STOPPIT-M

Study Protocol

Infant hypothalamic-pituitary-adrenal axis responses following antenatal corticosteroids and perinatal outcomes: a mechanism of action of health intervention study (STOPPIT-M)

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LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development - Joint office for The University of Edinburgh and Lothian Health Board
ACS	Antenatal Corticosteroids
CI	Chief Investigator
CRF	Case Report Form
GCP	Good Clinical Practice
HPA	Hypothalamic-pituitary-adrenal
ICH	International Conference on Harmonisation
NNU	Neonatal Unit
PI	Principal Investigator
QA	Quality Assurance
REC	Research Ethics Committee
SOP	Standard Operating Procedure

1 INTRODUCTION

1.1 BACKGROUND

The use of antenatal corticosteroids (ACS) in late preterm (35-36 weeks gestation) and early term (37 weeks gestation) infants is an area of substantial controversy in obstetrics (1-3). There is evidence that ACS reduce serious respiratory morbidity as well as short term neonatal unit (NNU) admissions in late preterm and early term born infants (4). However, the numbers needed to treat to prevent serious respiratory morbidity are high (5), and there is potential for both short (e.g. neonatal hypoglycaemia) and long-term harms (e.g. effects on neurodevelopment) (6). Practice is thus highly variable regarding ACS administration at late preterm and early term gestations.

The 2019 NICE guidelines recommend that twins are born in the late preterm and early term period (7). Despite limited data about effectiveness in twins, ACS are widely given to women with twin pregnancy having planned birth by induction of labour or caesarean section at these gestations. These slightly earlier, non-spontaneous births are at increased risk of respiratory, metabolic and haemodynamic morbidities leading to need for NNU admission. The main trial STOPPIT-3 (Funding reference: NIHR131352, Sponsor reference: AC21118) is a randomised controlled

trial (RCT) which will address the uncertainty regarding the effectiveness of ACS prior to planned birth of twins. The trial will determine the effect of ACS on neonatal morbidity: the primary outcome is the need for respiratory support within 72 hours of birth, and secondary outcomes include other neonatal morbidities and safety outcomes including severe respiratory morbidity, NNU admission, hypoglycaemia and Apgar score.

The STOPPIT-M trial will explore the mechanisms underlying ACS effects on the infant's endogenous hypothalamic-pituitary-adrenal (HPA) axis activity (i.e. the infant's own stress response regulatory system), and resultant effects on neonatal outcomes. Whilst we are studying the mechanisms of actions in twins, the findings will be relevant to both twin and singleton pregnancies.

The HPA axis plays vital regulatory physiological roles related to fetal maturation, metabolism, immune regulation, and coordination of the stress response (8). Endogenous glucocorticoids in the fetal circulation increase rapidly late in gestation to promote the maturation of fetal organs including the lungs, heart/cardiovascular system, kidney and brain. Throughout development, fetal exposure to maternal cortisol is tightly regulated, in part through activity of the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2) which converts cortisol to its inactive metabolite cortisone, as well as through active transport of cortisol from fetus to mother via ABC transporters (9).

A number animal and human studies have shown that the fetal HPA axis is highly sensitive to glucocorticoids with consequences for postnatal functioning (10). This may occur in part through alterations of brain regions that are both integral to the regulation of stress responses and vulnerable to exposure to glucocorticoid hormones. Vulnerable regions such as the hippocampus and amygdala undergo rapid development during fetal life. The predominant glucocorticoid, cortisol, has affinity to two receptors, glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). Both receptors types are present in the human hippocampus by 24 gestational weeks (11).

We and others have demonstrated that exposure to high levels of endogenous glucocorticoids in utero is associated with increased risk for disorders of HPA axis function later in life, as well as cardiovascular, reproductive and metabolic abnormalities (12).

ACS are administered to promote organ maturation in preterm birth. However, as an infant in the late preterm/early term stage of gestation may already have more mature organs, the potential harms of ACS administration may outweigh any possible benefits. Dexamethasone has pharmacological properties that differ from endogenous glucocorticoids. Dexamethasone is a fluorinated corticosteroid, more potent than cortisol, but also resistant to metabolism by HSD2, so able to cross the placenta and enter the fetal circulation, triggering GR-mediated signalling in the developing fetus.

