels in amniotic fluid correlated with gestational age in both term and prolonged pregnancies and were higher among prolonged pregnancies. Amniotic fluid EPO levels correlated with umbilical cord serum EPO levels, even after the stress caused by vaginal delivery. Nevertheless, neither amniotic fluid EPO nor umbilical cord serum EPO level correlated with umbilical artery pH or BE. In this relatively small study population of apparently low-risk term and prolonged pregnancies with induction of labor delivery, amniotic fluid EPO levels were not associated with adverse perinatal outcomes.

The primary indication for the induction of labor might have affected the perinatal outcomes. However, pregnancies complicated by conditions involving increased risk of fetal hypoxia and adverse outcomes were not included in this study. The number of umbilical cord serum EPO samples collected from the study patients was limited and this reduced the sample size considerably, thus possibly influencing the results. Nevertheless, the outcomes of the group lacking umbilical cord serum EPO samples and those of the study population were similar. The sample size was too limited to analyze rarely occurring adverse perinatal outcomes in low-risk population. This probably explains why this study failed to demonstrate that amniotic fluid EPO levels preceding onset of contractions could predict perinatal outcomes.

High levels of amniotic fluid EPO reflect chronic hypoxia and are associated with adverse perinatal outcomes in complicated pregnancies, such as those characterized by Rhesus alloimmunization, fetal growth restriction, pre-eclampsia, and maternal type 1 diabetes [7]. Fetal EPO levels are assumed to be relatively stable after the second trimester during normoxic conditions [11], but increased levels have been reported beyond 41 gestational weeks [12]. Consistent with this, we observed higher levels of amniotic fluid EPO in prolonged pregnancies compared to term pregnancies. This is in agreement with the assumption that incipient weakening of placental function and relative chronic hypoxia increase the risk of fetal compromise in prolonged pregnancies.

Adverse perinatal outcomes, such as low Apgar score and decreased arterial pH and BE levels, intrapartum distress, and meconium aspiration, are more common in late term and prolonged pregnancies [1,8,13]. However, in the current study both adverse outcome events and abnormal amniotic fluid EPO values were rare. This might reflect efficient fetal compensatory mechanisms among these pregnancies, which in part might explain why it was not possible to demonstrate an association between antenatal amniotic fluid EPO levels and adverse outcomes.

EPO levels in the fetal plasma begin to rise soon after a hypoxic event, several hours before they rise in the amniotic fluid [7]. Thus, elevated EPO levels in the umbilical cord blood may reflect subacute hypoxia caused by adverse events during delivery. Only a few abnormal blood gas values occurred in the current study, which probably explains the lack of correlation of umbilical cord serum EPO with pH and BE levels.

A correlation between the EPO level in amniotic fluid and that in fetal plasma has previously been observed during pregnancy and in cesarean deliveries performed prior to labor

contractions [7]. We demonstrated that this correlation persists even after variable fetal stress caused by contractions during vaginal delivery.

The mode of the delivery is known to affect umbilical cord serum EPO level, with higher values occurring following vaginal birth as compared to planned cesarean sections [7,14]. No such difference was found in the current study. This is biologically plausible, considering the exposure of all the neonates to delivery contractions. Umbilical cord serum EPO levels did not correlate with the duration of the labor nor that of the second stage of the labor in the current study. This is consistent with the findings of a recent study on term asphyxiated neonates [15].

Umbilical cord serum EPO levels correlated with gestational age, but the difference in this regard between term and prolonged pregnancies was not significant. This is reasonable, considering that the risk of adverse perinatal outcomes in term pregnancies increases gradually, without a specific gestational age threshold [16]. Abnormal umbilical cord serum EPO levels occurred in a substantial proportion of the study population, even though the neonatal outcomes were normal in most of the cases. Nevertheless, umbilical cord serum EPO levels were abnormal in all cesarean sections performed for fetal distress. The significance of the current study findings, along with an exploration of the normal range of umbilical cord serum EPO levels in low-risk populations, needs to be validated in further studies with larger sample size.

