PROTOCOL FOR A COHORT STUDY

Intelligence and neurodevelopmental outcome after prenatal exposure to labour epidural analgesia: a sibling matched clinical ambidirectional cohort study

Version number: v2 – **Date** 1/11/2024

Internal ref. nbr: S69530

Sponsor

University Hospitals Leuven (UZ Leuven)

Herestraat 49, B-3000 Leuven

Coordinating Investigator

Professor Dr. Sarah Devroe

LIST OF PARTICIPATING SITES

List Of Participating Sites	Principal Investigator
UZ Leuven, Herestraat 49, 3000 Leuven, Belgium	Prof. Dr. Sarah Devroe

Confidentiality Statement

The information in this document is strictly confidential and is available for review to Investigators, potential Investigators and appropriate Ethics Committees, Institutional Review Boards or Competent Authorities. No disclosure should take place without written authorization from the Sponsor

SIGNATURES

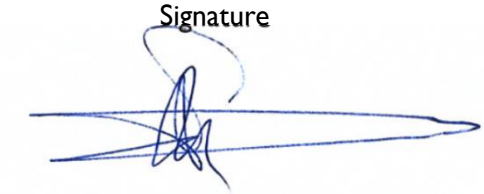
Title: Intelligence and neurodevelopmental outcome after prenatal exposure to labour epidural analgesia: a sibling matched clinical ambidirectional cohort study

Protocol: Labour epidural analgesia-IQ

The undersigned confirm that the above referenced protocol has been acknowledged and accepted, they agree to conduct the Study in compliance with the approved protocol, and will adhere to: the ICH guidelines, the most recent version of the Declaration of Helsinki, the EU General Data Protection Regulation 2016/679 (GDPR), relevant Belgian laws implementing the GDPR, the Belgian Law of August 22nd 2002 on patient rights, and any other regulatory requirements and Standard Operating Procedures (SOPs), as applicable.

The undersigned agree not to disclose the confidential information contained in this document for any purpose other than the evaluation or conduct of the Study, without prior written consent of the Sponsor.

Coordinating Investigator

Name & Title	Signature	Date
Prof. Dr. Sarah Devroe		1/11/2024

Principal Investigator (University Hospitals Leuven, Leuven, Belgium)

Name & Title	Signature	Date
Prof. Dr. Sarah Devroe		1/11/2024

Co-investigators

Name & Title	Signature	Date
Dr. Tom Bleeser		1/11/2024
Prof. Dr. Steffen Rex		1/11/2024
Prof. Dr. Jurgen Lemiere		1/11/2024
Prof. Dr. Jaan Toelen		1/11/2024

TABLE OF CONTENTS

FUNDING AND SUPPORT

Funder	Type of Financial or Non-Financial Support
ESRA (European Society for Regional Anaesthesia)	Grant of €10 000 to finance the intelligence tests and questionnaires

ROLES AND RESPONSIBILITIES

The Principal Investigator (PI) is responsible for the conduct of the Study at his/her Participating Site, and for protecting the rights, safety and well-being of Study participants. As such the PI must ensure adequate supervision of the Study conduct at the Participating Site. If any tasks are delegated, the PI will maintain a log of appropriately qualified persons to whom he/she has delegated specified Study-related duties. The PI will ensure that adequate training is provided and documented for all Study staff, prior to conducting assigned Study-related activities. Even when certain activities are delegated, the PI will ultimately remain responsible for the conduct of the Study at his/her Participating Site.

It is the Coordinating Investigator's (CI's) responsibility to supervise the general conduct (e.g. Study progress, communication, protocol training and support of the participating sites, annual reporting to the Ethics Committee (EC), end of Study notification(s) and results reporting…) of the Study. The CI fulfils both Investigator and Sponsor responsibilities, as outlined in International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) E6(R2) and applicable regulations.

PI and CI shall each be referred to as «Investigator(s)».

STUDY SYNOPSIS

Title of clinical Study («Study»)	Intelligence and neurodevelopmental outcome after prenatal exposure to labour epidural analgesia: a sibling matched clinical ambidirectional cohort study
Protocol Short Title Acronym	Labour epidural analgesia-IQ
Sponsor name	University Hospitals of Leuven (UZ Leuven)
Coordinating Investigator	Prof Dr. Sarah Devroe
Contact Address CI	Herestraat 49, 3000 Leuven, Belgium
Contact Email CI	sarah.devroe@uzleuven.be
Contact Phone CI	016/34.42.70
Public database nbr	NA
Principal Investigator	Prof Dr. Sarah Devroe
Medical condition or disease under investigation	Prenatal exposure to labour epidural analgesia
Study rationale	In Belgium, labour epidural analgesia (LEA) has been used for decades in the vast majority of vaginal deliveries (up to 83% of deliveries). Labour epidural analgesia is the most effective and safest way to allow pregnant women a nearly pain-free labour- and birth experience. Recent data showed that the use of LEA was associated with a reduced risk for severe maternal morbidity, neonatal resuscitation and with a reduced risk for low Apgar scores. However, prenatal exposure to labour epidural analgesia has been associated by several study groups with an increased risk for autism spectrum disorder (ASD) in the children, but these studies suffer from several important limitations which may have induced significant bias (e.g., not correcting for unmeasured confounders). Associations with other neurodevelopmental disorders (e.g., Attention Deficit Hyperactivity Disorder (ADHD), oppositional defiant disorder (ODD) and conduct disorder (CD)) and cognitive functioning have not been investigated yet. There is hence an urgent need for a study allowing definitive conclusions by overcoming the limitations of the previous studies. We will perform the first sibling-matched clinical cohort study using a prospective investigator-assessment of multiple domains of cognitive functions. We hypothesize that prenatal exposure to LEA will neither be associated with reduced intelligence nor with more neurodevelopmental disorders. Validating our hypothesis holds the potential to grant women the assurance to confidently receive the most efficacious method for labour analgesia, free from