Emerging data from animal and humans suggests there may be sex differences in HPA responses to exposures in utero, with increased vulnerability in females (13).

There are well documented placental differences between sexes and some data showing sexually dimorphic responses to ACS (14). Studying responses in twins has advantages to further explore potential sex differences in HPA responses whilst controlling for a common in utero environment.

This research fills a demonstrable evidence gap as the mechanisms of action of ACS in late preterm/early term babies are not well understood. Understanding mechanisms is key to be able to better target treatments in future. Understanding which mothers and babies are likely to benefit from ACS, and which do not require them and/or maybe more vulnerable of metabolic and neurodevelopmental side effects, is particularly important in the late preterm period where the number to treat for benefit in terms of serious respiratory morbidity is high.

Maturation of the infant's own stress response system (HPA axis activity and endogenous cortisol production) around the time of birth is a key driver of organ maturity in preparation for ex utero life. Exogenous, synthetic ACS mimic some of the effects of endogenous cortisol, hence reducing respiratory morbidity and other problems associated with organ immaturity (e.g. temperature regulation; glucose homeostasis). However, paradoxically, high doses of exogenous synthetic ACS (which cross the placenta) may suppress or dysregulate normal endogenous cortisol production at a crucial time, leading to increased morbidities.

We hypothesise that direct effects of ACS on the developing fetal HPA axis is a mechanism leading to infant HPA axis activity suppression with impaired glucocorticoid release, signalling and altered HPA axis maturation. This results in a reduced ability to mount an appropriate glucocorticoid 'stress' response in the early postnatal period and beyond.

1.2 RATIONALE FOR STUDY

Our proposed study aims to address the question 'Does infant hypothalamic-pituitary-adrenal (HPA) axis activity underpin respiratory morbidity and responses to antenatal corticosteroids (ACS) in later preterm and early term infants?'

The use of ACS in late preterm (35-36 weeks gestation) and early term (37 weeks gestation) is an area of substantial controversy in obstetrics. There is evidence that ACS reduce serious respiratory morbidity and neonatal unit admission but there is potential for both short (e.g. hypoglycaemia) and long-term harms (e.g. neurodevelopment) in some exposed babies. Practice is highly variable regarding ACS administration at later preterm and early term gestations.

STOPPIT-3 is a multicentre placebo-controlled trial to evaluate the effectiveness of ACS (dexamethasone phosphate) prior to planned birth of twins in an NHS setting. The primary outcome is respiratory support within 72 hours of birth. We propose a nested cohort study to explore the mechanisms underlying ACS effects on the infant's endogenous HPA axis responses, and resultant effects on neonatal outcomes.

We aim to compare the HPA axis in infants with

i) early neonatal morbidity (need for respiratory support; low APGAR score; hypoglycaemia; jaundice), in babies exposed and non-exposed to ACS (after unblinding)

ii) developmental delay aged 2 years

A secondary aim is to explore sex-specific effects given observational data suggesting that there is sexual dimorphism in responses to ACS and in neonatal outcomes per se.

Gaining a better understanding of the mechanisms of action of ACS is essential to improve targeted delivery of ACS – ensuring administration to mothers and babies who will benefit from ACS, but avoiding harm in those who will not benefit. The results will be communicated to the scientific community via publication and conference presentation alongside the main STOPPIT-3 trial, and to the public via links with charity partners, the University of Edinburgh press office, and social media.

An internal pilot phase is planned for the main STOPPIT-3 trial for the first 10 months of recruitment. This aims to recruit 159 women and will enable us to refine any protocols for recruitment to the mechanistic study and biological sample collection and will also enable us to demonstrate that sample quality is suitable for laboratory analyses.

During biological sample collection and laboratory analyses, we will remain blinded to treatment group.

2 STUDY OBJECTIVES

2.1 OBJECTIVES

2.1.1 Primary Objective

To understand whether the infant hypothalamic-pituitary-adrenal (HPA) axis activity underpins respiratory morbidity and responses to antenatal corticosteroids (ACS) in later preterm and early term infants.

