

STUDY PROTOCOL

Neonatal Outcomes After Acute Hypoxia: Re-evaluating Cardiotocography Traces Using Fetal Physiology-Based Interpretation and Eucapnic pH Assessment

ACRONYM: “NOAH” (Neonatal Outcomes after Acute Hypoxia)

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ABSTRACT

Cardiotocography (CTG) is widely used for fetal monitoring during labor, yet traditional pattern-based interpretation may fail to reflect actual fetal distress. A physiology-based approach has been proposed to enhance diagnostic accuracy, grounded in knowledge of the physiological fetal response to hypoxic stress.

Similarly, standard cord blood gas analysis may not adequately differentiate between respiratory and metabolic acidosis. The use of eucapnic pH, as proposed by Racinet et al., allows for correction of PCO_2 , thereby isolating the metabolic component.

This ambispective, single-center study will re-evaluate CTG tracings in all term neonates (≥ 37 weeks), integrating physiology-based CTG interpretation with pH, Base Excess, and pCO_2 from the umbilical artery blood gas, as well as the eucapnic pH derived from the Henderson-Hasselbalch equation. The study will include retrospective data from 2018 to 2025 and a prospective cohort of women who will deliver from 2026 onward. Findings from arterial blood analysis and CTG tracings, notably the type of hypoxia according to the physiological classification, will be integrated with maternal and labor characteristics.

Primary objectives are to assess whether physiology-based CTG interpretation better identifies fetal decompensation and to evaluate the relevance of the difference between measured and eucapnic pH. Secondary outcomes include correlations with neonatal outcomes and inter-rater reliability in CTG interpretation. Results may enhance understanding of fetal compromise and support the development of improved monitoring strategies during labor and delivery.

BACKGROUND AND RATIONALE

Labor is a complex physiological process characterized by regular, intense, and progressive uterine contractions that generate both mechanical stress (such as fetal head compression, cervical stretching, and umbilical cord pressure) and hypoxic stress (transient reductions in utero-placental oxygenation). In response to these events, the fetus activates a series of compensatory mechanisms essential for survival, including: 1. blood flow redistribution to vital organs (such as the heart, brain, and adrenal glands); 2. an increased catecholamine release, witnessed by an elevated baseline heart rate; 3. a reduction of the fetal movements¹. However, in the presence of predisposing clinical conditions—such as chronic utero-placental insufficiency, intrauterine infections, uterine hypercontractility, maternal hypotension, or acute sentinel events like placental abruption, uterine rupture, or cord prolapse—these adaptive responses may become insufficient². It has been estimated that acute sentinel events have an incidence of 0.5–1 per 1,000 live births. In such cases, the risk of developing hypoxia and metabolic acidosis rises significantly, as well as the risk of hypoxic-ischemic encephalopathy (1–3 cases per 1,000 live births), and, in the most severe scenarios, the risk of perinatal death (0.3–0.5 per 1,000 live births).

Cardiotocography (CTG) remains the cornerstone for intrapartum fetal surveillance. However, its interpretation has historically been limited by a pattern-recognition approach, which may not adequately reflect underlying fetal pathophysiology. Recent guidelines advocate for a physiology-based approach that integrates knowledge of fetal compensatory mechanisms, enabling a more accurate assessment of fetal well-being during labor³.

In parallel, evaluation of neonatal acid-base status at birth typically relies on analysis of umbilical cord blood gases, particularly pH and Base Excess. However, traditional interpretations do not always distinguish between the respiratory and metabolic components of acidosis. Racinet et al. introduced a novel method to calculate a "eucapnic pH" that corrects for the respiratory component by applying the Henderson-Hasselbalch equation with a standard PCO₂ value, thereby isolating the metabolic contribution to acidemia⁴. This offers an improved understanding of fetal compromise, particularly when assessing birth-related hypoxia^{5,6}.

Fetal-physiology-based interpretation could provide a better understanding of CTG patterns that reflect true fetal compromise, while using the eucapnic pH method may facilitate a clearer distinction between acute respiratory acidosis and chronic metabolic acidosis, thereby aiding correlation between CTG findings and the neonatal condition at birth.

This study aims to re-evaluate the CTG of full-term neonates admitted to labor (either spontaneous or induced) using physiological CTG interpretation guidelines and to correlate the type of hypoxia (acute, subacute, slowly evolving, and chronic) with findings from arterial cord blood gas analysis, including eucapnic pH. Together, these analyses could offer new insights into the pathophysiological events preceding adverse outcomes and contribute to the development of improved fetal surveillance strategies.