	apprehension that it might have adverse implications for their children's neurodevelopment.
Primary objective	To compare the intelligence of children prenatally exposed to labour epidural analgesia with that of sibling matched unexposed children.
Secondary objective(s)	To compare parentally reported traits of ASD, ADHD, ODD and CD of children prenatally exposed to labour epidural analgesia with that of sibling matched unexposed children.
Endpoints	-Primary endpoint: Wechsler full scale intelligence quotient -Secondary endpoints: multiple indexes assessed by the Wechsler intelligence scale: verbal comprehension index, visual spatial index, working memory index, fluid reasoning index and processing speed index. Total scores and subscores of the parental questionnaires "social responsiveness scale" (SRS) and "vragenlijst voor gedragsproblemen bij kinderen 6-16" (VvGK6-16) are additional secondary endpoints.
Sample size	At least 64 prenatally exposed children and 64 prenatally unexposed children
Summary of eligibility criteria	Dutch speaking sibling pairs discordant for prenatal exposure to labour epidural analgesia will be included. Children born from 1st January 2014 until 1st November 2019 will be included. Children prenatally exposed to maternal surgery, foetal surgery/interventions, chemotherapy, radiotherapy, radiology and oncologic pathology will be excluded. Children with a diagnosis of severe disability of genetic origin (e.g., Fragil X, Down syndrome,…), history of head trauma, major congenital birth defects, children born at a gestational age < 37 weeks or > 42 weeks, or with a birth weight < 2.5 kg will be excluded. Children exposed to general anaesthesia after birth, twins/triplets/multiple births will be excluded. Elective and intrapartum caesarean sections will be excluded.
Maximum duration a research subject remains in the study	Study will be conducted in 2024-2027. The end of the study for each individual participant is determined by the date at which the neuropsychological testing is planned. Neuropsychological testing usually takes no longer than 2 hours.
Participating research sites	UZ Leuven, Herestraat 49, 3000 Leuven, Belgium
Third parties	NA

1. Background and Rationale

In the European Union, nearly 4,100,000 children were born alive in 2021[1]. It can be estimated that during the delivery of more than 3,300,000 children, the mother received labour **epidural analgesia (LEA)** to manage labour pain[2-4]. This occurs during critical phases of foetal brain development[5]. Exposure of the developing brain to LEA has been associated by several study groups with autism spectrum disorder (ASD)[6-11]. To the best of our knowledge, currently, there is no data available on the potential association between LEA and other neurodevelopmental disorders, including attention-deficit/hyperactivity disorder (ADHD)[12], oppositional defiant disorder (ODD) en conduct disorder (CD). One of the most consistent findings is that neurodevelopmental disorders are characterized by lower and/or **discrepant intelligence profiles**[16, 17] (e.g., 30-70% of children with ASD have an intellectual disability)[18-23]. In general, these neurodevelopmental disorders affect 10% of the children worldwide and lead to impairments in various aspects of functioning, such as cognition, communication, social interaction, and motor skills[24]. While these deficits become frequently symptomatic in early childhood, they **affect individuals throughout their entire lifespan**[24, 25], can lead to a range of adverse outcomes, including **difficulties in academic and career development**, severe mental disorders, and even **premature death**[26, 27]. Neurodevelopmental disorders place a **significant economic burden on individuals, families, and societies** by increasing healthcare costs, educational costs, expenses for caregivers and social services, legal costs, and necessitating long-term care and mental care[25]. Families caring for a child with a neurodevelopmental disorder face higher financial costs and have reduced working hours with higher rates of poverty as a consequence[25]. In the European Union, the societal economic burden of ASD can be estimated to be as high as €140,000 billion[28, 29] and for ADHD, it can be estimated as €21,000 million[30, 31]. Therefore, gaining knowledge on the aetiology, pathophysiology and possible prevention strategies of neurodevelopmental disorders is a public health priority[25]. There is an **urgent need to further investigate LEA as a potential risk factor for long-term neurodevelopmental consequences**. We hypothesize that prenatal exposure to LEA will neither be associated with reduced intelligence nor with more neurodevelopmental disorders. Validating our hypothesis holds the potential to grant women the assurance to confidently receive the most efficacious method for labour analgesia, free from apprehension that it might have adverse implications for their children's neurodevelopment.

1.1 Labour pain and management

Labour pain is very severe pain caused by uterine contractions and perineal tissue damage, leading to intense maternal emotional distress and – if untreated – to a high risk of postpartum depression[32, 33]. Additionally, the maternal stress response to pain results in maternal hyperventilation, release of catecholamines and other vasoactive mediators, causing decreased uterine blood flow with foetal distress and neonatal asphyxia as potential consequences[32, 33]. **LEA is currently the gold standard** in the management of labour pain[33, 34]. LEA can be medically indicated, e.g., a high risk for caesarean section. In several European countries and the United States of America, **up to 83% of pregnant women receive LEA for delivery**[2-4, 32, 33]. In other countries (e.g., the UK), LEA is much less implemented[35, 36]. Patient satisfaction is higher for LEA when compared with other approaches, and LEA is considered safe during delivery for both the mother and the foetus[33, 34, 37, 38].

Recent data even showed that the use of LEA was associated with a reduced risk for severe maternal morbidity[36], neonatal resuscitation and with a reduced risk for low Apgar scores[39]. However, the investigation of long-term consequences of the use of LEA has been started only recently.

1.2 Neurodevelopmental effects of labour epidural analgesia

Observational studies

In 2020, a first retrospective clinical cohort study showed an **association between LEA and ASD** [hazard ratio (HR) 1.37, 95% confidence-interval (CI): 1.23-1.53][6]. Additionally, a clear dose-response relationship was reported, **raising a safety concern for public health[6]**. Thereafter, other retrospective observational studies corroborated this finding[7-11], even when taking several confounders into account (e.g., maternal age at delivery, parental education and income, birth weight, prematurity, pregnancy complications etc.). In contrast, other retrospective observational studies failed to confirm an association between LEA and ASD[40-43], especially when using a sibling matched design[8, 10, 40-42]. Likewise, the only prospective observational study (using caregiver questionnaires) performed so far failed to prove this association[44].