Secondary Objectives

To understand whether there are sex-specific effects in responses to ACS and neonatal outcomes.

2.2 ENDPOINTS

2.2.1 Primary Endpoint

To compare the HPA axis in infants with

i) early neonatal morbidity (need for respiratory support; low APGAR score; hypoglycaemia; jaundice), in babies exposed and non-exposed to ACS (after unblinding)

ii) developmental delay aged 2 years (as part of the main trial)

To assess the primary end point, paired maternal and cord blood samples, amniotic fluid and placenta collected at delivery for measurement of

i) endogenous glucocorticoids, dexamethasone and its metabolites (glucocorticoid release and response to ACS)

ii) glucocorticoid receptor in cord blood leukocytes (glucocorticoid signalling)

iii) placental genes regulating glucocorticoids (glucocorticoid metabolism and transfer)

iv) in a subset of infants in Edinburgh

a. salivary cortisol pre and post stressor event (glucocorticoid stress response)

b. hair cortisol (overall glucocorticoid secretion)

3 STUDY DESIGN

Observational/mechanistic study linked to the multicentre, double-blinded, randomised, placebo-controlled STOPPIT-3 trial of ACS for planned birth of twins. Paired maternal and cord blood samples, amniotic fluid and placenta will be collected at delivery using standardised protocols from women undergoing elective caesarean section.

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

We plan to recruit at least 543 women pregnant with twins enrolled in the STOPPIT-3 trial.

4.2 INCLUSION CRITERIA

- Women with a twin pregnancy with a planned caesarean birth scheduled between 35+0 and 38+6 weeks gestation who are enrolled in STOPPIT-3.

- Women aged 16 years or older and able to provide electronic or written consent

4.3 EXCLUSION CRITERIA

- Women taking prescribed corticosteroid medication (orally, injected, inhaled or topical) within the last 3 months.

- Women with planned vaginal birth.

4.4 CO-ENROLMENT

Women who take part in STOPPIT-M will be enrolled in STOPPIT-3 which is a CTIMP. Co-enrolment must follow the STOPPIT-3 protocol which states that co-enrolment in another study (either non-CTIMP or CTIMP) will be considered in line with the sponsor's policy on co-enrolment.

Co-enrolment in STOPPIT-M and another non-interventional research study (for example, sample only or questionnaire studies) is permitted and this does not require any formal written documentation.

Co-enrolment in STOPPIT-M and a CTIMP or interventional non-CTIMP (for example, diagnostic, device or surgical interventions) are permitted provided an assessment on the safety of study participants, interventions involved, participant burden and the potential impact on the study endpoints have been considered. This assessment will be performed and documented in line with the Sponsor policy on co-enrolment, and included within the site initiation visit (SIV) training during site set up.

5 PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

Women who agree to consent to participate in STOPPIT-3 and in whom birth is planned by elective caesarean section will be asked if they would like to participate in STOPPIT-M.

5.2 CONSENTING PARTICIPANTS

Women approached about the study will be given adequate time to read the patient information sheet. A summary booklet can be given at an appropriate time then the full participant information leaflet should be give once a birth plan in in place.. If the woman waives this opportunity for early information but still wishes to participate, consent may be taken after a shorter time interval. Women will then be asked to complete a consent form (electronically or on paper. Consent will be taken by a member of the maternity care (This may be a doctor, Research Midwife/ Nurse or suitably trained individual as delegated by the local PI). Details of the consent process, including the name of the

doctor confirming eligibility will be recorded in the woman's medical records.

Consent can be given on paper or electronically. If consent is given on paper, the original written consent form(s) will be stored in the Investigator Site File (ISF) file, a copy will be given to the woman and a copy added to the medical notes. Allowance for consent to be taken electronically has been included in the protocol. The software to be used for this will be reviewed and agreed with the sponsor, and necessary amendment approvals sought where required.

5.2.1 Withdrawal of Study Participants

Participants are free to withdraw from the study at any point or a participant can be withdrawn by the Investigator. If withdrawal occurs, the primary reason for withdrawal

will be documented in the participant's eCRF and medical records. The participant will have the option of withdrawal from:

i) all aspects of the trial but continued use of data and samples collected up to that point. To safeguard rights, the minimum personally-identifiable information possible will have been collected through the STOPPIT-3 trial
ii) all aspects of the trial with samples destroyed but continued use of data collected up to that point of withdrawal.