EPO levels in amniotic fluid samples collected at amniotomy proved to be consistent with those measured in samples obtained by amniocentesis, which, to the best of our knowledge, is a novel observation. The advantage of vaginally obtained samples is the noninvasive method. Even though it was not possible to demonstrate the predictive utility of amniotic fluid EPO in

terms of outcomes in this relatively low-risk population, its usefulness in this regard in high-

risk populations should be further evaluated.

Conclusion

An evaluation of EPO level in amniotic fluid can be safely performed from samples obtained

by amniotomy at induction of labor. In the current study amniotic fluid EPO levels correlated

with gestational age in term and prolonged pregnancies and were higher in prolonged

pregnancies compared to term pregnancies. Umbilical cord serum EPO levels correlated with

amniotic fluid EPO levels, even after vaginal delivery. In this study on apparently low-risk

pregnancies, however, amniotic fluid EPO was not related to adverse outcomes.

Consequently, an evaluation of EPO level in amniotic fluid may not be a useful routine for

the assessment of fetal well-being in low-risk pregnancies with induction of labor. Future

studies on amniotic fluid EPO assessment at amniotomy or spontaneous rupture of

membranes in selected high-risk pregnancies are warranted.

Acknowledgements

References

(1) Joseph KS. The natural history of pregnancy: diseases of early and late gestation. BJOG
2011 Dec;118(13):1617-1629.

(2) Zizzo AR, Kirkegaard I, Pinborg A, Ulbjerg N. Decline in stillbirths and perinatal
mortality after implementation of a more aggressive induction policy in post-date

pregnancies: a nationwide register study. Acta Obstet Gynecol Scand 2017 Jul;96(7):862-867.

(3) D'Antonio F, Patel D, Chandrasekharan N, Thilaganathan B, Bhide A. Role of cerebroplacental ratio for fetal assessment in prolonged pregnancy. Ultrasound Obstet Gynecol 2013 Aug;42(2):196-200.

(4) Coatleven F. Place of fetal heart rate monitoring and its computerized analysis during the surveillance of prolonged pregnancy. J Gynecol Obstet Biol Reprod (Paris) 2011 Dec;40(8):774-784.

(5) Kauppinen T, Kantomaa T, Tekay A, Makikallio K. Placental and fetal hemodynamics in prolonged pregnancies. Prenat Diagn 2016 Jul;36(7):622-627.

(6) Seikku L, Rahkonen L, Tikkanen M, Hamalainen E, Rahkonen P, Andersson S et al. Amniotic fluid erythropoietin and neonatal outcome in pregnancies complicated by intrauterine growth restriction before 34 gestational weeks. Acta Obstet Gynecol Scand 2015 Mar;94(3):288-294.

(7) Teramo KA, Widness JA. Increased fetal plasma and amniotic fluid erythropoietin concentrations: markers of intrauterine hypoxia. Neonatology 2009;95(2):105-116.

(8) Seikku L, Gissler M, Andersson S, Rahkonen P, Stefanovic V, Tikkanen M et al. Asphyxia, Neurologic Morbidity, and Perinatal Mortality in Early-Term and Postterm Birth. Pediatrics 2016 Jun;137(6):10.1542/peds.2015-3334.

(9) Singh T, Sankaran S, Thilaganathan B, Bhide A. The prediction of intra-partum fetal compromise in prolonged pregnancy. J Obstet Gynaecol 2008 Nov;28(8):779-782.

(10) Teramo KA, Hiilesmaa VK, Schwartz R, Clemons GK, Widness JA. Amniotic fluid and cord plasma erythropoietin levels in pregnancies complicated by preeclampsia, pregnancy-induced hypertension and chronic hypertension. J Perinat Med 2004;32(3):240-247.

(11) Forestier F, Daffos F, Catherine N, Renard M, Andreux JP. Developmental hematopoiesis in normal human fetal blood. Blood 1991 Jun 1;77(11):2360-2363.