Systematic reviews and meta-analyses

Recent systematic reviews found a significantly increased risk for ASD when confounders were not taken into account [HR 1.3, CI: 1.25-1.35[45] and HR 1.24, 95% CI: 1.14-1.34[32]]. When potential confounders were considered, the effect was smaller but still statistically significant [HR 1.13, 95% CI: 1.03-1.25[45] and HR 1.11, 95% CI: 1.06-1.16[32]]. When only studies using a sibling-matched design were analysed, one meta-analysis concluded that there was no significant association [HR 1.07, 95% CI: 0.99-1.16][45], whereas another meta-analysis demonstrated a significant association in sibling-matched designs [HR 1.10, 95% CI: 1.02-1.18], with however no dose-response effect[32]. It was concluded that the statistically significant **association between LEA and ASD may be explained by unmeasured confounders** (e.g., genetic factors)[45].

While nearly all studies focused on the association between LEA and *ASD*, other neurodevelopmental outcomes have been reported only in a limited number of studies. There was no evidence for an association between LEA and learning disorders[46], ADHD[8, 10], intellectual developmental disorder[8] and epilepsy[8]. A single retrospective cohort study observed that LEA was even associated with a *reduced* risk for developmental concerns (gross and fine motor function, communication and social functioning[39], while another study observed an increased risk[47].

Apart from being contradictory, the available studies suffer from several **important limitations which may have induced significant bias**. First, it is obvious that retrospective studies without sibling matching **cannot correct for unmeasured/unmeasurable confounders** such as genetic factors, which could contribute to the predisposition for ASD[45]. Second, with the exception of one study[44], **all studies assessed the outcomes retrospectively,** using already existing population-based datasets and administrative records (e.g., health insurance databases, which were obviously

not specifically designed to investigate this subject). Third, there are **no studies prospectively assessing investigator-assessed outcomes allowing an objective neurocognitive testing**. Fourth, in several studies[8, 39, 41], **both vaginal deliveries and caesarean sections** were included. Fifth, the **majority of studies focussed on ASD** and **did not assess other cognitive functions**. Last, the **neurophysiological mechanism** by which LEA could cause neurodevelopmental disorders **remains entirely elusive**.

LEA has been **used** for decades in the **vast majority of vaginal deliveries** (up to 83%[2-4, 32, 33]). LEA is the most effective and safest way to allow pregnant women a nearly pain-free labour- and birth experience[33, 34, 37, 38]. A detrimental effect on neurodevelopmental outcomes would be a massive public health issue, unsettle parents and caregivers, and put birthing persons at risk for pain and untoward maternal and neonatal outcomes. **There is hence an urgent need for a study allowing definitive conclusions by overcoming the majority of the above-mentioned limitations. We will perform the first sibling-matched clinical cohort study using a prospective investigator-assessment of multiple domains of cognitive functions**.

2. Study Objectives and Design

2.1 Study objectives

The primary objective of this clinical ambidirectional cohort study is to **compare the intelligence of children prenatally exposed to LEA with those of sibling-matched unexposed children**. In line with a recent systematic review investigating associations with ASD, and by appropriately taking confounders into account, we hypothesize that prenatal exposure to LEA will neither be associated with reduced intelligence nor with more neurodevelopmental disorders (ASS, ADHD)[45]. Validating our hypothesis holds the potential to grant women the assurance to confidently receive the most efficacious method for labour analgesia, free from apprehension that it might have adverse implications for their children's neurodevelopment.

The secondary objective is to compare parentally reported traits of ASD, ADHD, ODD and CD between exposed and unexposed children.

2.2 Primary Endpoints

Primary outcome will be the Wechsler full-scale intelligence quotient which will be assessed using the Wechsler Intelligence Scale for Children (WISC).

2.3 Secondary Endpoints

Secondary outcomes will include the multiple indices assessed by the Wechsler intelligence scale: verbal comprehension, working memory, visual spatial, fluid reasoning, processing speed.

The absolute risk reduction for a clinically relevantly decreased intelligence quotient, defined as an IQ<85, will be calculated.

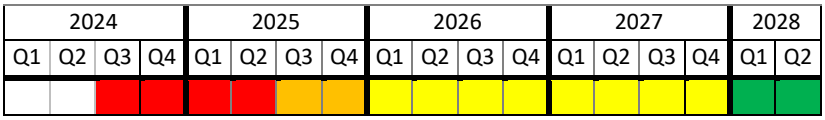
The total scores and subscores of the parental reported questionnaires "social responsiveness scale" (SRS) and "vragenlijst voor gedragsproblemen bij kinderen 6-16" (VvGK6-16) will be additional secondary outcomes. For the SRS questionnaire, the total score reflects the severity of parental reported social deficits in the autism spectrum. Additionally, five subscale scores will be analyzed: social awareness, social cognition, social communication, social motivation, and restricted interests and repetitive behavior. The VvGK6-16 is the Dutch versions of the "Disruptive Behavior Disorders Rating Scale" (DBDRS) and have four domains that will be analyzed: inattention, hyperactivity and impulsivity (ADHD), oppositional defiant disorder (ODD) and conduct disorder (CD).

2.4 Study Design

Ambidirectional cohort study with retrospective assessment of the exposure to prenatal labour analgesia and prospective assessment of the Wechsler intelligence quotient of the children and two parental questionnaires (SRS and VvGK6-16). Confounding bias will be reduced by using sibling matching and by adjusting for confounders in mixed-effects regression models.

2.5 Expected duration of the study

In the table below, the research timeline can be found.



	Identification of cohort
	Contacting participants to schedule neuropsychological testing
	Neuropsychological testing
	Data analysis, writing article and publication

The end of the study for each individual participant is determined by the date at which the neuropsychological testing is planned. Neuropsychological testing usually takes no longer than 2 hours.