To safeguard rights, the minimum personally-identifiable information possible will have been collected through the STOPPIT-3 trial. Data on the participant and babies' outcomes will be requested through the STOPPIT-3 trial and retained on the database, if the participant agrees to this.

6 STUDY ASSESSMENTS

6.1 STUDY ASSESSMENTS

There are no study assessments planned.

The site clinical team will be asked to take the following samples at delivery of twins:

- Paired maternal and cord blood samples (about 10mls)
- Amniotic fluid sample
- Placenta sample

In a subset of infants, in Edinburgh, the following samples will be taken:

- a saliva sample
- a hair sample

6.2 LONG TERM FOLLOW UP ASSESSMENTS

There is no long term follow up planned in STOPPIT-M. Follow up will be conducted through the STOPPIT-3 trial. We anticipate the data collected in the main trial will be available for us to use in the data analyses for STOPPIT-M. We will outline the use of these data in a separate data management plan.

6.3 STORAGE AND ANALYSIS OF SAMPLES

Staff with appropriate training will collect maternal and umbilical cord blood (about 10mls), amniotic fluid and placenta at the time of delivery. Blood samples will be collected in EDTA vials and centrifuged within two hours of collection. Blood and amniotic fluid will be stored at −80 °C until analysis.

We will use our established robust protocols for placental biopsy collection, tissue should be stored in RNA later for 24-72 hours in a 4-8°C fridge before discarding the RNA later and transfer tissue into microtubes and store at −80 °C until analysis.

In a subset of infants in Edinburgh (n=100), we will collect saliva samples before and after a stressor event. This can include but not limited to a physical exam, a blood glucose test or a heel-prick blood test (typically conducted at aged 5 days), using methods that we have established in our follow up studies of preterm infants. We will also collect a hair sample for measurement of cortisol using established methods in the neonatal period.
Corticosteroid concentrations: Laboratory analyses of corticosteroids in blood, amniotic fluid, saliva and placenta will be conducted at the University of Edinburgh Clinical Research Facility Mass Spectrometry Core. We have developed a robust method for plasma steroid extraction from plasma (100 µL) and tissues (15), with quantification of cortisol and related corticosteroids including cortisone, as well as dexamethasone and its metabolites, simultaneously by liquid chromatography tandem
mass spectrometry (LC-MS/MS), using a Sciex QTRAP® 6500 (Warrington, UK) operated in positive ion electrospray ionisation, with a Waters Acquity™ UPLC system (Manchester, UK).

Glucocorticoid signalling: RNA will be extracted from leukocytes and GR quantified by RT-PCR using well established methods.

Placental analyses of glucocorticoid regulating genes: mRNA levels of genes regulating glucocorticoid metabolism (including HSD2, HSD1, MR, GR, ABCB1, ABCC1) will be carried out using well established protocols (16). Briefly, total RNA will be extracted from placental tissue using QIAGEN RNeasy mini kits (Qiagen Ltd, West Sussex, UK). The RNA concentration and purity of all samples will be assessed using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, UK) and the integrity of RNA confirmed by separating ribosomal RNA (rRNA) using electrophoresis. cDNA synthesis will be carried out using the Access RT-PCR system (Promega, Southampton, UK). cDNA will be incubated in triplicate with gene-specific primers and fluorescent probes for each gene in RocheLightCycler 480 Probes mastermix using established methods (16). PCR cycling and detection of fluorescent signal will be carried out using a Roche LightCycler 480. A standard curve will be constructed for each primer-probe set using a serial dilution of cDNA pooled from all samples. Results will be corrected to the control gene TATA-binding

protein (TBP). Hair cortisol: will be measured by LC-MS/MS using our well established methods.

7 DATA COLLECTION

All other data collection for the mechanistic study will be done through the infrastructure of the main STOPPIT-3 trial.