(12) Jazayeri A, Tsibris JC, Spellacy WN. Elevated umbilical cord plasma erythropoietin levels in prolonged pregnancies. Obstet Gynecol 1998 Jul;92(1):61-63.

(13) Gulmezoglu AM, Crowther CA, Middleton P, Heatley E. Induction of labour for improving birth outcomes for women at or beyond term. Cochrane Database Syst Rev 2012 Jun 13;(6):CD004945. doi(6):CD004945.

(14) Widness JA, Clemons GK, Garcia JF, Oh W, Schwartz R. Increased immunoreactive erythropoietin in cord serum after labor. Am J Obstet Gynecol 1984 Jan 15;148(2):194-197.

(15) Summanen M, Seikku L, Rahkonen P, Stefanovic V, Teramo K, Andersson S et al. Comparison of Umbilical Serum Copeptin Relative to Erythropoietin and S100B as Asphyxia Biomarkers at Birth. Neonatology 2017;112(1):60-66.

(16) Caughey AB, Washington AE, Laros RK,Jr. Neonatal complications of term pregnancy: rates by gestational age increase in a continuous, not threshold, fashion. Am J Obstet Gynecol 2005 Jan;192(1):185-190.

Conflicts of interest

The authors declare no conflicts of interest.

Figure legends.

Figure 1. Flow chart of the study population

Figure 2. Amniotic fluid erythropoietin levels in relation to gestational weeks (n=93)

Table headings.

Table 1. The demographic and clinical characteristics of the study population (n = 93)

Table 2. A comparison of term and prolonged pregnancies in the vaginal delivery group (n = 78)

Table 1

Characteristics	n	(%)
Maternal age, *mean (SD)*	30.6	*(5.4)*
BMI, *mean (SD)*	24.1	*(5.3)*
Prolonged pregnancy ($\geq 41^{+0}$ GW)	63	*(67.7)*
Nulliparity	35	*(37.6)*
Gestational diabetes	11	*(11.8)*
In vitro fertilization	3	*(3.2)*
Smoking	9	*(9.7)*
Delivery mode		
Spontaneous vaginal	74	*(79.6)*
Vacuum extraction	4	*(4.3)*
Cesarean section	15	*(16.1)*
Adverse outcomes	18	*(19.4)*
Umbilical artery pH $\leq$ 7.15	13	*(14.0)*
Umbilical artery BE $\leq$ -12	2	*(2.2)*
1 or 5 min Apgar < 7	5	*(5.4)*
Cesarean section for fetal distress	3	*(3.2)*
Birth weight (g), *mean (SD)*	3797	*(422)*

SD: standard deviation
BMI: body mass index
GW: gestational weeks
BE: base excess

Table 2

	Term pregnancies 37^{+0} - 40^{+6} GW n = 23 *(29.5%)*		Prolonged pregnancies $\geq 41^{+0}$ GW n = 55 *(70.5%)*		
	median	*IR*	median	*IR*	*p*-value[a]
Amniotic fluid EPO	3.9	*2.7 to -6.2*	7.1	*4.6 to 11.3*	**0.026**
Umbilical artery pH	7.32	*7.24 to 7.36*	7.26	*7.16 to 7.32*	**0.017**
Umbilical artery BE	-4.5	*-6.1 to -2.2*	-5.5	*-7.0 to -3.1*	0.145
Umbilical serum EPO	20.3	*11.5 to 51.5*	34.0	*23.6 to 55.7*	0.057
Duration of the delivery (minutes)	290	*201 to 577*	427	*241 to 612*	0.070
Duration of the 2nd stage of the delivery (minutes)	18	*10 to 32*	17	*8 to 40*	0.900