2.6 Translational research

NA

3. Study Population / Eligibility Criteria

3.1 Inclusion criteria

Participants eligible for inclusion in this study must meet **all** of the following criteria:

Sibling pairs discordant for prenatal exposure to LEA will be included:

Exposed children	Unexposed children
• Children prenatally exposed to labour epidural analgesia	• Sibling of an exposed child • Prenatally not exposed to labour epidural analgesia
• Children born from 1st January 2014 until 1st November 2019 (In our centre, a new technique of LEA has been introduced in 2014 and thereafter, clinical practice has remained largely unchanged. Neurocognitive testing will start on 1st November 2025 and completion is anticipated for 31st December 2027. During this period, these children will have an age of 6-14 years. The Wechsler Intelligence Scale for Children is validated for children with this age.) • Parental informed consent	

3.2 Exclusion criteria

Participants eligible for this study must **not** meet any of the following criteria:

Exposed children	Unexposed children
• Children whose mothers underwent maternal surgery or foetal surgery/interventions during the same pregnancy. • Children exposed to general anaesthesia after birth • Children prenatally exposed to chemotherapy, radiotherapy, radiology, oncologic pathology • Children born at a gestational age < 37 weeks or > 42 weeks, or with a birth weight < 2.5 kg • Twins, triplets, multiple births • No Dutch-speaking children • Diagnosis of severe disability of genetic origin (e.g., Fragil X, Down syndrome,…), history of head trauma, major congenital birth defects • Elective caesarean section and intrapartum caesarean section (the number of caesarean sections will be reported and compared between both groups)	

- Foetus died before start of delivery

4. Assessment of Efficacy

See section "2. Objectives and Design".

5. Assessment of Safety

5.1 Specification, timing and recording of safety parameters

The sponsor will assess whether any relevant safety information that becomes available during the study should be reported ad hoc to the EC.

5.2 Treatment stopping rules

NA

6. Identification of study participants, contact with study participants and intelligence testing

6.1 Identification of study participants

In the period 2014-2024, approximately 14 000 parturients received LEA in our centre. Eligible sibling pairs discordant for prenatal exposure to LEA will be identified retrospectively in this period using the birth lists and hospital file system of our centre. For mothers having more than two eligible children with discordant prenatal LEA exposure (e.g., one exposed child and five unexposed children; two exposed children and four unexposed children), a computer random number generator will be used to select one exposed child and one unexposed child (i.e., sibling pairs) in these mothers. This will be done because a 1/1 ratio of exposed/unexposed children results in the sample size calculation in the lowest total number of children that should be included in this study. This 1/1 ratio with optimal statistical power will be used, as performance of the intelligence tests is time consuming: it is desirable for the participants and to limit financial resources.

6.2 Contact with study participants

After identification, we will send an invitation to the parents and their children by mail or e-mail with information on this study and our contact details. One week after receiving this invitation, we will start to contact the parents by telephone to ask for their willingness to participate, to send them the informed consent and to schedule the assessment of the Wechsler intelligence test. If the parents prefer to have more time before making a decision, we will send an e-mail with a link to an online planning tool to schedule the Wechsler intelligence test or to contact us again in case they decided not to participate. Depending on the results of this contact, the following actions will be undertaken:

- *The parents agree to participate and an intelligence test is scheduled*
  No further contact is needed.

- *The parents will contact us again to inform us on their decision*
  In case we do not receive a decision after one month, we will contact the parents again by telephone. When the parents are still interested in participating in the study, we will ask to schedule an intelligence test by telephone or resend the link for the online planning tool. We will continue this cycle of checking the responses after one month and contacting the parents again until they mention that they do not want to participate anymore. This method of continuing contact with the parents is important because practical problems can be a crucial reason for non-response, even for highly motivated parents.

- *No telephone number can be found in the hospital file system.*
  First, the child's patient file will be checked for a telephone number, followed by the mother, brothers or sisters' file.

- *Parents do not pick up the phone or a voicemail is heard.*
  In both cases, we will continue to call the parents until we succeed in talking directly to them. No message will be left in voicemails. Study information will be sent by e-mail or mail if telephone calls remain unanswered.

- *When the parents indicate that they are not interested in participation in the study at any stage of the phone calls, we will not contact them again anymore.*
  To allow estimating selection bias by passive non-response analysis, the active or passive reason for refusal of participation will be asked and one of the following categories will be registered:

  - No time
  - Too demanding
  - No interest in study
  - Health problem
  - Negative experience with delivery
  - Specific reason, i.e. …

6.3 Intelligence testing

The intelligence test will be performed by trained investigators. First, the investigators will follow the theoretic course on the assessment of intelligence testings to obtain a Pearson® certificate. Thereafter, the investigators will practice the assessment of the intelligence testing on volunteering children of friends and family under supervision and with feedback of Jurgen Lemiere, a pediatric neuropsychologist at UZ Leuven with extensive experience of intelligence testing. Only after achieving proficiency of the intelligence test assessment, they will start with the assessments for the study.

The intelligence test will be performed in UZ Leuven or can also be performed at home if it is not possible to come to the hospital.

First, the parents and the children will be given detailed written and oral information and will then be asked to sign the informed consent.

Thereafter, the intelligence testing will be performed while the parent(s) wait in a different room. During this time, the parent will be asked to complete the parental questionnaires.

Multiple investigators will assess the Wechsler intelligence quotient. Each of these investigators will always assess both siblings of each sibling pair.

After the test, child and the parent will receive a €25 voucher. This voucher is intended to cover travel expenses and to compensate for the time and effort invested in participating. Additionally, parents will be asked if they wish to be informed about the IQ score. This score, along with an explanation of how it compares to the general population, will be sent via mail after completion of the study. It will be emphasized that individual results and differences between the siblings should be interpreted with caution. Individual results can be affected by many factors, e.g., the efforts, concentration and motivation at the moment of testing. Furthermore, individual differences between siblings probably do not only represent the effects of labour epidural analgesia, but are also affected by many other factors. The study results will be discussed as well. The phrasing and communication of results will be done individually and will be conducted in collaboration with Jurgen Lemiere, a pediatric neuropsychologist at UZ Leuven. Our telephone number will be mentioned in the e-mail and the parents can call us personally in case of questions or concerns. These concerns will be discussed with Jurgen Lemiere and follow-up will be provided if desired.