STOPPIT-M may use the following data collected through the STOPPIT-3 trial:

- Screening/randomisation number (Study ID)
- Dosing of IMP in STOPPIT-3 – date and time of first and second doses
- Occurrence of any adverse events/SAEs (including description and duration)

- Medical history
- Obstetric history
- Estimated date of delivery
- Chorionicity
- Date of delivery
- Any concomitant therapy
- Reason for discontinuation/withdrawal, if applicable
- Maternal age at birth of twins
- Ethnicity
- Height and weight at pregnancy booking
- Smoking, alcohol and substance use at booking
- Obstetric history (parity, previous mode of birth)
- Medical conditions (hypertension, respiratory disease, cardiac disease)
- Pregnancy conditions (pre-eclampsia), medications (antihypertensives, steroids; oral/topical/inhaled)
- Previous ACS
- Twin complications (if there was ever suspected twin to twin transfusion syndrome [yes/no and Quintero stage]
- Fetoscopic laser ablation of placental anastomoses in pregnancy
- suspected twin anaemia-polycythaemia sequence
- Indication for scheduled birth and mode of birth of each twin.

Clinical data will be collected from case records and entered on a eCRF (REDCap database). We will outline the use of these data in a separate data management plan.

In the STOPPIT-3 trial, the trial data will be collected by members of the study team delegated by the PI.

Data will be reviewed regularly by the Central Trial Team for completeness and data queries sent for missing information.

7.1 Source Data Documentation

Source documents are those in which information is recorded and documented for the first time.

For each participant, the Investigator will indicate in the medical source records that the participant is in this trial and the date of obtaining the informed consent.

All source data for the mechanistic study will be done through the infrastructure of the main STOPPIT-3 trial. As participants are required to be in the main trial for recruitment for this trial, the only source data documentation we will obtain will be:

- Documentation of signed and dated Informed Consent
- Reason for discontinuation/withdrawal, if applicable

For withdrawals, all available e-CRF data should be monitored and source data verified. Source data verification (SDV) will be handled the same way for withdrawn patients as for completed patients.

7.2 Case Report Forms

An e-CRF system (REDCap) will be used for data capture. The system is validated and access at all levels to the system is granted/revoked following the ECTU's procedures, in accordance with regulatory and system requirements.

Data should be entered into the eCRF timely after the data becomes available, as applicable. Data will be entered by research staff at study sites.

Errors occurring in the e-CRF will be corrected electronically. Such corrections/modifications will be automatically tracked by an audit trail detailing the date and time of the correction and the name of the person making the correction.

8 DATA MANAGEMENT

8.1.1 Personal Data

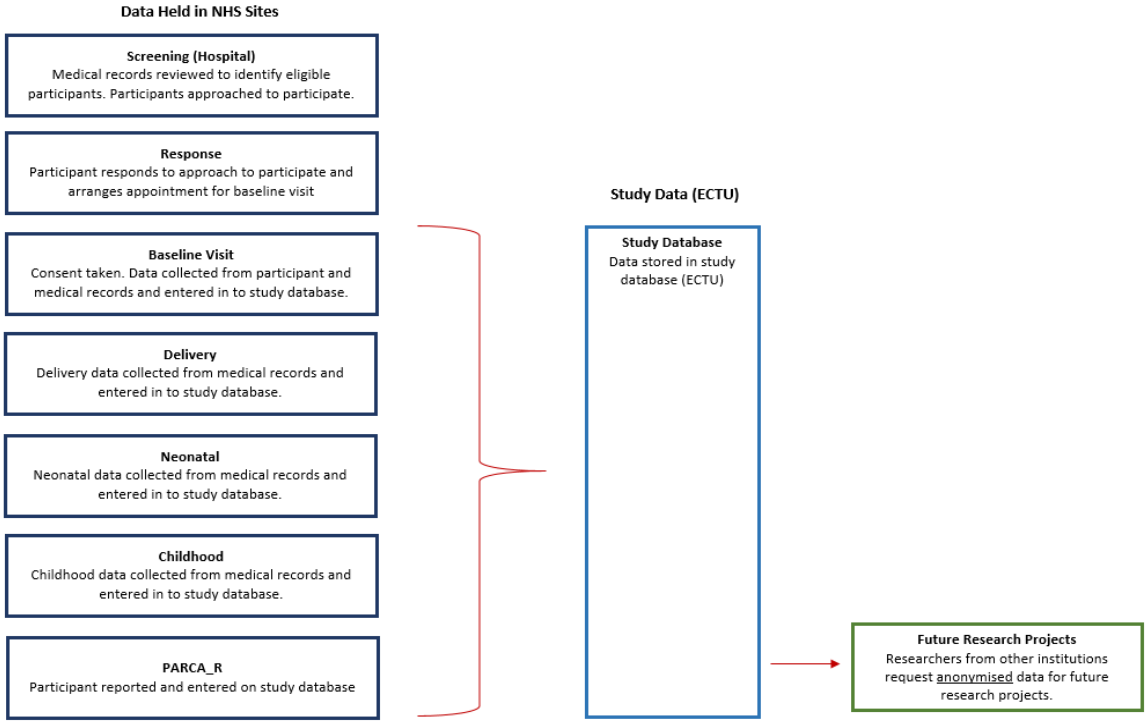
Personal data will be collected as part of the **main STOPPIT-3 trial for which consent will be obtained from participants through this trial.**