OBJECTIVES

Primary Objective

To evaluate the proportion of cases in which physiology-based CTG interpretation identifies features of fetal decompensation not detected by conventional interpretation, and to assess the association between the type and characteristics of hypoxia (e.g., duration and presence or absence of sentinel events) and arterial umbilical cord blood gas parameters, including eucapnic pH.

Secondary Objectives

- To examine the correlation between CTG features, eucapnic pH, and neonatal clinical outcomes.
- To describe the distribution of eucapnic pH values in comparison to measured pH in cases of sentinel events.
- To assess inter-rater agreement in CTG re-evaluation using a physiology-based approach.

METHODS

Study design

Ambispective, observational study (retrospective and prospective cohorts)

Population

- Retrospective cohort: retrospective collection of full-term neonates from singleton pregnancies delivered between January 2018 and December 2025
- Prospective cohort: consecutive inclusion of pregnant women delivering at term, undergoing CTG registration during labor

Study duration

24 months

Study Period:

- Retrospective arm: Review of cases from January 2018 to December 2025
- Prospective arm: Enrollment of eligible cases from January 2026 to December 2027

Population

Inclusion criteria

- Singleton pregnancies
- Term neonates (gestational age \geq 37 weeks)
- Availability of clinical data, such as
 - o CTG tracing within 90 minutes before delivery
 - o Umbilical cord blood gas analysis (arterial sample)
- Signed informed consent (only for the prospective arm)

Exclusion criteria

- Major fetal anomalies
- Incomplete or missing CTG or blood gas data
- Elective cesarean sections without labor

For the retrospective arm:

The Sponsor has conducted a Data Protection Impact Assessment (DPIA), which confirmed the adequacy of the technical and organizational measures adopted to ensure the protection of the rights and fundamental freedoms of data subjects, in accordance with Article 89 of Regulation (EU) 2016/679 and the guidance provided by the Italian Data Protection Authority. Data processing will be limited to the purposes of the study and carried out in accordance with the principles of data minimization, pseudonymization, and security, in compliance with the safeguards defined by the Supervisory Authority.

VARIABLES AND PROCEDURES

Case Identification:

Retrospective arm: Identification through electronic medical records and institutional delivery databases.

Prospective arm: Real-time identification and enrollment of cases meeting criteria by labor and delivery staff.

Data Collection:

For each case:

- Maternal and obstetric data (age, parity, previous cesarean, mode of delivery, indication)
- CTG tracings: archived digital files from the last 60–90 minutes before birth
- Cord arterial blood gas values (pH, PCO₂)
- Neonatal outcomes: Apgar scores, need for resuscitation, NICU admission, neonatal encephalopathy, early neonatal death

CTG Re-evaluation:

- Reinterpretation using the fetal physiology-based framework (Chandrasekaran et al.)
- Blinded assessment by at least two independent reviewers

Eucapnic pH Calculation:

Using the Henderson-Hasselbalch-based method:

$$pH_{\text{euc-n}} = pH + 7.28 - [7.62 + \text{LOG}_{10}(\text{HCO}_3^- \text{ expected}/p\text{CO}_2)]$$

ENDPOINTS

Primary endpoint

Proportion of cases in which physiology-based CTG interpretation identified features of fetal decompensation not evident in conventional interpretation.

Secondary endpoints

- Strength of association between CTG patterns, acid-base status, and neonatal outcomes.
- Mean difference between measured pH and eucapnic pH; frequency and distribution of metabolic vs. respiratory acidosis using eucapnic pH thresholds.
- Inter-rater agreement in CTG re-evaluation using a physiology-based interpretative approach will be assessed using Cohen's kappa coefficient (or weighted kappa if appropriate).

DATA COLLECTION AND MANAGEMENT

A password-protected database will be created using a study-specific Microsoft Excel spreadsheet, in which patient data collected during the study will be recorded.

The following data will be collected: date of birth, age at enrollment, date of last menstrual period, ethnicity, smoking status, method of conception, obstetric history, gestational age at delivery, comorbidities, pharmacological treatments, pregnancy-related complications, cardiotocographic (CTG) monitoring for at least 90 minutes prior to delivery or prior to a sentinel event, mode and timing of delivery, neonatal birth weight, Apgar score, umbilical

cord blood gas analysis, admission to the neonatal intensive care unit (NICU), and neonatal complications

STATISTICAL ANALYSIS PLAN

Sample size calculation

To determine the required sample size for the study, we considered the comparison between physiology-based CTG interpretation and conventional CTG interpretation for the identification of fetal decompensation. It was assumed that physiology-based interpretation would detect additional features not evident with conventional CTG in approximately 20% of cases, while discordances favoring conventional CTG would occur in about 5% of cases. A clinically significant difference between the two methods was estimated at approximately 15%.