Withdrawal of participation

In case one sibling or its parents decide to withdraw his/her participation to the study, the results of the other sibling will still be included in the study.

7. Statistics and Data Analysis

Analysis

A non-inferior sibling matched study design will be used. The inferiority margin represents the largest difference acceptable to show non-inferiority. The inferiority margin will be set at half of the standard deviation of the normative score for each instrument in a normal population, which is shown in the table below. The difference of the outcome in the unexposed group minus the outcome in the exposed group will be investigated. The null hypothesis states that this difference is greater than or equal to the inferiority margin, i.e., LEA is inferior to no LEA. The alternative hypothesis states that this difference is smaller than the inferiority margin, i.e., LEA is non-inferior to no LEA. Non-inferiority is defined as the upper limit of the 95% confidence interval for the difference of the outcomes between exposed and unexposed children being lower than the inferiority margin.

	Standard deviation in a normal population	Inferiority margin
Wechsler intelligence test	15	7.5
Social responsiveness scale (SRS)	10	5
Vragenlijst voor gedragsproblemen bij kinderen 6-16 (VvGK6-16)	3	1.5

A sibling matched study design is used to take unmeasured (e.g., genetic) confounders into account.

It will be compared whether following measured confounders are different between exposed and unexposed children: gender of the child, birth weight, gestational age at delivery, exposure of the mother during pregnancy to smoking, alcohol, illegal drugs, paritus history, pregnancy diabetes, pregnancy induced hypertension, pre-eclampsia, eclampsia, premature rupture of membranes, antepartum hemorrhage[6-11, 39-42, 47].
The analyses of the primary and secondary outcomes will be adjusted for these measured confounders with statistically significant differences ($p < 0.05$) between both groups.

The primary and secondary outcomes will be analysed in mixed-effects regression models with a random intercept to take the clustering of the siblings into account. The primary/secondary outcome will be the dependent variable; the group (exposed/unexposed) and the measured confounders (previous paragraph) will be the independent variables. The Wechsler full scale intelligence quotient scores, primary indexes of the Wechsler intelligence scale, and the total scores and subscores of SRS and VvGK6-16 will be analyzed using linear mixed regression models. The absolute risk reduction for IQ-scores <85 (the definition of a clinically relevantly decreased intelligence) will be analysed by using Poisson mixed regression models with the identity link[51].

A dose-response effect will be investigated by adding the duration of prenatal exposure to LEA as an independent variable to the regression model.

The results of the adjusted matched analysis will be compared with an unmatched analysis without taking confounders into account (two sample t-test).

The characteristics of the study participants (see section 8.2) will be compared between both groups.

The potential selection bias caused by non-response rate will be assessed by comparing all known demographic variables (see section 8.2) between the subjects with response with these of the subjects without response, by wave analysis and passive non-response analysis [52, 53].

A significance level of 0.05 two-sided will be used. Strong claims will be made only for the primary outcome in the primary analysis (i.e., primary objective). Secondary outcomes will be analyzed without correction for multiple testing.

SAS software (SAS System for Windows version 9.4, SAS Institute Inc, USA) will be used.

In case of missing data, first an attempt will be done to complete these data by reviewing the hospital files, contacting the parents again etc. Therefore, and because this is a prospective study in which the investigators will collect all data, we expect a low number of missing data, especially for the confounders and study characteristics. Participants/sibling pairs with missing data for the intelligence/neurodevelopmental outcomes will be included in the analyses and the number of missing data will be reported for each outcome.

Sample size

For the primary outcome, **assessment of the Wechsler intelligence quotient in 64 sibling pairs will allow to show non-inferiority of LEA in comparison with no LEA, with the inferiority margin being** 7.5 [being half the standard deviation and half the difference between the average IQ (100) and the clinical cut-off value for a decreased IQ (85)][12, 17, 54-56]. For this sample size calculation, a one-sided alpha of 0.025 and 80% power is used, it is expected that there will be no difference between the population average of exposed and unexposed children, and it is assumed that the standard deviation of the primary outcome will be comparable to that of a normative population of children (i.e., 15)[12, 17, 54-56]. While 64 sibling pairs are sufficient to show non-inferiority, all children agreeing to participate in the entire cohort will be included in the study to further increase the power of the study. Additionally, this sample size calculation assumes the worst-case scenario of absence of correlation between the intelligence quotient of siblings. As observing a certain correlation within these pairs would be likely, this would also further increase the power of the study.

8. Data handling

8.1 General data handling information

Data collection, handling and processing for the purpose of this Study will be performed in compliance with applicable regulations, guidelines for clinical studies and internal procedures. It remains the responsibility of the Investigator to check that all data relating to the Study, as specified in the Study protocol, are entered into the electronic Case Report Form ((e)CRF) in accordance with the instructions provided and that the forms are filled out accurately, completely and in a timely manner.

(e)CRFs are provided by the Sponsor for each participant. The Study data will be transcribed from the source records into an (e)CRF by Study Staff.

The (e)CRFs shall under no circumstances capture personal data such as but not limited to the participant or their relative(s) name, home address, contact details, full date of birth medical record number (e.g. UZ Leuven EAD number), social security number etc.

When the researchers (co-investigators) enter the data in RedCap, a record ID is generated, which is a unique pseudonymized code that is associated with a specific trial participant. In RedCap, no identifiers will be entered. On a secured departmental server, an Excel file will be stored, containing the identifiers required to contact the participants.

8.2 Study specific data handling information

Redcap will be used to capture study related data.