STOPPIT-M will not collect any further personal data however, we will use data such as age and pregnancy details for the analysis.

Personal data collected as part of the STOPPIT-3 trial will be stored by the research team in the Edinburgh Clinical Trials Unit, The University of Edinburgh for a minimum of 25 years. Study reports will contain only summary data. Identifiable data will not be released to any third party.

8.1.2 Data Information Flow

The collection, use and deletion of personal data is shown in the example diagram below.



8.1.3 Transfer of Data

Data collected or generated by the study (including personal data collected through the main STOPPIT-3 trial) will not be transferred to any external individuals or organisations outside of the Sponsoring organisation(s) as part of this study.

The dataset will be retained and access may be given to other researchers from other institutions providing appropriate approvals are in place.

Personal data collected as part of the main STOPPIT-3 trial will be retained (with consent from the main STOPPIT-3 trial) for use in future studies into the long term outcomes of ACS. It is necessary to keep personal information for both mother and baby to allow record linking of trial data (treatment group) to long term outcomes (NHS records/school records). STOPPIT-M will used linked anonymised data from STOPPIT-3 in our analysis, Any future studies would be subject to separate funding, and the relevant research governance approvals would be obtained.

The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site)

8.1.4 Data Controller

A data controller is an organisation that determines the purposes for which, and the manner in which, any personal data are processed.

The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site)

8.1.5 Data Breaches

Any data breaches will be reported to the University of Edinburgh and NHS Lothian Data Protection Officers who will onward report to the relevant authority according to the appropriate timelines if required.

9 STATISTICS AND DATA ANALYSIS

9.1 SAMPLE SIZE CALCULATION

Our data indicate 70% (n=1086) of the STOPPIT-3 sample (n=1552) will have elective caesarean section and be eligible for inclusion. The study is powered assuming samples are collected from ~50% of eligible women.

9.2 PROPOSED ANALYSES

A comprehensive statistical analysis plan will be finalised before the data are released from the main study for the final analysis of the mechanistic sub-studies.

Statistical significance will be at the 5% level with corresponding 95% confidence intervals (CI) presented. Randomised groups of participants in the mechanistic study will be described at baseline and follow-up using mean (SD), median (IQR) and counts (with percentages) as appropriate and data will be compared with characteristics of eligible participants who declined to participate, if relevant.

For the primary outcome of differences in cord blood cortisol concentrations we will test for differences between:

1) infants with respiratory morbidities within 72 hours after birth compared with those who do not;

2) infants with other neonatal morbidities within 72 hours after birth compared with those who do not;

3) infants exposed to ACS compared to non-exposed (mechanism of response to ACS);

4) females compared to males. We will adjust for gestation of birth, recruiting centre and chorionicity with linear regression.

To account for the clustering effect within twin pairs a random effects regression model will be used by fitting the mother as a random effect. Recruiting centre will also be included as an additional random effect.