Calculations indicate that, to detect this difference with high reliability (95% statistical power and a 5% significance level), approximately 228 patients per group would be required. To provide a sufficient margin for potential exclusions due to incomplete or non-interpretable CTG recordings, the sample size was increased to approximately 500 patients per group.

This sample size not only ensures high statistical power but also allows for accurate estimation of the proportion of additional detections by physiology-based interpretation and enables exploratory analyses of factors associated with discordant interpretations.

Furthermore, a retrospective review suggests that approximately 500 neonates from singleton pregnancies, delivered between January 2018 and December 2025 and meeting the inclusion/exclusion criteria, can be enrolled, making this sample size feasible and achievable.

The sample will be described in terms of its clinical and demographic characteristics using descriptive statistics techniques.

Continuous variables will be summarized as mean and standard deviation or median and interquartile range (IQR), as appropriate, based on data distribution assessed using the Shapiro–Wilk test. Categorical variables will be presented as absolute frequencies and percentages.

The proportion of cases in which physiology-based CTG interpretation identified features of fetal decompensation not evident on conventional interpretation will be calculated and reported with corresponding 95% confidence intervals (CIs).

The primary analysis will compare the proportion of fetal decompensation events detected by physiology-based CTG versus conventional CTG. Differences between groups will be assessed using Chi-square tests or Fisher’s exact test for categorical outcomes and t-tests or Mann-Whitney U tests for continuous outcomes, as appropriate.

Exploratory analyses will evaluate clinical and intrapartum factors associated with discordant interpretation (i.e., decompensation identified by physiology-based interpretation only) using univariable and multivariable logistic regression models. Results will be reported as odds ratios (ORs) with 95% CIs.

The association between the type and characteristics of hypoxia (e.g., duration of hypoxic exposure and presence or absence of sentinel events) and arterial umbilical cord blood gas parameters, including eucapnic pH, will be examined using Pearson or Spearman correlation coefficients, as appropriate.

The mean difference between measured pH and eucapnic pH will be assessed using paired Student's t test or the Wilcoxon signed-rank test, depending on data distribution. Agreement between measured and eucapnic pH values will be further explored using Bland–Altman analysis. The frequency and distribution of metabolic versus respiratory acidosis, classified according to eucapnic pH thresholds, will be described and compared using χ^2 or Fisher's exact test, as appropriate.

The relationships among cardiotocography (CTG) features, eucapnic pH, and neonatal clinical outcomes will be evaluated using linear or logistic regression models, depending on the nature of the outcome variables. The discriminative performance of eucapnic pH compared with measured pH will be explored using receiver operating characteristic (ROC) curve analysis, where appropriate.

The distribution of eucapnic pH values will be described and compared with measured pH values in cases involving sentinel events using Student's t test or the Mann–Whitney U test for independent samples, as appropriate.

Inter-rater agreement in CTG re-evaluation using a physiology-based interpretative approach will be assessed using Cohen's kappa coefficient (or weighted kappa, where appropriate). Kappa values will be interpreted according to established conventions.

All statistical tests will be two-sided, and a p value < 0.05 will be considered statistically significant. Statistical analyses will be performed using standard statistical software (e.g., R, SPSS, or Stata).

ETHICS APPROVAL AND CONSENT

For the retrospective arm of the study, informed consent will not be required, as the data collection involves only the review of existing clinical records and does not include any direct patient interaction or intervention. In accordance with applicable regulations for observational studies using anonymized or previously collected data, and consistent with institutional ethical standards, the use of retrospective data does not necessitate informed consent from patients. All data will be handled in compliance with privacy and confidentiality regulations, ensuring that patient identifiers are removed and that the analysis is conducted exclusively for research purposes.

For the prospective collection, a signed consent form will be used to share personal and medical data for clinical and research purposes. Consent to participate in the study will be sought from each participant only after a full explanation has been provided, an information leaflet has been offered, and sufficient time has been allowed for consideration. Signed participant consent will be obtained only for this group of patients. The participant's right to refuse to participate without providing a reason will be respected.

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