The following data will be collected in the (e)CRF :

Characteristics of study participants, recruitment information *(These data are required to describe the characteristics of the study participants and to contact and recruit the participants. The data will be obtained from the medical files, unless specified otherwise below.)*

- e-mail address (only visible for researchers involved in sending e-mails, *from medical file or phone calls*)
- Group (exposed/unexposed)
- Age group (2,5-6/7-13)
- Theoretical date of last menstruation
- Age child
- Age mother at delivery
- Gender child *(Required for adjustment for confounders)*
- Child deceased (yes/no) *(Required to avoid contacting parents of deceased children for intelligence testing. Also required to construct the flowchart of the included and excluded participants in the study.)*
- Additional notes
- Will participate/refuses to participate in study *(Obtained in phone calls)*
- Reason for refusal *(Obtained in phone calls)*
- Additional notes on answer during telephone call *(Obtained in phone calls)*
- All data is complete (yes/no)
- Exclusion of this patient (yes/no)

Pregnancy *(These data are required to adjust for confounders, as these factors could affect primary and secondary endpoints. These data will be obtained from the medical files.)*

- Partus history
- exposure of the mother during pregnancy to smoking
- exposure of the mother during pregnancy to alcohol
- exposure of the mother during pregnancy to drugs
- pregnancy diabetes
- pregnancy induced hypertension
- pre-eclampsia
- eclampsia
- premature rupture of membranes
- antepartum hemorrhage
- data complete (yes/no)

Delivery *(These data are required to describe the characteristics of the study participants. This is important as these factors could be a potential consequence of the exposure to LEA and could be a cause of impaired primary and secondary outcomes[57, 58]. These data will be obtained from the medical files.)*

- Gestational age at delivery *(Required for adjustment for confounders)*
- Birth weight *(Required for adjustment for confounders)*
- Mode of delivery (vaginal/caesarean section)
- Emergency caesarean section (yes/no)

- Type of labour analgesia (CSE/epidural/none)
- Duration of labour analgesia
- Duration of delivery
- Use of forceps or vacuum extraction
- Fetal presentation (cephalic versus all other presentations)
- Dystocia
- Birth trauma
- Umbilical cord compression
- Labour induction
- Neonatal resuscitation
- Admission to the neonatal intensive care unit after delivery
- Foetal pH at birth
- Apgar score at birth

Intelligence testing

- date when intelligence testing was completed
- neuraxial analgesia during delivery
- Did your child receive anaesthesia?
    - Type of anaesthesia (general/local)
- Remarks of parents
- Results of Wechsler intelligence score testing
    - Full-scale intelligence quotient
    - Primary index scores
        - verbal comprehension index
        - visual spatial index (WPPSI-IV and WISC-V)
        - working memory index
        - fluid reasoning index (WPPSI-IV, WISC-V)
        - processing speed index
- Result of the SRS questionnaire
    - Total score
    - Subscores
        - social awareness
        - social cognition
        - social communication
        - social motivation
        - restricted interests and repetitive behavior
- Result of the VvGK6-16 questionnaire
    - Inattention
    - hyperactivity and impulsivity
    - oppositional defiant disorder (ODD)
    - conduct disorder (CD)

Immediately before the start of the data analysis, the RedCap database will be exported to SAS software (SAS 9.4, SAS Institute Inc., Cary, NC, USA) for the statistical analysis.

8.3 Direct Data Access

The investigator(s) and the institution(s) will permit trial-related monitoring, audits, EC review, and regulatory inspections (where appropriate) by providing direct access to source data and other documents.

9. Ethical and Regulatory Considerations

9.1 Ethics Committee (EC) review & reports

Before the start of the Study, this protocol and other related documents will be submitted for review to the EC for Study authorization. The Study shall not commence until such approvals have been obtained and until other relevant essential Study documents, such as duly signed contract agreements, evidence of adequate Study financing etc. are in place.

9.2 Protocol / GCP compliance

The Study must be performed in accordance with the protocol, current ICH and ICH-GCP guidelines, and applicable regulatory and country-specific requirements. ICH guidelines are an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of Study participants are protected, consistent with the principles that originated in the most recent version of the Declaration of Helsinki, and that the Study data are credible, reliable and reproducible.

9.3 Data protection and participant confidentiality

The Study will be conducted in compliance with the requirements of the EU General Data Protection Regulation 2016/679 (GDPR), and the relevant Belgian laws implementing the GDPR including the Belgian Privacy Act of 30 July 2018 on the protection of privacy in relation to the processing of personal data. Any collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with the aforementioned personal data protection laws (cfr. Data Processing Annex (DPA) in Appendix).

Any personal data shall be treated as confidential at all times including during collection, handling and use or processing, and the personal data (including in any electronic format) shall be stored securely at all times and with all technical and organizational security measures that would be necessary for compliance with EU and national data protection legislation (whichever is more stringent). The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

10. Research Registration, Dissemination of Results and Publication Policy

The Declaration of Helsinki (latest version) and European and Belgian regulations require that every research Study involving human participants be registered in a publicly accessible database before recruitment of the first participant. The CI is responsible for registering the Study.

In addition, the CI will fulfil their ethical obligation to disseminate and make the research results publicly available. As such the CI is accountable for the timeliness, completeness and accuracy of the reports. Researchers, authors, Sponsors, editors and publishers must adhere to accepted guidelines for ethical reporting. Negative and inconclusive, as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in publication.

Publications will be coordinated by the CI. Authorship to publications will be determined in accordance with the requirements published by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

11. Insurance/Indemnity

In accordance with the Belgian Law relating to experiments on human persons dated May 7, 2004, Sponsor shall assume, even without fault, the responsibility of any damages incurred by a Study Patient and linked directly or indirectly to the participation to the Study, and shall provide compensation therefore through its insurance."