For the prognostic modelling, we will use a mixed effects logistic regression model (e.g. on the outcome of respiratory distress within 72 hours (Y/N); or for cause specific or non-cause specific neonatal unit admission (Y/N). The above covariates (gestational age at birth, chorionicity) and other pre-specified baseline factors will be included, and the incremental role of cord-blood cortisol will be explored. Full details, including any adaptions in the light of the internal pilot study, will be specified in the Statistical Analysis Plan.

10 ADVERSE EVENTS

No adverse events are expected from this study.

11 OVERSIGHT ARRANGEMENTS

11.1 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit trial related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

11.2 STUDY MONITORING AND AUDIT

The ACCORD Sponsor Representative will assess the study to determine if an independent risk assessment is required. If required, the independent risk assessment will be carried out by the ACCORD Quality Assurance Group to determine if an audit should be performed before/during/after the study and, if so, at what frequency.

Risk assessment, if required, will determine if audit by the ACCORD QA group is required. Should audit be required, details will be captured in an audit plan. Audit of Investigator sites, study management activities and study collaborative units, facilities and 3rd parties may be performed.

12 GOOD CLINICAL PRACTICE

12.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).

Before the study can commence, all required approvals will be obtained and any conditions of approvals will be met.

12.2 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

12.2.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person, and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s).

The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant will receive a copy of this document and a copy filed in the Investigator Site File (ISF) and participant's medical notes (if applicable).

12.2.2 Study Site Staff

The Investigator must be familiar with the protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the protocol and their trial related duties.

12.2.3 Data Recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site.

12.2.4 Investigator Documentation

The Principal Investigator will ensure that the required documentation is available in local Investigator Site files ISFs.

12.2.5 GCP Training

For non-CTIMP (i.e. non-drug) studies all researchers are encouraged to undertake GCP training in order to understand the principles of GCP. However, this is not a mandatory requirement unless deemed so by the sponsor. GCP training status for all investigators should be indicated in their respective CVs.

12.2.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose

other than performance of the study, any data, record, or other unpublished information, which is confidential or identifiable, and has been disclosed to those individuals for the purpose of the study.. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

12.2.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the appropriate data protection legislation (including the General Data Protection Regulation and Data Protection Act) with regard to the collection, storage, processing and disclosure of personal information.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data and be of a form where individuals are not identified and re-identification is not likely to take place

STUDY CONDUCT RESPONSIBILITIES

12.3 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator and NIHR.

Amendments will be submitted to a sponsor representative for review and authorisation before being submitted in writing to the appropriate REC, and local R&D for approval prior to participants being enrolled into an amended protocol.

12.4 MANAGEMENT OF PROTOCOL NON COMPLIANCE

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, and local R&D for review and approval if appropriate.

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to

the sponsor within 3 days of becoming aware of the violation. All protocol deviation logs and violation forms should be emailed to QA@accord.scot

Deviations and violations are non-compliance events discovered after the event has occurred. Deviation logs will be maintained for each site in multi-centre studies. An alternative frequency of deviation log submission to the sponsors may be agreed in writing with the sponsors.

12.5 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (seriousbreach@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to research ethics committees as necessary.

12.6 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 25 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

12.7 END OF STUDY

The end of study is defined as the last participant's sample collection.

The Investigators or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, and R+D Office(s) and co-sponsors within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the co-sponsors via email to resgov@accord.scot

A summary report of the study will be provided to the REC within 1 year of the end of the study.

12.8 CONTINUATION OF TREATMENT FOLLOWING THE END OF STUDY

This is not applicable as no intervention within the mechanistic trial.

12.9 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused

by poor protocol design by the Chief Investigator and researchers employed by the University.

- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's National Health Service will have the benefit of NHS Indemnity.
- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.

13 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

13.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared in accordance with ICH guidelines. The Co-applicants on the grant will be named as authors and wider members of the main STOPPIT-3 trial will be invited to be co-authors if they have contributed to recruitment to STOPPIT-M.

The STOPPIT publication and dissemination policy describes how trial outputs will be managed, reviewed and disseminated. Investigators have the right to publish orally or in writing the results of the study trial, but must do so in accordance with the publication policy.

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