[a]*Mann Whitney U-test*

GW: gestational weeks, IR: interquartile range, BE: base excess, EPO: erythropoietin
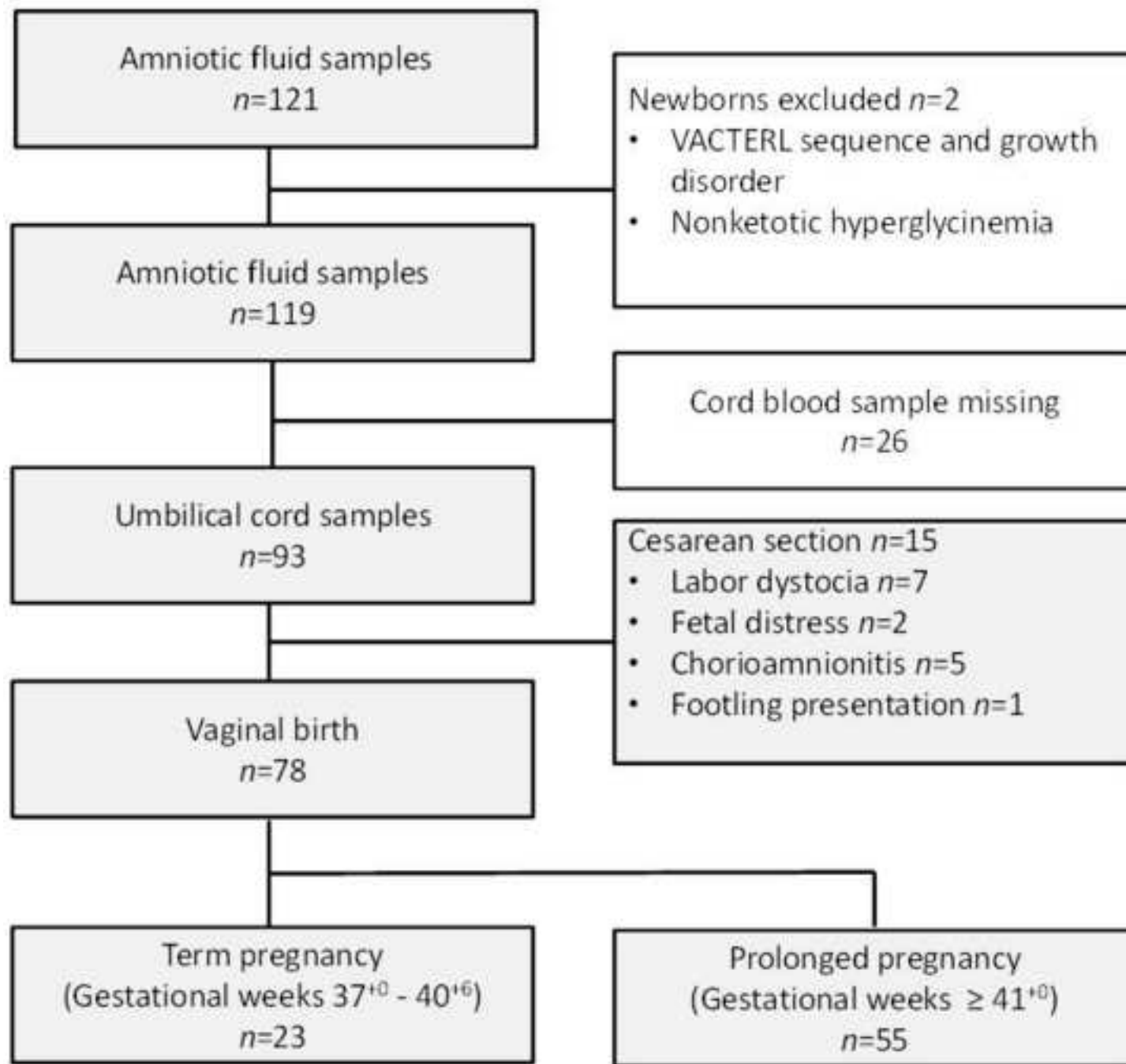
Figure 1

Figure 2