12. References

1 Eurostat. Fertility statistics. 2022. Available from https://ec.europa.eu/eurostat/databrowser/view/tps00204/default/table?lang=en 2022)
2 Chassard D, Langlois-Jacques C, Naaim M, et al. Anesthesia practices for management of labor pain and cesarean delivery in France (EPIDOL): A cross-sectional survey. *Anaesth Crit Care Pain Med* 2023; **42**: 101302
3 Devroe S, Bleeser T, Van de Velde M, et al. Anesthesia for non-obstetric surgery during pregnancy in a tertiary referral center: a 16-year retrospective, matched case-control, cohort study. *Int J Obstet Anesth* 2019; **39**: 74-81
4 Seijmonsbergen-Schermers AE, van den Akker T, Rydahl E, et al. Variations in use of childbirth interventions in 13 high-income countries: A multinational cross-sectional study. *PLoS Med* 2020; **17**: e1003103
5 Silbereis JC, Pochareddy S, Zhu Y, Li M, Sestan N. The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. *Neuron* 2016; **89**: 248-68
6 Qiu C, Lin JC, Shi JM, et al. Association Between Epidural Analgesia During Labor and Risk of Autism Spectrum Disorders in Offspring. *JAMA Pediatr* 2020; **174**: 1168-75
7 Straub L, Huybrechts KF, Mogun H, Bateman BT. Association of Neuraxial Labor Analgesia for Vaginal Childbirth With Risk of Autism Spectrum Disorder. *JAMA Netw Open* 2021; **4**: e2140458
8 Ren T, Zhang J, Yu Y, et al. Association of labour epidural analgesia with neurodevelopmental disorders in offspring: a Danish population-based cohort study. *Br J Anaesth* 2022; **128**: 513-21
9 Murphy MSQ, Ducharme R, Hawken S, et al. Exposure to Intrapartum Epidural Analgesia and Risk of Autism Spectrum Disorder in Offspring. *JAMA Netw Open* 2022; **5**: e2214273
10 Hegvik TA, Klungsøyr K, Kuja-Halkola R, et al. Labor epidural analgesia and subsequent risk of offspring autism spectrum disorder and attention-deficit/hyperactivity disorder: a cross-national cohort study of 4.5 million individuals and their siblings. *Am J Obstet Gynecol* 2023; **228**: 233.e1-.e12
11 Qiu C, Carter SA, Lin JC, et al. Association of Labor Epidural Analgesia, Oxytocin Exposure, and Risk of Autism Spectrum Disorders in Children. *JAMA Netw Open* 2023; **6**: e2324630
12 Hu D, Flick RP, Zaccariello MJ, et al. Association between Exposure of Young Children to Procedures Requiring General Anesthesia and Learning and Behavioral Outcomes in a Population-based Birth Cohort. *Anesthesiology* 2017; **127**: 227-40
13 Wilder RT, Flick RP, Sprung J, et al. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. *Anesthesiology* 2009; **110**: 796-804
14 Flick RP, Katusic SK, Colligan RC, et al. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. *Pediatrics* 2011; **128**: e1053-61
15 Bong CL, Allen JC, Kim JT. The effects of exposure to general anesthesia in infancy on academic performance at age 12. *Anesth Analg* 2013; **117**: 1419-28
16 Glatz P, Sandin RH, Pedersen NL, Bonamy AK, Eriksson LI, Granath F. Association of Anesthesia and Surgery During Childhood With Long-term Academic Performance. *JAMA Pediatr* 2017; **171**: e163470
17 Backeljauw B, Holland SK, Altaye M, Loepke AW. Cognition and Brain Structure Following Early Childhood Surgery With Anesthesia. *Pediatrics* 2015; **136**: e1-12
18 Maenner MJ, Warren Z, Williams AR, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. *MMWR Surveill Summ* 2023; **72**: 1-14
19 Mouga S, Café C, Almeida J, Marques C, Duque F, Oliveira G. Intellectual Profiles in the Autism Spectrum and Other Neurodevelopmental Disorders. *J Autism Dev Disord* 2016; **46**: 2940-55

20 Kanai C, Hashimoto R, Itahashi T, et al. Cognitive profiles of adults with high-functioning autism spectrum disorder and those with attention-deficit/hyperactivity disorder based on the WAIS-III. *Res Dev Disabil* 2017; **61**: 108-15
21 Takeda T, Nakashima Y, Tsuji Y. Discrepancies in Wechsler Adult Intelligent Scale III profile in adult with and without attention-deficit hyperactivity disorder. *Neuropsychopharmacol Rep* 2020; **40**: 166-74
22 Jin WY, Wu LL, Hu LF, et al. Intelligence profiles and adaptive behaviors of high-functioning autism spectrum disorder and developmental speech and language disorders. *Front Pediatr* 2022; **10**: 972643
23 Rommelse N, Langerak I, van der Meer J, et al. Intelligence May Moderate the Cognitive Profile of Patients with ASD. *PLoS One* 2015; **10**: e0138698
24 Thapar A, Cooper M, Rutter M. Neurodevelopmental disorders. *Lancet Psychiatry* 2017; **4**: 339-46
25 Zwicker J, Lamsal R. Evaluating the Economic Impact of Neurodevelopmental Disability Intervention on the Family and Community. In: Zwicker J, ed. *Neurodevelopmental Pediatrics*: Springer, 2023; 773–88
26 Sun S, Kuja-Halkola R, Faraone SV, et al. Association of Psychiatric Comorbidity With the Risk of Premature Death Among Children and Adults With Attention-Deficit/Hyperactivity Disorder. *JAMA Psychiatry* 2019; **76**: 1141-9
27 Aro T, Eklund K, Eloranta AK, Närhi V, Korhonen E, Ahonen T. Associations Between Childhood Learning Disabilities and Adult-Age Mental Health Problems, Lack of Education, and Unemployment. *J Learn Disabil* 2019; **52**: 71-83
28 Rogge N, Janssen J. The Economic Costs of Autism Spectrum Disorder: A Literature Review. *J Autism Dev Disord* 2019; **49**: 2873-900
29 Zeidan J, Fombonne E, Scorah J, et al. Global prevalence of autism: A systematic review update. *Autism Res* 2022; **15**: 778-90
30 Olesen J, Gustavsson A, Svensson M, Wittchen HU, Jönsson B. The economic cost of brain disorders in Europe. *Eur J Neurol* 2012; **19**: 155-62
31 De Ridder T, Bruffearts R, Danckaerts M, Bonnewyn A, Demyttenaere K. [The prevalence of ADHD in the Belgian general adult population: an epidemiological explanatory study]. *Tijdschr Psychiatr* 2008; **50**: 499-508
32 Hung KC, Chen JY, Hsing CH, et al. Association of labor epidural analgesia exposure with long-term risk of autism spectrum disorder in offspring: A meta-analysis of observational studies. *Autism* 2023; **27**: 1177-89
33 Wang X, Li J, Liu D. Effects of epidural analgesia exposure during parturition on autism spectrum disorder in newborns: A systematic review and meta-analysis based on cohort study. *Front Psychiatry* 2022; **13**: 974596
34 Hawkins JL. Epidural analgesia for labor and delivery. *N Engl J Med* 2010; **362**: 1503-10
35 Devroe S, Rex S, Lucas N. Unlocking maternal health: labour epidurals and severe morbidity. *Bmj* 2024; **385**: q1053
36 Kearns RJ, Kyzayeva A, Halliday LOE, Lawlor DA, Shaw M, Nelson SM. Epidural analgesia during labour and severe maternal morbidity: population based study. *Bmj* 2024; **385**: e077190
37 Wang TT, Sun S, Huang SQ. Effects of Epidural Labor Analgesia With Low Concentrations of Local Anesthetics on Obstetric Outcomes: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Anesth Analg* 2017; **124**: 1571-80
38 Anim-Somuah M, Smyth RM, Cyna AM, Cuthbert A. Epidural versus non-epidural or no analgesia for pain management in labour. *Cochrane Database Syst Rev* 2018; **5**: Cd000331
39 Kearns RJ, Shaw M, Gromski PS, Iliodromiti S, Lawlor DA, Nelson SM. Association of Epidural Analgesia in Women in Labor With Neonatal and Childhood Outcomes in a Population Cohort. *JAMA Netw Open* 2021; **4**: e2131683

40 Hanley GE, Bickford C, Ip A, et al. Association of Epidural Analgesia During Labor and Delivery With Autism Spectrum Disorder in Offspring. *Jama* 2021; **326**: 1178-85
41 Mikkelsen AP, Greiber IK, Scheller NM, Lidegaard Ø. Association of Labor Epidural Analgesia With Autism Spectrum Disorder in Children. *Jama* 2021; **326**: 1170-7
42 Wall-Wieler E, Bateman BT, Hanlon-Dearman A, Roos LL, Butwick AJ. Association of Epidural Labor Analgesia With Offspring Risk of Autism Spectrum Disorders. *JAMA Pediatr* 2021; **175**: 698-705
43 Isik OG, Junaid S, Guo L, et al. Behavioural and neuropsychological outcomes in children exposed in utero to maternal labour epidural analgesia. *Br J Anaesth* 2024
44 Sim MA, Shen L, Ti LK, et al. Association between maternal labour epidural analgesia and autistic traits in offspring. *J Clin Anesth* 2023; **89**: 111162
45 Fang LL, Zhou YY, Jiang HY, Shi YD. Labor epidural analgesia and risk of autism Spectrum disorders in offspring: A systematic review and meta-analysis. *Front Pediatr* 2022; **10**: 965205
46 Flick RP, Lee K, Hofer RE, et al. Neuraxial labor analgesia for vaginal delivery and its effects on childhood learning disabilities. *Anesth Analg* 2011; **112**: 1424-31
47 Shima M, Tokuda N, Hasunuma H, et al. Association of epidural analgesia during labor with neurodevelopment of children during the first three years: the Japan Environment and Children's Study. *Environ Health Prev Med* 2022; **27**: 37
48 Walkden GJ, Pickering AE, Gill H. Assessing Long-term Neurodevelopmental Outcome Following General Anesthesia in Early Childhood: Challenges and Opportunities. *Anesth Analg* 2019; **128**: 681-94
49 Rosenbaum P, Rubin D. The central role of the propensity score in observational studies for causal effects. *Biometrika* 1983; **70**: 41-55
50 Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res* 2011; **46**: 399-424
51 Curtis LH, Hammill BG, Eisenstein EL, Kramer JM, Anstrom KJ. Using inverse probability-weighted estimators in comparative effectiveness analyses with observational databases. *Med Care* 2007; **45**: S103-7
52 Halbesleben JR, Whitman MV. Evaluating survey quality in health services research: a decision framework for assessing nonresponse bias. *Health Serv Res* 2013; **48**: 913-30
53 Phillips AW, Reddy S, Durning SJ. Improving response rates and evaluating nonresponse bias in surveys: AMEE Guide No. 102. *Med Teach* 2016; **38**: 217-28
54 Gano D, Andersen SK, Glass HC, et al. Impaired cognitive performance in premature newborns with two or more surgeries prior to term-equivalent age. *Pediatr Res* 2015; **78**: 323-9
55 McCann ME, de Graaff JC, Dorris L, et al. Neurodevelopmental outcome at 5 years of age after general anaesthesia or awake-regional anaesthesia in infancy (GAS): an international, multicentre, randomised, controlled equivalence trial. *Lancet* 2019; **393**: 664-77
56 Warner DO, Zaccariello MJ, Katusic SK, et al. Neuropsychological and Behavioral Outcomes after Exposure of Young Children to Procedures Requiring General Anesthesia: The Mayo Anesthesia Safety in Kids (MASK) Study. *Anesthesiology* 2018; **129**: 89-105
57 Callahan EC, Lee W, Aleshi P, George RB. Modern labor epidural analgesia: implications for labor outcomes and maternal-fetal health. *Am J Obstet Gynecol* 2023; **228**: S1260-s9
58 Halliday L, Kinsella M, Shaw M, Cheyne J, Nelson SM, Kearns RJ. Comparison of ultra-low, low and high concentration local anaesthetic for labour epidural analgesia: a systematic review and network meta-analysis. *Anaesthesia* 2022; **77**: 910-8